CLINAM

European Foundation for Clinical Nanomedicine

14/2023

Basel, October 8 – 11

SUMMIT PROCEEDINGS

Clinical Nanomedicine 2023: Fulfilling the Global Potential

Crossing the Horizon towards Novel Possibilities, Existing and Evolving Products, Technologies, Research and Strategies for Global Health

THE SUPPORTERS OF CLINAM'S GOALS AND STRATEGY

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Non Biological Complex Drugs working group

BIO PEOPLE
Denmark's Life Science Cluster

SPHN Swiss Personalized Health Network

FDA

ISNM International Society for Nanomedicine

Health Canada

EPMA

EF GCP

E·MRS

nanomedicine EUROPEAN TECHNOLOGY PLATFORM

eppm

University of Basel

European Commission

FAG Freiwillige Akademische Gesellschaft Basel

APV

DIA DEVELOP INNOVATE ADVANCE

EUROPEAN MEDICINES AGENCY SCIENCE MEDICINES HEALTH

imi innovative medicines initiative

CIMT Cancer Immunotherapy

EURO nanotox LETTERS

PAUL SCHERRER INSTITUT PSI

Empa Materials Science and Technology

Swiss Biotech Association

BNN

NIHS

Nanosafety Lab. CAS

NCL Alliance for Nanotechnology

Nanomedicines ALLIANCE

ESNAM European Society for Nanomedicine

NCNST

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THE SPONSORS OF THE CLINAM SUMMIT

EXHIBITORS 2023

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EDITORIAL

CLINAM – **Back to the Roots**

Welcome to the 14th edition of the European Summit for Clinical Nanomedicine and related fields!

The coronavirus pandemic has instantly changed our lives in many ways. Mask-shadowed faces, stopping to shake hands when meeting and a distance in groups by 2 meters became the new standard. We experienced that most conferences and seminars were only virtual. Even always being online with zero mistakes during the three days of the CLINAM Summit of 2022 left some qualms: Science was excellent, meeting in Zoom rooms was almost authentic, and even some good humour, such as "let's make the best of it". However, how many personal bonds were made? How many projects were initiated by this virtual Summit? How many friendships were renewed by a glimpse in each other's eyes?

Since 2007, CLINAM's goal has been to invite excellent speakers from all generations, bringing them together to discuss and bring knowledge forward. A summit to provoke people to individually speak to each other in a great atmosphere, shaping new projects and making bonds for cooperation.

Soon after the summit, we received many letters in which participants congratulated, yet in most cases, added that they deplored that it was a virtual meeting. They wrote us that they expect that we will soon offer again a face-to-face meeting.

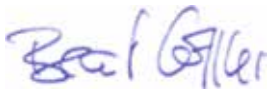
After the pandemic, in 2023 CLINAM had practically no funds for such a summit. When Novartis offered to make use of their magnificent auditorium, we decided to have this summit on this Campus in October. We started out and wrote a programme and then started to find funds. It was a run for everything, and finally, we found great helping sponsors; we met all hurdles of the size of the halls, since we had now instead of five halls to conduct the meeting in two halls in a very dense mode.

We have expected the usual 5-8 exhibitors. Instead, 20 parties have applied, and fifteen of them will be in the Foyer. Instead of the expected 50 posters, we have received 130 posters (to be presented in A3 format and online) and now have 110 in the foyer, where the catering and exhibition will also be.

We are overwhelmed by the understanding, cooperation, and the will of the community of speakers and poster presenters to make this summit a success.

In the last years, we often addressed our participants as "Dear CLINAM Family." Today, we say it again and thank you all cordially for sharing these three days. We have done everything possible to create a well-being atmosphere for every participant.

We look forward to meeting you in person again. And welcome those who come for the first time to CLINAM!



Dr. med. h.c. Beat Löffler, MA
CEO of the CLINAM-Foundation

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CURRICULA VITAE SPEAKERS

Khuloud Al-Jamal

Chair of Drug Delivery & Nanomedicine, King's College London

I am a Chair of Drug Delivery & Nanomedicine and Head of Medicines Developments at the Institute of Pharmaceutical Science, King's College London. I completed my PhD at the Centre for Drug Delivery Research and postdoctoral training at the Nanomedicine Lab, The School of Pharmacy, University of London (2000-2010). I undertook my pre-registration training at University College London Hospitals (2005). I joined KCL as a Lecturer in 2011 and was promoted to Professor in 2016. My research focusses on developing nanomedicines to improve treatment of neurological diseases and cancer. I published over 150 research articles with H-index 50. I was named one of the World's Top 2% researchers (2022) according to Stanford University analysis.

I am a recipient of the Royal Pharmaceutical Society of Great Britain Science Medal, Maplethorpe Fellowship for the promotion of pharmaceutical education and excellence in research, BBSRC New Investigator Award, the Controlled Release Society Nanomedicine Young Investigator Award. I am a three-time winner of the Wellcome Trust Science Image Awards. I am an editorial board member for Journal of Controlled Release, Biomaterials Science, Scientific Reports, MedBioMed and Journal of Drug Targeting. I am a Fellow of Royal Society of Chemistry and The Royal Pharmaceutical Society.

RECENT PUBLICATIONS

- Qin Y, Walters AA, Rouatbi N, Wang JT, Abdel-Bar HM, Al-Jamal KT*. Evaluation of a DoE based approach for comprehensive modelling of the effect of lipid nanoparticle composition on nucleic acid delivery. Biomaterials. 2023 Aug;299:122158. doi: 10.1016/j.Biomaterials.2023.122158. Epub 2023 May 15.
- Qin Y, Walters AA, Al-Jamal KT*. Plasmid DNA cationic non-viral vector complexes induce cytotoxicity-associated PD-L1 expression up-regulation in cancer cells *in vitro*. Int J Pharm. 2023 Jan 25;631:122481. doi: 10.1016/j.ijpharm.2022.122481.
- Walters AA*, Santacana-Font G, Li J, Routabi N, Qin Y, Claes N, Bals S, Wang JT and Al-Jamal KT*. (2021) Nanoparticle Mediated in Situ Molecular Reprogramming of Immune Checkpoint Interactions for Cancer Immunotherapy. ACS Nano, doi.org/10.1021/acsnano.1c04456
- Abdel-Bar HM, Walters AA, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, Osman R, Wang J, Al Jamal KT*. (2021) An "Eat me" Combinatory Nano-formulation for Systemic Immunotherapy of Solid Tumours. Theranostics. doi:10.7150/thno.56936; doi:10.7150/thno.56936.
- Abdel-Bar H, Walters Adam, Wang JT-W, Al-Jamal KT* (2021) Combinatory Delivery of Etoposide and siCD47 in a Lipid Polymer Hybrid Delays Lung Tumor Growth in an Experimental Melanoma Lung Metastatic Model. Advanced Healthcare Materials. doi: 10.1002/adhm.202001853.

Christoph Alexiou

Assistant Medical Director, Else Kröner-Fresenius-Foundation-Professorship, Head Section of Experimental Oncology and Nanomedicine (SEON), Universitätsklinikum Erlangen.

Prof. Dr. Christoph Alexiou, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the Universityhospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the Universitätsklinikum Erlangen. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF) and Bavarian State Ministry of the Enviroment and Consumer Protection and is a member of the Executive Board of the European Technology Platform for Nanomedicine (ETPN). His research is addressing the emerging fields of Diagnosis, Treatment and Regenerative Medicine using magnetic nanoparticles and the translation from basic research into clinical trials. He received for his research several national and international renowned awards.

RECENT PUBLICATIONS

- Genç H, Cianciosi A, Lohse R, Stahlhut P, Groll J, Alexiou C, Cicha I, Jüngst T: Adjusting Degree of Modification and Composition of gelAGE-Based Hydrogels Improves Long-Term Survival and Function of Primary Human Fibroblasts and Endothelial Cells in 3D Cultures. Biomacromolecules 24:1497-1510, 2023
- Vogel P, Rückert MA, Friedrich B, Tietze R, Lyer S, Kampf T, Hennig T, Dölken L, Alexiou C, Behr VC: Critical Offset Magnetic PArticle SpectroScopy for rapid and highly sensitive medical point-of-care diagnostics. Nature Communications 13:7230, 2022
- Stein R, Pfister F, Friedrich B, Blersch PR, Unterweger H, Arkhypov A, Mokhir A, Kolot M, Alexiou C, Tietze R: Plasmid-DNA Delivery by Covalently Functionalized PEI-SPIONs as a Potential 'Magnetofection' Agent. Molecules 27:7416, 2022
- Scarletti M, Park H, Wirth J, Englisch S, Eigen A, Drobek D, Vivod D, Friedrich B, Tietze R, Alexiou C, Zahn D, Zubiri B, Spiecker E, Halik M: The remediation of nano-/microplastics from water. Mat. Today 48: 38-46, 2021
- Blümler P, Friedrich RP, Pereira J, Baun O, Alexiou C, Mailänder V: Contactless Nanoparticle-Based Guiding of Cells by Controllable Magnetic Fields. Nanotechnology, Science and Applications 14: 91–100, 2021

Theresa (Terry) Allen

M, PhD, FRSC, FCRS

Dr. Allen is Professor Emerita, retired a decade ago after a 40+ year career in drug discovery and drug delivery. Career highlights include research into the development of long-circulating liposomes (Doxil), ligand-targeted liposomes and ligand-targed LNPs. She continues to be active in consulting and strategic advising. Currently, she is a visiting professor at UBC, the chair of the Research Advisory Committee of the Nanomedicines Innovation Network (NMIN) and an organizer of LRD 2022.

CITATION CLASSICS:

- Drug delivery systems: entering the mainstream # of citations 5261; TM Allen, PR Cullis Science 303 (5665), 1818-1822, 2004
- Liposomal drug delivery systems: from concept to clinical applications # of citations 4186; TM Allen, PR Cullis Advanced drug delivery reviews 65 (1), 36-48, 2013
- Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. # of citations 2125;D Papahadjopoulos, TM Allen, A Gabizon, E Mayhew, K Matthay,

...Proceedings of the National Academy of Sciences 88 (24), 11460-11464, 1991

- Ligand-targeted therapeutics in anticancer therapy # of citations 2113; TM Allen, Nature Reviews Cancer 2 (10), 750-763, 2002
- Liposomes containing synthetic lipid derivatives of poly (ethylene glycol) show prolonged circulation half-lives *in vivo* # of citations 1792;TM Allen, C Hansen, F Martin, C Redemann, A Yau-YoungBiochimica et Biophysica Acta (BBA)-Biomembranes 1066 (1), 29-36, 1991

Marianne Ashford

Advanced Drug Delivery, Pharmaceutical Sciences , AstraZeneca

Marianne Ashford, PhD, is a Senior Principal Scientist in a global role in Advanced Drug Delivery Department within Pharmaceutical Sciences at AstraZeneca. Marianne is responsible for applying drug delivery approaches which enable the progression of innovative medicines and is working to enable novel targets through intracellular delivery of new modalities such as nucleic acid based drugs.

Marianne has been instrumental in introducing nanomedicines to improve therapeutic index into the AstraZeneca Oncology clinical portfolio. She has initiated several collaborations and the building of the internal capability in nanomedicines, drug targeting and intracellular delivery receiving several internal awards for this work.

Previously Marianne led a Preformulation and Biopharmaceutics Group which was responsible for influencing candidate drug design from a product perspective and providing support across the portfolio in solid state science and biopharmaceutics.

Marianne has also held project management roles leading pharmaceutical teams and influencing the global product strategy of various AstraZeneca oncology compounds.

Marianne has published over 65 peer reviewed papers and reviews, six book chapters and holds several patents. She has delivered invited talks, keynotes and plenaries in nanomedicine and advanced drug delivery worldwide. Marianne holds Honorary Professor roles at the Universities of Nottingham and Manchester and is a Fellow of the Controlled Release Society. Marianne has served on numerous academic and industrial scientific committees and advisory boards in the field of drug delivery.

Marianne is passionate about using her scientific knowledge and experience to improve therapies for patients and applying drug delivery science to enable medicines of the future.

RECENT PUBLICATION

- Designing Highly Stable Poly(sarcosine)-Based Telodendrimer Micelles with High Drug Content Exemplified with Fulvestrant
- Qing Yu, Richard M. England, Anders Gunnarsson, Robert Luxenhofer, Kevin Treacher, and Marianne B. Ashford, Macromolecules 2022 55 (2), 401-412
- Highway to success Highway to Success—Developing Advanced Polymer Therapeutics
- Marianne B. Ashford,Richard M. England,Nadim Akhtar, Advanced Therapeutics, March 2021
- Patterson, C.M., Balachander, S.B., Grant, I. et al. Design and optimisation of dendrimer-conjugated Bcl-2/xL inhibitor, AZD0466, with improved therapeutic index for cancer therapy. Commun Biol 4, 112 (2021).

Anthony Attama

Ph.D, FAS, FPCPharm., FPSN, JP.

Anthony Amaechi Attama is a Professor of Pharmaceutics, University of Nigeria, Nsukka since 2009, where he currently holds the position of the Director, Institute for Drug, Herbal Medicine and Excipient Research and Development. He is the immediate past Dean, Faculty of Pharmaceutical Sciences and former Director, Education Innovation Unit of the University. Prof. Attama has research focus on novel drug delivery systems that has attracted many research grants and awards. He was a visiting Professor at the Hebrew University of Jerusalem, Israel and a fellow of the Alexander von Humboldt Foundation. His postdoctoral programme in Germany resulted in a grant to start a nanomedicines laboratory in Nigeria in 2008. He has developed novel methods of enhancing drug incorporation in lipid nanoparticles involving mixed crystals and mixtures of crystals using natural biodegradable lipids which have resulted in the development of several nanomedicines for malaria and other diseases, including two patents, one in Nigeria and the other in Australia. He has locally developed a novel ex vivo method and modified an equation for assessing the mucoadhesive properties of drug formulations. Prof. Attama has successfully supervised 23 PhD and 43 master's degree students in pharmaceutical sciences. He has 317 publications in research articles and book chapters, and a high Google Scholar Citation of 5495, h-index 33 and i10-index 117. He has served as external examiner and assessor for 12 universities and is on the Editorial Board of more than 9 journals. He is an alumnus of Global Young Faculty (GYF I, 2009-2011) that developed a framework for 'Optimal Healthcare', organised by Stiftung Mercator, Volkswagen Foundation and the University Alliance Ruhr. He is a registered pharmacist and a fellow of the Nigerian Academy of Science, a fellow of West African Postgraduate College of Pharmacists, fellow, Pharmaceutical Society of Nigeria and Member, Institute for Public Analysts of Nigeria (IPAN).

Lajos (Lou) P. Balogh

Editor-in-Chief, Precision Nanomedicine

Dr. Lajos (Lou) P. Balogh, Ph.D., is the Editor-in-Chief of Precision Nanomedicine, the official journal of the European Foundation for Nanomedicine (CLINAM). Lou and 44 other Board members are on the list of the World's top 2% of Scientists (DOI: 10.17632/btchxktzyw.5). Balogh published more than 200 scientific papers, gave>230 invited lectures, and was awarded 12 patents. His publications have been cited over 9300 times (22 articles with > 100 citations, 12 >200, and two over 1000 times). He is considered an international expert in Nanomedicine and scholarly publications. Dr. Balogh is one of the five American Society for Nanomedicine Founders and a Fulbright Scholar. He serves on the Science Board of several international and US national organizations. Lou has held faculty positions at the Kossuth University Debrecen, Hungary, the UMass Lowell, the U-of-M, Ann Arbor, MI, and the Roswell Park Cancer Institute, Buffalo, NY. For more information, see http://www.linkedin.com/in/lajosbalogh and Google Scholar: https://scholar.google.com/citations?user=jbDlqSwAAAAJ&hl=en

RECENT PUBLICATIONS

- Balogh, LP, Are we there yet? Precis. Nanomed. 2023 March; 5(1):1007-1012, https://doi.org/10.33218/001c.74009

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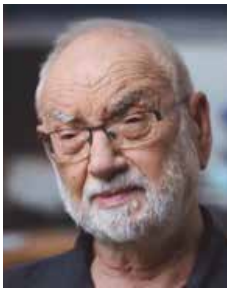

Yechezkel Barenholz

PhD

Professor Emeritus Yechezkel Barenholz is at the Hebrew-University faculty since 1968, received Ph.D. in 1971, became a Professor in 1981. During 1973 to 2006 he was a visiting Professor and teaching, at the University of Virginia , He was also teaching at leading Universities world-wide (US, UK, Italy, Denmark, Japan, China, Australia). Barenholz current research focuses on development of liposome-based nano-drugs, and vaccines as best exemplified by the anticancer drug Doxil®, the first nano-drug approved by the FDA (November 1995) and generic Doxil on 2021 over 1,000,000 cancer-patients treated so-far world-wide. The generic Doxil is produced in Israel by Ayana Pharma (one of the companies founded by Barenholz, It is sold in the US and Israel. Recently Barenholz initiated a program based on liposomal angiotensin receptor blocking agents (ARBs) on “normalizing tumor microenvironment to improve therapeutic efficacy of anticancer drugs, nanodrugs and immune check point inhibitors that seems highly promising. The other three most advanced Barenholz’s and his team liposomal nano-drugs developed are: MMII liposomes for osteoarthritis treatment Which ended phase II with very encouraging results leading the way to Phase III clinical studies. Nano-Mupirocin against resistant-bacteria is ready for clinical trials; and the local anesthetic LBL-100 is now in the scale-up stage in preparation for clinical trials. Development of vaccines based on genetically engineered defined protein antigens as well as on “synthetic Biology” in both approaches the vaccines require the use liposomes and lipid nanoparticles based on novel cationic lipids as a delivery systems and adjuvant. Barenholz success stems from his major achievements in membrane biophysics including the concept of membrane “fluidity” and nanotechnology. Barenholz is author in >435 publications (cited >39,000 times, h-index 98), Based on Standard evaluation Barenholz is among the best 5000 scientists among almost 1,000,000 scientists. Barenholz research was supported by major grant agencies (NIH, EU, ISF, BSF, GIF Israel Ministry of Health, Israel Ministry of Science and others from various industries.). He is a co-inventor in >55 granted patents, half of them licensed, and founder of six start-ups: NasVax (now Therapix), Moebious, PolyPid, LipoCureRX, Ayana Lipovation and Vivac). He received many national and international awards related to Nanomedicine, Nanotechnology and drug delivery including the 2012 CRS founder award and the 2020 Israeli Prime Minister EMET Prize for Exact Sciences (nanotechnology).

Among Barenholz many contributions are: The “Barenholz Prize” to encourage excellence and innovation of Israeli Ph.D. students in Applied Sciences, being a founder and the head of the steering committee of the new BioMed-.MBA program at the Hebrew-University School of Business-administration, On 2021 this School, the Israel Medical Association and 8400 organization together with Barenholz initiated a program of Directors with expertise in BioMed aim to serve in boards of BioMed/biotech industries .All the latter aim at building the network needed for enabling a thriving Israeli BioMed ecosystem. Professor Barenholz is married to Dr Hanna Barenholz together they have 4 daughters and 12 grandchildren.

Matthias Barz

Professor for BioPharmacy, Head of the BioTherapeutics Division, LACDR

Matthias Barz studied chemistry at the Johannes Gutenberg-University Mainz (Germany) and Seoul National University (South Korea). After graduation in 2006 he received his PhD in polymer chemistry from the Johannes Gutenberg-University Mainz (Germany) working under the supervision of Prof. R. Zentel. Afterwards he worked in the laboratories of Dr. Maria J. Vicent at the CIPF (2010-2011) and Prof. T. Kirchhausen at Boston Children’s Hospital, Harvard Medical School (2011-2013) before returning to Mainz.

In 2013 he became independent junior research group leader and started his habilitation at the Institute of Organic Chemistry at the Johannes Gutenberg-University Mainz (Germany). After having received the venia legendi for organic chemistry in 2016 he joined the Leiden Academic Center for Drug Research (LACDR) as Full Professor for Biotherapeutic Delivery in 2020 and became Head of the Division of BioTherapeutics in 2022. His research focusses on the development of functional nanoparticles base on polypept(o)ides, polymers combining poylpeptides with polyppetoids, for diagnosis and therapy. For his independent research he received numerous awards, including the highly prestigious Chemiedozentenpreis (Young Faculty Award of the German Industry Fonds), the Young Faculty Scholarship of the Dr. Hermann-Schnell-Foundation, (German Chemical Society, Devision of Macromolecular Chemistry) and was named ACS PMSE Young Investigator in 2018.

RECENT PUBLICATIONS

- Birke A, Ling J*, Barz M*. Polysarcosine Containing Copolymers: Synthesis, Characterization, Self-Assembly, and Applications. Prog. Polym. Sci. 2018, 81, 163-208.
- Bauer TA, Schramm J, Fenaroli F, Siemer S, Seidl CI, Rosenauer C, Bleul R, Stauber R, Koynov K, Maskos M, Barz M*. Complex Structures Made Simple - Continuous Flow Production of Core Cross-Linked Polymeric Micelles for Pro-Drug-Delivery. Adv. Mat. 2023, 2210704.
- Dal NJK, Schäfer G, Thompson AM, Schmitt S, Redinger N, Alonso-Rodriguez N, Johann K, Ojong J, Wohlmann J, Best A, Koynov K, Zentel R, Schaible UE, Griffiths G, Barz M, Fenaroli F. Π-Π interactions stabilize PeptoMicelle-based formulations of Pretomanid derivatives leading to promising therapy against tuberculosis in zebrafish and mouse models. J. Control. Release 2023, 354, 851-868.
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Mario Benn

Group Leader

Dr. Mario C. Benn, a distinguished dual-degree holder (Dr. med. vet. & Dr. sc. ETH), leads a dynamic research group within Prof. Viola Vogel's Laboratory of Applied Mechanobiology at the Institute of Translational Medicine, ETH Zurich. After gaining broad-spectrum clinical experience in human medicine during his work as a paramedic with the German Red Cross, his academic journey began at Justus-Liebig University Giessen, Germany, where he secured his degree in Veterinary Medicine. Pursuing his passion for surgical oncology, he underwent specific training at world-renowned animal clinics in the University of Tennessee, USA, and Ottawa, Canada.

Dr. Benn carried out his Doctoral research in Veterinary Medicine at the Musculoskeletal Research Unit (MSRU) and the Center for Applied Biotechnology and Molecular Medicine (CABMM), Vetsuisse Faculty, University Zurich. His interdisciplinary work involved pre-clinical *in vivo* research including orthopaedic and soft tissue surgery in varying animal testing models, focusing on investigation of musculoskeletal regeneration. Concurrently, he explored the integration of *in vitro* tissue engineering approaches, cultivating a profound interest in mechanobiological cues.

During his subsequent Doctorate in Science at the Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Dr. Benn further developed a pioneering *in vitro* 3D µTissue platform. This platform elucidates the intricate dynamics of de novo tissue growth and maturation, shedding light on the regulation by tensional state, cell phenotypes, and extracellular matrix composition with an unprecedented spatiotemporal resolution.

Dr. Benn is deeply committed to advancing mechanomedicine, aiming to refine, reduce, and replace animal experiments. His work builds a critical bridge between *in vitro* research and clinical application, marking significant strides towards the development of novel mechanomedicine strategies.

RECENT PUBLICATIONS

- Benn MC, Pot SA, Moeller J, Yamashita T, Fonta CM, Lickert S, Orend G, Kollmannsberger P, Vogel V. How the mechanobiology orchestrates the iterative and reciprocal ECM-cell cross-talk that drives microtissue growth. Science Advances. AAAS; 2023. https://doi.org/10.1126/sciadv.add9275
- Pot SA, Lin Z, Shiu J, Benn MC, Vogel V. Growth factors and mechano-regulated reciprocal crosstalk with extracellular matrix tune the keratocyte–fibroblast/myofibroblast transition. Scientific Reports. Nature Research; 2023. https://doi.org/10.1038/s41598-023-37776-9
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- Flück M, Kasper S, Benn MC, Clement Frey F, von Rechenberg B, Giraud MN, Meyer DC, Wieser K, Gerber C. Transplant of Autologous Mesenchymal Stem Cells Halts Fatty Atrophy of Detached Rotator Cuff Muscle After Tendon Repair: Molecular, Microscopic, and Macroscopic Results From an Ovine Model. The American Journal of Sports Medicine. SAGE Publications; 2021. https://doi.org/10.1177/03635465211052566
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Gerrit Borchard

Professor, President of the Schoolof Pharmaceutical Sciences, University of Geneva

Gerrit Borchard is a licensed pharmacist and obtained his Ph.D. in pharmaceutical technology from the University of Frankfurt (Germany). After holding several academic positions at Saarland (Germany) and at Leiden University (The Netherlands), he joined Enzon Pharmaceuticals, Inc. (USA) as Vice President Research. In 2005, he was appointed Full Professor of Biopharmaceutics at the University of Geneva (Switzerland), and in 2015, he was an invited professor at Graz University (Austria). He was president of the Swiss Academy of Pharmaceutical Sciences (SAPhS) from 2014 to 2022 and serves as president of the School of Pharmaceutical Sciences at the University of Geneva since 2022. Prof. Borchard's research interests lie in the fields of biopharmaceutical sciences, nanomedicine and vaccine development. He has published more than 150 scientific papers (13052 citations, h-factor 57) and 23 book chapters, he edited two books, is named as inventor on 10 patents, and gave over 230 invited lectures.

In 2012 he joined the Non-Biological Complex Drugs (NBCD) working group hosted at Lygature (Utrecht, The Netherlands), joining its steering committee in 2015. He joined the External Advisory board of the EU-Nanotechnology Characterization Laboratory (EU-NCL) in 2016 and was Chair of the NBC working party at the European Directorate for the Quality of Medicines & Health Care (EDQM) from 2016 to 2023. In November 2022 he was appointed Chair of the mRNA Vaccine working party at EDQM.

Having been exposed to a variety of different work environments, he became fluent in the Dutch, English, French and German languages. Being an enthusiastic long-distance runner, he loves to roam the trails and by-roads of the Swiss mountains.

RECENT PUBLICATIONS

- M. Petrovic, S. Tankov, M. Kiening, Y. Chakradhar, D. Rafael P.R.R. Walker, G. Borchard and O. Jordan, How to outsmart the cold tumor microenvironment: Design of STING ligand nanoparticles for improved cancer immunotherapy. OpenNano 12 (2023) 100157. doi: 10.1016/j.onano.2023.100157
- C. Marques, M.J. Javad, C. Marets, A. Oudot, R. Safavi-Sohi, M. Guillemin, G. Borchard, O. Jordan, L. Saviot and L. Maurizi, Identification of the Proteins Determining the Blood Circulation Time of Nanoparticles, ACS Nano 17 (2023) 12458–12470. doi: 10.1021/acsnano.3c02041
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- A. Peletta, E. Prompetchara, K. Tharakhet, P. Kaewpang, S. Buranapraditkun, T. Techawiwattanaboon, T. Jbilou, P. Krangvichian, S. Sirivichayakul, S. Manopwisedjaroen, A. Thitithanyanont, K. Patarakul, K. Ruxrungtham, C. Ketloy and G. Borchard, Toward a more equitable SARS-CoV-2 vaccine distribution: a cationic liposome formulation enhances the immunogenicity of a SARS-CoV-2 DNA vaccine candidate. Vaccines 9 (2021) 874. doi: 10.3390/vaccines9080874.

Donald Bruce

Dr Donald Bruce, Managing Director,
Edinethics Ltd.,
1 Peinsoraig, Uig, Isle of Skye, IV51 9YD, Scotland, UK
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Dr Donald Bruce holds doctorates in chemistry and in theology. He is managing director of the independent consultancy Edinethics Ltd., working on the ethics and public engagement of emerging technologies. After working 15 years as a chemist in nuclear energy research, risk regulation, and energy policy, he became Director of the Church of Scotland's Society, Religion and Technology Project (SRT) from 1992-2007. In this role he did pioneering ethical assessment of many emerging technologies including GM crops and animals, cloning and stem cells. He has worked extensively on nanomedicine and related technologies from 2003 in EC projects Nano2Life, NanoBio-Raise, NanoMedRound, Ethentech (on human enhancement), and NanoAthero. An integral part of this work has been in developing and writing public engagement tools with Perry Walker formerly of the New Economics Foundation. He helped develop the Democs/Decide card games and Open-up argument map concepts, on such issues as GM crops, synthetic biology, human enhancement, and stem cells for therapy and for toxicity testing. He created a Democs game on nanomedicine for the NanoAthero EC FP7 project on nanodevices to detect and treat atherosclerosis. He has a diverse range of ethical research interests, including the ethics of genome editing in food animals and in humans, ethics and livestock breeding in the context of global warming and changing food habits (EC H2020 BovReg project), the role of PGD in addressing genetic conditions, end of life issues, and ethical investment. He is a member of the UK Animals in Science Committee, which advises the UK government on animal research. He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council, the Public Affairs advisory group of Biotechnology Research Council, and of the Advisory Board of the Institute of Nanotechnology. He advises the Scottish Episcopal Church on ways to work towards net zero carbon emissions in diverse rural contexts. He and his wife Ann live in the beautiful Isle of Skye in Scotland where they run his family croft, with a flock of Cheviot hill sheep.

Thomas Bruckdorfer

CSO & VP Business Development

I was born on April 1st in 1963 in the town Roth in Bavaria, Germany. To school i went in Schwabach and studied chemistry in Erlangen. All towns are around Nuremberg in Northen Bavaria in Germany. Aftern university I finished an MBA study and after a few positons at various companies I joind the fonder of Iris Biotech GmbH in 2002, in order to build and raise the company.
In 2013 I co-founded the company Iris Biotech Laboratories GmbH, located in Willstätt, Germany, which serves as a maunufacturing unit of Iris Biotech producing unusual building blocks and linkers.
In spring this year, in March 2023, I co-founded the company B4 PharmaTech GmbH, located in Berlin, which can produce biomolecules such as membrane enzymes, antibodies and antibody formats (e.g. nanobodies), including points of connectivity suitable for Linkerology.
I am member of the Brain-8-Network of the Biopark Regensburg, supporting with coaching and consulting within the topics chemistry, biotechnology, pharma, diagnostics, and analytics.

RECENT PUBLICATIONS

- co-autor of some 20 publications about peptide synthesis, PEGylation and related topics

Bastiaan Buddingh

Business Development Manager
Nanomedicines

I am from the Netherlands, where I attended Radboud University and obtained an MSc in Molecular Life Sciences, focusing on nanomedicine and bioconjugation. As part of my degree I spend a semester doing research in the lab of Prof. Carolyn Bertozzi at UC Berkeley, who won the Nobel prize in 2022 for Bioorthogonal chemistry. I got my Ph.D. degree from Eindhoven University of Technology, working with lipid- and polymer-based (nano/micro)particles. This was followed by several years in Paris (France) working as a post-doc and as a Research Scientist for a start-up that develops microparticles for diagnostic applications. Currently, I am both Senior Scientist and Business Development Manager at Ardena for their Nanomedicines business unit in the Netherlands, where I combine my broad experience in nanoparticles and bioconjugation strategies to help biotech companies bring their investigational drugs to the clinic.

Dirk Bumann

Professor

I was born in Berlin, Germany, in 1967. I studied both chemistry and biology at the Free University Berlin. I finished my Masters in 1989 (Chemistry) and 1992 (Biology). From 1990 to 1994, I did my PhD under supervision of Dieter Oesterhelt at the Max-Planck-Institute for Biochemistry in Martinsried near Munich, Germany. From 1995 to 1997, I was a post-doctoral researcher at the Marine Biology Laboratory, Woods Hole, USA, in the lab of Alan Kuzirian. From 1997 to 2004 I was a team leader at the Max-Planck-Institute for Infection Biology, Berlin, Germany, in the department of Thomas F. Meyer. From 2004 to 2007 I was an independent junior group leader at Hannover Medical School, Hannover, Germany. In 2007 I became Associate Professor it the Biozentrum, University of Basel, Switzerland, and was promoted to full professor in 2014. From 2007 to 2013, I was also a guest professor in pharmaceutical biotechnology at University of Freiburg, Germany. the In 2020, I became deputy director of the National Competence Center for Research "AntiResist", a network of ~20 research groups with funding for 12 year

RECENT PUBLICATIONS

- Tissue compartmentalization enables Salmonella persistence during chemotherapy. Li J, Claudi B, Fanous J, Chicherova N, Cianfanelli FR, Campbell RAA, Bumann D. Proc Natl Acad Sci U S A. 2021 118:e2113951118.
- Outer membrane permeability: Antimicrobials and diverse nutrients bypass porins in Pseudomonas aeruginosa. Ude J, Tripathi V, Buyck JM, Söderholm S, Cunrath O, Fanous J, Claudi B, Egli A,

Schleberger C, Hiller S, Bumann D. Proc Natl Acad Sci U S A. 2021 118:e2107644118.
- Host resistance factor SLC11A1 restricts Salmonella growth through magnesium deprivation. Cunrath O, Bumann D. Science. 2019 366:995-999.

Luigi Calzolai

Project leader

I was born in a town close to Florence and I went to the University of Florence for a MS in Chemistry and University of Siena for a PhD in chemistry. After a Postgraduate Research at the University of California, Davis, I joined, in 1998, the Swiss Institute of Technology in Zurich, in the laboratory of the then Nobel laureate Kurth Wuthrich, where I determined the three dimensional structure of prion proteins responsible of neurological disorders, such as Mad Cow Disease. In 2007, I moved to the University of Kent (UK) as Senior Lecturer in biochemistry. In 2008 I joined the Joint Research Centre of the European Commission where my research focuses on the development of methods for the characterization of nanomedicines and biotherapeutics.

RECENT PUBLICATIONS

- Simon Jr, C. G., et al. "Orthogonal and complementary measurements of properties of drug products containing nanomaterials." Journal of Controlled Release 354 (2023): 120-127.
- Guerrini, Giuditta, et al. "Characterization of nanoparticles-based vaccines for COVID-19." Nature Nanotechnology 17.6 (2022): 570-576.
- Guerrini, Giuditta, et al. "Monitoring anti-PEG antibodies level upon repeated lipid nanoparticle-based COVID-19 vaccine administration." International Journal of Molecular Sciences 23.16 (2022): 8838.
- De Gasparo, Raoul, et al. "Bispecific IgG neutralizes SARS-CoV-2 variants and prevents escape in mice." Nature 593.7859 (2021): 424-428.
- Caputo, Fanny, et al. "Measuring particle size distribution of nanoparticle enabled medicinal products, the joint view of EUNCL and NCI-NCL. A step by step approach combining orthogonal measurements with increasing complexity." Journal of Controlled Release 299 (2019): 31-43.

Patrick Couvreur

Patrick COUVREUR is Full Professor of Pharmacy at the Paris-Sud University, member of the Académie des Sciences and holder of the chair of "Innovations Technologiques" (2009-2010) at the prestigious « Collège de France ». He is appointed as a Senior Member of the "Institut Universitaire de France" since 2009. He is also the recipient of an "ERC Advanced Grant" (2010-2015) and of an "ERC Proof of Concept" (2015-2016). He has hold many important national and international academic positions as Director of the UMR CNRS 8612 (a CNRS associated department gathering together more than 120 researchers in the drug delivery field), Director of the Doctoral School "Therapeutic Innovation" (over 300 PhD students at Paris-Sud University), founder member of the pole of competitivity MEDICEN, Extraordinary Professor at the University of Louvain (Belgium), member of the board of governors of many international scientific organizations (ie. The International Pharmaceutical Federation FIP, the Controlled Release Society CRS, the European Federation of Pharmaceutical Scientists, APGI etc.). He is the chair of the LS-7 panel of the European Research Council (ERC consolidator grant) and has served in many scientific committees in France (Institut Pasteur, ENS Cachan, Academic Council of Paris-Saclay University, Scientific Committee of the Région Centre, Comité National of the CNRS, Conseil National des Universités CNU etc.).
Prof Patrick COUVREUR's contributions in the field of drug delivery, nanomedicine and drug targeting are highly recognized around the world with more than 520 peer review research publications (Google Scholar H-index 120 and Thomson Reuters H-index 89), some of them in prestigious journals (Nature Nanotechnology, Nature Materials, Nature Communications, Proceedings of the National Academy of Sciences, Angewandte Chemie, Cancer Research, Journal of the American Chemical Society etc.). His research is interdisciplinary, aiming at developing new nanomedicines for the treatment of severe diseases. This research is at the interface between Physico-Chemistry of Colloids, Polymer Chemistry, Material Science, Cellular and Molecular Biology and Experimental Pharmacology.
Patrick COUVREUR's research has led to the funding of two start-up companies (Bioalliance and Medsqual). Bioalliance (now ONXEO) entered the stock market in 2005 and a nanomedicine invented in Couvreur's lab is currently finishing phase III clinical trial for the treatment of the hepatocarcinoma.
The major scientific contribution of Patrick COUVREUR to the Pharmaceutical Sciences is also recognized by numerous international (the "2004 Pharmaceutical Sciences World Congress Award", the prestigious "Host Madsen Medal", the "European Pharmaceutical Scientist Award" of the European Federation of Pharmaceutical Sciences, the European Inventor Award 2013 given by the European Patent Office, the Speiser's award from ETH and the Higuchi Award 2015, Japan) and national awards (The Grand Prix de l'Innovation of « L'USINE NOUVELLE » 2008, the "Prix Galien 2009" and the "Médaille de l'Innovation 2012 of the CNRS). His appointment as a member of eight academies (Académie des Sciences, Académie des Technologies, Académie Nationale de Médecine and Académie Nationale de Pharmacie in France, as well as the Académie Royale de Médecine in Belgium, the Royal Academy of Pharmacy in Spain, the United States National Academy of Medicine and the United States National Academy of Engineering) is another recognition of major scientific and scholarly contributions of Patrick COUVREUR.

Daan Crommelin

Professor-emeritus

Daan J.A. Crommelin, Ph.D.
Prof. Daan Crommelin is professor emeritus from the Department of Pharmaceutics at Utrecht University. Until December 2011 he was scientific director of the Dutch Top Institute Pharma – a public private partnership - in Leiden. He was adjunct professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is co-founder of OctoPlus (1995), a Leiden based company specialized in the development of pharmaceutical (mainly protein based) product formulations and advanced drug delivery systems. He published over 380 scientific articles, many book chapters and edited a number of books among those 6 (between 1995 and 2023) editions of the textbook 'Pharmaceutical Biotechnology: Fundamentals and Applications'. He was Editor-in-Chief of the AAPS book series 'Advances in the Pharmaceutical Sciences'. He advises venture capital groups and acts as a consultant/expert witness for big pharma companies and SME's. He is past president of the European Federation of Pharmaceutical Sciences (EUFEPS)

and past vice-chair of the scientific advisory board of the European Innovative Medicines Initiative (IMI).
His research interests include pharmaceutical technology and physical pharmacy in general and more specifically formulation aspects of small and large molecule drug products. Over the years his interest has focused on: site specific drug delivery with colloidal-nanosized carrier systems/self-assembling systems (in combination with monoclonal antibodies), purification, characterization and stabilization of monoclonal antibodies and other pharmaceutical proteins, receptor-ligand interactions, parenteral systems for (sustained) release of small molecules, proteins and nucleic acids, pharmaceutical aspects of vaccines (adjuvanticity/antigen presentation and delivery), immunogenicity of pharmaceutical proteins.

RECENT PUBLICATIONS

- (Oude Blenke, E., Örnskov, E., Schöneich, C., Nilsson, G.A., Volkin, D.B., Mastrobattista, E., Almarsson, Ö., Crommelin, D.J.A.; The Storage and In-Use Stability of mRNA Vaccines and Therapeutics: Not A Cold Case; J. Pharm. Sci. (2023) 112 (2), pp. 386-403. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85142835395&doi=10.1016%2fj.xphs.2022.11.001&partnerID=40&md5=6736da6d235e93ff9da7b9874bdc22d7
- Schoenmaker, L., Witzigmann, D., Kulkarni, J.A., Verbeke, R., Kersten, G., Jiskoot, W., Crommelin, D.J.A.; mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability; Int. J. Pharmaceutics (2021) 601, art. no. 120586,. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85104283670&doi=10.1016%2fj.ijpharm.2021.120586&partnerID=40&md5=a4797b043387d4b85d2a46b3fe8d502a
- Crommelin, D.J.A., Anchordoquy, T.J., Volkin, D.B., Jiskoot, W., Mastrobattista, E.; Addressing the Cold Reality of mRNA Vaccine Stability; J. Pharm. Sciences (2021) 110 (3), pp. 997-1001. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85099496618&doi=10.1016%2fj.xphs.2020.12.006&partnerID=40&md5=c5230dfe325472be7695f10dfc05051d
- Crommelin, D.J.A., Volkin, D.B., Hoogendoorn, K.H., Lubiniecki, A.S., Jiskoot, W.; The Science is There: Key Considerations for Stabilizing Viral Vector-Based Covid-19 Vaccines; J. Pharm. Sciences (2021) 110 (2), pp. 627-634. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85097223005&doi=10.1016%2fj.xphs.2020.11.015&partnerID=40&md5=0cc25dc3d7905229ee2116195884e2a9
- Crommelin, D.J.A., van Hoogevest, P., Storm, G.; The role of liposomes in clinical nanomedicine development. What now? Now what?; J. Controlled Release (2020) 318, pp. 256-263. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85076672583&doi=10.1016%2fj.jconrel.2019.12.023&partnerID=40&md5=81d0e5b35466762f1727ae719c06c6f5text)

Pieter Cullis

Professor

Pieter R. Cullis, Professor, Department of Biochemistry and Molecular Biology, University of British Columbia. Dr. Cullis and co-workers have been responsible for fundamental advances in the development of nanomedicines employing lipid nanoparticle (LNP) technology for cancer therapies, gene therapies and vaccines. This work has contributed to five drugs that have been approved by the FDA, the EMA and other regulatory bodies. These include Onpattro (the first RNAi drug to receive regulatory approval) to treat the previously fatal hereditary condition transthyretin-induced amyloidosis (hATTR), as well as Comirnaty, the Pfizer/BioNTech COVID-19 mRNA vaccine.
Dr. Cullis has also co-founded eleven biotechnology companies that now employ over 400 people, has published over 400 scientific articles and is an inventor on over 100 patents. He co-founded two Canadian National Centre of Excellence networks, the Centre for Drug Research and Development (now AdMare) in 2004 and the NanoMedicines Innovation Network in 2019. Dr. Cullis has received many awards including the Order of Canada in 2021 and the Vin-Future Prize (Vietnam), the Prince Mahidol Award (Thailand), the Gairdner International Award (Canada) and the Tang Prize (Taiwan) in 2022. Professor Cullis was elected to be a Fellow of the Royal Society (London) in 2023

Ramin Darvari

Pharmaceutical Scientist

Ramin Darvari is a Research Fellow in Drug Product Design & Development group at Pfizer; contributing to the strategic and tactical planning for evaluation of external delivery technologies and internal delivery formulation & process development, with a focus on collaborative partner engagement. Ramin's expertise in particle engineering and matrix-based drug delivery systems have led to evaluation and development of variety of particle-based modalities & applications, including his role as the drug product project lead for Pfizer-BioNTech Covid-19 Vaccine. He is currently leading the mRNA/LNP drug product platform development at Pfizer.

Jon de Vlieger

Strategy Director at Lygature & Coordinator NBCD Working Group

Foundation Lygature
Non-Biological Complex Drugs Working Group

Jon de Vlieger obtained his doctoral degree in bio analytical chemistry from the VU University in Amsterdam. In 2011 he joined Lygature (former Top Institute Pharma), an independent not-for-profit organization based in the Netherlands that catalyzes the development of new medical solutions by driving public-private collaboration between academia, industry, and society. Dr. de Vlieger is director of business development at Lygature and a frequent guest lecturer on science & business topics related to public private partnerships. He coordinates several international public private partnerships, such as the European Lead Factory on early drug discovery and the Non Biological Complex Drugs Working Group on regulatory innovation. He serves as a board member at the Federation for Innovative Drug Research Netherlands. He is a co-editor of the book on NBCDs in the AAPS Advances in the Pharmaceutical Sciences Series, co-author on a series of key-papers related to regulatory challenges for NBCDs and publishes on the value of public-private partnerships in drug discovery and development.

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www.lygature.org
https://nbcds.info/

RECENT PUBLICATIONS

- The EU regulatory landscape of non-biological complex drugs (NBCDs) follow-on products: Observations and recommendations. Klein K, Stolk P, De Bruin ML, Leufkens HGM, Crommelin DJA, De Vlieger JSB.Eur J Pharm Sci. 2019 May 15;133:228-235. doi: 10.1016/j.ejps.2019.03.029. Epub 2019 Apr 3.

- A pragmatic regulatory approach for complex generics through the U.S. FDA 505(j) or 505(b)(2) approval pathways. Klein K, Borchard G, Shah VP, Flühmann B, McNeil SE, de Vlieger JSB.Ann N Y Acad Sci. 2021 Oct;1502(1):5-13. doi: 10.1111/nyas.14662. Epub 2021 Jul 22.
- Editorial: Public-Private Partnerships as Drivers of Innovation in Healthcare. de Vrueh RLA, de Vlieger JSB, Crommelin DJA.Front Med (Lausanne). 2019 May 31;6:114. doi: 10.3389/fmed.2019.00114. eCollection 2019.

Paolo Decuzzi

Professor and Senior Scientist

I am currently a Senior Scientist and Professor of Biomedical Engineering at the Italian Institute of Technology (IIT) in Genova where, in July 2015, I founded the Laboratory of Nanotechnology for Precision Medicine. I have been serving as an Associate Professor of Biomedical Engineering at The University of Texas Health Science Center (Houston, TX-USA) from 2007 to 2010 and Professor of Biomedical Engineering and Translational Imaging at the Methodist Hospital Research Institute (Houston, TX-USA), until July 2015. My Laboratory in IIT is involved in the rational design of multi-functional nanoconstructs for the treatment and imaging of cancer, cardiovascular and neurodegenerative diseases; the fabrication of microfluidic chips for the rapid screening of novel molecular and nano-based therapeutic agents; the development of multi-scale, hierarchical computational models for predicting the transport and therapeutic efficacy of nanoconstructs; as well as in the organization of dissemination activities at the interface between engineering and biomedical sciences. In this context, I have published over 200 peer-reviewed journal articles and book chapters, generated multiple patents and patent applications. My research activities have been funded by multiple organizations, including NCI, DOD, State of Texas, ESF, ERC, MSCA, and private enterprises, totaling over $15 million. I have been serving on review panels for the NIH, ERC, ESF and for several national Research Agencies.

RECENT PUBLICATIONS

- Palange, A.L., Mascolo, D.D., Ferreira, M., Gawne, P.J., Spanò, R., Felici, A., Bono, L., Moore, T.L., Salerno, M., Armirotti, A., Decuzzi, P. Boosting the Potential of Chemotherapy in Advanced Breast Cancer Lung Metastasis via Micro-Combinatorial Hydrogel Particles. (2023) Advanced Science, 10 (10), art. no. 2205223
- Gawne, P.J., Ferreira, M., Papaluca, M., Grimm, J., Decuzzi, P. New opportunities and old challenges in the clinical translation of nanotheranostics (2023) Nature Reviews Materials, in press
- Di Mascolo, D., Guerriero, I., Pesce, C., Spanò, R., Palange, A.L., Decuzzi, P. µMESH-Enabled Sustained Delivery of Molecular and Nanoformulated Drugs for Glioblastoma Treatment (2023) ACS Nano, in press
- Di Mascolo, D., Palange, A.L., Primavera, R., ...Grant, G.A., Decuzzi, P. Conformable hierarchically engineered polymeric micromeshes enabling combinatorial therapies in brain tumours. Nature Nanotechnology, 2021, 16(7), pp. 820–829
- Bedingfield, S.K., Colazo, J.M., Di Francesco, M., ...Decuzzi, P., Duvall, C.L. Top-Down Fabricated microPlates for Prolonged, Intra-articular Matrix Metalloproteinase 13 siRNA Nanocarrier Delivery to Reduce Post-traumatic Osteoarthritis. ACS Nano, 2021, 15(9), pp. 14475–14491

Neil Desai

PhD

Dr. Desai is the founder and Executive Chairman of Aadi Bioscience, where he has served as President and CEO since 2011. He is the inventor of the Company's foundational nab technology and the mTOR inhibitor nab-sirolimus (FYARRO) and has led the organization through the drug development process leading to approval as well as commercialization of FYARRO, the first FDA approved therapy for advanced malignant perivascular epithelioid cell tumor (PEComa). In August 2021 he steered the company to a transition into the public market (AADI: Nasdaq) with a raise of $155 million and since then has helped to guide the organization through the launch of the Phase 2 PRECISION 1 tumor-agnostic trial targeting TSC1 and TSC2 inactivating alterations, and most recently led the Company through an additional financing in September 2022, raising approximately $73 million in part to support further expansion the development of additional therapies utilizing the nab technology. Dr. Desai previously served as the SVP of global R&D at Abraxis Bioscience, where he invented the nab technology, Abraxane and ABI-009 (nab-sirolimus), leading the Abraxane team through all drug development stages and approvals in Breast, Lung and Pancreatic cancer. Dr. Desai is also the Founder and Chairman of Aadigen, LLC, a company focused on gene modulation using peptide-based delivery of nucleotide therapeutics. Prior roles during his 25+ year career include senior positions in strategic development at Celgene and varying roles at American BioScience, Inc., VivoRx, Inc. and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient. Dr. Desai has over 100 issued patents, over 40 peer-reviewed publications and book chapters, and over 200 presentations at scientific meetings. He was an active participant in FDA and EU Nanotechnology initiatives and a member of the Steering Committee for the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer. Dr. Desai recieved a M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

Julie Devallière

Biology Team leader at Curadigm

Julie Devalliere is the biology team leader at the early-stage company Curadigm in Paris, France. She holds an engineer's degree in Bioengineering and a doctorate in Cell Biology. Julie has broad experience in nanomedicine acquired during postdoctoral training at Yale and Harvard Universities, where she developed innovative delivery systems for proteins and nucleic acids. Julie worked on multiple aspects of nanoparticle development including particle design, targeting, biological activity or toxicity assessment and has authored more than 20 peer-reviewed publications.

RECENT PUBLICATIONS

- Mannose-modified hyaluronic acid nanocapsules for the targeting of tumor-associated macrophages. Fernández-Mariño I, Anfray C, Crecente-Campo J, Maeda A, Ummarino A, Teijeiro-Valiño C, Blanco-Martinez D, Mpambani F, Poul L, Devalliere J, Ger-

main M, Correa J, Fernandez-Villamarin M, Allavena P, Fernandez-Megia E, Alonso MJ, Andón FT. Drug Deliv Transl Res 2023 Jul;13(7):1896-1911
- A Nanoprimer To Improve the Systemic Delivery of siRNA and mRNA. Saunders N., Paolini M., Fenton O., Poul L., Devalliere J., Mpambani F., Darmon A., Bergere M., Jibault O., Germain M., Langer R. Nano Lett. 2020 Jun 10;20(6):4264-4269.
- Improving functional re-endothelialization of acellular liver scaffold using REDV cell-binding domain. Devalliere J, Chen Y, Dooley K, Yarmush ML, Uygun BE Acta Biomater. 2018 Sep 15;78:151-164.
- Co-delivery of a growth factor and a tissue-protective molecule using elastin biopolymers accelerates wound healing in diabetic mice. Devalliere J, Dooley K, Hu Y, Kelangi SS, Uygun BE, Yarmush ML.Biomaterials. 2017 Oct;141:149-160.

Mustafa Diken

Dr. Mustafa Diken received his Ph.D. in tumor immunology from Johannes Gutenberg University, Mainz under the provision of Prof. Ugur Sahin and is currently serving as Deputy Director Immunotherapy Development Center at Translational Oncology Institute (TRON) as well as Vice President Vaccines & Immunology at BioNTech SE. His research focuses on the development of novel cancer vaccines based on antigen-encoding messenger RNA (mRNA) and the elucidation of immunomodulatory mechanisms for cancer immunotherapy. His other scientific interests include assay development for preclinical evaluation of cancer vaccines. His research led to novel mRNA vaccines which are currently being tested in several clinical trials and development of an mRNA vaccine against SARS-CoV-2. Dr. Diken is also a board member and the scientific program director of the Association for Cancer Immunotherapy (CIMT), a non-profit organization aimed at advancing cancer immunotherapy.

Gilles Divita

CEO of DivInCell / Chief Scientist at Aadigen LLC.

Dr. Gilles DIVITA is founder and CEO of DivInCell (FR) and Chief Scientist at Aadigen LLC, California (USA). a BioPharmaceutical NanoMedicine Start-up pioneering a novel drug delivery technology for the treatment of cancer and genetic diseases. Dr. DIVITA has over 25 years of experience in drug delivery systems, peptide-drugs and oligonucleotide therapeutics. Dr Divita's work focuses on strategies to probe and perturb the behavior of biomolecules in physiological and pathological settings. He is the pioneer of the "non covalent cell penetrating peptide-based strategy" for therapeutic delivery. Dr. DIVITA is author of over 200 articles in peer reviewed scientific journals and of 25 patents. Dr. DIVITA holds a Ph.D. in Biochemistry/Biophysics from the University in Lyon, France. From 1992-1994, he worked as an Associate Scientist at the Max Planck Institute for Medical Research in Heidelberg-Germany and as Associate Professor at the SCRIPPS Research Institute, La Jolla, USA. In 1996, Dr. DIVITA joined the French National Center for Scientific Research (CNRS) and was Research Director, Head of Chemical Biology and Nanotechnology for the Therapeutics Team at the CNRS in Montpellier-France from 1999 to 2016.)

Marina Dobrovolskaia

Director of Operations, Nanotechnogy Characterization Lab

Dr. Dobrovolskaia is the Director of Operations and the Head of Immunology Section at the Nanotechnology Characterization Laboratory (NCL). In her role as the Director of Operations, Dr. Dobrovolskaia leads the NCL operations to provide preclinical nanoparticle characterization services to the nanotechnology research community, advance the translation of promising nanotechnology concepts from bench to the clinic, and contribute to the education of the next generation of scientists in the field of preclinical development of nanotechnology-based products, the activities emphasized in the NCL mission. She also directs the performance of Immunology, Client Relations and Administrative sections of the NCL. Closely integrated functioning of these sections plays a critical role in advancing the NCL's key strategic goals, and in supporting the missions of the Frederick National Laboratory for Cancer Research. In her role as the Head of the Immunology Section, Dr. Dobrovolskaia leads a team conducting preclinical studies to monitor nanoparticles' toxicity to the immune system both *in vitro* and *in vivo* using variety of immune function animal models. Prior to joining the NCL, Dr. Dobrovolskaia worked as a Research Scientist in a GLP laboratory at PPD Development, Inc. in Richmond, VA, where she was responsible for the design, development and validation of bioanalytical ligand-binding assays to support pharmacokinetic and toxicity studies in a variety of drug development projects. She received her M.S. degree from the Kazan State University in Russia; Ph.D. from the N.N. Blokhin Cancer Research Center of the Russian Academy of Medical Sciences in Moscow, Russia; and MBA from the Hood College in Frederick, MD. Since 2016, she is also a member of Project Management Institute and a certified Project Management Professional.

RECENT PUBLICATIONS

- Shi D, Beasock D, Fessler A, Szebeni J, Ljubimova JY, Afonin KA,Dobrovolskaia MA. To PEGylate or not to PEGylate: Immunological properties ofnanomedicine's most popular component, polyethylene glycol and its alternatives. Adv Drug Deliv Rev. 2022 Jan;180:114079.
- Dobrovolskaia MA, Afonin KA. Use of human peripheral blood mononuclear cells to define immunological properties of nucleic acid nanoparticles. Nature Protocols. 2020 Nov;15(11):3678-3698.
- Neun BW, Cedrone E, Potter TM, Crist RM, Dobrovolskaia MA. Detection of Beta-Glucan Contamination in Nanotechnology-Based Formulations. Molecules. 2020 Jul 24;25(15):3367.
- Dobrovolskaia MA. Nucleic Acid Nanoparticles at a Crossroads of Vaccines and Immunotherapies. Molecules. 2019 Dec 17;24(24):4620.
- Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. Roadmap and strategy for overcoming infusion reactions to nanomedicines. Nature Nanotechnol. 2018 Dec;13(12):1100-1108.

Simon Drescher

Managing Director at Phospholipid Research Center Heidelberg

After studying pharmacy at the Martin Luther University (MLU) Halle-Wittenberg in Halle, Germany, Dr. Drescher completed 2008 his PhD there in the field of pharmaceutical chemistry and physical chemistry

on the synthesis and aggregation behavior of bipolar phospholipids (bolaamphiphiles). He has dedicated himself to this topic at various places of work over 10 years; and finally received the Habilitation and Venia Legendi in Pharmaceutical Chemistry at MLU in 2017 on the topic of "Artificial phospholipids: syntheses, properties, and applications". After two semesters as deputy professor for Pharmaceutical Bioanalytics at the University of Greifswald, Germany, he joined the Phospholipid Research Center Heidelberg in December 2019, initially as deputy managing director and, since February 2021, as managing director.

Dr. Drescher's main interests are (i) the synthesis of artificial, i.e. non-naturally occurring, mono- and bipolar phospholipids, including fully synthetic and partial biochemical approaches, (ii) the physicochemical characterization of lipids in 2D and 3D assemblies and their miscibility, mainly using calorimetric methods, infrared spectroscopy, X-ray and neutron scattering techniques, electron microscopy, and mass spectrometry, and (iii) the application of liposomes for (oral) drug delivery.

RECENT PUBLICATIONS

- Li F, Harvey RD, Modicano P, Hamdi F, Kyrilis F, Müller S, Gruhle K, Kastritis P, Drescher S, Dailey LA, Investigating bolalipids as solubilizing agents for poorly soluble drugs: Effects of alkyl chain length on solubilization and cytotoxicity. Colloids Surf. B 2022, 212, 112369.
- Korn P, Schwieger C, Gruhle K, Garamus VM, Meister A, Ihling C, Drescher S, Azide- and diazirine-modified membrane lipids: Physicochemistry and applicability to study peptide/lipid interactions via cross-linking/mass spectrometry. Biochim. Biophys. Acta Biomembr 2022, 1864, 184004.
- van Hoogevest P, Tiemessen H, Metselaar JM, Drescher S, Fahr A, The Use of Phospholipids to make Pharmaceutical Form Line Extensions. Eur. J. Lipid Sci. Technol. 2021, 2000297.
- Hoffmann M, Drescher S, Schwieger C, Hinderberger D, Influence of a Single Ether Bond on Assembly, Orientation, and Miscibility of Phosphocholine Lipids at the Air-Water Interface. Phys. Chem. Chem. Phys. 2021, 23, 5325-5339.
- Dorner J, Korn P, Gruhle K, Ramsbeck D, Garamus VM, Lilie H, Meister A, Schwieger C, Ihling C, Sinz A, Drescher S, A Diazirine-Modified Membrane Lipid to Study Peptide/Lipid Interactions - Chances and Challenges. Chem. Eur. J. 2021, 27, 14586-14593.

Alexander Eggermont

Prof. Immunotherapy University Medical Center Utrecht & Chief Scientific Officer Princess Maxima Center, Utrecht, the Netherlands; Board Comprehensive Cancer Center München of the Technical University Munich & the Ludwig Maximiliaan University, Munich, Germany

Prof. Alexander M.M. Eggermont, MD, PhD is an internationally recognized expert in surgical oncology, immunotherapy, melanoma, sarcoma and cancer drug development.

Prof. Eggermont was previously Director General of Gustave Roussy Comprehensive Cancer Center, Villejuif, France (2010-2019) & Professor of Oncology (Classe Exceptionnelle) at the University Paris-Saclay; and Professor of Surgical Oncology (2003-2016) and Endowed Professor of International Networking in Cancer Research (2011-2018) at Erasmus University MC, Rotterdam, the Netherlands. He holds a PhD in tumor immunology from Erasmus University and is Fellow of the National Institutes of Health's National Cancer Institute (Dept Surgical Oncology; Head Steven Rosenberg) (NIH-NCI).

Prof. Eggermont has served as President of European Academy of Cancer Sciences, President of ECCO, President of the EORTC, Chairman of EORTC Melanoma Group, was a member of the Board of Directors of ASCO, served on the Editorial Board of the Journal of Clinical Oncology, and is currently Editor-in-Chief of the European Journal of Cancer. Prof. Eggermont has published more than 1000 peer-reviewed papers and his expertise has been acknowledged by many professional awards throughout his career.

RECENT PUBLICATIONS

- Neo-adjuvant immunotherapy emerges as best medical practice, and will be the new standard of care for macroscopic stage III melanoma. van Akkooi A, Blank C, Eggermont A. Eur J Cancer. 2023;182:38-42. 2) Pembrolizumab versus placebo as adjuvant therapy in completely resected stage IIB or IIC melanoma (KEYNOTE-716): a randomised, double-blind, phase 3 trial. Luke JJ, Rutkowski P, Queirolo P,…., & Eggermont AMM Lancet. 2022;399:1718-1729.

Eldad Elnekave

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CERTIFICATIONS: Diagnostic Radiology, American Board of Radiology & Israel Medical Association
Interventional Radiology Fellowship (2012), Memorial Sloan-Kettering Cancer Center

2023- Present	Director of the Unit for Interventional Oncology, Shaare Zedek Medical Center, Jerusalem, Israel
2023- Present	Co-Founder and Chief Medical Officer, Listen Therapeutics
2014- 2022	Director of Interventional Oncology Clinic, Davidoff Cancer, Rabin Medical Center, Israel.
2014- 2022	Interventional Radiologist, Department of Radiology, Rabin Medical Center, Israel
2014- 2021	Founding Chief Medical Officer, Zebra Medical Vision

TRAINING

2015	GCP Training and Certificate
2011- 2012	Memorial Sloan-Kettering Cancer Center, New York, NY; Fellowship, Vascular and Interventional Radiology
2006-2011	Albert Einstein Medical Center, Philadelphia, PA; Internship and Residency, Diagnostic Radiology
2000-2006	Tufts University School of Medicine, Boston, MA
2003- 2005	Howard Hughes Medical Institute-National Institutes of Health, Bethesda, MD; HHMI Scholar at the Institute for Allergy and Infectious Diseases

RECENT PUBLICATIONS

- Selective Intra-Arterial Doxorubicin Eluting Microsphere Embolization for Desmoid Fibromatosis: A Combined Prospective and Retrospective Study. Elnekave E, Ben Ami E, Shamai S, Peretz I, Tamir S, Bruckheimer E, Stemmer A, Erinjeri J, Abu Quider A, Seidensticker M, Wildgruber M, Ricke J, Anazodo A, Fung KF, Zer A, Ash S. Cancers (Basel). 2022 Oct
- Improving cardiovascular disease prediction using automated coronary artery calcium scoring from existing chest CTs. N. Barda; N. Dagan; A. Stemmer; J. Yuval; E. Bachmat; E. Elnekave *; R. Balicerv*; J Digit Imaging. 2022 Aug;35
- Using Machine Learning to Identify Intravenous Contrast Phases on Computed Tomography; R. Muhamedrahimov, A. Bar, J. Laserson, A.Akselrod-Ballin, E. Elnekave; Computer Methods and Programs in Biomedicine, Dec 2021
- Stemmer A, Shadmi R, Bregman-Amitai O, Chettrit D, Blagev D, Orlovsky M, Deutsch L, Elnekave E.; Using machine learning algo-

rithms to review computed tomography scans and assess risk for cardiovascular disease: Retrospective analysis from the National Lung Screening Trial (NLST). PLoS One. Aug 2020
- Bavli Y, Chen BM, Roffler SR, et al. PEGylated Liposomal Methyl Prednisolone Succinate does not Induce Infusion Reactions in Patients: A correlation between *in vitro* immunological and *in vivo* clinical studies.. Molecules 2020

Bengt Fadeel

Professor, Vice Chairman

BENGT FADEEL is Professor of Medical Inflammation Research at the Institute of Environmental Medicine, Karolinska Institutet. He obtained his M.D. and Ph.D. from Karolinska Institutet and his board certification at the Karolinska University Hospital. He currently serves as Vice Chairman of the Institute of Environmental Medicine. Prof. Fadeel is also Chair of the steering group of the national nanosafety platform SweNanoSafe, and he has been engaged in several EU-funded projects on nanosafety in FP7 and H2020. He is also a member of the Health & Environment workpackage of the GRAPHENE Flagship (2013-2023), and a member of the European Innovation Council project PERSEUS which is focused on nanomaterials for cancer therapy. Prof. Fadeel is a Fellow of the Academy of Toxicological Sciences (ATS) (since 2012).

RECENT PUBLICATIONS

- Peng G, Sinkko HM, Alenius H, Lozano N, Kostarelos K, Bräutigam L, Fadeel B. Graphene oxide elicits microbiome-dependent type 2 immune responses via the aryl hydrocarbon receptor. Nat Nanotechnol. 2023;18(1):42-48.
- Peng G, Fadeel B. Understanding the bidirectional interactions between two-dimensional materials, microorganisms, and the immune system. Adv Drug Deliv Rev. 2022;188:114422.
- Peng G, Keshavan S, Delogu L, Shin Y, Casiraghi C, Fadeel B. Two-dimensional transition metal dichalcogenides trigger trained immunity in human macrophages through epigenetic and metabolic pathways. Small. 2022;18(20):e2107816.
- Mukherjee SP, Gupta G, Klöditz K, Wang J, Rodrigues AF, Kostarelos K, Fadeel B. Next-generation sequencing reveals differential responses to acute versus long-term exposures to graphene oxide in human lung cells. Small. 2020;16(21):e1907686.
- Fadeel B, Bussy C, Merino S, Vázquez E, Flahaut E, et al. Safety assessment of graphene-based materials: focus on human health and the environment. ACS Nano. 2018;12(11):10582-10620.

Ilise Feitshans

LLM Student Georgetown Uniiversity

I was born in Manhattan New York City and raised there, with education from premiere schools including Columbia University. I am a lawyer, public health professional and former UN staff at the diplomatic level. In 2014 i completed the first Swiss doctorate in the law of nanotechnology, which was awarded a universitywide prize for Best Research in Social Medicine and Prevention and then became the book Global Health Impacts of NANOTECHNOLOGY Law (English and French on amazon.com) I have a Masters of Science from Johns Hopkins University School of Public Health about informed consent in genetic testing. Now i am studying governance and law of nanotechnologies for the Masters of Law (LLM) in global health law in Georgetown University law center Washington DC. I serve as Inagural Chair, Special Session on Law and Ethics of Nanotechnology Safety and Health in Food for Nanotexnology 2023, and Chair, Committee on Science and Technology Law Virginia Mountain Valley Lawyers Alliance, and Director, ESI SAFERNANO European Scientific Institute, Archamps France and also
Virginia Mountain Valley Lawyers Alliance Representative for Lawyers Abroad
LLM Student O'Neill Institute for National and Global Health Law, Georgetown University Law Center Washington DC https://oneill.law.georgetown.edu/experts/ilise-feitshans/

RECENT PUBLICATIONS

- https://orcid.org/ 0000-0002-6931-314X; Ilise L Feitshans Global Health Technology Transforming Intellectual Property and Global Commerce Precision Nanomedicine online open access journal December 2022
- Ilise L Feitshans"3D Printing of Medical Devices: Issues of Patient Safety" Medicine and Law Journal World Association for Medical Law, Vol 41 March 2022 p53-65, André Dias Pereira Editor.
- Ilise Feitshans, Philippe Sabatier,. Global Health Impacts Of Nanotechnology Law: Advances In Safernano Regulation. Mater. Today Proc. 2022, 67, 985 994.
- Ilise Feitshans "Nanoethics for safe work; philosophical foundations of safer nanodesign protecting workplace health" 12.1 "International efforts to harness nanomaterials under law" p209-211 IN Marcel Van de Voorde and Gunjan Jeswani Handbook of Nanoethics Walter de Gruyter GmbH Berlin/ Boston 2021 ISBN 978-3-11066923-7
- Janeck Fordsmand, ilise feitshans et al, "Bridging international approaches on environmental, health and safety aspects of nanotechnology" Nature Nanotechnology May 2021 DOI10.1038/s41565-021-00912-5.

Michael Fichter

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Michael Fichter received his Bachelor's and Master's degree in Applied Life Sciences at the University of Applied Sciences in Kaiserslautern in 2009 and 2011, respectively. For his Bachelor's thesis he evaluated the influences of breast milk components on the growth and differentiation of enteric neurons in the group of Prof. Karl-Herbert Schäfer in Zweibrücken. For his Master's thesis he characterized enteropancreatic interactions and the neuronal regulation of the islets of Langerhans with Sanofi in Frankfurt.
Subsequently, Michael Fichter joined Prof. Stephan Gehring's lab at the University Medical Center Mainz as a PhD student focusing on the modulation of intrahepatic immune responses through nanoparticle-mediated delivery of drugs, adjuvants, and antigens. After completing his PhD in 2016, he received a fellowship grant by the German Research Foundation and joined Prof. Darrell Irvine's lab at the Massachusetts Institute of Technology as a postdoctoral researcher. His main research focus was the development of cytokine-based nanogel formulations for the T cell-mediated treatment of liver tumors.
In 2020 he joined Prof. Volker Mailänder's and Prof. Katharina Landfester's groups where is currently working on the antibody- and

nanobody-based targeting of nanoparticles to dendritic cell subtypes as well as on the therapeutic use of adjuvant- and antigen-loaded nanocapsules for the treatment of melanoma within the SFB1066 in Mainz.

RECENT PUBLICATIONS

- Simon, J.*, Fichter, M.*, Kuhn, G.*, Brückner, M., Kappel, C., Schunke, J., Klaus, T., Grabbe, S., Landfester, K., and Mailänder, V., Achieving dendritic cell subset-specific targeting *in vivo* by site-directed conjugation of targeting antibodies to nanocarriers. Nano Today, 2022. 43: p. 101375. *shared first authors
- Ma, L., Dichwalkar, T., Chang, J.Y.H., Cossette, B., Garafola, D., Zhang, A.Q., Fichter, M., Wang, C., Liang, S., Silva, M., Kumari, S., Mehta, N.K., Abraham, W., Thai, N., Li, N., Wittrup, K.D., and Irvine, D.J., Enhanced CAR-T cell activity against solid tumors by vaccine boosting through the chimeric receptor. Science, 2019. 365(6449): p. 162-168.
- Fichter, M., Piradashvili, K., Pietrzak-Nguyen, A., Pretsch, L., Kuhn, G., Strand, S., Knuf, M., Zepp, F., Wurm, F.R., Mailander, V., Landfester, K., and Gehring, S., Polymeric hepatitis C virus non-structural protein 5A nanocapsules induce intrahepatic antigen-specific immune responses. Biomaterials, 2016. 108: p. 1-12.
- Fichter, M.*, Dedters, M.*, Pietrzak-Nguyen, A., Pretsch, L., Meyer, C.U., Strand, S., Zepp, F., Baier, G., Landfester, K., and Gehring, S., Monophosphoryl lipid A coating of hydroxyethyl starch nanocapsules drastically increases uptake and maturation by dendritic cells while minimizing the adjuvant dosage. Vaccine, 2015. 33(7): p. 838-46. *shared first authors
- Fichter, M., Baier, G., Dedters, M., Pretsch, L., Pietrzak-Nguyen, A., Landfester, K., and Gehring, S., Nanocapsules generated out of a polymeric dexamethasone shell suppress the inflammatory response of liver macrophages. Nanomedicine, 2013. 9(8): p. 1223-34.

Holger Frey

University Professor, Department of Chemistry, Johannes Gutenberg University Mainz

Prof. Holger Frey was born 1965 and has been a Full Professor of Organic and Polymer Chemistry at the Johannes Gutenberg University Mainz since 2003. His main research interest lies in precise polymerization methods as well as new polymers for biomedical materials. In his research he places a special emphasis on polyethylene glycol (PEG)-based materials and also the synthesis of new PEG-derived polyethers. In 2022 he was awarded an ERC Adv. Grant for the project RandoPEGMed. He has (co)authored more than 410 peer-reviewed original research papers and review articles and is (co)inventor of 45 patent applications. His publications have been cited more than 27,000 times.
Website: www.ak-frey.chemie.uni-mainz.de

RECENT PUBLICATIONS

- V. Müller, R. Matthes, M. Wagner, M. Bros, P. Dreier, H. Frey, "Tailoring thermoresponsiveness of biocompatible polyethers: copolymers of linear glycerol and ethyl glycidyl ether" Polymer Chemistry, 2023, 14, 2599-2609.
- M. Scharfenberg, J. Hilf, H. Frey, "Functional Polycarbonates from Carbon Dioxide and Tailored Epoxide Monomers: Degradable Materials and Their Application Potential", Adv. Funct. Mater. 2018, 28, 1704302.
- J. Herzberger, K. Fischer, D. Leibig, M. Bros, R. Thiermann, H. Frey, "Recent advances in the use of nanoparticles for allergen-specific immunotherapy", H. Pohlit, I. Bellinghausen, H. Frey, J. Saloga, Allergy 2017, 72, 1461–1474.
- J. Herzberger, K. Fischer, D. Leibig, M. Bros, R. Thiermann, H. Frey, "Oxidation-responsive and "clickable" poly(ethylene glycol) via copolymerization of 2-(methylthio)ethyl glycidyl ether", J. Am. Chem. Soc. 2016, 138, 9212-9223.
- J. Herzberger, K. Niederer, H. Pohlit, J. Seiwert, M. Worm, F. R. Wurm, H. Frey, "Polymerization of Ethylene Oxide, Propylene Oxide and Other Alkylene Oxides: Synthesis, Novel Polymer Architectures and Bioconjugation", Chem. Rev. 2016, 116 (4), 2170-2243.

Wolfgang Frieß

Professor for Pharmaceutics and Biopharmaceutics

Dr. Wolfgang Frieß holds a position as Professor for Pharmaceutical Technology and Biopharmaceutics at the LMU Munich since 2001. He received his PhD in Pharmaceutical Technology in 1993 and his Pharmacy degree in 1989 from the University of Erlangen. His primary research goals are protein formulation, drug delivery and biomaterials, in particular new analytical tools for protein formulations, freeze-drying of proteins and different local delivery routes. He has worked for several years in academia both in Germany and the US. He is co-editor of the European Journal of Pharmaceutics and Biopharmaceutics and has published over 200 research papers, patents and book chapters.

RECENT PUBLICATIONS

- 1.Marschall C, Witt M, Hauptmeier B, Friess W, Drug Product Characterization of High Concentration Non-Aqueous Protein Powder Suspensions, J Pharm Sci 2023, 112(1), 61-75.
- Deiringer N, Friess W, Proteins on the Rack: Mechanistic Studies on Protein Particle Formation During Peristaltic Pumping. J Pharm Sci 2022, 111(5), 1370-1378.
- Trenkenschuh E, Richter M, Heinrich E, Koch M, Fuhrmann G, Friess W, Enhancing the Stabilization Potential of Lyophilization for Extracellular Vesicles. Adv Healthc Mater 2022, 11, 2100538, 1-16.
- Bluemel O, Buecheler JW, Hauptmann A, Hoelzl G, Bechtold-Peters K, Friess W, Scaling Down Large-Scale Thawing of Monoclonal Antibody Solutions: 3D Temperature Profiles, Changes in Concentration, and Density Gradients. Pharm Res 2021, 38(11), 1977-1989.
- Domnowski M, Maruno T, Enomoto K, Kummer F, Kulakova A, Harris P, Uchiyama S, Jaehrling J, Friess W, A Multi-Method Approach to Assess the Self-Interaction Behavior of Infliximab. J Pharm Sci 2021, 110(5), 1979-1988.

Lorenza Fugazza

Head of RLT Technical Research and Development

I'm a chemist by education and, after working in the academic and "classic" pharmaceutical development environment for 5 years, I have approached the radiochemistry and radiopharmaceutical disciplines when I have started working in the Nuclear Medicine Department of the National Cancer Institute in Milano in 2005. This gave me the opportunity to connect with the ultimate purpose of developing drugs to treat the patients in need.
In 2008, I have joined Advanced Accelerator Applications, a company focusing on developing, producing and commercializing nuclear medicine products, which at that time was about to acquire the rights to develop the first approved theragnostic pair for the treat-

ment of NET tumors. Since then I have been working in the development of radioligand therapy/radioligand imaging products contributing to establishing formal drug development processes and standardized quality in the radiopharmaceutical field. Since 2018, following the acquisition of Advanced Acceleration Applications by Novartis, I'm leading the RLT Technical Research and Development team accountable for developing innovative and high quality RLT products for an expanding ranges of tumors.

Alberto Gabizon

Alberto Gabizon received his M.D. degree from the School of Medicine, University of Granada, Spain (1974), and his Ph.D. in Cancer Immunology from the Weizmann Institute of Science, Rehovot, Israel (1979). He then completed his training and certification in Radiation and Medical Oncology at Hadassah-Hebrew University Medical Center, Jerusalem, Israel (1985). During his research fellowship at the Cancer Research Institute of UCSF Medical Center, San Francisco, CA (1986-89), he pioneered the development of a new generation of long-circulating liposomes known as Stealth liposomes which have greatly improved stability and selective accumulation in tumors.

Dr. Gabizon's inventorship and research contribution played a key role in the development of DOXIL® (pegylated liposomal doxorubicin, also known as Caelyx and Lipodox), a unique anticancer formulation extensively used in the clinic (ovarian cancer, breast cancer, and other cancer types) with important pharmacologic and safety advantages over conventional chemotherapy. Gabizon was one of the first researchers to identify the cardioprotective of liposome delivery in doxorubicin-based chemotherapy. His recent inventions include two liposome products for cancer therapy: Promitil® (pegylated liposomal mitomycin-C prodrug), a formulation currently in clinical studies with improved safety over the parent drug mitomycin C, that may be particularly useful in DNA repair-deficient tumors; and, Pegylated liposomal alendronate of doxorubicin (acronym: PLAD), a hybrid therapeutic product with chemotherapeutic and immunotherapeutic properties currently in the research and development phase. He has founded two start-up companies in the field of cancer nanomedicine: Lipomedix Pharmaceuticals (2011), aimed at developing Promitil®, and Levco Pharmaceuticals (2020) to advance PLAD.

Dr. Gabizon has received the National Prize of Medicine of Spain Ministry of Education (1975), the Research Career Award of the Israel Cancer Research Fund (1989), the Hebrew University Kaye Innovation Award (1997) for the invention "Liposomal Doxorubicin for Cancer Treatment", the Tel Aviv University Sarnat Lectureship (2000), the Professorship Award of the Israel Cancer Research Fund (2008), and the Alec Bangham Life Time Achievement Award of the International Liposome Research Society (2010).

Dr. Gabizon is active in the medical oncology field in clinical practice and early phase clinical trials including 3 first-in-man studies, as well as in preclinical pharmacology with special emphasis on applications of liposomes in drug delivery, targeting of drugs, and experimental cancer therapy. He has published over 180 articles and specialized book chapters, and is an inventor of 15 USPTO and EPO-approved patents.

In 2002, Dr. Gabizon was appointed full Professor at the Hebrew University-Faculty of Medicine in Jerusalem. Between 2001 and 2019, Dr. Gabizon served as Chairman of the Shaare Zedek Oncology Institute. He is currently head of the Nano-oncology Research Center at Shaare Zedek Medical Center in Jerusalem, Israel, and Medical Advisor of Nextar Chempharma (Ness Zyona, Israel).

Ruth Gabizon

Ph.D

Department of Neurology
Hadassah University Hoospital
gabizonr@hadassah.org.il

After getting my PhD from the Hebrew University Medical School I went to Dr. Prusiner's lab in UCSF for my postdoctoral studies on prion diseases. In 1988, I established my own prion lab in the Neurology department of the Hadassah University Hospital. Since then, I investigate features of neurodegenerative diseases and in particular those of genetic prion diseases, since the E200K PrP mutation is common among Jews of Libyan origin living in my country. In the last years, we are generating nanotechnology based formulations of natural antioxidants that can prevent/delay the outbreak of neurodegenerative diseases in at risk individuals. A few years ago, I founded Granalix Biotechnologies, together with Prof Magdassi, and we have established our first product, GranaGard, a nanotechnology based formulation of Pomegranate seed oil.

Jérôme Galon

Le Dr Jérôme Galon est Directeur de Recherche de Classe Exceptionnelle à l'INSERM (Institut national de la santé et de la Recherche Médicale), et chef du laboratoire d'immunologie et Cancérologie Intégratives, à Paris. Le Dr Galon a été formé comme immunologiste à l'Institut Pasteur et à l'Institut Curie (Paris, France). Il est titulaire d'un doctorat en immunologie (1996). Entre 1997 et 2001, il a travaillé au NIH (National Institute of Health, Bethesda, USA). Depuis sa titularisation à l'INSERM en 2001, il dirige des programmes de recherche interdisciplinaires sur l'immunologie des tumeurs. Il est directeur associé et cofondateur de l'Académie européenne d'immunologie des tumeurs (EATI) et a été directeur du conseil Scientifique de la Société pour l'immunothérapie du cancer américaine (SITC). Ses travaux sur l'analyse intégrative du microenvironnement tumoral et le rôle des cellules T dans le cancer humain ont conduit à la démonstration de l'importance de l'immunité adaptative préexistante, et au concept de contexture immunitaire du cancer. Il a été le pionnier de l'Immunoscore. Il est le cofondateur de HalioDx, maintenant société Veracyte dont il est SVP et directeur scientifique exécutif. Le Dr Galon a publié plus de 300 articles dans des revues scientifiques de premier plan et a donné plus de 350 conférences invitées dans le monde entier. Ses contributions ont été reconnues par de nombreux prix, dont le prix William B. Coley, un prix international qui récompense les meilleurs scientifiques dans le domaine de l'immunologie fondamentale et de l'immunologie du cancer, ainsi que des prix de l'Académie des Sciences et de l'Académie de Médicine. Il a remporté le prestigieux prix de l'inventeur Européen dans la catégorie Recherche en 2019, le prix Jeantet-Collen (Suisse) en 2021, le prix Galien 2021 et le prix Duquesne en 2022.

Robert Geertsma

Senior Scientist, Centre for Health Protection, National Institute for Public Health and the Environment (RIVM)

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for more than twentyfive years. As a senior scientist and project leader he is responsible for the provision of scientific advice to regulators on quality and safety of medical technology and nanomedicine. He works on multiple research projects on opportunities as well as risks of nanotechnologies and nanomaterials in medical applications, performing both desk research and experimental research. He participated in the recently finalised H2020 project REFINE (Regulatory Science Framework for Nano(bio)material-based Medicinal Products and Medical Devices). He is also one of the experts of the Risks of Nanotechnology Knowledge and Information Centre (KIR nano), a Dutch government-supported observation organisation based at RIVM. His areas of expertise include risk management, biological safety, nanotechnology and emerging medical technologies. He participates actively in international ISO/CEN Standards Committees on these subjects and he is chairman of the joint CEN/CENELEC/TC3 responsible for horizontal standards on topics like quality and risk management systems. He was a member of the SCENIHR WG that wrote the Scientific Opinion "Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices". He is chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials, and he is a member of the Nanomedicines WG of the International Pharmaceutical Regulators Programme. Furthermore, he frequently represents the Dutch competent authority in European Commission's working groups such as the New Technologies WG, of which he was appointed co-Chair in 2009. He is a member of the European Society for Nanomedicine and the European Technology Platform Nanomedicine.

RECENT PUBLICATIONS

- Jong WH de, Geertsma RE, Borchard G. Regulatory safety evaluation of nanomedical products: key issues to refine. Drug Delivery and Translational Research 2022; 12: 2042–2047.
- Halamoda-Kenzaoui B, Geertsma R, Pouw J, Prina-Mello A, Carrer M, Roesslein M, Sips A, Weltring KM, Spring K, Bremer-Hoffmann S. Future perspectives for advancing regulatory science of nanotechnology-enabled health products. Drug Delivery and Translational Research 2022; 12: 2145–2156.
- Zabeo A, Rosada F, Pizzol L, Caputo F, Borgos SE, Parot J, Geertsma RE, Pouw JJ, Vandebriel RJ, Ibarrola Moreno O, Hristozov D. A Decision Support System for preclinical assessment of nanomaterials in medical products: the REFINE DSS. Drug Delivery and Translational Research 2022; 12: 2101–2113.
- Halamoda-Kenzaoui B, Box H, Elk M van, Gaitan S, Geertsma RE, Gainza Lafuente E, Owen A, Del Pozo A, Roesslein M, Bremer-Hoffmann S. Launching stakeholder discussions on identified regulatory needs for nanotechnology-enabled health products. Prec. Nanomed. 2020 July;3(3):608-621.
- Giannakou C, Park MVDZ, Bosselaers IEM, Jong WH de, Laan JW van der, Loveren H van, Vandebriel RJ, Geertsma RE. Nonclinical regulatory immunotoxicity testing of nanomedicinal products: Proposed strategy and possible pitfalls. WIREs Nanomed Nanobiotechnol. 2020;e1633.

Marc Gitzinger

CEO BioVersys AG and President BEAM Alliance

Chief Executive Officer and co-founder of BioVersys with over 10 years of experience in the biotech industry, having launched a university spin-off in the field of antimicrobial resistance and growing it into a multi-asset clinical stage company. Some of these assets will address significant unmet medical needs in infectious conditions such as tuberculosis and hospital acquired Acinetobacter infections. Marc has raised over $70 mio in equity financing and secured over $30 mio in non-dilutive funding. He has also established several important partnerships with a Big Pharma and other development organizations. Multi-award-winning Biotech CEO, having received amongst others the Swiss Technology Award 2011, Venture Kick 2009 and Venture Leaders 2008 and 2017 awards for his work in founding and advancing BioVersys. He is also President of the Board of the BEAM Alliance, a European association representing over 70 European and international SMEs active in antimicrobial research and development and Board member of AMR Industry Alliance. Marc is a young thought leader in the field of antimicrobial research and development. He is passionate about next generation antimicrobial therapies and leads a highly motivated team striving to bring life-saving antimicrobial therapies to patients in need. He is also co-author on several high ranked scientific publications and patents in the field.

RECENT PUBLICATIONS

- Rifabutin for infusion (BV100) for the treatment of severe carbapenem-resistant Acinetobacter baumannii infections. Trebosc V, Kemmer C, Lociuro S, Gitzinger M, Dale GE. Drug Discov Today. 2021 Sep;26(9):2099-2104. doi: 10.1016/j.drudis.2021.07.001. Epub 2021 Jul 6. PMID: 34242796 Review.
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Daniel Gonzalez Carter

'La Caixa' Junior Leader Research Fellow/ Senior Scientist

Dr. Daniel Gonzalez-Carter's research focuses on developing nanotechnology-based strategies to deliver therapies to the brain.

He carried out his Ph.D. studies at the Department of Brain Sciences in Imperial College London (ICL) under a scholarship from the Mexican National Council of Science and Technology (CONACyT), where he focused on investigating pharmacological strategies to modulate microglial activity to combat neuronal death in Parkinson's disease. During his first post-doctoral position at the Department of Materials, ICL, he applied his cellular neuroscience background to study the interaction of nanomaterials (in particular carbon nanotubes and gold/silver nanoparticles) with the brain, focusing on their ability to cross the blood-brain barrier (BBB) and modulate brain inflammation. After moving to Japan to work at the Innovation Center of Nanomedicine (iCONM) under Prof. Kazunori Kataoka, he won an 'Early Career Scientist' research grant from the Japanese Society for the Promotion of Science (JSPS) to examine the potential of glucose-functionalized polymeric nanomicelles to deliver therapies against Alzheimer's disease across the BBB. He is currently a 'La Caixa' Junior Leader research fellow at the Institute for Bioengineering of Catalonia (IBEC), Barcelona, where he is developing a novel strategy to target nanoparticles to the brain by exploiting the impermeability of the BBB to generate artificial brain targets.

RECENT PUBLICATIONS

- D. Gonzalez-Carter*, X. Liu, T. Tockary et al., (2020) Targeting nanoparticles to the brain by exploiting the blood-brain barrier impermeability to generate brain specific targets. PNAS, 117 (32): 19141-19150
- J. Xie, D. Gonzalez-Carter, T. Tockary et al., (2020) Dual-sensitive nanomicelles enhancing systemic delivery of therapeutically active antibodies specifically into the brain. ACS Nano, 14 (6): 6729-6742
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- D. Gonzalez-Carter*, B. Leo, P. Ruenraroengsak, S. Chen, A. Goode, et al. (2017). Silver nanoparticles reduce brain inflammation and related neurotoxicity through induction of H2S-synthesizing enzymes. Scientific Reports, 7:42871.

Stephan Grabbe

Stephan Grabbe, MD (born 1961), is a Dermatologist and currently holds the position as Director and Chairman of the Department of Dermatology, University of Mainz Medical Center (UMMC), Germany.

He received his medical and scientific education at the University of Münster, Germany (Department of Dermatology (1987-2003), as well as at Harvard Medical School (Research fellowships at the Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, USA (1989-1992) and at the Department of Dermatology, Brigham and Womens' Hospital, Harvard Medical School, Boston, USA (1998)). Before being appointed to his current position, Stephan Grabbe was Director and Chairman, Dept. of Dermatology, University of Essen Medical center (2003-2007). His clinical focus is on skin oncology and immune-mediated skin dieseases. Currently, he is also Head of the UMMC Skin Cancer Center, Member of the board of the University of Mainz Cancer Center (UCT) , Adjunct Clinician Scientist of the Institute for Molecular Biology of the Johannes Gutenberg University, and co-speaker of the Research Center Immunotherapy (FZI) of the University of Mainz.
Stephan Grabbes scientific focus is in the field of cellular immunology and immunotherapy, dendritic cells, as well as nanoparticle-mediated immunomodulation. In this respect, he is speaker of the collaborative research center SFB 1066 of the German Research Council ("Nanoparticle-mediated immunotherapy"), and deputy speaker of the collaborative research center SFB TR156 ("Skin imunology"). Stephan Grabbe has published more than 250 original papers in peer-reviewed journals, has been cited more than 18.000 times and has an h-index of 58.

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- Kappel, C., Seidl, C., Medina-Montano, C., Schinnerer, M., Alberg, I., Leps, C., Sohl, J., Hartmann, A.-K., Fichter, M., Kuske, M., Schunke, J., Kuhn, G., Tubbe, I., Paßlick, D., Hobernik, D., Bent, R., Haas, K., Montermann, E., Walzer, K., Diken, M., Schmidt, M., Zentel, R., Nuhn, L., Schild, H., Tenzer, S., Mailänder, V., Barz, M., Bros, M., Grabbe, S. (2021). Density of Conjugated Antibody Determines the Extent of Fc Receptor Dependent Capture of Nanoparticles by Liver Sinusoidal Endothelial Cells. ACS Nano. 15, 15191-15209.
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Heinrich Haas

Dr. Haas is in the group of Prof. Dr. Helmuth Möhwald at Johannes-Gutenberg Universität Mainz. He researches lipid membranes and organized bio-molecular systems. In the pharmaceutical industry (Munich Biotech, Medigene, BioNTech) he developed different types of nanoparticle products to clinical stage. Focus on advanced approaches for nanoparticle development and control.

Stefan Halbherr

CEO / Country Manager InnoMedica

Stefan Halbherr studied biochemistry and immunology at the university of Bern in Switzerland. During his PhD, he developed genetically engineered self-amplifying RNA vaccines against influenza A viruses. He played a crucial role in the founding team of InnoMedica and restructured the company into a cutting-edge nanodrug company. Since 2013 he led the R&D department and brought research concepts to a marketable product for patients. In 2019 Stefan Halbherr took the role as Country Manager and President of the Board of InnoMedica Switzerland AG, while also leading the R&D team to the creation of a well-diversified clinical stage product pipeline. Today, the portfolio of InnoMedica encompasses innovative solutions for oncology with Talidox at the forefront, addressing the fact that many frequently used drugs unfortunately still cause severe adverse effects with yet limited antitumor efficacy. Stefan Halbherr was also the inventor and patent author of Talineuren, a new nanodrug in neurology, currently in phase IIa testing for treatment of Parkinson's disease. Until to date, Stefan Halbherr has helped InnoMedica to grow into an advanced clinical stage nanopharmaceutical company with >CHF65M of funds raised and 50+ employee strong team.

Heiko Heerklotz

Professor

After my degree in Physics at Leipzig University, I finished my PhD there in 1996 on lipid-detergent interactions. My interdisciplinary approach is highlighted by the subsequent postdocs in physical chemistry (Leipzig), biochemistry (McMaster) and biophysical chemistry (Basel Biocenter). In Basel, I also received my venia legendi and started my own, SNF-funded research group. Our data challenged the hypothesis that so-called detergent-resistent membranes would represent biological membrane domains referred to as "rafts". In 2007, I moved to the University of Toronto for an associate (and later full) professorship in pharmaceutics. Since 2015, I'm holding the chair of pharmaceutics at the University of Freiburg, still maintaining a status professorship at UofT. Since 2021 I am serving as the Associate Editor of the "Membranes" section of the Biophysical Journal. Current research topics are (i) fundamental phenomena governing lipid-and detergent-based drug delivery systems, (ii) antimicrobial lipopeptides considered for clinical and established for agricultural applications and (iii) effects of lipid asymmetry and heterogeneity of membrane bilayers.

RECENT PUBLICATIONS

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- Complex Micellization Behavior of the Polysorbates Tween 20 and Tween 80; H. Knoch, M.H. Ulbrich, J.J. Mittag, J. Buske, P. Garidel and H. Heerklotz (2021); Molecular Pharmaceutics https://doi.org/10.1021/acs.molpharmaceut.1c00406
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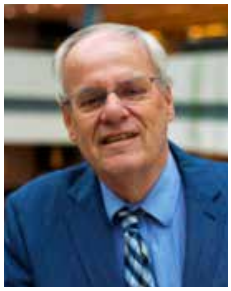
- Primary and secondary binding of exenatide to liposomes.Stulz, A., Breitsamer, M., Winter, G., Heerklotz, H. (2020) Biophys. J. 118:600-611. https://doi.org/10.1016/j.bpj.2019.12.028



Wim Hennink

Professor at Utrecht Uniiversity

Professor Wim Hennink obtained his Ph.D. degree in 1985 at the Twente University of Technology on a thesis of a biomaterials research topic. From 1985 until 1992 he had different positions in the industry. In 1992 he was appointed as professor at the Faculty of Pharmacy of the University of Utrecht. From 1996 till 2020 he was head of the Department of Pharmaceutics. From 1997 till 2021 he was editor of the Journal Controlled Release. From 2012-2015 he was the scientific director of the Utrecht Institute for Pharmaceutical Sciences and from September 2015 his retirement in august 2022 he was head of the Department of Pharmaceutical Sciences. His main research interests are in the field of polymeric drug delivery systems. He published over 600 papers and book chapters and is the inventor of 20 patents.

RECENT PUBLICATIONS

- Yan Wang, Mies J van Steenbergen, Nataliia Beztsinna, Yang Shi, Twan Lammers, Cornelus F van Nostrum, Wim E Hennink. Biotin-decorated all-HPMA polymeric micelles for paclitaxel delivery. Journal of Controlled Release 328, 970-984, 2020.
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Nina Hentzen

Nina Hentzen is a Senior Scientist in Early Development Bioconjugates at Lonza in Visp, Switzerland. She is responsible for leading preclinical bioconjugation projects and establishing collaborations with technology partners to enhance innovation in bioconjugates development and manufacturing. Nina is an organic chemist by training and holds a PhD in peptide chemistry from ETH Zurich. Prior to joining Lonza, she was a Swiss National Science Foundation postdoctoral fellow at UC San Francisco where she was working on computational protein design.

Inge Herrmann

Assistant Professor and Group Leader,
ETH Zurich and Empa

Inge Herrmann is a chemical engineer with Inge Herrmann is a chemical engineer with additional training in (pre)clinical research. After graduating with a PhD from ETH Zurich, she underwent further training at the University Hospital Zurich (USZ), the University of Illinois (US) and the Imperial College London (UK). Since 2015, she is heading a research group at Empa specialized on nanoscale materials and devices for healthcare. In 2019, Inge Herrmann joined the Department of Mechanical and Process Engineering at ETH Zurich where she is heading the Nanoparticle Systems Engineering Lab. She is an expert in nanoparticle synthesis and characterization, spectromicroscopy and translational nanomedicine. She has spearheaded several translational nanomedicine projects, and serves as a scientific advisor of the spin-off companies hemotune, anavo and veltist commercializing technologies emerging from her lab. Inge Herrmann has won various prestigious awards, including the ETH Dandelion Award 2021, the 2022 Smoluchowski Award, the Bayer Healthcare Award and the Johnson & Johnson Award, the Swiss National Science Foundation Eccellenza Award, the Empa Innovation Award and has been named Emerging Investigator 2021 by the Royal Society of Chemistry and 2022 Rising Star by the American Chemical Society (ACS). Students under her supervision have won major awards, including the SCS DPCI Award, the Hilti Award, the ETH Medal, ETH's best doctoral thesis in the area of materials and processes (MaP, 3x), ETH Pioneer Fellowships (2x) and several best presentation awards at international conferences. She is principle investigator (PI) of several national and international projects supported by the Swiss National Science Foundation, the Personalized Health and Related Technologies Initiative (PHRT), the Novartis FreeNovation program and several medical foundations (incl. the Swiss Heart Foundation, Krebsliga and many others).

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- I.K. Herrmann*, M.J.A. Wood, G. Fuhrmann*, Extracellular Vesicles as Next Generation Drug Delivery Platform, Nature Nanotechnology, 2021.
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Martin Hingle

Early Product Development

Dr. Martin Hingle is an Associate Director of the Early Phase Product Development group at Novartis Pharmaceuticals AG. In his current role, he leads the biopharmaceutics group supporting formulation development across all dosing routes and is responsible for guiding biopharmaceutics risk assessments and characterization, PBBM, bridging strategies, biowaivers and supporting clinically relevant drug product specifications. He has previously held various positions at Elan Biotechnology Research and GSK within physical properties, oral and inhaled formulation development, predictive technologies and Biopharmaceutics. Martin participates in consortia IQ and AgePOP and is co-supervising two PhD students and a Postdoc. Martin studied Pharmaceutical Science at the University of Greenwich and obtained his PhD from the University of Brighton in Pharmaceutics.

RECENT PUBLICATIONS

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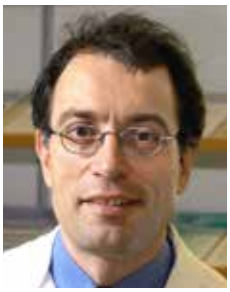

Patrick Hunziker

Prof. Dr.med.

Patrick Hunziker has studied Medicine at the University of Zurich, Switzerland and has received a doctoral decree based on thesis work in experimental immunology from the University of Zurich in 1989. He started his research with experimental hematology and throughout his career remains involved in clinical as well as in laboratory research. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. Professional activities in the Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future. As a fellow at the Massachusetts General Hospital, Harvard Medical School, he worked on imaging technologies in humans and in animals in a joint project with the Massachusetts Institute of Technology, Cambridge and in animal models of acute myocardial infarction including early efforts toward cell transplantation in myocardial infarction. Focusing his research on application of high-tech methods to improved diagnosis and therapy, he became lecturer at the University of Basel in the field of cardiology based on his work on innovative imaging modalities based on computer vision, which led to the prize of the Swiss Heart Foundation, the most prestigious prize in cardiology in Switzerland. He also won the Pfizer research prize for his work in improved diagnosis of cardiovascular disease. In 2008, he received the title of Professor from the University of Basel. He remains active in the field of computational modeling, computational imaging and artificial intelligence. In cardiovascular disease, he pioneered the use of catheter based mechanical heart pump support in cardiogenic shock.

Foreseeing in the late 90'ies that significant advances in medicine disease would be brought by the advances in the nanosciences, he became involved in medical applications of nanoscience in the late nineties, combining his experience in experimental research and his knowledge in the problems of clinical medicine, as the physician at the Swiss National Center of Competence Nanotechnology in 2001. His nanoscience research has been cited as a "Nanoscience milestone of the year" by MIT technology review in 2007 for the development of nanosize polymer carriers with switchable functionality. He has worked in the medical applications of atomic force microscopy, nano-optics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting, including cell-culture and animal research in transgenic animal models.
Applying his research to poverty diseases, he has, led one of the largest epidemiologic field trials on Schistosomiasis in Congo, Africa. He is the founding president of the International Society of Nanomedicine, has initiated together with Beat Löffler (CLINAM Foundation for Nanomedicine) the annual European Conference for Clinical Nanomedicine, the leading event in its field, and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital of Basel, Switzerland. He is frequently invited as speaker to international conferences in the topic of nanomedicine and has gained a high visibility (google:"Hunziker Nano") within the field. He was invited to shape the future research policy of the European Commission in the field of Nanomedicine and has been member of various strategic committees on a university, national and continental level.
Patrick Hunziker has authored >230 scientific publications, has received >13000 citations and has a current Hirsch Index of 52, as shown in
https://scholar.google.com/citations?user=vc6l6sAAAAAJ&hl=de&oi=ao .

Jörg Huwyler

Professor

Prof. Dr. Jörg Huwyler is full professor and head of the Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel. His research interests are in the field of drug delivery and drug targeting using particulate drug carriers. Important professional milestones after his PhD in biochemistry and a habilitation in pharmacy were appointments at the University Hospital of Basel and the Brain Research Institute, UCLA School of Medicine, Los Angeles. From 1999 to 2006 he joined the pharmaceutical industry, where he worked as DMPK project leader for F. Hoffmann-La Roche Ltd. in Switzerland. He joined the University of Basel in the year 2010.)

RECENT PUBLICATIONS

- Alter, C.L. et al., 2023. High efficiency preparation of monodisperse plasma membrane derived extracellular vesicles for therapeutic applications. Commun Biol 6, 1–17. https://doi.org/10.1038/s42003-023-04859-2
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- Sieber, S. et al., 2017. Zebrafish as an early stage screening tool to study the systemic circulation of nanoparticulate drug delivery systems *in vivo*. Journal of Controlled Release 264, 180–191. https://doi.org/10.1016/j.jconrel.2017.08.023

Sarah Ibrahim

Sarah Ibrahim is the Associate Director for Stakeholder and Global Engagement in the Office of Generic Drugs (OGD)/ Center of Drug Evaluation and Research (CDER) at the U.S. Food and Drug Administration (FDA). In this role, Dr. Ibrahim develops OGD strategies to address identified and emerging regulatory challenges in relation to the international nature of the generic drug industry. In collaboration with other CDER and FDA offices, she supports stakeholder engagement concerning issues related to globalization of the generic pharmaceutical supply and harmonization of regulatory approaches for generic drugs. Dr. Ibrahim received her PhD in Biopharmaceutics/ Pharmaceutics from the School of Pharmacy, University of Cincinnati and a B.S. in Pharmacy and Pharmaceutical Sciences from Cairo University, Egypt. Dr. Ibrahim started her career at the FDA in 2014 as a scientific reviewer in the Office of Pharmaceutical Quality. Prior to her FDA career, she has years of experience in the US pharmaceutical industry in the area of pharmaceutical development. As an assistant professor, along with the founding faculty, Dr. Ibrahim established the pharmaceutical sciences department for the second school of pharmacy in the state of New Jersey.

Lloyd Jeffs

PhD.
Senior Director of Biopharma Services

Dr. Lloyd Jeffs joined Precision NanoSystems (PNI) in June 2018. His Biopharma Services team is responsible for developing and executing custom programs to meet the clinical manufacturing needs of PNI's clients. Lloyd is an expert in developing lipid-based nanotherapeutics and has over 20 years of experience in this field, including formulation and process development, scale-up and technology transfer.
Dr. Jeffs received his PhD. in Applied Microbiology from the University of Saskatchewan and has B.Sc. and M.Sc. degrees from the University of British Columbia. He is a co-author of numerous peer-reviewed publications dealing with the development of lipid nanoparticle therapeutics and is a co-inventor for key patents in this field.

Michael Johnston

Research Scientist

Dr. Johnston's (PhD, University of British Columbia) has over 20 years of experience in the formulation of liposomes and lipid nanoparticles (LNP) and is a research scientist within Health Canada. He and his laboratory's current focus is on the development of model LNP vaccines as a tool for regulatory improvement of these products. Dr. Johnston also heads the Health Products and Food Branches nanotechnology working group, previously co-chaired the International Pharmaceutical Regulators Forum Nanomecidines working group and is a member of the Government of Canada's Interdepartmental Nanotechnology working group.

RECENT PUBLICATIONS

- 2023 Bivalent vaccines effectively protect mice against influenza A and respiratory syncytial viruses, Emerging Microbes & Infections, 12:1, DOI: 10.1080/22221751.2023.2192821
- 2023* DNA lipid nanoparticle vaccine targeting outer surface protein C affords protection against homologous Borrelia burgdorferi needle challenge in mice, Front. Immunol., Vol. 14, DOI: 10.3389/fimmu.2023.1020134
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Michael Keller

Michael Keller studied Chemistry & Biochemistry at the ETH Zürich from 1989-1994. The award of the ETHZ-Imperial College London exchange scholarship 1994 enabled him to pursue a MSC/DIC in Chemical Research at Imperial College London, before joining the Research group of Professor Manfred Mutter at the University of Lausanne where he carried out a PhD in Bioorganic Chemistry. After a year as lecturer at the same Institute, he joined Imperial College London Genetic Therapies Centre as Academic Visitor specializing in nonviral delivery systems for nucleic acids. He co-founded the Anglo/Japanese Biotech company IC-Vec Ltd. in 2002 developing novel cationic lipids and nanomedicines for siRNA delivery, before joining Novartis Pharma AG Basel to build up siRNA formulation in Technical Research & Development. He was awarded the Novartis Leading Scientist Award in 2009 for his work on siRNA delivery. In late 2017 he joined the Therapeutic Modality function at pRED/Hoffmann-La Roche Ltd. Basel to work on nucleic acid based medicines (NABM), with a particular focus on disruptive concepts to enable efficient delivery of nucleic acids to non-hepatic tissues.

Andreas Kjaer

Professor, chief physician, MD, PhD

Dr. Andreas Kjaer is a professor at the University of Copenhagen and a chief physician at the Department of Clinical Physiology and Nuclear Medicine at Rigshospitalet, the National University Hospital of Denmark.
Professor Kjaer is a former president of the Scandinavian Society of Clinical Physiology and Nuclear Medicine (SSCPNM) and served on the Scientific Committee of the Danish Cancer Society and the European Association of Nuclear Medicine (EANM) Oncology Committee. He is currently a member of the executive committee of the European Neuroendocrine Tumor Society (ENETS). Professor Kjaer is the founding Editor-in-Chief of Diagnostics (Basel), head of the Cluster for Molecular Imaging, and director of the Postgraduate School for Medical and Molecular Imaging at the Faculty of Health Sciences, University of Copenhagen.
Scientific achievements include development of new PET tracers of which 5 have so far entered clinical use or clinical testing. He has published more than 600 peer-reviewed articles, filed more than 20 patents and received numerous prestigious scientific awards over the years. He is an elected member of the Danish Academy of Technical Sciences and received Knighthood from her Majesty the Queen of Denmark.

RECENT PUBLICATIONS

- Persson M, Skovgaard D, Brandt-Larsen M, …, Kjaer A. First-in-human uPAR PET: Imaging of cancer aggressiveness. Theranostics. 2015; 5: 1303-16
- Fosbøl MØ, Kurbegovic S, Johannesen HH, …, Kjaer A. Urokinase Plasminogen Activator Receptor (uPAR) PET/MRI of Prostate Cancer for Non-invasive Evaluation of Aggressiveness: a Prospective Phase II Clinical Trial Comparing with Gleason Score. J Nucl Med. 2020; 62: 354-359.
- Risør LM, Clausen MM, Ujmajuridze Z, ….. Kjaer A. Prognostic value of Urokinase-type Plasminogen Activator Receptor (uPAR)-PET/CT in Head and Neck Squamous Cell Carcinomas and Comparison with 18F-FDG-PET/CT: A single-center prospective study. J Nucl Med. 2021 (online first Dec 2)
- Juhl K, Christensen A, Rubek N, …., Kjaer A. Improved surgical resection of metastatic pancreatic cancer using uPAR targeted in vivo fluorescent guidance: comparison with traditional white light surgery. Oncotarget 2019; 10: 6308-6316.
- Persson M, Juhl K, Rasmussen P, Brandt-Larsen M, ..., Kjaer A. uPAR targeted radionuclide therapy with 177Lu-DOTA-AE105 inhibits dissemination of metastatic prostate cancer. Mol Pharm. 2014; 11: 2796-806.

Kostas Kostarelos

Professor Kostas Kostarelos
http://www.nanomedicinelab.com

Kostas Kostarelos currently holds the Chair of Nanomedicine at the Faculty of Biology, Medicine & Health and the National Graphene Institute (NGI) of the University of Manchester (UK) and is the Severo Ochoa Distinguished Professor at the Catalan Institute of Nanoscience and Nanotechnology (ICN2) in Barcelona (Spain).
Kostas read Chemistry at the University of Leeds and obtained his Diploma in Chemical Engineering and PhD from the Department of Chemical Engineering at Imperial College London, studying the

steric stabilization of liposomes using block copolymer molecules. He carried out his postdoctoral training in various medical institutions in the United States and worked closely in his career with Professors Th.F. Tadros (ICI plc, UK), P.F. Luckham (Imperial College London), D. Papahadjopoulos (UCSF, USA), G. Sgouros (Memorial Sloan-Kettering, NY, USA), R.G. Crystal (Weill Medical College of Cornell University, NY, USA).
He was Assistant Professor of Genetic Medicine & Chemical Engineering in Medicine at Cornell University Weill Medical College when he relocated to the UK as the Deputy Director of Imperial College Genetic Therapies Centre in 2002. In 2003 Professor Kostarelos joined the Centre for Drug Delivery Research at the UCL School of Pharmacy as the Deputy Head of the Centre. He was promoted to the Personal Chair of Nanomedicine and Head of the Centre in 2007. The entire Nanomedicine Lab was embedded within the Faculty of Medical and Human Sciences and the National Graphene Institute at the University of Manchester in 2013.
He has been invited Fellow of the Royal Society of Chemistry (FRSC), Fellow of the Royal Society of Medicine (FRSM), and Fellow of the Royal Society of Arts (FRSA) all in the United Kingdom. In 2010 he was awarded the Japanese Society for the Promotion of Science (JSPS) Professorial Fellowship with the National Institute of Advanced Industrial Science and Technology (AIST) in Tsukuba, Japan. Kostas is the Founding and Senior Editor of the journal Nanomedicine (Future Medicine) and sits on the Editorial Advisory Board of ACS Nano (ACS), Nanoscale Horizons (RSC), npj 2D Materials & Applications (NPJ), Archives in Toxicology (Springer), Cell Reports Physical Science (Cell Press). He was included in the Highly Cited Researcher 2018 list in the Cross-Field category.

Silke Krol

Senior editor with European Research Services, Munster, Germany

CEO with encytos B.V, Enschede, The Netherlands

Visiting researcher with University of Twente, Enschede, The Netherlands.

RECENT PUBLICATIONS

- 1. Reshamwala D, Shroff S, Sheik Amamuddy O, Laquintana V, Denora N, Zacheo A, Lampinen V, Hytonen VP, Tastan Bishop Ö, Krol S, Marjomäki V. Polyphenols Epigallocatechin Gallate and Resveratrol, and Polyphenol-Functionalized Nanoparticles Prevent Enterovirus Infection through Clustering and Stabilization of the Viruses. Pharmaceutics. 2021 Jul 31;13(8):1182. doi: 10.3390/pharmaceutics13081182. PMID: 34452144; PMCID: PMC8398301.2. Centonze M, Berenschot EJW, Serrati S, Susarrey-Arce A, Krol S. The Fast Track for Intestinal Tumor Cell Differentiation and In Vitro Intestinal Models by Inorganic Topographic Surfaces. Pharmaceutics. 2022 Jan 17;14(1):218. doi: 10.3390/pharmaceutics14010218. PMID: 35057113; PMCID: PMC8781367.
- Dituri F, Centonze M, Berenschot EJW, Tas NR, Susarrey-Arce A, Krol S. Complex Tumor Spheroid Formation and One-Step Cancer-Associated Fibroblasts Purification from Hepatocellular Carcinoma Tissue Promoted by Inorganic Surface Topography. Nanomaterials (Basel). 2021 Nov 28;11(12):3233. doi: 10.3390/nano11123233. PMID: 34947582; PMCID: PMC8706479.)

Martin Kuentz

Professor of Pharmaceutical Technology

I started at the University of Applied Sciences and Arts Northwestern Switzerland in 2007 where I became a professor of Pharmaceutical Technology in 2008. My research group is in the field of oral delivery of poorly water-soluble drugs by means of lipid-based systems and solid dispersions and there is a more recent interest in computational modeling in this field of Pharmaceutics. Prior to my academic assignment, I spent nearly seven years at F. Hoffmann-La Roche Ltd. in pharmaceutical research and development of oral drug products. The preceding PhD thesis was about the physics of pharmaceutical compaction under the supervision of Prof. Leuenberger at the University Basel where I studied earlier Pharmacy with additional semesters in Physical Chemistry to graduate in 1995 as a pharmacist. I published more than 160 articles in peer-reviewed journals and have given numerous scientific presentations at international conferences and workshops. I'm a work package leader in the EU projects PEARRL and InPharma and serve on the editorial board/advisory board of the European Journal of Pharmaceutical Sciences, Journal of Pharmaceutical Sciences, "Die Pharmazie". Moreover, I'm menber of the science advisory board of SweDeliver (Uppsala, Sweden) as well as a member of AAPS, APV, GSIA, and CISDEM (Cátedra Iberoamericana-Suiza de Desarrollo de Medicamentos)

RECENT PUBLICATIONS

- Gautschi, N., Van Hoogevest, P., Kuentz, M., 2017. Molecular Insights into the Formation of Drug-Monoacyl Phosphatidylcholine Solid Dispersions for Oral Delivery. Eur. J. Pharm. Sci. 108, 93-100.
- Kuentz, M., Holm, R., Kronseder, Ch., Saal, Ch., Griffin, B.T., 2021. Rational Selection of Bio-Enabling Oral Drug Formulations – A PEARRL Commentary. J. Pharm. Sci. 110, 1921-1930.
- Wyttenbach, N., Niederquell, A., Kuentz, M., 2020. Machine Estimation of Drug Melting Properties and Influence on Solubility Prediction. Mol. Pharm. 17(7), 2660-2671.
- Wyttenbach, N., Niederquell, A., Ectors, Ph., Kuentz, M., 2022. Study and Computational Modeling of Fatty Acid Effects on Drug Solubility in Lipid-Based Systems. J. Pharm. Sci. 111(6), 1728-1738.
- Holm, R., Kuentz, M., Ilie-Spiridon, A.R., Griffin, B.T., 2023. Lipid Based Formulations as Supersaturating Oral Delivery Systems: from Current to Future Industrial Applications. Eur. J. Pharm. Sci. (submitted)

Ulrich Lächelt

Ass. Professor

Ulrich Lächelt studied pharmaceutics at the University of Heidelberg. He received license as pharmacist in 2011 and a doctoral degree at the LMU Munich in 2014 for his work on non-viral gene vectors together with Prof. Ernst Wagner. Since then, he continued his research on drug delivery and nanomedicine as junior research group leader and obtained the habilitation (venia legendi) in 2021. In October 2021 he was appointed as assistant professor for Preclinical Medicines Development at the University of Vienna. His research focusses on the intracellular delivery and therapeutic applications of biomacromolecules as well as the development of inorganic-organic hybrid nanopharmaceuticals.
Until now, he has authored 54 publications, 3 book chapters and 2 patent applications. He received a prize for excellent exam, an

AbbVie Doctoral Thesis Award and the Galenus Technology Prize. He is extraordinary member of the Center for NanoScience at the LMU Munich, and editorial board member of the European Journal of Pharmaceutics and Biopharmaceutics as well as Pharmaceutical Nanotechnology.

RECENT PUBLICATIONS

- Lin Y., Wilk U., Pöhmerer J., Hörterer E., Höhn M., Luo X., Mai H., Wagner E., Lächelt U., Folate Receptor-Mediated Delivery of Cas9 RNP for Enhanced Immune Checkpoint Disruption in Cancer Cells, Small, 2022, 2205318.
- Kuhn J., Lin Y., Krhac Levacic A., Al Danaf N., Peng L., Höhn M., Lamb D.C., Wagner E., Lächelt U., Delivery of Cas9/sgRNA Ribonucleoprotein Complexes via Hydroxystearyl Oligoamino Amides, Bioconj. Chem., 2020, 31(3), 729-742.
- Kuhn J., Klein P.M., Al Danaf N., Nordin J.Z., Reinhard S., Loy D.M., Höhn M., El Andaloussi S., Lamb D.C., Wagner E., Aoki Y., Lehto T., Lächelt U., Supramolecular Assembly of Aminoethylene-Lipopeptide PMO Conjugates into RNA Splice-Switching Nanomicelles, Adv. Funct. Mater., 2019, 29, 1906432.
- Röder R., Preiß T., Hirschle P., Steinborn B., Zimpel A., Höhn M., Rädler J.O., Bein T., Wagner E., Wuttke S., Lächelt U., Multifunctional nanoparticles by coordinative self-assembly of His-tagged units with metal-organic frameworks, J. Am. Chem. Soc., 2017, 139(6), 2359–2368.
- Lächelt U., Wagner E., Nucleic Acid Therapeutics Using Polyplexes: A Journey of 50 Years (and Beyond), Chem. Rev. (Washington, DC, U. S.) 2015, 115, 11043-11078.

Twan Lammers

Professor, Head of Department

Twan Lammers obtained a D.Sc. in Radiation Oncology from Heidelberg University in 2008 and a Ph.D. in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nano-medicine and Theranostics group at RWTH Aachen University. In 2014, he was promoted to full professor of medicine at RWTH Aachen University Hospital. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided (theranostic) drug delivery systems are being developed, as well as materials and methods to monitor tumor growth, angiogenesis, inflammation, fibrosis and metastasis. He received multi-ple scholarships and awards, including ERC starting, consolidator and proof-of-concept grants, the CRS Young Investigator Award, the Adritelf International Award, the Belgian Society for Pharma-ceutical Sciences International Award, and the JNB Trailblazer Award. He serves on the editorial board of 10 journals, and is associate editor for JCR, DDTR and MIB. Since 2019, he has been in-cluded in the Clarivate Analytics list of Highly Cited Researchers.

RECENT PUBLICATIONS

- Sun Q, Baues M, Klinkhammer B, Ehling J, Djudjaj S, Drude N, Daniel C, Amann K, Kramann R, Kim H, Saez J, Weiskirchen R, Onthank D, Botnar R, Kiessling F, Floege J, Lammers T, Boor P. Elastin imaging enables non-invasive staging and treatment monitoring of kidney fibrosis. Science Translational Medicine 11, eaat4865, 2019.
- Van der Meel R, Sulheim E, Shi Y, Kiessling F, Mulder W, Lammers T. Smart cancer nanomedicine. Nature Nanotechnology 14, 1007-1017, 2019.
- Biancacci I, De Lorenzi F, Theek B, Bai X, May J, Consolino L, Baues M, Moeckel D, Gremse F, von Stillfried S, El Shafei A, Benderski K, Shalmani A, Wang A, Momoh J, Pena Q, Buhl E, Buyel J, Hennink Kiessling F, Metselaar J, Shi Y, Lammers T. Monitoring EPR effect dynamics during nanotaxane treatment with theranostic polymeric micelles. Advanced Science e2103745, 2022.
- Bhatia S, Chen X, Dobrovolskaia M, Lammers T. Cancer nanomedicine. Nature Reviews Cancer 22, 550-556, 2022.
- Dasgupta A, Sun T, Palomba R, Rama E, Zhang Y, Power C, Moeckel D, Liu M, Sarode A, Weiler M, Motta A, Porte C, Magnuska Z, El-Shafei A, Barmin R, Graham A, McClelland A, Rommel D, Stickeler E, Kiessling F, Pallares R, De Laporte L, Decuzzi P, McDannold N, Mitragotri S, Lam-mers T. Nonspherical ultrasound microbubbles. Proceedings of the National Academy of Sciences, 120, e2218847120, 2023.

Dong Soo Lee

Professor

As a nuclear medicine physician and nanomedicine specialist, I recently introduced imaging of cerebrospinal fluid (CSF)- lymphatic drainage using Cu-64 labelled albumin Positron Emission Tomopgraphy (PET). CSF-lymphatic drainage of exosomes could also be elucidated using the same technology of PET and Cu-64 click labeling of exosomes. We aim to investigate the roles of CSF-lymphatic interaction using this method to investigate pathogenesis of neurodegenerative diseases and novel immunomodulatory therapy to treat them.

RECENT PUBLICATIONS

- Lee, D. S., Suh, M., Sarker, A., & Choi, Y. (2020). Brain glymphatic/lymphatic imaging by MRI and PET. Nuclear Medicine and Molecular Imaging, 54, 207-223.
- Sarker, A., Suh, M., Choi, Y., Park, J. Y., Kwon, S., Kim, H., ... & Lee, D. S. (2022). [64Cu] Cu-Albumin Clearance Imaging to Evaluate Lymphatic Efflux of Cerebrospinal Space Fluid in Mouse Model. Nuclear Medicine and Molecular Imaging, 56(3), 137-146.
- Sarker, A., Suh, M., Choi, Y., Park, J. Y., Lee, Y. S., & Lee, D. S. (2023). Intrathecal [64Cu] Cu-albumin PET reveals age-related decline of cerebrospinal fluid (CSF)-lymphatic efflux. bioRxiv, 2023-01.

Jean-Marie Lehn

ISIS, Université de Strasbourg
8 allée Gaspard Monge,
F-67000 Strasbourg
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E-mail: lehn@unistra.fr

- Professor at the University of Strasbourg Institute for Advanced Study (USIAS), Chair of Chemistry of Complex Systems
- Honorary Professor at the Collège de France, Paris
- Emeritus Professor at the University of Strasbourg, Director
- Director of ISIS (Institut de Science et d'Ingénierie Supramoléculaires), Strasbourg, 1997–2004.
- Director of the Laboratoire de Chimie Supramoléculaire, ISIS, Université de Strasbourg
- Director at the Nanotechnology Institute of the Karlsruhe Institute of Technology, since 1998
- Honorary Director, "Lehn Institute of Functional Materials", Sun Yat Sen University, Guangzhou, since 2010

EDUCATION

Undergraduate Studies, University of Strasbourg: Licence ès-Sciences (Bachelor of Sciences), Strasbourg, 1960; Graduate work on

"Conformational Studies of Triperpenes" with Professor Guy OURISSON, University of Strasbourg; Doctorat-ès-Sciences (Ph.D.), University of Strasbourg, 1963; Post-Doctoral Research Fellow at Harvard University, 1964: work on Vitamin B12 total synthesis with Professor Robert B.WOODWARD.

NOBEL PRIZE

Nobel Prize in Chemistry, 1987; Sigillum Magnum, University of Bologna, 1988.

SCIENTIFIC WORK

985 publications ; 3 books

- "Chemia Supramolekularna", Collection of publications by J.-M. LEHN, organised and translated into Polish under the direction of Janusz Lipkowski, Institute of Physical Chemistry, Polish Academy of Sciences, 1985.
- B. DIETRICH, P. VIOUT, J.-M. LEHN, "Aspects de la chimie des composés macrocycliques", InterEditions/Editions du CNRS, 1991. English Version: "Macrocyclic Chemistry – Aspects of Organic and Inorganic Supramolecular Chemistry", VCH, Weinheim, 1993.
- J.-M. LEHN, "Supramolecular Chemistry – Concepts and Perspectives", VCH, 1995. French Version : "La chimie supramoléculaire : Concepts et perspectives", Translated from the English original text by A. Pousse, De Boeck Université, Bruxelles, 1997. Portuguese Version, translated by M.J. Calhorda, R. Delgado, A.M. Martins, V. Gageiro Machado, N. Miranda, 2007; Japanese Version, translated by Y. Takeuchi, Kagaku Dojin, Tokyo, 1997; Russian Version, translated by E.V. Boldyreva ; coeditors, V.V. Vlassov and A.A. Varnek; Nauka, Novosibirsk, 1998; Chinese Version, translated by X. Shen, Peking University, Beijing, 2002.

Claus-Michael Lehr

Professor & Head of department

Claus-Michael Lehr is Professor at Saarland University as well as cofounder and head of the department "Drug Delivery" at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). He has also been cofounder of Across Barriers GmbH and PharmBioTec GmbH.
Claus-Michael Lehr's research focus is on drug delivery across biological barriers, which involves advanced (nano-)carriers as well as human cell culture models of epithelial barriers (lung, gut, skin). He is co-editor of the European Journal of Pharmaceutics and Biopharmaceutics and has been the initiator of the International Conference on "Biological Barriers", which takes place biennially at Saarland University. In August 2023, he will be hosting the 24th Congress of the International Society of Aerosols in Medicine (ISAM).

RECENT PUBLICATIONS

- Carius P, Jungmann A, Bechtel M, Grißmer A, Boese A, Gasparoni G, Salhab A, Seipelt R, Urbschat K, Richter C, Meier C, Bojkova D, Cinatl J, Walter J, Schneider-Daum N, Lehr C-M (2023) A Monoclonal Human Alveolar Epithelial Cell Line ("Arlo") with Pronounced Barrier Function for Studying Drug Permeability and Viral Infections. Adv. Sci. 2023, 2207301
- Huck B, Thiyagarajan D, Bali A, Boese A, Besecke K, Hozsa C, Gieseler R, Furch M, Carvalho-Wodarz C, Waldow F, Schwudke D, Metelkina O, Titz A, Huwer H, Schwarzkopf K, Hoppstädter J, Kiemer A K, Koch M, Loretz B, Lehr C-M (2022) Nano-in-Microparticles for Aerosol Delivery of Antibiotic-Loaded, Fucose-Derivatized, and Macrophage-Targeted Liposomes to Combat Mycobacterial Infections: In Vitro Deposition, Pulmonary Barrier Interactions, and Targeted Delivery. Adv Healthcare Mater. 2022, 11, 2102117
- Horstmann J, Laric A, Boese A, Yildiz D, Röhrig T, Empting M, Frank N, Krug D, Müller R, Schneider-Daum N, Carvalho-Wodarz C, Lehr C-M (2022) Transferring Microclusters of P. aeruginosa Biofilms to the Air-Liquid Interface of Bronchial Epithelial Cells for Repeated Deposition of Aerosolized Tobramycin. ACS Infect. Dis. 2022, 8, 137-149
- Schütz C, Ho D-K, Hamed M M, Abdelsamie A S, Röhrig T, Herr C, Hany A M, Rox K, Schmelz S, Siebenbürger L, Wirth M, Börger C, Yahiaoui S, Bals R, Scrima A, Blankenfeldt W, Horstmann J, Christmann R, Murgia X, Koch M, Berwanger A, Loretz B, Hirsch A K H, Hartmann R W, Lehr C-M, Empting M (2021) A New PqsR Inverse Agonist Potentiates Tobramycin Efficacy to Eradicate Pseudomonas aeruginosa Biofilms. Adv. Sci. 2021, 8, 2004369
- Ho D-K, Murgia X, Rossi C de, Christmann R, Hüfner de Mello Martins, A.G., Koch M, Andreas A, Herrmann J, Müller R, Empting M, Hartmann RW., Desmaele D, Loretz B, Couvreur P, Lehr C-M (2020) Squalenyl Hydrogen Sulfate Nanoparticles for Simultaneous Delivery of Tobramycin and an Alkylquinolone Quorum Sensing Inhibitor Enable the Eradication of P. aeruginosa Biofilm Infections. Angew. Chem. Int. Ed. 59:10292–10296.

Beat Löffler

Beat Löffler, MD h.c. MA studied after a study visit in the USA, Philosophy, Communication Sciences and Politics at the University the Freie Universität in Berlin, graduating with a Master of Arts. 2007 he absolved the training of the European Center of Pharmaceutical Medicine (ECPM). In 2014 he received an MD h.c. from the University of Basel. He founded the European Foundation for Clinical Nanomedicine in 2007 together with Patrick Hunziker. The aim of the foundation is the research and development of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in nanomedicine and related fields. Today is his 14th programme and organization of the scientific summit on clinical nanomedicine. The foundation launched the European Journal of Nanomedicine PRNANO and founded the European Society for Nanomedicine and the International Society for Nanomedicine.

PUBLICATIONS

- INSIDE EMRS Vol. 1 | No. 3 December 2022. The Landscape of Nanomedicine for Global Health
- Editor of the Proceedings 1 -14 of the European Summit for European and Global Health
- Regular contributions to PRNANO https://precisionnanomedicine.com/

Robert Luxenhofer

Professor for Soft Matter Chemistry

Robert Luxenhofer completed his PhD in 2007 at the TU München in polymer chemistry developing a novel polymer functionalization approach since developed further by Serina Therapeutics to introduce the first-in-human poly(2-oxazoline)-drug conjugates. As a postdoc with Alexander V. Kabanov at the University of Nebraska Medical Center, he discovered ultra-high loaded drug formulations and investigated structure dependent endocytosis of polymer amphiphiles. Returning to Germany in 2009, he started to investigate polysarcosine and polypeptoids as biomaterials at the TU Dresden. In 2012, he joined the Julius-Maximilians Universität

as an Associate Professor, where he continued working on polypeptoids and ultra-high drug formulations, but also started investigating biofabrication and 3D printing using melt electrowriting. In 2019, he joined the University of Helsinki as a Full Professor. He holds 7 patents and is co-founder of two companies focusing on developing novel polymers for medical applications)

PUBLICATIONS

- J. Kehrein, E. Gürsöz, R. Luxenhofer, A. Bunker. Unravel the tangle: atomistic insight into ultrahigh curcumin-loaded poly(2-oxazoline) and poly(2-oxazine)-based micelles. Small 2023, 2303066. DOI: 10.1002/smll.202303066
- S. Endres, S. Ehrmanntraut, L. Meier, K. Can, K. Kraft, T. Rasmussen, R. Luxenhofer, B. Böttcher, B. Engels, A.-C. Pöppler, Detailed structural investigation of the effect of guest loading of poly(2-oxazoline) based micelles on their interaction with fed state simulated intestinal fluids. ACS Biomaterials Science and Engineering 2023, in print. DOI: 10.1021/acsbiomaterials.3c00645.
- Q. Yu, R. M. England, A. Gunnarsson, R. Luxenhofer, K. Treacher, M. Ashford, Designing Highly Stable Poly(sarcosine)-based Telodendrimer Micelles with High Drug Content Exemplified with Fulvestrant, Macromolecules 2022, 2100331. DOI: 10.1002/macp.202100331
- S. Hasselmann, L. Hahn, T. Lorson, E. Schätzlein, I. Sébastiani, M. Beudert, Tessa Lühmann, G. Sextl, R. Luxenhofer*, D. Heinrich*, Freeform direct laser writing of versatile topological 3D scaffolds enabled by intrinsic support hydrogel, Materials Horizons 2021, 8, 3334-3344. DOI: 10.1039/d1mh00925g.
- M.S. Haider, M. M. Lübtow, S. Endres, S. Forster, V.J. Flegler, B. Böttcher, V. Aseyev, A.-C. Pöppler, R. Luxenhofer*, Think Beyond the Core: The Impact of the Hydrophilic Corona on the Drug Solubilization Using Polymer Micelles. ACS Appl. Mat. Interfac. 2020, 12, 24531–24543. DOI: 10.1021/acsami.9b22495.

Volker Mailänder

Group leader (Univ.-Prof.)

Volker Mailänder studied medicine at the University of Ulm supported by a stipend from the Studienstiftung des Deutschen Volkes and was in the graduate program "Molecular Biology". He worked in the Blume/Negrin lab at Stanford, California, on natural killer cells and was involved in patient care in the bone marrow transplantation unit. Afterward, he received training in internal medicine (hematology/oncology) at the Charité Hospital in Berlin. After relocating to the Institute for Clinical Transfusion Medicine, University Clinic of Ulm, he worked on stem cell manipulation, the interaction of nanoparticles with cells, and especially uptake mechanisms and the intracellular pathway. He was board certified in transfusion medicine. Further work focused on using polymeric nanoparticles to label or manipulate stem cells and other cell types. Since 2008 he is leading a joint research group between the University Medical Clinic and the MPI for Polymer Science in Mainz. He has been appointed a professorship dealing with the translation of nanocarriers into medical applications. He is proficient in the procedures of manipulating, freezing, and storing stem and immune cells for patient care as the head of production and qualified person. He is active in several cooperative projects (SFB1066 "Nanodimensional polymeric therapeutics for tumor therapy", BMBF projects) and is vice speaker of the center BiomaTiCS (Biomaterials, Tissues, and Cells in Science) of the University Medical Center. Since 1.1.2016 he has been a W2 professor at the University Medicine Mainz, associated with the Dermatology department, and heads the Center for Translational Nanomedicine – CTN. He is especially interested in understanding and overcoming the hurdles of applying nanocarriers for use in clinical applications. Therefore, protein corona, targeting and GMP-conform production of nanocarriers are the main focus of his research.

PUBLICATIONS

- Simon, J., Fichter, M., Kuhn, G., Brückner, M., Kappel, C., Schunke, J., Klaus, T., Grabbe, S., Landfester K., Mailänder, V. Achieving dendritic cell subset-specific targeting *in vivo* by site-directed conjugation of targeting antibodies to nanocarriers. Nano Today (2022), 43,2022,101375
- Prawatborisut, M.; Oberländer, J.; Jiang, S.; Graf, R.; Avlasevich, Y.; Morsbach, S.; Crespy, D.; Mailänder, V.; Landfester, K., Temperature-Responsive Nanoparticles Enable Specific Binding of Apolipoproteins from Human Plasma. Small (2021), e2103138.
- M Tonigold, J Simon, D Estupiiñán, M Kokkinopoulou, J Reinholz, U Kintzel, A Kaltbeitzel,P Renz, MP Domogalla, K Steinbrink, I Lieberwirth, D Crespy, K Landfester, and V Mailänder: Pre-adsorption of antibodies enables targeting of nanocarriers despite a biomolecular corona; Nature Nanotechnology (2018), 13(9): 862-+
- J Simon, T Wolf, K Klein, K Landfester, FR Wurm, and V Mailänder: Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers; Angewandte Chemie International Edition (2018) 57(19): 5548-555
- Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailänder V, Wurm FR.: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. Nat Nanotechnol. 2016 Apr;11(4):372-7

Ulrich Massing

Apl. Prof. (Inst. Pharm. Sciences, University of Freiburg, Germany), Head Dept. F&E Lifescience Applications (Andreas Hettich GmbH & Co KG).

Ulrich Massing, Chemist, performed his Ph.D. studies at the Max Planck Institute for Biophysical Chemistry (Göttingen) studying the substrate recognition of phospholipase C & sphingomyelinase. After a postdoctoral training position (development of a new class of sPLA2-inhibitors), he moved to Freiburg in 1993 to become a principal scientist at the newly established Tumor Biology Center. Research focus of his department/group was on PLA2-inhibitors to prevent metastases, development of Alkylphosphocholines to induce apoptosis of cancer celss, development of new lipid nanoparticles (liposomes) carrying anticancer drugs to improve cancer therapy, investigation of the role of LysoPC in metastases, development of new cationic lipids for gene transfer, and recently the development of new methods to easily produce new anticancer liposomes using dual centrifugation (in-vial homogenization). Ulrich Massing obtained his Habilitation in 2000 at the university of Heidelberg (pharmaceutical chemistry), where he teached for about 10 years. In 2010, he started teaching at the University of Freiburg at the Institute for Pharmaceutical Sciences (instrumental analysis, stereochemistry, modern pharmaceutical strategies in cancer therapy, nutrition, etc.). When the Tumor Biology Center closed in 2015, Prof. Massing moved to Andreas Hettich GmbH & Co KG leading a newly established department focusing on the development of new lab devices, with a strong focus on dual centrifugation, which meanwhile become the quasi-standard for the fast and easy preparation of all kinds of liposomes, emulsions and nanocrystals. Prof Massing is still active in teaching and supervising PhD, Master and Bachelor students in Freiburg and Heidelberg. Prof. Massing was awarded with the innovation award of Freiburg as well as of the state of Baden-Württemberg.

RECENT PUBLICATIONS

- K Koehler, J Schnur, H Heerklotz, U Massing; Screening for Optimal Liposome Preparation Conditions by Using Dual Centrifugation and Time-Resolved Fluorescence Measurements, Pharmaceutics 2021, 13(12), 2046

- JK Koehler, LGedda, L Wurster, J Schnur, K Edwards, H Heerklotz, U Massing; Tailoring the Lamellarity of Liposomes Prepared by Dual Centrifugation, Pharmaceutics 2023, 15(2), 706
- LA Taylor, J Arends, AK Hodina, C Unger, U Massing; Plasma lysophosphatidylcholine concentration is decreased in cancer patients with weight loss and activated inflammatory status Lipids in Health and Disease 2007, 6, 17
- A Raynor, P Jantscheff, T Ross, M Schlesinger, M Wilde, S Haasis, T Dreckmann, G Bendas, U Massing; Saturated and mono-unsaturated lysophosphatidylcholine metabolism in tumour cells: a potential therapeutic target for preventing metastases, Lipids in Health and Disease (2015) 14, 69)

Stephane Mazlan

Regional Business Director (EMEA) of Izon Science

I am the Regional Business Director (EMEA) for Izon Science Europe and I focus on developing collaborative projects with academic and industry partners across the Europe, Middle East and Africa (EMEA) region in the diagnostic and therapeutic space. This entails scalable nanoparticle purification in terms of both volume and throughput using Izon's qEV technology.

Prior to Izon Science, I embarked on my extracellular vesicle (EV) journey a decade ago in the field of cardiovascular diseases where I was managing the lab and leading the large animal surgical team in developing heart failure models in the Cardiovascular Research Institute at the National University of Singapore. This was then followed by the completion of my doctoral studies at the University of Paris (Sorbonne Paris Cité) on the role of EVs in the context of myocardial infarction and diabetes type II.

Born and raised in Singapore, my EV experience spans cross-functional technology solutions in terms of both EV characterisation as well as EV isolation.

Spencer McGrath

Spencer McGrath, MA, is a publishing professional with more than 15 years of experience managing scientific journals for commercial publishers and not-for-profit associations in both Europe and the United States . Since 2018, he has worked as Director of Scientific Publications for The American Association of Thoracic Surgery and as Managing Editor of the Journal of Thoracic and Cardiovascular Surgery. He is responsible for the editorial operations of six peer-reviewed journals in the field of cardiothoracic surgery, publishing more than 1,200 papers each year and receiving over 4,000 submissions annually.

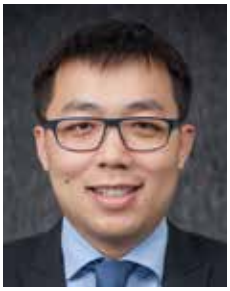
Scott McNeil

Professor

Prof. Dr. Scott McNeil is the Head of the Nanopharmaceutical and Regulatory Science Group within the Department of Pharmaceutical Sciences at the University of Basel. He received his B.S. degree in Chemistry from Portland State University and his Ph.D. in Cell Biology and Anatomy from Oregon Health Sciences University. Scientists within his group develop and characterize novel nano-based formulations, with the goal of improving the therapeutic index of active pharmaceutical ingredients. Prof. McNeil's research involves Regulatory Science topics in nanomedicine and using nanomedicines to deliver enzymes for the treatment of lysosomal storage diseases. In collaboration with other research institutions, the Group also identifies and investigates the critical quality attributes (CQAs) of nanopharmaceuticals and nanosimilars, such as mechanisms of action, safety, and practical application issues. Prior to joining the University of Basel in 2020, he was the Director of the Nanotechnology Characterization Laboratory (NCL) at the National Cancer Institute. In addition to his academic career, McNeil served for twenty years in the US Army.

RECENT PUBLICATIONS

- Eva Hemmrich and Scott McNeil. "Active ingredient vs excipient debate for nanomedicines." Nature Nanotechnology (2023): 1-4.
- Klein, K., Borchard, G., Shah, V.P., Flühmann, B., McNeil, S.E. and de Vlieger, J.S.B. (2021), A pragmatic regulatory approach for complex generics through the U.S. FDA 505(j) or 505(b)(2) approval pathways. Ann. N.Y. Acad. Sci., 1502: 5-13



Huan Meng

Dr. Huan Meng received a bachelor's degree in pharmaceutical science at Peking University and a PhD degree in bioinorganic chemistry in Chinese Academy of Sciences. Dr. Meng is a Professor at the National Center for Nanoscience and Technology (NCNST) in China. Before joining NCNST, Dr. Meng was an Associate Professor of Medicine at University of California, Los Angeles (UCLA). Huan has extensive experience in drug delivery, nanomedicine, nano/bio interface, and nano safety. In the capacity of PI, Dr. Meng has received various research awards such RO1 grants from NIH, and since the relocation to Beijing, his lab was funded by Chinese NSF and Ministry of Science and Technology. He has contributed to >110 peer-review publications, leading to a total of ~18, 000 citations and an H-factor of 55. The impact of Dr. Meng's work was recognized by Clarivate Analytics' inclusion as a "Highly Cited Researcher". Meng is an associate editor of Biomedical Microdevices, and the editor of Nano Today.

PUBLICATIONS

- X Liu, P Lin, I Perrett, J Lin, Y Liao, CH Chang, J Jiang, N Wu, T Donahue, Z Wainberg, A Nel*, H Meng*, Tumor-Penetrating Peptide Enhances Transcytosis of Silicasome-based Chemotherapy for Pancreatic Cancer, The Journal of Clinical Investigation, 2017, 127, 2007–2018
- X Liu, J Jiang, H Meng*, Transcytosis – An effective targeting strategy that is complementary to "EPR effect" for pancreatic cancer nano drug delivery, Theranostics, 2019, 9: 8018–8025
- X Liu, J Jiang, R Chan, Y Ji, J Lu, YP Liao, M Okene, J Lin, P Lin, CH Chang, X Wang, I Tang, E Zheng, W Qiu, ZA. Wainberg, A. Nel,*

and H Meng*, Improved efficacy and reduced toxicity using a Custom-Designed Irinotecan-Delivering silicasome for orthotopic colon cancer, ACS Nano, 2019, 13, 38-53

- Y Ji, X Liu, J Li, X Xie, M Huang, J Jiang Y-P Liao, T Donahue, H Meng*, Use of ratiometrically designed nanocarrier targeting CDK4/6 and autophagy pathways for effective pancreatic cancer treatment. Nature Communications, 2020, 11, 4249.
- S Dong, Z Feng, R Ma, T Zhang, J Jiang, Y Li, Y Zhang, S Li, X Liu, X Liu*, H Meng*, Engineered Design of a Mesoporous Silica Nanoparticle-Based Nanocarrier for Efficient mRNA Delivery In Vivo, Nano Letters, 2023, in press.

Nathalie Mignet

Team Leader

Dr Nathalie MIGNET is Research Director at the National Center for Scientific Research (CNRS) in France. She is the head of the Laboratory Chemical and Biological Technologies for Health located at Université de Paris, also supported by CNRS and INSERM. After a PhD in France in organic chemistry, Dr Mignet was hired by the company Lynx Therapeutics in San Francisco. She then joined the University of Sheffield in UK. In 1998, she was hired by the French biotech company Capsulis to work on onion-based nanoparticles called spherulites. She joined the CNRS as a research Scientist in 2000 to work on non-viral gene delivery. Since then, she expanded her domain of interest from drug delivery systems to nanomedicine designed for triggered delivery or imaging.
With her lab (#Mignet Lab), she is interested in nanomedicine for delivery or imaging going from fundamental to preclinical studie mostly in cancer.
She is also the founder and the president of the French Society for Nanomedecine, SFNano.

RECENT PUBLICATIONS

- Do H et al, Combination of thermal ablation by focused ultrasound, IL-12 pFAR4 plasmid transfection and lipidic adjuvant provide a distal immune response Exploration of Targeted Antitumor Therapy, Explor Target Antitumor Ther. 2022;3:398–413; doi: 10.37349/etat.2022.00090
- Thébault CJ et al. Theranostic MRI liposomes for magnetic targeting and ultrasound triggered release of the antivascular CA4P. J Control Release. 2020, 322:137-148. doi: 10.1016/j.jconrel.2020.03.003.
- Martin B, Seguin J, Annereau M, Fleury T, Lai-Kuen R, Neri G, Lam A, Bally M, Mignet N, Corvis Y. Preparation of parenteral nanocrystal suspensions of etoposide from the excipient free dry state of the drug to enhance *in vivo* antitumoral properties. Science Report 2020 Oct 22;10(1):18059. doi: 10.1038/s41598-020-74809-z
- K. Lemdani, N. Mignet, V. Boudy, J. Seguin, E. Oujagir, O. Bawa, F. Peschaud, J-F. Emile, C. Capron, R. Malafosse Local immunomodulation combined to radiofrequency ablation results in a complete cure of local and distant colorectal carcinoma, Oncoimmunology. 2019;8(3):1550342.
- Manta S, Renault G, Delalande A, Couture O, Lagoutte I, Seguin J, Lager F, Houzé P, Midoux P, Bessodes M, Scherman D, Bureau MF, Marie C, Pichon C, Mignet, N. Cationic Microbubbles and Antibiotic-Free Plasmid for sustained Ultrasound-mediated Transgene Expression in liver. J. Controlled Rel. 2017, 262:170-181.

Hannes Mikula

University Professor

Hannes Mikula is Full Professor (Univ. Prof.) of Chemical Biology at the Institute of Applied Synthetic Chemistry at TU Wien (Vienna, Austria). His research focuses on the development of biocompatible chemical reactions with unmatched performance and unique capabilities. One of the main goals of his group is to design new chemical tools for ultrafast bioorthogonal bond-cleavage and application of this concept in chemical biology and molecular targeting. Hannes trained as a synthetic chemist, finishing his studies in Technical Chemistry in 2008. Following a 1-year career break (parental leave), he received his Ph.D. in 2014. Fascinated and inspired by the field of bioorthogonal chemistry, he then joined the Center for Systems Biology at the Massachusetts General Hospital & Harvard Medical School as a postdoctoral fellow in the group of Prof. Ralph Weissleder. Hannes returned to TU Wien in 2016 as a young principal investigator and has been leading the research group 'Molecular Chemistry & Chemical Biology' since 2018.

RECENT PUBLICATIONS

- Kuba, W.; Sohr, B.; Keppel, P.; Svatunek, D.; Humhal, V.; Stöger, B.; Goldeck, M.; Carlson, J. C. T.; Mikula, H.*, Oxidative Desymmetrization Enables the Concise Synthesis of a trans-Cyclooctene Linker for Bioorthogonal Bond-Cleavage. Chem. Eur. J. 2023, 29, e202203069, doi: 10.1002/chem.202203069
- Ko, J.; Wilkovitsch, M.; Oh, J.; Kohler, R. H.; Bolli, E.; Pittet, M. J.; Vinegoni, C.; Sykes, D. B.; Mikula, H.; Weissleder, R.; Carlson, J. C. T., Spatiotemporal multiplexed immunofluorescence imaging of living cells and tissues with bioorthogonal cycling of fluorescent probes. Nat. Biotechnol. 2022, 40, 1654–1662, doi: 10.1038/s41587-022-01339-6
- Svatunek, D.; Wilkovitsch, M.; Hartmann, L.; Houk, K. N.; Mikula, H.*, Uncovering the Key Role of Distortion in Bioorthogonal Tetrazine Tools that Defy the Reactivity/Stability Tradeoff. J. Am. Chem. Soc. 2022, 144, 8171-8177, doi: 10.1021/jacs.2c01056
- Wilkovitsch, M.; Haider, M.; Sohr, B.; Herrmann, B.; Klubnick, J.; Weissleder, R.; Carlson, J. C. T.; Mikula, H.*, A Cleavable C2-Symmetric trans-Cyclooctene Enables Fast and Complete Bioorthogonal Disassembly of Molecular Probes. J. Am. Chem. Soc. 2020, 142, 19132-19141, doi: 10.1021/jacs.0c07922
- Schwarz, M.; Skrinjar, P.; Fink, M.; Kronister, S.; Mechtler, T.; Koukos, P. I.; Bonvin, A.; Kasper, D.; Mikula, H.*, A click-flipped enzyme substrate boosts the performance of the diagnostic screening for Hunter syndrome. Chem. Sci. 2020, 11, 12671-12676, doi: 10.1039/D0SC04696E

Moein Moghimi

Moein Moghimi is a Professor of Pharmaceutics and Nanomedicine at the School of Pharmacy, and Translational and Clinical Research Institute, Newcastle University (UK), and an Adjoint Professor at the Skaggs School of Pharmacy, University of Colorado, Denver. He is co-founder of S M Discovery Group Inc. and S M Discovery Group Ltd. He further serves as an Associate Editor of Molecular Therapy (the flagship journal of the American Society of Gene Therapy) and Drug Delivery (Taylor and Francis). Previously, he was Chair of Nanomedicine at Durham University (UK), Professor of Nanomedicine at Copenhagen University, Director of the Centre for Pharmaceutical Nanotechnology and Nanotoxicology (Copenhagen University), Visiting Professor at the University of Padova (Italy) and

Affiliate Professor at Houston Methodist Research Institute (Texas). He graduated with Honors in Biochemistry from the University of Manchester (UK) in 1985 and completed his PhD in Biochemistry at Charing Cross and Westminster Medical School (Imperial College). He is widely published and reported in the press, and recognised for his contribution to fundamental and translational research in nanomedicine and drug delivery, especially in mechanistic understanding of nanoparticle-mediated adverse reactions and complement activation processes, and as an inventor of many tissue-specific drug delivery systems and therapeutic platforms (e.g., Nano-Ligand Carriers for crossing the blood-brain barrier and targeting neurons and microglial cells). A 2021 study conducted by Stanford University list Moghimi among the top 0.1% of world's leading scientists across in all fields, and top 60 in pharmacology in the world.

SELECTED REPRESENTATIVE PUBLICATIONS:

Nature Nanotechnology 2023 (in press) | Nature 2022, 603:228 | Nature Communications 2021, 12:4858 | Nature Nanotechnology 2019, 14:260 | Nature Communications 2019, 10:4635 | Nature Nanotechnology 2017, 12:387 | Nature Nanotechnology 2017, 12:589

Roger Molto Pellares

Junior Group Leader

Roger Molto Pallares obtained a PhD in Materials Science from the University College London (UK) in 2017. He has worked as a visiting researcher at NTT Basic Research Laboratories (Japan), visiting doctoral student at A*STAR (Singapore), postdoctoral scholar at Northwestern University (IL, USA), and project scientist at Lawrence Berkeley National Laboratory (CA, USA). In 2021, Roger joined the Institute for Experimental Molecular Imaging (ExMI) at RWTH Aachen University Clinic as a junior group leader, and established the research group "Biohybrid Nanomedical Materials", which aims to develop bio-inspired nanomaterials for diagnostics and therapeutics. He has been recognized with multiple scholarships and awards, including the Vulcanus in Japan fellowship, RWTH JPI Fellowship, and Umbrella Award. He also serves as a leadership committee member of the Molecular Imaging in Nanotechnology and Theranostics (MINT) Interest Group of the World Molecular Imaging Society (WMIS).

RECENT PUBLICATIONS

- 1. R. Zhang, F. Kiessling, T. Lammers, and R. M. Pallares*. Clinical Translation of Gold Nanoparticles. Drug Delivery and Translational Research, 13, 378 (2023).
- R. M. Pallares,* F. M. Mottaghy, V. Schulz, F. Kiessling, T. Lammers. Nanoparticle Diagnostics and Theranostics in the Clinic. Journal of Nuclear Medicine, 63, 1802 (2022).
- R. Barmin, A. Dasgupta, A. Rix, M. Weiler, L. Appold, S. Rutten, F. Padilla, A. Kuehne, A. Pich, L. De Laporte, F. Kiessling, R. M. Pallares,* and T. Lammers. Enhanced stable cavitation and nonlinear acoustic properties of PBCA polymeric microbubbles after bioconjugation. ACS Biomaterials Science & Engineering, DOI: 10.1021/acsbiomaterials.2c01021 (2022).
- R. Barmin, A. Dasgupta, C. Bastard, L. De Laporte, S. Rutten, M. Weiler, F. Kiessling, T. Lammers, and R. M. Pallares*. Engineering the acoustic response and drug loading capacity of PBCA-based polymeric microbubbles with surfactants. Molecular Pharmaceutics, 19, 3256 (2022).
- R. M. Pallares, D. Faulkner, D. D. An, S. Hebert, A. Loguinov, M. Proctor, J. A. Villalobos, K. A. Bjornstad, C. J. Rosen, C. Vulpe and R. J. Abergel. Genome-wide toxicogenomic study of the lanthanides sheds light on the selective toxicity mechanisms associated with critical materials. Proceedings of the National Academy of Sciences (PNAS), 118, e2025952118 (2021).

Fotios Mpekris

Post Doctoral Fellow and Lecturer, Cancer Biophysics Laboratory, University of Cyprus

I earned a BS degree (with an excellent GPA) in Physics from the University of Cyprus in 2012 and the same year, I joined the Department of Mechanical and Manufacturing Engineering at the University of Cyprus and the Cancer Biophysics Laboratory in particular as a PhD student. I defended my PhD thesis in November 2016, and since then I have been a Postdoctoral fellow and now a Senior Research Fellow at the Cancer Biophysics Laboratory and a Part time Lecturer at the University of Cyprus. My research activity focuses on the study of the mechanical forces generated during tumor progression and how taming these forces can improve therapeutic outcomes in many cancer. During my research career, I was trained as a biomedical engineer and mathematical modeler, received experimental training on the biomechanical characterization of solid tumors and other biological tissues and polymers and I was extensively and successfully trained in murine tumor models, small laboratory animal handling and surgical procedures, as well as in anticancer drug treatments. Additionally, I have gained profound knowledge of tumor biology as well as ultrasound imaging techniques in small laboratory animals. Furthermore, I have expertise in bioluminescence and fluorescence imaging on animals and tissue specimens for preclinical research and for the *in vivo* detection of nano-drugs and metastasis in tumors. The implementation of my research has led to the publication of a remarkably large number of articles in high impact journals. I have co-authored 31 scientific articles in peer-reviewed journals (h-index=17, >1,700 citations, Google Scholar).

RECENT PUBLICATIONS

- Mpekris F., Panagi M., Michael C., Voutouri C., Tsuchiya M., Wagatsuma C., Kinoh H., Osada A., Akinaga S., Yoshida S., Martin J.D., Stylianopoulos T. (2023). Translational nanomedicine potentiates immunotherapy in sarcoma by normalizing the microenvironment, J Controlled Release, 353: 956-964. DOI: 10.1016/j.jconrel.2022.12.016
- Panagi M.*, Mpekris F.*, Chen P.*, Voutouri C., Nakagawa Y., Martin J.D., Hiroi T., Hashimoto H., Philippos D., Pierides C., Samuel R., Fukushima S., Georgiou P., Papageorgis P., Papaphillipou P.C., Michael C., Koumas L., Costeas P., Ishii G., Kojima M., Kataoka K., Cabral H., Stylianopoulos T. (2022). Polymeric micelles increase tumor microenvironment reprogramming efficiency to potentiate nano-immunotherapy. Nature Communications 13: 7165. DOI: 10.1038/s41467-022—34744-1. * Equal contribution.
- Mpekris F., Panagi M., Voutouri C., Martin J.D., Samuel R., Takahashi S., Gotohda N., Suzuki T., Papageorgis P., Demetriou P., Pierides C., Koumas L., Costeas P., Kojima M., Ishii G., Constantinidou A., Kataoka K., Cabral H., Stylianopoulos T. (2021). Normalizing the microenvironment overcomes vessel compression and resistance to nano-immunotherapy in breast cancer lung metastasis. Advanced Science 8(3): e2001917. DOI:10.1002/advs.202001917
- Mpekris F., Voutouri C., Baish J.W, Duda D.G., Munn L.L., Stylianopoulos T., Jain R.K. (2020). Combining microenvironment normalization strategies to improve cancer immunotherapy. PNAS 117(7):3728-3737. DOI:10.1073/pnas.1919764117

5. Mpekris F., Baish J.W., Stylianopoulos T., Jain R.K. (2017). Role of vascular normalization in benefit from metronomic chemotherapy. PNAS, 114(8):1994-1999. DOI:10.1073/pnas.17003401

Willem Mulder

As a chemistry student, I started working in the field of nanomedicine in 1999. Since then, I have been captivated by the application of nanotechnology in medicine, particularly in immunology. After a 15-year tenure at Mount Sinai, I returned to the Netherlands in the beginning of 2021 to established a unique multidisciplinary ecosystem at the Radboud University Medical Center and the Eindhoven University of Technology. It allows young scientists from diverse backgrounds to flourish and mature into the engineers, scientists, and medical doctors of tomorrow, by participating in and driving innovative science today. Through exploration of the biological, chemical and experimental knowledge, we interconnect nanotechnology and immunology with the overarching goal to develop nanomedicine strategies for detrimental immune-mediated diseases and translate them to the clinic through entrepreneurship.

Bert Müller

Director Biomaterials Science Centerr

Bert Müller holds the Thomas Straumann Chair for Materials Science in Medicine at the University of Basel, Switzerland and is founding director of the Biomaterials Science Center. He received his Master degree in Physics from the Dresden University of Technology, Germany, his Ph.D. in Experimental Physics from the University of Hannover, Germany and his Habilitation in Experimental Physics from ETH Zurich, Switzerland. Since April 2001, Bert Müller teaches at the Physics Department of ETH Zurich. His current research interests include hard X-ray imaging down to nanometer scale and physics-based research in medicine and dentistry. He authored more than 400 publications including several patents. More recently, Bert Müller became an entrepreneur, being co-founder and advisor of Bottmedical AG, Acthera Therapeutics AG, and Bottneuro AG, all located in Basel, Switzerland. He is named as the 2022 recipient of the SPIE Biophotonics Technology Innovator Award..

RECENT PUBLICATIONS

- M. Joodaki, B. Müller, H. Schift, A. Nallathambi, B. Osmani: Micro-patterned cellulose films for flexible electrodes in medical implants, Micro and Nano Engineering 16 (2022) 100162, doi: 10.1016/j.mne.2022.100162
- G. Rodgers, G. R. Sigron, C. Tanner, S. E. Hieber, F. Beckmann, G. Schulz, A. Scherberich, C. Jaquiery, C. Kunz, B. Müller: Combining high-resolution hard X-ray tomography and histology for stem cell-mediated distraction osteogenesis, Applied Sciences 12(12) (2022) 6268, doi: 10.3390/app12126286
- R. Ammann, C. Tanner, G. Schulz, B. Osmani, P. Nalabothu, T. Töpper, B. Müller: Three-dimensional analysis of aligner gaps and thickness distributions using hard x-ray tomography with micrometer resolution, Journal of Medical Imaging 9(3) (2022) 031509, doi: 10.1117/1.JMI.9.3.031509 - with featuring coverpage
- A. Migga, G. Schulz, G. Rodgers, M. Osterwalder, C. Tanner, H. Blank, I. Jerjen, P. Salmon, W. Twengtröm, M. Scheel, T. Weitkamp, C. M. Schlepütz, J. S. Bolten, J. Huwyler, G. Hotz, S. Madduri, B. Müller: Comparative hard x-ray tomography for virtual histology of zebrafish larva, human tooth cementum, and porcine nerve, Journal of Medical Imaging 9(3) (2022) 031507, doi: 10.1117/1.JMI.9.3.031507
- G. Rodgers, C. Bikis, P. Janz, C. Tanner, G. Schulz, P. Thalmann, C.A. Haas, B. Müller: 3D X-ray histology for the investigation of temporal lobe epilepsy in a mouse model, Microscopy and Microanalysis (2023), doi: 10.1093/micmic/ozad082

Anette Müllertz

Professor

Anette Müllertz is professor in oral drug delivery and industrial relations at the University of Copenhagen, Denmark (UCPH) and head of Bioneer:FARMA, a business unit of Bioneer A/S, which is a research-based, non-for-profit service provider within the area of biomedicine and pharmaceutical development. She is heading the Physiological Pharmaceutics Research Group at UCPH, focusing on developing oral lipid-based drug delivery systems and predictive biopharmaceutics tools. She has >250 publications in international, peer-reviewed journals (h-index: 68, 14211 citations, (Google Scholar 16/8-23). She is / has been supervising 12 post docs, 53 PhD students and numerous master students, primarily at the University of Copenhagen, but also at other universities. She is/has been involved in multiple national and international research consortia, e.g. the EU sponsored Innovative Medicines Initiative Consortium Oral Biopharmaceutics Tools (OrBiTo; http://www.orbitoproject.eu) and Horizon 2020 MSCA training network COLOTAN.
She is a Fellow at the American Association of Pharmaceutical Scientists (2022), Fellow at the controlled Release Society (2023) and recipient of the AAPS Lipid Based Drug Delivery Award (2005). She is editor of Journal of Drug Delivery Science and Technology (IF2023: 5.062).

RECENT PUBLICATIONS

- Liu X, Berthelsen R, Bar-Shalom D, Lind TK, Doutch J, Müllertz A. Amphotericin B and monoacyl-phosphatidylcholine form a stable amorphous complex. Int J Pharm, 122601, 2023
- Nora GI, Venkatasubramanian R, Strindberg S, Siqueira-Jørgensen SD, Pagano L, Romanski FS, Swarnakar NK, Rades T, Müllertz A. Combining lipid based drug delivery and amorphous solid dispersions for improved oral drug absorption of a poorly water-soluble drug. J Cont Rel. 349, 206-212, 2022
- Pedersen PB, Berthelsen R, Rades T, Jørgensen SA, Vilmann P, Bar-Shalom D, Baldursdottir S, Müllertz. Physico-chemical characterization of aspirated and simulated human gastric fluids to study their influence on the intrinsic dissolution rate of cinnarizine. Int J Pharm, 622, 121856, 2022
- Strindberg S, Plum J, Bagger C, Janfelt C, Müllertz A*. Visualizing the Journey of Fenofibrate through the Rat Gastrointestinal Tract by Matrix-Assisted Laser Desorption/Ionization–Mass Spectrometry Imaging. Mol Pharm 18 (6), 2189-2197, 2021
- Kubackova J, Holas O, Zbytovska J, Vranikova B, Zeng G, Pavek P, Müllertz A. Oligonucleotide Delivery across the Caco-2 Monolayer: The Design and Evaluation of Self-Emulsifying Drug Delivery Systems (SEDDS). Pharmaceutics, 13, 4, 459-467, 2021. (text)

Lior Nissim

Assistant Professor, Head of the Biomedical Synthetic Biology Group, the Hebrew University of Jerusalem

Dr. Nissim is a pioneer in the field of synthetic biology. He has multidisciplinary training in bioengineering, cancer biology, and immunology. He is focused on developing synthetic biology platforms that provide effective and efficient solutions to biomedicine, biotechnology, and basic research. He received his Ph.D. at the Weizmann Institute of Science, where he developed the very first synthetic gene circuit for the precise targeting of cancer cells. During his postdoctoral studies at the Synthetic Biology Center at MIT, he developed innovative platforms for cancer immunotherapy and precise targeting of cell states. His technologies are already implemented in several companies in Israel and the US, including anti-viral vaccines, cancer immunotherapy, adoptive cell therapies, and cultivated meat.

RECENT PUBLICATIONS

- Katzman*, C.; Israely, T.; Melamed, S.; Politi, B.; Sittner, A.; Yahalom-Ronen, Y.; Weiss, S.; Abu Rass, R.; Zamostiano, R.; Bacharach, E.; Ehrlich, M.; Paran, N.; Nissim, L.** Modeling SARS-CoV-2 Infection in Mice Using Lentiviral hACE2 Vectors Infers Two Modes of Immune Responses to SARS-CoV-2 Infection. Viruses 2022, 14, 11. https://doi.org/10.3390/v14010011
- Wu MR*, Nissim L*, Stupp D, Pery E, Binder-Nissim A, Weisinger K, Enghuus C, Palacios SR, Humphrey M, Zhang ZZ, Novoa EM, Kellis M, Weiss R, Rabkin SD, Tabach Y, Lu TK. A high-throughput screening and computation platform for identifying synthetic promoters with enhanced cell-state specificity (SPECS). Nature Communications. 2019; PMID: 31253799
- Nissim L*, Wu MR, Pery E, Binder-Nissim A, Suzuki HI, Stupp D, Wehrspaun C, Tabach Y, Sharp PA, Lu TK. Synthetic RNA-Based Immunomodulatory Gene Circuits for Cancer Immunotherapy. Cell. 2017; PMID: 29056342
- Morel M, Shtrahman R, Rotter V, Nissim L**, Bar-Ziv RH**. Cellular heterogeneity mediates inherent sensitivity-specificity tradeoff in cancer targeting by synthetic circuits. Proc Natl Acad Sci USA. 2016; PubMed PMID: 27385823;
- Nissim L*, Perli SD*, Fridkin A, Perez-Pinera P, Lu TK. Multiplexed and programmable regulation of gene networks with an integrated RNA and CRISPR/Cas toolkit in human cells. Mol Cell. 2014; PMID: 24837679

Lutz Nuhn

Chair of Macromolecular Chemistry, Institute of Functional Materials and Biofabrication, Julius-Maximilians-Universität Würzburg, Germany

Prof. Dr. Lutz Nuhn studied biomedical chemistry at the Johannes Gutenberg-University Mainz (Germany) and received his diploma degree in 2010. In 2008/09, he practiced first research experience in the laboratories of Prof. Robert Langer (MIT, USA). For his doctoral degree he studied in the group of Prof. Rudolf Zentel, and during summer 2013 also in the group of Prof. Kazunori Kataoka (University of Tokyo, Japan). In 2014, he was awarded a PhD with distinction from Johannes Gutenberg-University Mainz. For his postdoctoral research, he moved to Belgium and worked together with Prof. Bruno De Geest and Prof. Richard Hoogenboom at Ghent University as a Feodor-Lynen fellow of the Alexander-von-Humboldt Foundation. In summer 2017, Lutz Nuhn returned to Germany and joined the group of Tanja Weil at the MPIP as a Liebig fellow of the Fonds der Chemischen Industrie (FCI), and since 2019 as Emmy Noether group leader supported by the German Research Foundation (DFG). In 2022 he was appointed as full professor by the Julius-Maximilians-Universität Würzburg and is now leading the Chair of Macromolecular Chemistry at Institute of Functional Materials and Biofabrication in Würzburg.
Lutz Nuhn is a member of the Gradute School of Life Science at Würzburg University (GSLS) and received scholarships and awards from the Controlled Release Society (CRS), German National Academic Foundation, the Alexander-von-Humboldt-Foundation, the Research Foundation Flanders ("Fonds Wetenschappelijk Onderzoek Vlaanderen, FWO"), the "Fonds der Chemischen Industrie" (FCI), the "DECHEMA - Gesellschaft für Chemische Technik und Biotechnologie" and the "Gesellschaft Deutscher Chemiker" (GDCh). He is currently also leading two projects in the interdisciplinary Collaborative Research Center "Nano-Sized Polymer Therapeuctis for Tumor Immunotherapy" (CRC/SFB 1066).
His research focuses on multi-responsive and degradable polymeric nanocarriers, especially for advanced immunotherapies.

RECENT PUBLICATIONS

- C. Czysch, C. Medina-Montano, Z. Zhong, A. Fuchs, J. Stickdorn, P. Winterweber, S. Schmitt, K. Deswarte, M. Raabe, M. Scherger, F. Combes, J. De Vrieze, S. Kasmi, N. N. Sandners, S. Lienenklaus, K. Koynov, H.-J. Räder, B. N. Lambrecht, S. A. David, M. Bros, H. Schild, S. Grabbe, B. G. De Geest, K. Nuhn – "Transient Lymph Node Immune Activation by Hydrolysable Poly(carbonate) Nanogels", Advanced Functional Materials 2022, 32, 202203490.
- L. Kaps, A. Huppertsberg, N. Choteschovsky, A. Klefenz, F. Durak, B. Schrörs, M. Diken, E. Eichler, S. Rosigkeit, S. Schmitt, C. Leps, A. Schulze, F. Foerster, E. Bockamp, B. G. De Geest, K. Koynov, H.-J. Räder, S. Tenzer, F. Marini, D. Schuppan, L. Nuhn – "PH-Degradable, Bisphosphonate-Loaded Nanogels Attenuate Liver Fibrosis by Repolarization of M2-type Macrophages", Proceedings of the National Academy of Sciences 2022, 119, e2122310119
- J.Stickdorn, L. Stein, D. Arnold-Schild, J. Hahlbrock, C. Medina-Montano, J. Bartneck, T. Ziß, E. Montermann, C. Kappel, D. Hobernik, M. Haist, H. Yurugi, M. Raabe, A. Best, K. Rajalingam, M. P. Rasak, S. A. David, K. Koynov, M. Bros, S. Grabbe, H. Schild, L. Nuhn – "Systemically Administered TLR7/8-Agonist- and Antigen Conjugated Nanogels Govern Immune Responses against Tumors", ACS Nano 2022, 16, 4426-4443.
- A. Huppertsberg, L. Kaps, Z. Zhong, S. Schmitt, J. Stickdorn, K. Deswarte, F. Combes, C. Czysch, J. De Vrieze, S. Kasmi, N. Choteschovsky, A. Klefenz, C. Medina-Montano, P. Winterwerber, C. Chen, M. Bros, S. Lienenklaus, N. N. Sanders, K. Koynov, D. Schuppan, B. N. Lambrecht, S. David, B. G. De Geest, L. Nuhn – "Squaric Ester-Based, pH-Degradable Nanogels: Modular Nanocarriers for Safe, Systemic Administration of Toll-Like Receptor 7/8 Agonistic Immune Modulators", J. Am. Chem. Soc. 2021, 143, 9872-9883.
- E. Bolli, M. Scherger, S. M. Arnouk, A. R. Pombo Antunes, D. Straßburger, M. Urschbach, J. Stickdorn, K. De Vlaminck, K. Movahedi, H. J. Räder, S. Hernot, P. Besenius, J. A. Van Ginderachter, L. Nuhn – "Targeted Repolarization of Tumor-associated Macrophages via Imidazoquinoline-linked Nanobodies", Adv. Sci. 2021, 8, 202004574.

Marisa Papaluca Amati

Pharmaceuticals regulatory science expert with interest in science and innovative technologies, immunology, oncology, cardiometabolic and rare conditions. Former European Medicines Agency's Senior Scientific Advisor and Honorary Professor at Imperial College London.

PROFESSIONAL PROFILE

September 2021 to date: independent consultant Pharmaceuticals Regulatory Science. Special interest in biologicals and advanced therapies medicinal products for immunology driven diseases, oncology, cardiometabolic and rare diseases. Experience in Pipelines review and innovative health technologies.

September 2019 to date: Honorary Professor Imperial College London – Faculty of medicine -Department of Public Health and primary Care

August 2013 - March 2019 EMA Senior Scientific Advisor (SSA) Contributing to the analyses of the scientific working parties operations pilot EMA Regulatory Science Observatory (RSO) leading to the initial draft of EMA regulatory Science Strategy to 2025.

October 1994 – August 2013. EMA Senior Scientist European Medicines Agency. Active role in initiating many areas of activities Medicines EU Marketing Authorisations centralised procedure, European Public Assessment Reports, Biotechnology Working Party. Established "safe harbour" platforms (Innovation Task Force, EMA Business Pipeline), the biomarkers qualification process, the EU-Innovation Network, I have been very instrumental for EMA (international) activities on medical innovations (pharmaceuticals h-r-DNA technologies, gene and cell therapy, nanotechnology, biosimilars, pharmacogenomics, personalised medicine, biomarkers novel clinical designs)

April 1984 – September 1994 – Medical Director at the Pharmaceuticals Department of the Italian Ministry of Health.

Pre-clinical and clinical assessor, as medical director I introduced international-grade processes and standards, including guidelines on assessment reports, electronic reporting to the WHO ADRs monitoring centre, coordination Office for Centralised Community Procedures (OCCP), multinational scientific advice for large clinical trials in EU.

1978 – 1994 – Clinical consultant in internal medicine and metabolic diseases

Dan Peer

Professor and Director, Laboratory of Precision NanoMedicine, Vice President for Research. Tel Aviv University.

Dan Peer pioneered the field of Active Cellular Targeting of RNA payloads. His lab studies novel ways to manipulate cells' function with RNA, predominantly in subset of immune cells that are notoriously hard to transfect. These molecules include siRNAs, modified mRNA, self-amplifying RNA and circular RNA to upregulate missing proteins or mutated ones or to downregulate or completely knockout gene expression in specific cell types.

His lab was the first to show systemic delivery of mRNA in a cell specific manner; the frst to show efficienct genome editing in a cell specific manner and the first to develop bacterial mRNA vaccines for antibiotic resistance strains.

Prof. Peer is a elected to the Israel Young Academy (2014) and to the US National Academy of Engineering (2023).

RECENT PUBLICATIONS

- Kedmi R., Viaga N. Ramishetti S, Goldsmith M, Rosenblum D, Dammes N, Hazan-Halevy I, Nahary L, Leviatan-Ben-Arye S, Harlev M, Behlke M, Benhar I, Lieberman J, and Peer D (2018). A modular platform for targeted RNAi therapeutics. Nature Nanotechnology. 13(3):214-219. * A universal, cell specific platform for RNAi therapeutics exemplifies in mantle cell lymphoma.
- Rosenblum D., Gutkin A., Kedmi R., Ramishetti S., Veiga N., Jacobi A.M, Schubert M.S, Friedmann-Morvinski D., Cohen Z.R, Behlke M.A, Liberman J, Peer D.CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. SCIENCE Advances, 2020, 6:EABC9450, pp.1-12. (Cover)* The first systemic, highly efficient, cell specific therapeutic genome editing in cancer.
- Dammes N., Goldsmith M., Ramishetti S., Dearling J.L.J., Veiga N., Pacjard A.B., Peer D. Conformation-sensitive targeting of lipid nanoparticles for RNA therapeutics. Nature Nanotechnology., 2021, doi: 10.1038/s41565-021-00928-x. * The first conformation sensitive targeting approach for RNA payloads.
- Yong SB, Ramishetti S., Goldsmith M., Diesendruck Y., Hazan-Halevy I., Chatterjee S, Gonna SN, Ezra A, Peer D. Dual-Targeted Lipid Nanotherapeutic Boost for Chemo-Immunotherapy of Cancer. Advanced Materials 2022. e2106350. doi: 10.1002/adma.202106350. (Cover). * A single targeted nanoparticle that acts in boosting immunotherapy and chemotherapy.
- Chatterjee S., Naidu GS., Hazan-Halevy I., Grobe H., Ezra A. Sharma P., Goldsmith M., Ramishetti S., Sprinzak D., Zaidel-Bar R., Peer D. Therapeutic Gene Silencing of CKAP5 leads to lethality in genetically unstable cancer cells. SCIENCE Advances 2023. * A new potential therapeutic target in genetically unstable cancers.

Ling Peng

Research director

I'm currently a research director in the Interdisciplinary Center on Nanoscience in Marseille (CINaM) at the French National Scientific Research Center (CNRS) in France. I undertook my PhD program with Prof. Albert Eschenmoser at Swiss Federal Institute of Technology in Zurich, Switzerland, and my postdoctoral research with Prof. Maurice Goeldner at Louis Pasteur University of Strasbourg in France. I was recruited as a research scientist in CNRS in 1997, promoted as a research director since 2008.

I have been working actively at the interface of chemistry and biology, and in particular, developing functional dendrimers for biomedical applications. Our group has established bio-inspired structurally flexible dendrimers for nucleic acid delivery. Recently, we have inaugurated the concept of self-assembling supramolecular dendrimers for the delivery of anticancer drugs, nucleic acid therapeutics and imaging agents. Our team has been labelled by La Ligue contre Le Cancer in France since 2016, and myself was awarded with the Prize of Dr & Mme Henri Labbé of the French Academy of Sciences in 2017 and the Distinguished Member of French Chemical Society in 2020.

RECENT PUBLICATIONS:

- Jiang Y, Lyu Z, Ralahy B, Liu J, Roussel T, Ding L, Tang J, Kosta A, Giorgio S, Tomasini R, Liang X-J, Dusetti N, Iovanna J, Peng L, "Dendrimer nanosystems for adaptive tumour-assisted drug delivery via extracellular vesicle hijacking", Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2215308120.
- Chen J, Zhu D, Lian B, Shi K, Chen P, Li Y, Lin W, Ding L, Long Q, Wang Y, Laurini E, Lan W, Li Y, Tintaru A, Ju C, Zhang C, Pricl S, Iovanna J, Liu X, Peng L, "Cargo-selective and adaptive delivery of nucleic acids by bola-amphiphilic dendrimers", Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2220787120.

- Chen J, Zhu D, Liu X, Peng L, “Amphiphilic dendrimer vectors for RNA delivery: state-of-the-art and future perspective”, Acc. Mater. Res. 2022, 3, 5, 484-497.
- Chen J, Ellert-Miklaszewska A, Garofalo S, Dey AK, Tang J, Jiang Y, Clément F, Marche PN, Liu X, Kaminska B, Santoni A, Limatola C, Rossi J, Zhou J, Peng L, “Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells”, Nat. Protoc. 2021, 16, 327.
- Garrigue P, Tang J, Ding L, Bouhlel A, Tintaru A, Laurini E, Huang Y, Lyu Z, Zhang M, Fernandez S, Balasse L, Lan W, Mas E, Marson D, Weng Y, Liu X, Giorgio S, Iovanna J, Pricl S, Guillet B, Peng L, “Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors”, Proc. Natl. Acad. Sci. USA, 2018, 115, 11454

Nathalie Pinkerton

Assistant Professor

Dr. Nathalie Pinkerton joined the Chemical and Biomolecular Engineering Department at NYU Tandon School of Engineering as an assistant professor in 2020. She is a member of the NYU Pain Research Center and is also on the scientific advisory board of Endosome Therapeutics. Dr. Pinkerton received her bachelors degree in Chemical Engineering from MIT. She went on to receive her Ph.D. in Chemical Engineering from Princeton University, where she worked under the guidance of Dr. Robert K. Prud’homme developing new designs and processing methods for polymeric drug delivery vehicles and imaging agents. While at Princeton, Dr. Pinkerton was a Francis Upton Fellow and received a National Science Foundation Graduate Research Fellowship and a National Defense Science and Engineering Graduate Fellowship. After graduation, Nathalie was a postdoctoral fellow at L’Institut des Technologies Avancées en sciences du Vivant (ITAV), an interdisciplinary CNRS research institute in Toulouse, France. At ITAV, she was part of Dr. Stefan Chassaing’s organic chemistry group and Dr. Bernard Ducommun cancer biology group. While at ITAV, she received a Recherche et Innovation Thérapeutique en Cancérologie (RITC) foundation fellowship. In 2016, she was recruited to Pfizer’s Early Discovery Oncology Research Unit to help establish their new cancer nanomedicine research team. As a senior scientist and research project leader, she led two cross-functional teams focused on developing nanoparticle-based cancer therapies. While at Pfizer, Nathalie was a recipient of the Pfizer W.E. Upjohn Prize in 2017, 2018 and 2019. At NYU Tandon, Nathalie leads an interdisciplinary research lab focused on the conception, development, and translation of highly engineered nanomaterials for the detection and treatment of disease with a focus on cancer and pain. Her lab is grateful for funding support from the NIH and DoD.

RECENT PUBLICATIONS

- “Calcitonin Related Polypeptide Alpha Mediates Oral Cancer Pain” N.H Tu, K. Inoue, P.K. Lewis, A. Khan, J.H, Hwang, V. Chokshi, B.B. Dabovic, S. Selvaraj, A. Bhattacharya, Z. Dubeykovskaya, N.M. Pinkerton, N.W. Bunnett, C.A. Loomis, D.G. Albertson, B.L. Schmidt, Cells, 12, 1675-1690, June 2023. https://doi.org/10.3390/cells12131675
- “Quench Ionic Flash Nanoprecipitation (qiFNP) as a Simple and Tunable Approach to Decouple Growth and Functionalization for the One-Step Synthesis of Functional LnPO4-Based Nanoparticles in Water” N.M. Pinkerton, K. Hadri, B. Amouroux, L. Behar, C. Mingotaud, M. Destarac, I. Kulai, S. Mazières, S. Chassaing, J.D. Marty, Chemical Communications, 54, 9438-9441, July 2018. https://doi.org/10.1039/C8CC041
- “Ionic Flash NanoPrecipitation for the Facile One-Step Synthesis of Inorganic-Organic Hybrid Nanoparticles in Water” N.M. Pinkerton, L. Behar, B. Amouroux, K. Hadri, C. Mingotaud, D.R. Talham, S. Chassaing, J.D. Marty, Nanoscale, 4, 1403-1408, December 2016. https://doi.org/10.1039/C6NR09364G
- “Single-step assembly of multimodal imaging nanocarriers: MRI and long-wavelength fluorescence imaging” N.M. Pinkerton, M.E. Gindy, V.L. Calero-DdelC, R.F. Pagels, D. Adler, D. Gao, S. Li, M. Zevon, N. Yao, C. Pacheco, M.J. Therien, C. Rinaldi, P.J. Sinko, R.K. Prud’homme, Advanced Healthcare Materials, 4, 1376-1385, April 2015. https://doi.org/10.1002/adhm.201400766
- “Formation of stable nanocarriers by in situ ion pairing during block-copolymer-directed rapid precipitation” N.M. Pinkerton, A. Grandeury, A. Fisch, J. Brozio, B.U. Riebesehl, R.K. Prud’homme, Molecular Pharmaceutics, 10, 319-328, December 2012. https://doi.org/10.1021/mp300452g

Sebastian Pomplun

Assistant Professor - Novel Chemical Modalities in Drug Discovery

Sebastian Pomplun (born 15-06-1985, LACDR-UL) is Assistant Professor in bioorganic and medicinal chemistry. His research focuses on using combinatorial chemistry and rational design to develop novel chemical modalities that address challenging drug targets. After obtaining is PhD in Medicinal Chemistry at the Max-Planck-Institute in Munich in 2015, S. Pomplun pursued postdoctoral research at Roche Diagnostics (Penzberg) and at the Massachusetts Insititute of Technology (MIT, Boston). Since 2021 he started his independent research group with a tenure track position at Leiden University. S. Pomplun is recipient of a DFG Walter Benjamin Fellowship (2019), an ERC-StG (2021), the Volkswagen Freigeist (2021) and an NWO-M1 grant (2022).

RECENT PUBLICATIONS

- Pomplun, S.; Gates, Z.P.; Zhang, G.; Quartararo, A.J.; B. L. Pentelute, B.L. Discovery of nucleic acid binding molecules from combinatorial biohybrid nucleobase peptide libraries. J. Am. Chem. Soc. 2020, 14, 19642. Showing the use of combinatorial libraries of biohybrid nucleobase peptides to target structured-noncoding RNA.
- Pomplun, S.; Jbara, M.; Schissel, C.; Boija, A.; Wilson Hawken, S.; Klein, I.; Pentelute, B.L. Parallel automated flow synthesis of covalent protein complexes that inhibit MYC-driven transcription. ACS Cent. Sci. 2021, 7, 1408. The design of non-functional analogs of the MYC/MAX complex to displace oncogenic MYC from its DNA binding site.
- Pomplun, S.; Jbara, M.; Quartararo, A.J.; Zhang, G.; Brown, J.; Lee, Y.; Ye, X.; Hanna, S.; Pentelute, B.L. De novo discovery of high affinity peptide binders for the SARS-CoV-2 spike protein; ACS Cent. Sci. 2021, 7, 156. A combinatorial discovery platform is used to rapidly identify high affinity ligands for the SARS-CoV-2-Spike-protein.

Adrian Press

Professor for Molecular Medicine of Life-Threatening Infection

Adrian Press, born in 1989, studied molecular medicine for his bachelor’s and master’s degrees at Furtwangen and the Friedrich Schiller University in Jena, Germany. He then obtained his doctoral de-

gree at the medical faculty in Jena researching novel theranostic nanoparticles. During his postdoc, he specialized in researching biophotonic technologies to characterize and model septic organ failure and translate this knowledge to generate novel personalized therapeutics and diagnostics. After research internships at the Janelia Research Campus of the Howard Hughes Medical Institute in Ashburn, USA, the Tokyo University of Science and Technology and the Innovation Center of NanoMedicine (iCONM), Japan, Lund University, Sweden, and Zhejiang University, PR China till 2020 he accepted the call for a position as Junior Professor at the Friedrich Schiller University for Molecular Medicine of Life-Threatening Infection.

RECENT PUBLICATIONS

- Area: Molecular pathophysiology; Hoff J, Xiong L, Kammann T, Neugebauer S, Micheel JM, Gaßler N, Bauer M, Press AT. RIPK3 promoter hypermethylation in hepatocytes protects from bile acid-induced inflammation and necroptosis. Cell Death Dis. 2023;14(4):275. DOI: 10.1038/s41419-023-05794-0
- Area: Molecular medicine of life-threatening infection; Press AT, Babic P, Hoffmann B, Müller T, Foo W, Hauswald W, Benecke J, Beretta M, Cseresnyes Z, Hoeppner S, Nischang I, Coldewey SM, Gräler MH, Bauer R, Gonnert F, Gaßler N, Wetzker R, Figge MT, Schubert US, Bauer M, Targeted delivery of a phosphoinositide 3-kinase γ inhibitor to restore organ function in sepsis through dye-functionalized lipid nanocarriers, EMBO Molecular Medicine, 2021, 13(10):e14436 DOI: https://doi.org/10.15252/emmm.202114436
- Area: Drug Delivery; Muljajew I, Husche S, Ramoji A, Cseresnyes Z, Hoeppener S, Nischang I, Foo Wanling, Popp J, Figge MT, Weber C, Bauer M, Schubert US*, Press AT*, Stealth Effect of Short Polyoxazolines in Graft Copolymers: Minor Changes of Backbone End Group Determine Liver Cell-Type Specificity, ACS Nano, 2021, 15(7): 12298–12313, DOI: https://doi.org/10.1021/acsnano.1c04213
- Area: Biophotonics; Foo W, Wiede A, Bierwirth S, Heintzmann R, Press AT*, Hauswald W*, *shared senior authorship. Automated multicolor mesoscopic imaging for the 3-dimensional reconstruction of fluorescent biomarker distribution in large tissue specimens. Biomedical Optics Express 2022, 13(7): 3723-3742. DOI: https://doi.org/10.1364/BOE.455215
- Area: Infection research; Sommerfeld O, Anna Medyukhina A, Neugebauer S, Ghait M, Ulferts S, Lupp A, König R, Wetzker R, Schulz S, Figge MT, Bauer M, Press AT, Targeting Complement C5a receptor 1 is a theranostic target for the treatment of immunosuppression in sepsis, Molecular Therapy, 2020, 29(1):338-346. DOI: https://doi.org/10.1016/j.ymthe.2020.09.008

Benjamin Rengstl

Director Clinical Development,
BioNTech SE

Dr. Rengstl develops cell- and RNA-based immunotherapies and further takes technology agnostic approaches to create new strategies for controlled modulation of the immune system. Dr. Rengstl began specializing in this area during his postdoctoral training at the Medical School of Goethe University Frankfurt, where he developed chimeric antigen receptor (CAR)-engineered T-cell therapies against lymphomas and got trained in clinical pathology. In 2017, he joined BioNTech SE located in Mainz to develop a clinical CAR-T candidate for treatment of solid tumors. To improve CAR-T therapy, his team pioneered an *in vivo* expansion concept based on a liposomally formulated RNA vaccine (CARVac) for systemic delivery of CAR antigen. A FIH clinical trial assessing BioNTech´s novel CLDN6-CAR in combination with CARVac is currently ongoing. Furthermore, Dr Rengstl is leading programs on next generation cell & gene therapies that besides oncology focus on infectious disease.

RECENT PUBLICATIONS

- Reinhard K1*, Rengstl B1*, Oehm P1*, Michel K1, Billmeier A1, Hayduk N1, Klein O1, Kuna K1, Ouchan Y1, Wöll S1, Christ E1, Weber D2, Suchan M2, Bukur T2, Birtel M1, Jahndel V1, Mroz K1, Hobohm K1, Kranz L1, Diken M2, Kühlcke K1, Türeci Ö1#, Sahin U1,2,3#. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science. 2020 Jan 24;367(6476):446-453. doi: 10.1126/science.aay5967. Epub 2020 Jan 2.
- Birtel M, Voss RH, Reinhard K, Rengstl B, Ouchan Y, Michel K, Hayduk N, Tillmann B, Becker R, Suchan M, Theobald M, Oehm P, Türeci Ö, Sahin U. A TCR-like CAR Promotes Sensitive Antigen Recognition and Controlled T-cell Expansion Upon mRNA Vaccination. Cancer Res Commun. 2022 Aug 18;2(8):827-841. doi: 10.1158/2767-9764.CRC-21-0154. PMID: 36923303; PMCID: PMC10010320.

Bernd Riebesehl

Executive Director Technical Project and Portfolio Managemen, Novartis AG, Baselt

At Novartis he is leading the the early technical development of several parenteral and topical drug products. Externally Dr. Riebesehl has been serving the Focus Group Drug Delivery of International Association for Pharmaceutical Technology (APV). He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig. 1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development.
In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Base

RECENT PUBLICATIONS

Smart design of patient centric long-acting products: from preclinical to marketed pipeline trends and opportunities, https://doi.org/10.1080/17425247.2022.2106213

Cristianne Rijcken

Founder and CSO of Cristal Therapeutics

Dr. Cristianne Rijcken is the founder of Cristal Therapeutics, and serves as Chief Scientific Officer of the company. She is (co-) author of ~ 50 scientific publications.
As of October 2022, she is also an independent consultant to early stage life science startups, with general company and CMC being main expertise area's. Dr. Rijcken is pharmacist by training and holds a PhD degree in Pharmaceutics from Utrecht University (The Netherlands).
Cristal Therapeutics was a clinical stage pharmaceutical company developing the next generation nanomedicines based on its proprietary CriPec® platform to treat various diseases, including cancer. CriPec® is perfectly suited for the development of highly customizable (targeted) nanomedicines with superior efficacy and safety

profiles. The most advanced product in development has been CriPec® docetaxel for the treatment of solid tumours. Next to CriPec® docetaxel, the product portfolio comprised various early-stage products in various therapeutic areas. Cristal Therapeutics developed these products independently, as well as in collaboration with third parties. Currently, the main focus is to commercially exploit CliCr® as a next generation class of metal-free click reagents. CliCr® has a higher stability, enables faster reactions, and generates optimal bioconjugate products. Our mission is to offer our CliCr® technology to partners under license agreements in different diagnostic and therapeutic areas. (see also www.cristaltherapeutics.com)

RECENT PUBLICATION

- IA Boere, I Vergote, R Hanssen, M Jalving, C Gennigens, PB Ottevanger, Y. van de Wouw, CJ. Rijcken, RHJ Mathijssen, JA Ledermann, CINOVA: a phase II study of CriPec® nanoparticles docetaxel in patients with platinum resistant recurrent ovarian cancer, International Journal of Gynecological Cancer, 2023
- CJF Rijcken, F de Lorenzi, I Biancacci, RJM Hanssen, M Thewissen, Q Hu, Florence Atrafi, RMJ Liskamp, RHJ Mathijssen, IHC Miedema, CW Menke- van der Houven van Oordt, GAMS van Dongen, D Vugts, M Timmers, WE Hennink, T Lammers, Design, development and clinical translation of CriPec®-based core-crosslinked polymeric micelles, ADDR, 2022
- IHC Miedema, GJC Zwezerijnen, M Huisman, E Doeleman, RHJ Mathijssen, T Lammers, Q Hu, GAMS van Dongen, CJF Rijcken, DJ Vugts, CW Menke-van der Houven van Oordt, PET-CT imaging of polymeric nanoparticle tumour accumulation in patients, Advanced Materials 2022
- F Atrafi, H Dumez, RHJ Mathijssen, CW Menke - van der Houven van Oordt, J Costermans, CJF Rijcken, R Hanssen, FALM Eskens, P. Schöffski, A phase I dose-escalation and pharmacokinetic study of a nanoparticle with entrapped docetaxel (CPC634) in patients with advanced solid tumours, Napoly Journal Controlled Release 2020
- J Weterings, CJF Rijcken, H Veldhuis, T Meulemans, D Hadavi, M Timmers, M Honing, H Ippel, RMJ Liskamp, TMTHSI, a superior 7-membered ring alkyne containing reagent for strain-promoted azide-alkyne cycloaddition reactions, Chemical Science 2020

Umberto Romeo

Development Director

Umberto Romeo has 15+ years of experience in drug product process design, development and manufacturing of chemical and biological compounds throughout all stages of development up to commercialization. He is currently the Development Director at Corden Pharma Caponago site leading formulation development (including nanoparticles based formulations/ATMPs), analytical development, validation and industrialization activities for injectable drug products as well as clinical manufacturing operations and MS&T. Prior to his current role, he worked as Project and Alliance Manager in Evotec managing complex projects (from Discovery to Phase 3) to successful completion, covering all phases of the project management process (initiation, planning, execution, monitoring and control, and closure) leading project teams to deliver results within the constraints of schedule.
He worked in UCB as Drug Product Process Design and Development Principal Scientist where he was responsible of CMC development activities of Biopharmaceuticals, with a focus on Drug Product Process Design, Development and Technology Transfer to GMP manufacturing sites. During his mission he established the Drug Product Process Design and Development department supporting the CMC development program of the whole UCB biological pipeline.
He worked in Patheon Inc. as Technology Transfer-Project Manager where he managed successfully the technology transfer of more than 15 drug product manufacturing processes of oral, injectable lyophilized and liquid drug products for European, US, Japanese and ROW markets at Phase 3 /commercial scale into Monza manufacturing plant.
Umberto graduated with a degree in Pharmaceutical Biotechnologies in Milano State University.

Meike Roskamp

Head of Business Management
Biopharma at BASF

Meike Roskamp earned her Diploma in Chemistry from Philipps-University Marburg, followed by a PhD in Natural Sciences from the Free University Berlin, where her doctoral research focused on the surface functionalization of nanoparticles. In 2023, she obtained an EMBA from ESSEC & Mannheim Business School.
Meike started her industrial career in 2010 with the development of high-throughput assay technologies for detecting biomolecular interactions. In 2014, she transitioned to working on nanoparticle-based delivery systems for chemotherapy and vaccines. In 2021, she joined BASF Pharma Solutions as an R&D Lab Leader, overseeing the development of new excipients and processing aids for biologics. Since July 2023 she serves as the Head of Business Management Biopharma at BASF.

RECENT PUBLICATION

- H.J. Becker, R. Ishida, A. C. Wilkinson, T. Kimura, M. S. J. Lee, C. Coban, Y. Ota, Y. Tanaka, M. Roskamp, T. Sano, A. Tojo, D. G. Kent, S. Yamazaki: “Controlling genetic heterogeneity in gene-edited hematopoietic stem cells by single-cell expansion”, Cell Stem Cell 2023.
- M. Bednarczyk, C. Medina-Montano, F.J. Fittler, H. Stege, M. Roskamp, M. Kuske, C. Langer, M. Vahldieck, E. Montermann, I. Tubbe, N. Röhrig, A. Dzionek, S. Grabbe, M. Bros: “Complement-Opsonized Nano-Carriers are Bound by Dendritic Cells (DC) via Complement Receptor (CR)3, and by B Cell Subpopulations via CR-1/2, and Affect the Activation of DC and B-1 Cells”, Int. J. Mol. Sci. 2021, 22, 2869.
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Gaurav Sahay

Gaurav Sahay is Professor in the Department of Pharmaceutical Sciences and co-Director for the Center of Innovative Drug Delivery and Imaging (CIDDI), at the College of Pharmacy at Oregon State University. Dr. Sahay’s lab is developing novel nanotechnology-based platforms including lipid-based nanoparticles for ef-

fective delivery of messenger RNA therapeutics for treatment of cystic fibrosis, retinal degeneration and against SARS-CoV2. He has done pioneering work to dissect the intracellular transport essential for nucleic acid delivery to the cytosol and developed methods to overcome endosomal barriers. He has more than 60-peer-reviewed publications in top tier journals including Science Advances, Nature, Nature Communications, Nature Biotechnology, Nature Nanotechnology, Journal of Controlled Release, Nano Letters etc. He is the winner of a 2013 American Association of Pharmaceutical Scientists (AAPS) Postdoctoral Fellow Award, the 2015 Controlled Release Society (CRS) T. Nagai Award, a 2016 American Association of Colleges of Pharmacy (AACP) New Investigator Award, a 2019 Oregon Health & Sciences University (OHSU) Distinguished Faculty Senate Award for Collaboration, 2020 Phi Kappa Phi OSU Emerging Scholar Award and 2020 CMBE Young Innovator Award. He serves as the Principal Investigator on awards funded through the National Institutes of Health, Cystic Fibrosis Foundation and biotech companies. He serves as a consultant and scientific advisory board member to several biotech and venture capital firms. He is co-founder of Enterx Bio and RNAvax Bio. He was the Chair of the 2018 NanoMedicine and Drug Delivery Symposium (NanoDDS, Portland, OR) and is standing section member for Innovative in NanoSystems and Nanotechnology. Dr. Sahay completed his postdoctoral research with Prof. Robert Langer and Prof. Daniel Anderson at the Koch Institute for Integrative Cancer Research at MIT and received his Ph.D. from the University of Nebraska Medical Center under the mentorship of Prof. Alexander Kabanov.

Anna Salvati

Associate Professor

Anna Salvati is an associate professor at the Department of Nanomedicine & Drug Targeting of the Groningen Research Institute of Pharmacy of the University of Groningen (the Netherlands). After graduating in biology and obtaining a PhD in physical chemistry from Florence University (Italy, 2007), she joined the Centre of Bionano Interactions, University College Dublin (Ireland). In 2014 she was awarded a Rosalind Franklin Fellowship to establish her group in Groningen University. Since then, she has been awarded several grants, including among others an ERC Starting Grant (NanoPaths) where she has investigated the mechanisms of nanoparticle uptake into cells.
Her research is focused on characterizing how nano-sized drug carriers interact with and are processed by cells in order to understand how to improve their design for nanomedicine applications. Using methods in cell biology, genetic screening and proteomics, she is studying the early interactions of nanoparticles with cell receptors and the subsequent mechanisms of uptake and intracellular trafficking by cells. She also studies how nanomedicines are modified in biological environments, for instance in blood, where a biomolecule corona adsorbs on their surface and the consequence of corona formation on targeting and nanomedicine interactions with cells. Additionally, she is developing novel methods to study these mechanisms and exploring the use of more complex *in vitro* systems for addressing these questions.

Kirsten Sandvig

Professor

Prof. Kirsten Sandvig is associated with Dept. of Biosciences, University of Oslo, Norway and she has for many years been heading a research group in Department of Molecular Cell Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital. The Norwegian Radium Hospital is the main cancer hospital in Norway. Sandvig has for many years been interested in mechanisms of endocytosis, intracellular transport and secretion both of soluble molecules and extracellular vesicles. In some of our studies we have been using protein toxins such as ricin and Shiga toxin, which are well established as markers for studies of membrane traffic, and which can be used as agents in cancer diagnosis and therapy. Our expertise is also applied to investigate uptake of nanoparticles, and we obtained a large grant (Biodegradable nanoparticles in cancer diagnosis and therapy) from the Norwegian Research Council to build national competence in nanomedicine (running to spring 2019). This project involved collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, *in vitro* and *in vivo* biology studies, *in vivo* imaging and clinical studies. In addition, international collaboration was included. The Sandvig group was also involved in an INNO INDIGO granted project, which lasted until autumn 2019. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research. Sandvig and collaborators have future support from the Norwegian Cancer Society and the Health Region to study nanoparticles and extracellular vesicles *in vitro* and *in vivo*. Moreover, we have an EEA grant, a collaboration between Norway and Romania. We characterize exosomes from prostate cancer cells and prostate cancer patients and we characterize the exosomes as well as their mechanisms of formation and secretion. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. Sandvig and collaborators have extensive national and international collaboration.
EDUCATION: M.Sci. from The Technical University of Norway, Trondheim; Ph.D. from the Medical Faculty, University of Oslo, Norway. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.
SCIENTIFIC ACTIVITY: Sandvig has published 354 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 79.
AWARDS AND HONOURS: Anders Jahres Medical Prize for young researchers, 1989 (first woman to receive this prize); The Norwegian Research Councils research prize, 1990; Member of the Norwegian Academy of Science and Letters, 1993; Stiansens Biomedical Research Prize, 1995; King Olav V´s Cancer Research Prize, 1998; Member of EMBO (European Molecular Biology Organization), 1998; Member of Academia Europea from 2002; Honorary Doctor at the University of Copenhagen, Denmark, 2007; Member of the American Academy of Microbiology, 2010; The Fridjof Nansen Award for outstanding research in science and medicine, 2014; Oslo University Hospital Prize for excellent research, 2017.

Bruno Sarmento

Principal Investigator / Associate Professor

Bruno Sarmento is Principal Investigator, Group Leader and member of the Board of Directors at i3S. Bruno Sarmento is also invited Associated professor at IUCS-CESPU. He was Visiting Professor at UniOeste (BR) between 2015-2017 and is Visiting Professor at Shanghai Jiao Tong University School of Medicine (CH).
He has supervised/co-supervised 15 Post-Docs (13 completed), 51 PhD students (40 completed) and 40 MSc students (39 completed), and 15 researcher assistants. He attracted direct competitive funding worth more than 24 M€, at national and international levels. Bruno Sarmento has a strong involvement in EU projects, being WP coordinator in HORIZON-RIA 101057491-GENEGUT, 814558-2 RESTORE and ERA-Chair 951723-MOBILIsE, and coordinator of Litwin IBD Pioneers Program 937924.
His research is focused on developing functionalized nanomedicines, namely nanoformulations for mucosal and target permeability. He has also specialized in mucosal tissue engineering models to validate functionalized nanomedicines and to perform *in vitro/in vivo* correlation. He published 450 papers in international journals (total citations in Scopus 18000; H-index 68).

RECENT PUBLICATION

- Rui Pedro Moura, Eva Carvalho, Cláudia Martins, Anne des Rieux, Ana Paula Pêgo, Bruno Sarmento *, Functionalized retinoic acid lipid nanocapsules promotes a two-front attack on inflammation and lack of remyelination on neurodegenerative disorders, Journal of Controlled Release, in press
- Claudia Martins, Bruno Sarmento*, Multi-ligand functionalized blood-to-tumor sequential targeting strategies in the field of glioblastoma nanomedicine, WIREs Nanomedicine and Nanobiotechnology, in press
- Claudia Martins, Marco Araújo, Alessio Malfanti, Catarina Pacheco, Stuart J. Smith, Bernard Ucakar, Ruman Rahman, Jonathan W. Aylott, Véronique Préat, Bruno Sarmento*, Stimuli-responsive multifunctional nanomedicine for enhanced glioblastoma chemotherapy augments multistage blood-to-brain trafficking and tumor targeting, Small, 2300029, 2023
- Claudia Martins, Catarina Pacheco, Catarina Moreira-Barbosa, Ângela Marques-Magalhães, Sofia Dias, Marco Araújo, Maria Oliveira, Bruno Sarmento*, Glioblastoma immuno-endothelial multicellular microtissue as a 3D *in vitro* evaluation tool of anticancer nano-therapeutics, Journal of Controlled Release, 353, 77-95, 2023
- Renata Castro, Flavia Carmo, Cláudia Martins, Alice Simon, Valeria Sousa, Carlos Rodrigues, Lucio Cabral, Bruno Sarmento*, Clofazimine functionalized polymeric nanoparticles for brain delivery in the tuberculosis treatment, International Journal of Pharmaceutics, 602, 120655, 2021

Ronit Satchi-Fainaro

Ronit Satchi-Fainaro is a full Professor at Tel Aviv University, where she is head of the Cancer Research & Nanomedicine Laboratory, Director of Tel Aviv University Cancer Biology Research Center, Director of the TAU Kahn 3D BioPrinting Initiative, and holds the Kurt and Herman Lion Chair in Nanosciences and Nanotechnologies.

Prof. Satchi-Fainaro received her B. Pharm. from the Hebrew University in Jerusalem, her Ph.D. from the University of London, and a Postdoctoral Research Fellowship at Harvard University and Children's Hospital Boston. She joined Tel Aviv University in 2006 as an Alon fellow.
Prof. Satchi-Fainaro serves on the Board of Directors of Teva Pharmaceutical Industries Ltd. and the Board of Governors of Tel Aviv University, and is a member of the Scientific Advisory Board (SAB) of the Blavatnik Center for Drug Discovery, the Israel Cancer Association, the University of Lisbon, the Hospital Universitari Vall d'Hebron - Institut de Recerca, The Rothschild and Fulbright Fellowships Committees, several VCs, and editorial boards of scientific journals.
Her multidisciplinary research laboratory focuses on basic research elucidating the mechanisms underlying the switch from cancer dormancy leading to the discovery of new molecular targets to interrupt tumor-host interactions. Her approach is followed by the design of highly selective targeting molecules integrating biology, chemistry, medicine, data science, engineering, and nanotechnology to selectively guide drugs into pathological sites. Throughout, she has maintained an interest in understanding the biological rationale for the design of nanomedicines suitable for transfer into clinical testing. She has published more than 150 manuscripts, 13 book chapters, edited two books, is named inventor on 70 patents, some of which were licensed to Pharmaceutical and Biotech companies, and has delivered over 500 lectures worldwide. Prof. Satchi-Fainaro received more than 80 awards including The 2019 CRS Translational Research Award, The 2020 Youdim Family Prize for Excellence in Cancer Research, The 2020 Kadar Family Award for Outstanding Research, the 2020 Michael Bruno Memorial Award, the 2020 Humboldt Foundation Bessel Research Prize, The 2021 3D Printing Industry Award- Medical application of the year, The 2021 Salisbury Award for Entrepreneurial Translational Research by the National Foundation for Cancer Research (NFCR), and elected to the 2022 CRS College of Fellows. She founded three spin-off companies and is actively engaged in translational research with several industry partners and in science outreach.

RECENT PUBLICATIONS

- Conniot J*, Scomparin A*, et al.,..Satchi-Fainaro R*, Florindo H*, Immunization with mannosylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators, Nature Nanotechnology, 14(9):891-901 (2019) *Corresponding authors.
- Florindo, et al.,..Satchi-Fainaro, Immune-mediated approaches against COVID-19, Nature Nanotechnology, 15(8):630 (2020).
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- Neufeld, et al.,..Satchi-Fainaro, Microengineered perfusable 3D-bioprinted glioblastoma model for *in vivo* mimicry of tumor microenvironment, 7(34):eabi9119, Science Advances (2021).
- Neufeld, et al.,..Satchi-Fainaro, 3D-Bioprinted cancer models: from basic biology to drug development, Nature Reviews Cancer, 22(12):679-692 (2022).

Raymond Schiffelers

Professor of nanomedicine
University Medical Center Utrecht
Utrecht, The Netherlands
Group website:
Nanomedicinelab.eu

Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD at Erasmus University Rotterdam on liposomal targeting of antimi-

crobial agents (1996-2001). Subsequently he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA) he moved to delivery of RNA. After his return to Utrecht University he became assistant and then associate professor. He received an ERC Consolidator Grant in 2010 to investigate extracellular vesicles as biological drug delivery systems for RNA. After he moved to University Medical Center Utrecht in 2011 he became professor of nanomedicine working on bio-inspired and synthetic drug delivery systems. He coordinates two H2020 projects, B-SMART (6 M€) and EXPERT (15 M€), a Horizon Europe project, NANO-ENGINE (3 M€), and an NWA-ORC project NANOSPRESSO-NL (9 M€), all devoted to RNA delivery. He is editor for the International Journal of Pharmaceutics, Journal of Controlled Release and Journal of Extracellular Vesicles, and is founder of EXCYTEX-an extracellular vesicle-based company. Since 2021 he also works part-time for Nanocell Therapeutics as VP Preclinical R&D and has been elected president of the European Technology Platform Nanomedicine since 2021.

Ruth Schmid

Retired, Consultancy, Member of scientific advisory boards

Dr. Schmid is retired, former Vice President Marketing at SINTEF Industry in Trondheim, Norway with special responsibility for the area of medical technology, including nanomedicine. SINTEF is one of Europe's largest independent non-profit research institute. Her research activities included the preparation and characterisation of micro- and nanoparticles by various technologies and from a wide variety of materials (including biodegradable polymers and hybrid materials), as well as the surface modification of polymers and polymer particles by wet-chemistry, to introduce tailor-made properties. Focus has been on the encapsulation and immobilisation of liquids and solids from emulsions, for protection and controlled release. Examples of encapsulated substances are liquid crystals, magnetic iron oxides, insect repellents and fragrances. Another focus has been on coating of biomaterials by self-assembling methods and covalent attachment with biocompatible, biomimetic and functional coatings, e.g. for introduction of antimicrobial properties, for increased osseo-integration or for immobilisation of biological molecules. There was a special focus on applied research and product orientated solutions. Fields of special interest were the emerging fields of nanomedicine, targeted drug delivery and release, nanotechnology-based diagnostics and regenerative medicine, with special focus on applications based on particle technology and surface modification. Application of encapsulation technologies and controlled release in various industrial segments, e.g. medicine, animal health, cosmetics, house-hold and body care products, food and beverages, agriculture, etc. were another field of interest.

Dr. Schmid is a Swiss citizen living in Norway since 1979. She gained her Diploma (1975) and PhD (1979) in Natural Sciences (physical organic chemistry) at ETH Zürich, Switzerland. She is a member of ACS, CRS (member of the board of directors (2009-2018), President (2016-2017) and the European Technology Platform in Nanomedicine (Chair 2019-2021). Dr. Schmid is author/co-author of 60 scientific publications and 25 patents and patent applications (h-index 24, 2719 citations, Google Scholar 2023).

RECENT PUBLICATION

- 1. A. Hyldbakk, Y. Mørch, S. Snipstad, A.K.O. Åslund, G. Klinkenberg, V. To Nakstad, A.M.Wågbø, R. Schmid & P.P. Molesworth, International Journal of Pharmaceutics: X 4 (2022) 100124; https://doi.org/10.1016/j.ijpx.2022.100124. "Identification of novel cyanoacrylate monomers for use in nanoparticle drug delivery systems prepared by miniemulsion polymerization – A multistep screening approach."
- K. Spring, K.M.Weltring, A. Prina-Mello & R. Schmid, Drug Delivery and Translational Research (2022) 12:2039-2041; https://doi.org/10.1007/s13346-022-01209-3. "REFINE special issue."
- A.K.O. Åslund, R.J. Vandebriel, F. Caputo, W.H. de Jong, C. Delmaar, A. Hyldbakk, E. Rustique, R. Schmid, S. Snipstad, I. Texier, K. Vernstad & S.E.F. Borgos, Drug Delivery and Translational Research (2022) 12:2114-2131; https://doi.org/10.1007/s13346-022-01157-y. "A comparative biodistribution study of polymeric and lipid-based nanoparticles."
- M. Germain, F. Caputo, S. Metcalfe, G. Tosi, K. Spring, AKO Åslund, A. Pottier, R. Schiffelers, A. Ceccaldi & R. Schmid, Journal of Controlled Release, 326, 164-171 (2020). "Delivering the power of nanomedicine to patients today."
- JP. Martins, J. das Neves, María. de la Fuente, C. Celia, H. Florindo, N. Günday-Türeli, A. Popat, JL. Santos, F. Sousa, R. Schmid, J. Wolfram, B. Sarmento & HA. Santos, Drug Delivery and Translational Research, 10, 726-729 (2020); https://doi.org/10.1007/s13346-020-00743-2. "The solid progress of nanomedicine."

Simo Schwartz

MD PhD

I am strategy director of biobanking and biomodels at the Vall d'Hebron Hospital Barcelona Campus, and Head of Research and Innovation of its Clinical Biochemistry Service (Clinical Labs). Member of the legal and compliance committee of the Vall d'Hebron Research Institute (VHIR). Former General Director of the Banc de Sang i Teixits (Blood and Tissue Bank) of Catalonia. A public agency of the Catalan Department of Health whose mission is to guarantee the supply and proper use of human blood and tissue in Catalonia. It is also the leading centre in the field of immunodiagnostics and advanced therapy development. Former Director and Board member of CIBBIM-Nanomedicine of VHIR, fostering research on new biomedical advanced therapies and nanotechnology-based applications for the clinical practice. In particular, advanced cell therapies, new biomaterials and drug delivery systems in the areas of oncology and rare diseases. Former Director Assistant of Translational Research at VHIR, and member of the Science Advisory Board of the European Nanomedicine Characterization Laboratory (EU-NCL). Holding 17 patents, most transferred to leading companies of the biotech and pharma sectors and coauthors more than 120 papers in high impact factor journals. Expert reviewer for more than 20 journals in the field and for international projects and EU calls. Coordinator and collaborator of several research projects directly related with the obtention and validation of therapeutic drug delivery systems. Among them are international and EU projects involving SMEs and big biotech and pharma industry. Member of the Nanomedicine Spanish Platform (Nanomed-Spain) and of the "European Platform for Nanomedicine". Member of the Steering Committee and Nanomedicine Coordinador of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers top groups of national excellence in the field of nanotechnology and nanomedicine at the national level. I was appointed as Deputy Director and technology transfer coordinator. I was also Cofounder and Science Advisor of ARGON Pharma Ltd (2008-2015), a Spin-Off company at the Barcelona Science Park with the mission to develop innovative therapies for unmet medical needs in oncology, and of BSure Medical Ltd. (created in 2020), a Spin-Off company from VHIR that commercializes tests to prevent severe immune adverse reactions to implanted materials. Member of the editorial Board of Precision Nanomedicine, J. Nanotechnology and of J. of Nanotheranostics. Former editorial Board member of Eur. J. Nanomedicine

and Nanomedicine-NBM and Science Advisor and Consultant of SOM BIOTECH, U-Cell Therapeutics and CELGENE. Former member of the Advisory Board of The Lundbeck Foundation Center of Excellence NanoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics), Southern Denmark Univ. Recently appointed as President of the European Society of Nanomedicine and Executive Board member of the International Society of Nanomedicine. Appointed expert of the NTWP Operational Expert Group (OEG) on nanomedicines - EMA (European Medicines Agency).

G.V. Shiva Shivashankar

G.V. "Shiva" Shivashankar is currently a Full Professor of Mechano-Genomics at the Department of Health Sciences and Technology, ETH Zurich. He also heads the Laboratory of Nanoscale Biology at the Paul Scherrer Institute, Switzerland. Shivashankar carried out his PhD at the Rockefeller University (1994-1999) and Postdoctoral research at NEC Research Institute, Princeton USA (1999-2000). He was a tenured faculty at the National Center for Biological Sciences, NCBS-TIFR- Bangalore, India (2000-2009) before relocating to the National University of Singapore (NUS) in 2010. He was the Deputy Director of the Mechanobiology Institute at NUS (2011-2019) and was the IFOM-NUS Chair Professor (2014-2019) before joining ETH. His scientific awards include the Birla Science Prize in 2006, the Swarnajayanthi Fellowship in 2007, and he was elected to the Indian Academy of Sciences in 2010 and to the EMBO membership in 2019.

Marco Siccardi

Dr Marco Siccardi received his PhD in pharmacology from the University of Liverpool in 2011 where it continued his academic career being appointed as Associate Professor in 2015. He focused his research on pharmacokinetics and pharmacodynamics, working in collaboration with several international research centres and companies to develop pharmacokinetic models for the simulation of relevant clinical scenario, to characterise the distribution of nanomedicine and to optimise novel drug delivery strategies. Dr. Siccardi joined Labcorp in 2021 as the Head of Toxicokinetics, Modeling and Simulation, leading the application of different quantitative approaches for the characterization of drug distribution. He is the author of over 130 articles on molecular pharmacology, modelling and clinical pharmacokinetics.

Daniel Siegwart

Professor

Daniel J. Siegwart is a Professor in the Department of Biomedical Engineering, Department of Biochemistry, and the Simmons Comprehensive Cancer Center (SCCC) at the University of Texas Southwestern Medical Center. He holds the W. Ray Wallace Distinguished Chair in Molecular Oncology Research and serves as the Director of the Program in Genetic Drug Engineering, Director of the Drug Delivery Program in Biomedical Engineering, and Co-leader of the Chemistry and Cancer Program in the NCI-designated SCCC. He received a B.S. in Biochemistry from Lehigh University (2003), and a Ph.D. in Chemistry from Carnegie Mellon University (2008), studying with Professor Krzysztof Matyjaszewski. He also studied as a Research Fellow at the University of Tokyo with Professor Kazunori Kataoka (2006). He then completed a Postdoctoral Fellowship at MIT with Professor Robert Langer (2008-2012). His research laboratory utilizes materials chemistry to enable targeted nanoparticle delivery of genomic medicines. Their efforts led to an understanding of the essential physical and chemical properties of synthetic carriers required for therapeutic delivery of siRNA, miRNA, tRNA, pDNA, mRNA, and gene editors. His lab has been at the forefront in the design of synthetic carriers for gene editing and has applied these technologies for correction of genetic diseases and treatment of cancer. They reported the first non-viral system for *in vivo* CRISPR/Cas gene editing. Recently, they developed Selective ORgan Targeting (SORT) lipid nanoparticles (LNPs), which was the first strategy for predictable tissue specific mRNA delivery and gene editing. They ultimately aspire to utilize chemistry and engineering to make a beneficial impact on human health.

RECENT PUBLICATION

- Cheng, Q., Wei, T., Farbiak, L., Johnson, L.T., Dilliard, S.A. & Siegwart, D.J. Selective ORgan Targeting (SORT) nanoparticles for tissue specific mRNA delivery and CRISPR/Cas gene editing. Nat. Nanotechnol. 15, 313-320 (2020).
- Dilliard, S.A., Cheng, Q. & Siegwart, D.J. On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. Proc. Natl. Acad. Sci. USA 118, e2109256118 (2021).
- Liu, S., Cheng, Q., Wei, T., Yu, X., Johnson, L.T., Farbiak, L. & Siegwart, D.J. Membrane-destabilizing ionizable phospholipids for organ-selective mRNA delivery and CRISPR-Cas gene editing. Nat. Mater. 20, 701-710 (2021).
- Zhang, D., Wang, G., Yu, X., Wei, T., Farbiak, L., Johnson, L.T., Taylor, A.M., Xu, J., Hong, Y., Zhu, H. & Siegwart, D.J. Enhancing CRISPR/Cas gene editing through modulating cellular mechanical properties for cancer therapy. Nat. Nanotechnol. 17, 777-787 (2022).
- Dilliard, S.A., Sun, Y., Brown, M.O., Sung, Y.C., Chatterjee, S., Farbiak, L., Vaidya, A., Lian, X., Wang, X., Lemoff, A. & Siegwart, D.J. The interplay of quaternary ammonium lipid structure and protein corona on lung-specific mRNA delivery by selective organ targeting (SORT) nanoparticles. J Control Release 361, 361-372 (2023).

Dmtri Simberg

Professor of Nanomedicine and Nanosafetyr

Dr. Simberg earned his Ph.D. in Biochemistry from the Hebrew University of Jerusalem, Israel, where he conducted his thesis research on the biochemical and biophysical mechanisms of lipofection using cationic lipids. He then pursued a postdoctoral study on tumor targeting of iron oxide nanoparticles at the Burnham Institute in La Jolla, California. In 2013, Dr. Simberg joined the faculty of the Skaggs School of Pharmacy at the University of Colorado, where he currently serves as a full tenured professor.

Dr. Simberg has authored or co-authored over 85 research papers, reviews, perspectives, and book chapters and has received funding exceeding $12M. His research interests are in development of nanoparticles and cells for drug delivery and imaging, mechanisms of immune recognition of nanomedicines, and mechanisms of drug carrier accumulation in tumors and healthy tissues.)

RECENT PUBLICATION

- Li, Y.; Lofchy, L.; Wang, G.; Gaikwad, H.; Fujita, M.; Simberg, D. Pegylated Liposomes Accumulate in the Areas Relevant to Skin Toxicities Via Passive Extravasation across "Leaky" Endothelium. ACS Nano 2022, 10.1021/acsnano.2c00423,

Tore Skotland

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the marked (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products.

Skotland has since 2009 been a senior researcher (emeritus since 2020) at the Institute for Cancer Research at The Norwegian Radium Hospital, the main cancer hospital in Norway, being part of Oslo University Hospital. He is there a member of the Sandvig group studying exosomes, endocytosis and intracellular transport of protein toxins and nanoparticles. Quantitative lipidomic studies have been important parts of several recent publications, where the goal has been to learn more about the importance of specific lipid species on membrane structure and function.

During the period 2013-2018 the group headed a 5-year national competence building project in Norway entitled "Biodegradable nanoparticles for cancer diagnosis and therapy". Skotland co-ordinated the *in vivo* studies in this project, which has members from academia, university hospitals, research institutes and pharmaceutical industry. The 10 groups involved have expertise in nanoparticle syntheses and characterization, *in vitro* studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models for biodistribution and efficacy studies.

Skotland is co-author of more than 140 publications (H-index 41) and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging.

RECENT PUBLICATIONS

Selected review articles in the field of nanoparticle and extracellular vesicle research:

- Iversen TG, Skotland T & Sandvig K: Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today 6 (2011) 176-185.
- Skotland T, Iversen TG & Sandvig K: Development of nanoparticles for clinical use. Nanomedicine (Future Medicine) 9 (2014) 1295-1299.
- Skotland T, Sagini K, Sandvig K & Llorente: An emerging focus on lipids in extracellular vesicles. Adv Drug Del Rev 159 (2020) 308-321.
- Skotland, T & Sandvig, K: Transport of nanoparticles across the endothelial cell-layer. Nano Today 36 (2021) 101029.
- Skotland T, Iversen TG, Llorente A & Sandvig K: Biodistribution, pharmacokinetics and excretion studies of intravenously injected nanoparticles and extracellular vesicles: Possibilities and challenges. Adv Drug Del Rev 186 (2022) 114326.

Alexandros Marios Sofias

Group Leader / Principal Investigator at RWTH Aachen University

Dr. Alexandros Marios Sofias is the head of the "Immune Cell Targeting and Imaging" Research Group, at the Institute for Experimental Molecular Imaging (ExMI), RWTH Aachen University Hospital, and affiliated with the Center for Integrated Oncology (CIO, Aachen, Germany), and the Norwegian University of Science and Technology (NTNU, Trondheim, Norway). In addition, he is the Co-Chair of the Image-Guided Drug Delivery (IGDD) Study Group of the European Society for Molecular Imaging (ESMI), and Theme Editor (Advances in Image-Guided Drug Delivery) for the Advanced Drug Delivery Reviews.

His research focuses on (i) unraveling the intricate *in vivo* fate of cancer nanomedicine via multimodal and multiscale imaging (e.g., PET/CT, FLT/CT, Intravital Confocal / Multiphoton Microscopy), and (ii) developing nanoimmunotherapeutics for targeting and manipulating the cancer-associated immune system, inside and outside the tumor microenvironment. Currently he investigates the ability of nanomedicines to target and modulate specific myeloid cell populations in primary triple negative breast cancer and metastasis, myeloproliferative neoplasms, and hepatocellular carcinoma.

His research is funded by the prestigious Junior Principal Investigator Fellowship by the Excellence Initiative of the German Research Foundation (DFG), and research grants by the German Cancer Aid (Krebshilfe), the Clinical Research Unit (CRU) program of the German Research Foundation (DFG, CRU 344), the Collaborative Research Centers (SFB) program of the German Research Foundation (DFG, SFB 1066), and the EuroBioimaging.

RECENT PUBLICATION

- Sofias AM*, Toner YC, Meerwaldt AE, […], Pérez-Medina C, Mulder WJM, Hak S* (2020). Tumor Targeting by αvβ3-Integrin-Specific Lipid Nanoparticles Occurs via Phagocyte Hitchhiking. ACS Nano, 14(7), 7832–7846.
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Nicole Steinmetz

Professor and Director

Dr. Steinmetz is a Professor and Vice Chair of NanoEngineering at the University of California, San Diego (2018-present). She is the founding Director of the Center for Nano-ImmunoEngineering (nanoIE), the Co-Director for the Center for Engineering in Cancer within the Institute for Engineering in Medicine, and she serves on the Leadership Team for a UC San Diego Materials Research Science and Engineering Center (MRSEC), an $18M NSF-funded research center. She started her independent career at Case Western Reserve University School of Medicine in the Department of Biomedical Engineering (in 2010-2018), where she was promoted through the ranks of Assistant, Associate, and Full Professor. Dr. Steinmetz trained at The Scripps Research Institute, La Jolla, CA

where she was a NIH K99/R00 awardee and AHA post-doctoral fellow (2007-2010); she obtained her PhD in Bionanotechnology from the University of East Anglia where she prepared her dissertation as a Marie Curie Early Stage Training Fellow at the John Innes Centre, Norwich, UK (2004-2007). Her early training was at the RWTH-Aachen University in Germany. Dr. Steinmetz's research program focuses on the engineering of plant virus-based nanomaterials targeting human and plant health applications, such as drug and pesticide delivery, vaccines and immunotherapies. Dr. Steinmetz has authored more than 250 journal articles (H index >65). Dr. Steinmetz has served as standing member on the NIH NANO study section (2016-2021) and she serves on the Advisory Editorial Board for ACS Nano, Molecular Pharmaceutics, Journal of Materials Chemistry B, Materials Advances, Advanced Therapeutics. Dr. Steinmetz is a Fellow of the National Academy of Inventors, the Biomedical Engineering Society, the International Association of Advanced Materials, the Royal Society of Chemistry, and the American Institute of Medical and Biological Engineering. Dr. Steinmetz's research program is supported through grants from NIH, NSF, NIFA, CDMRP as well as ACS, Susan G. Komen, AHA, amongst other agencies. Over the past 10+ years, Dr. Steinmetz has been awarded grants as PI and Co-PI totaling $45+ million in total costs.

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Gert Storm

Professor Pharmaceutics

Gert Storm is a (bio)pharmaceutical scientist and professor (Nanomedicine/Targeted Drug Delivery) at the Department of Pharmaceutics of the Utrecht University (UU), The Netherlands. He keeps also professor positions (Targeted Therapeutics) at the Department Advanced Organ Bioengineering and Therapeutics of the University of Twente (UT), Enschede and the Department of Surgery at the National University of Singapore (NUS). He is the (co-)author of > 600 publications (H-index 112, Google Scholar, January 2022), and since 2014 every year in the Highly Cited Researcher lists of Clarivate Analytics (Researcher ID: O-8696-2016).

Alexander Sturm

Chief Scientific Officer of Resistell

I joined the Resistell team in the summer of 2020 to lead the Microbiology RnD for a rapid diagnostic test of antibiotic susceptibility using the novel in-house developed nanomotion technology platform - currently under clinical evaluation in two studies on sepsis patients.

Before joining Resistell, I spent most of my professional career on the research-heavy American East Coast in Boston and New York, where I worked for most of the time on the world's deadliest infectious disease: tuberculosis (briefly second to Covid-19) at the Broad Institute in Cambridge, MA, and Harvard Medical School, Boston, MA. Here, I used omics technologies to investigate antibiotic tolerance of non-growing Mycobacterium tuberculosis, which plays a major role in latent infections. Before that, I investigated bacterial spores and their resilience to all sorts of drugs at Columbia University, NY. My interest in antibiotics and the mechanisms bacteria use to deal with them was founded here. I hold a Ph.D. from ETH Zurich on Salmonella virulence.

Lu Su

Assistant Professor

Lu Su was born in 1989 in Jiaxiang (China). She studied Polymer Chemistry and Physics and obtained her Ph.D. in 2014 at Fudan University under the supervision of Prof. Ming Jiang and Prof. Guosong Chen. Her Ph.D. research was on glycopolymer-based self-assembly. Then she moved to Texas A&M University as a postdoctoral fellow under the direction of Prof. Karen L. Wooley, where she worked on the functional and degradable poly(glucose carbonate)s and polyphosphoesters towards biomedical applications. Switching to supramolecular chemistry, she joined TU Eindhoven in 2017 as a postdoctoral fellow to work with Prof. Bert Meijer and discovered the dilution-induced gel-sol-gel-sol transition in the multicomponent supramolecular system. In 2022, she was appointed as Assistant Professor at Leiden university where her research focusses on the development of (supramolecular) polymeric droplets for the delivery of biotherapeutics.

RECENT PUBLICATION

- Rijns, L.; Su, L.*, Maxeiner, K.; Morgese, G.; Ng, D.Y.W.; Weil, T.; Dankers, P.Y.W.* "Introducing carbohydrate patterning in mannose-decorated supramolecular assemblies and hydrogels" Chem. Commun. 2023, 59, 2090-2093.
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Ralf Sudbrak

Deputy Director of Global AMR R&D Hub

Biotext: Dr. Ralf Sudbrak (male) is Deputy Director of the Global AMR R&D Hub in Berlin. He joined the Hub in February 2019. The main goal of the HUB is to promote high-level coordination among governments and upstream funders from different world regions, in order to better align national and international R&D efforts in the fight against AMR. The central deliverable of the GLOBAL AMR R&D HUB will be a close to real-time Dynamic Dashboard providing information and analysis at a high level on current initiatives, funding flows and activities in the field of AMR R&D. He is a biologist by training and specialist in genetic and genomics as well as personalised medicine. From 2014 to 2018 he was involved as an independent researcher in tenders of the European Commission about the assessment EU framework programmes FP7 and Horizon2020. From 2013 to 2014 he worked in a young Berlin-based SME that has been specialised in the development of novel processes and technologies in personalised medicine in cancer patients. Until 2013 he used to work at the Max Planck Institute for Molecular Genetics in Berlin, Germany for over 15 years. He was involved in the Human Genome project and later was project leader in the 1000 Genome project. He participated as principal investigator, workpackage leader and coordinator in several EU and national funded projects. He was head of the coordination and communication office of the EC FET (Future & Emerging Technologies) Flagship IT Future of Medicine pilot project. He is used to coordinate and organise large research consortia. Before that, he worked at a Postdoctoral research fellow in the laboratory of Anthony Monaco at the Wellcome Trust Centre for Human Genetics of the University of Oxford. He published his research findings in over 50 publications in peer-reviewed journals including, Nature, Nature Genetics and Science.

RECENT PUBLICATION

- Ogilvie L, Sudbrak R, Schwarz S (2022) The impact of AMR on cancer care – reinvigorating the R&D pipeline. AMR Control Supplement The Challenge for the Cancer Community: 18-22.
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Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., professor at Semmelweis and Miskolc Universities, in Hungary. Immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, Hungary, and CEO of a contract research CRO "SeroScience". He has held various guest professor and scientific positions in Hungary and abroad, mostly in the USA where he lived for 22 years and worked at the University of Arizona, NIH, MGH/ Harvard, and Walter-Reed Army Institute of Research (MD). His research on various themes in hematology (GVHD), membrane biology (liposomes), and immunology (complement and allergy) resulting in >200 publications including peer-reviewed papers, book chapters, patents, etc. (citations: >13,300, H index: 58). Editor of a book on the complement system (2004) and co-editor of another on immune aspects of biopharmaceuticals and nanomedicines (2019). Principal investigator in 13 European and Hungarian grants raising >5M USD, and led ~40 CRO projects over the past ~20 years. Speaker at 2-8/ international conferences/year for the past ~30 years, and ad hoc consultant for the FDA/EMA. Three research fields stand out where he has been most active: artificial blood, liposomes, and the complement system. His original works led to the "CARPA" concept, i.e., that complement activation underlies numerous drug-induced (pseudo)allergic (infusion) reactions. CARPA has been included in an EMA guideline as a recommended preclinical safety test for liposomal drugs.

Ying Tam

Chief Scientific Officer

Dr. Tam obtained his M.Sc. and Ph.D. University of Waterloo, Canada. He has held several senior academic and industry positions including being one of the founding scientists of Acuitas Therapeutics in 2009. He is now Chief Scientific Officer at Acuitas, a company with a leadership position in the development and application of lipid nanoparticle (LNP) technology for delivery of nucleic acid therapeutics, in particular, messenger RNA medicines. Dr. Tam has contributed to over 100 publications in peer-reviewed journals relating to LNP technology and development of pharmaceutical products.

RECENT PUBLICATION

- Schiepers et al., Molecular fate-mapping of serum antibody responses to repeat immunization. Nature, 615, 2023.
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Rob Tempest

Sales and Applications Manager (EMEA)

Rob Tempest (PhD) is the Sales and Applications Manager (EMEA) at NanoFCM, based in Nottingham UK. He has worked in multiple industrial labs including in R&D, CRO work and supervision of teams performing Covid-19 PCR testing during the pandemic. Rob holds an undergraduate degree in Biotechnology, a master's degree in Molecular and Cell Biology, and a PhD from Sheffield Hallam University focused on extracellular vesicle signalling in the tumour microenvironment between colorectal cancer and resident adipocytes. Since his PhD, Rob has built upon this knowledge to become a specialist in the field of nanoparticles and nano-flow cytometry, now working with a range of particle types such as EVs, LNPs, Liposomes and viruses. He joined NanoFCM in November 2021, a company which commercialises high-end analytical instruments based on nano-flow cytometry, for the quantitative and multi-parameter analysis and characterisation of EVs, nanomedicines and gene therapy vectors.

Carolin Tetyczka

Senior Scientist

I was born on 30.03.1989 in Spittal an der Drau and received my matura at the Bundesgymnasium Porcia in 2007. Following this, I studied pharmacy at the University of Graz and graduated in July 2013. From September 2013 until July 2021, I worked as a Scientific Project Assistant at the University of Graz, Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology and Biopharmacy. In 2018, I started working on my PhD thesis with the title "(Bio)-Pharmaceutical Investigations of Nano Drug Delivery Systems" under the supervision of Univ.-Prof. Mag.pharm. Dr.rer.nat. Eva Roblegg. I graduated from the doctoral studies in July 2021 and started to work as Senior Scientist at Research Center Pharmaceutical Engineering GmbH in August 2021. My main field of research is nanotechnology. This includes the physico-chemical profiling of active pharmaceutical ingredients, the formulation development and manufacturing of different nano drug delivery systems (i.e., lipid based nanosystems, polymeric nano drug delivery systems, nanocrystals, etc.), the comprehensive characterization of the nanosystems as well as process technologies to transfer the nano drug delivery systems into medications.

RECENT PUBLICATION

- Rump A., Tetyczka C., Littringer E., Kromrey M.-L., Bülow R., Roblegg E., Weitschies W. and Grimm M., In Vitro and In Vivo Evaluation of Carbopol 71G NF-Based Mucoadhesive Minitablets as a Gastroretentive Dosage Form. Molecular Pharmaceutics, 2023, 20, 3, 1624-1630, doi.org/10.1021/acs.molpharmaceut.2c00835
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- Tetyczka C., Hodzic A., Kriechbaum M., Juraić K., Spirk C., Hartl S., Pritz E., Leitinger G., Roblegg E., Comprehensive characterization of nanostructured lipid carriers using laboratory and synchrotron X-ray Scattering and Diffraction, European Journal of Pharmaceutics and Biopharmaceutics, 2019. pii: S0939-6411(18)31334-1. doi: 10.1016/j.ejpb.2019.03.017.

Peter van Hoogevest

Consultant, CEO and Owner at PHARMANOVATION, Rheinfelden (Baden), Germany

Peter van Hoogevest, is a pharmacist by training (Utrecht University in The Netherlands), who got his PhD degree in biochemistry 1984 at the Utrecht University in The Netherlands. In 1994 he received the degree of Privatdozent (adjunct professor) in pharmacy at the University of Basel, Switzerland. His industrial career started at the Biovet Group of the Animal Health Division of Ciba-Geigy Ltd. (Basel) in 1984. Shortly thereafter he obtained a position at the Novel Dosage Form Department of Pharmaceutical Development of the Pharmaceuticals Division of Ciba-Geigy Ltd. After having several positions at this department at Ciba Ltd. and Novartis Ltd. he founded in 1998 together with colleagues of the Pharmaceutical Development Department and reputed industrial managers and scientists the company ADD Advanced Drug Delivery Technologies (Muttenz, CH) and became CEO of this company and was member of the Board of Directors. In 2000 he joined Phares Drug Delivery AG (Muttenz, CH), a company specialized in the delivery of poorly water soluble drug substances, as Managing Director and COO and member of the Board of Directors. From 2012 till 2021 he was Managing Director of the Phospholipid Research Center, Heidelberg and Head of the Scientific Department (including the Development Department) of Lipoid GmbH, Ludwigshafen am Rhein, Germany). He runs from 2021 on his own consulting business PHARMANOVATION, based in Rheinfelden (Baden), Germany.
His drug delivery expertise, especially in the (phospho)lipid research and development area, is underscored by 79 scientific publications, including 8 book chapters, 33 symposium posters, co-promotion of 48 PhD Theses, 13 patents and 45 patent applications.

RECENT PUBLICATIONS

- Van Hoogevest, P., Review-Non-Aqueous Phospholipid Concentrates for Increasing the Bioavailability of Poorly Soluble Compounds, DOI: 10.1002/ejlt.201900411.
- Van Hoogevest, P., Verwendung natürlicher Phospholipide als pharmazeutische Hilfsstoffe, Pharm. Ind. 82, Nr 5, 641-652 (2020).
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Sander van Kasteren

Professor of Molecular Immunolgoy

The research of Sander van Kasteren bridges the fields of chemistry and immunology. He completed his PhD in 2007 in the group of Prof. Benjamin G. Davis in Oxford, where he worked on carbohydrate total synthesis and its application to the development of MRI- and histological probes for the detection of early brain inflammation. This was followed by a period in the lab of Prof. Colin Watts at the University of Dundee. Here he worked on the development of protease inhibitors to improve antigen cross-presentation in dendritic cells. A second postdoctoral position in the groups of Huib Ovaa and Jacques Neefjes brought him back to the Netherlands and to chemistry, working on deubiquitinase inhibitors.
In 2012, he started his own group at Leiden University. In 2014 he joined the institute of chemical immunology of which he is now a board member and in 2018 was promoted to associate professor, and in 2021 he was promoted to full professor. He work has been funded by, amongst others, a Sir Henry Wellcome Fellowship, a Veni fellowship of the Netherlands Council for Scientific Research, and 2 ERC Grants (Starting/Consolidator). He was also awarded the 2012 Early Career Investigator Award by the British Biochemical Society.

RECENT PUBLICATION

- Riera, R. et al. Single-molecule imaging of glycan–lectin interactions on cells with Glyco-PAINT. Nature chemical biology 17, 1281-1288 (2021).

María J. Vicent

Scientific Director, Coordinator
Cancer Program and Head of the Polymer Therapeutics Lab at Prince Felipe Research Center (CIPF)

María's research group (http://www.VicentResearchLab.com) focuses on the development of novel nanopharmaceuticals for different therapeutic and diagnostic applications - in particular the application of Polymer Therapeutics in unmet clinical needs. María has been funded by both national and European grants (including an ERC Consolidator grant-MyNano, ERC-PoC-Polymmune, ERC-PoC-Polybraint and Fund Health La Caixa-NanoPanTher and NanoGBA) from academia as well as industry. María received several prizes including several Idea and Women in Science. She is fellow of the American Institute for Medical and Biological Engineering (AIMBE) College of Fellows 2019 and Controlled Release Society (CRS) College of fellows 2021. María has co-authored >150 peer-reviewed papers and 16 patents. 4 patents have been licensed to the pharmaceutical industry, one being used for co-founding the spin-off company 'Polypeptide Therapeutic Solutions S.L.' (Valencia, Spain) in 2012, now Curapath after acquisition by Arcline in 2021.

RECENT PUBLICATION

- T. Melnyk, E. Masia, O. Zagorodko, I. Conejos-Sánchez*, M.J. Vicent*. Rational Design of Poly-L-glutamic acid-Palbociclib Conjugates for Pediatric Glioma Treatment. J. Controlled Rel. 2023, 355,385-394
- S. Vicente-Ruiz, A. Armiñan*, K. Maso, E. Gallon, O. Zagorodko, J. Movellan, F. Rodríguez-Otormín, M. Baues, J-N. May, F. De Lorenzi, T. Lammers, M. J. Vicent*. Poly-l-glutamic acid modification modulates the bio-nano interface of a therapeutic anti-IGF-1R antibody in prostate cancer. Biomaterials 2023, https://doi.org/10.1016/j.biomaterials.2023.122280
- Y. Fernández, J. Movellan, L. Foradada, V. Giménez, N. GarcV≠a-Aranda, S. Mancilla, A. Armiñán, S. E. Borgos, A. Hyldbakk, A. Bogdanska, O.L. Gobbo, A. Prina-Mello, O. Zagorodko, E. Gallon, A. Niño-Pariente, A. Paul, S. Schwartz Jr., I. Abasolo*, M.J. Vicent* In vivo anti-tumor and metastatic efficacy of a polyacetal-based paclitaxel conjugate for prostate cancer therapy. Adv. Healthcare Mat. 2022, 11(7) 2101544
- E. Giraldo, V. Nebot, O. Zagorodko, Raquel Requejo-Aguilar, A. Alastrue-Agudo, A. Armiñan, B. Martinez-Rojas, Pablo Bonilla-Villamil, S Dordevic, M.J. Vicent*, V. Moreno-Manzano* A rationally designed self-immolative linker enhances the synergism between a polymer-rock inhibitor conjugate and neural progenitor cells in the treatment of spinal cord injury. Biomaterials 2021, 276, 121052
- A. Duro-Castano, C. Borras, V. Herranz-Pérez, M.C. Blanco-Gandía, I. Conejos-Sánchez, A. Armiñan, C. Mas-Bargues, M. Inglés, J. Miñarro, M. Rodríguez-Arias, J. M. García-Verdugo, J. Viña, M.J. Vicent* Targeting Alzheimer's disease with Multimodal Polypeptide-based Nanoconjugates. Science Advances 2021, 7, eabf9180.

Viola Vogel

Professor
Laboratory of Applied Mechanobiology,
Institute of Translational Medicine,
Department of Health Sciences and Technology, ETH Zurich, Switzerland

Viola Vogel is Professor in the Department of Health Sciences and Technology (D-HEST) and is directing the Laboratory of Applied Mechanobiology at ETH Zurich, Switzerland. She holds a PhD in Physics from the University of Frankfurt (1987) based on her research conducted at the Max-Planck Institute for Biophysical Chemistry in Göttingen (1980-88). After her postdoctoral studies in the Department of Physics at UC Berkeley in nonlinear optics, she started her academic career at the University of Washington Seattle in Bioengineering (1990-2004) and was the founding Director of the Center for Nanotechnology (1997-2003). When moving to ETH Zurich in 2004, she initially joined the Department of Materials, and then co-founded (2012) the Department Health Sciences and Technology (D-HEST), in which she later served as Vice Chair (2016-2018) and then Chair (2018-2020). As most knowledge in Biology and Medicine is based on equilibrium structures of proteins, even today, she co-founded the ETH start-up Company Tandem Therapeutics in 2023 with the goal to exploit emerging knowledge in Mechanobiology for medical applications, with a focus in regenerative medicine. In this context, she also became an Einstein Visiting Fellow at Charité Berlin in 2018.
With her background in Physics and Bioengineering, she pioneered the rapidly growing field of Mechanobiology and its medical applications, as she discovered many structural mechanisms how mechanical forces can turn proteins into mechano-chemical switches. Such mechanisms are exploited by bacteria, as well as by mammalian cells and tissues to sense and respond to mechanical forces, and if abnormal, can cause various diseases. Her research was recognized by major awards, including an ERC Advanced Grant on "Proteins as Mechano-Chemical Switches" (2008-13), the International Solvay Chair in Chemistry Brussels 2012. She serves on various international advisory boards in the fields of nanotechnology and bioengineering, including on the White House panel that finalized the US National Nanotechnology Initiative under the Clinton administration (1999), as well as for the Max-Planck Society, A*STAR and CREATE in Singapore and the Wyss Institute in Boston. She was awarded an Honorary Doctor of Philosophy from Tampere University, Finland (2012), she served on the Board of Regents of the Ludwig Maximilian University in Munich (2011-19), serves on the Board

of Trustees of the Gordon Research Conference Organisation since 2018. She is an elected member of the National German Academy Leopoldina since 2018 and of the Berlin-Brandenburg Academy of Sciences since 2019, as well as of the National Academy of Engineering USA (NAE) since 2018 and of the National Academy of Sciences USA (NAS) since 2020. She is Member of the Jury of the Queen Elizabeth Prize for Engineering since 2014.

RECENT PUBLICATIONS

- V. Vogel, Unraveling the mechanobiology of extracellular matrix, Annual Review Physiology, 80 (2018) 353-387.
- C. M. Fonta, S. Arnoldini, D. Jaramillo, A. Moscaroli, A. Oxenius, M. Behe, V. Vogel, Fibronectin fibers are highly tensed in healthy organs in contrast to tumors and virus-infected lymph nodes, Matrix Biology Plus 8 (2020) 100046.
- S. Arnoldini, A. Moscaroli, M. Chabria, M. Hilbert, S. Hertig, R. Schibli, M. Béhé and V. Vogel, Novel peptide probes to assess the tensional state of fibronectin fibers in cancer, Nature Communications, 8 (2017) 1793.
- M. C. Benn, S. A. Pot, J. Moeller, T. Yamashita, C. M. Fonta, G. Orend, P. Kollmannsberger, V. Vogel, How the mechanobiology orchestrates the iterative and reciprocal ECM-cell crosstalk that drives microtissue growth, Science Advances, 9 (2023) eadd9275
- C. Fonta, T. Loustau, L. Chengbei, S. P. Surendran, U. Hansen, D. Murdamoothoo, M. Benn, I. Velazquez-Quesada, R. Carapito, G. Orend and V. Vogel, Infiltrating CD8+ T cells and M2 macrophages are retained in cancer-associated tracks enriched in low tension fibronectin fibers, Matrix Biology, 116 (2023) 1-27.

Andreas Wagner

Head Liposome Technology

(Dr Andreas Wagner
is Head of Liposome Technology at Polymun Scientific GmbH. He has significant expertise formulation of liposomes and LNPs and development of the respective processes for their clinical use. He and the team at Polymun Scientific have significantly contributed to the 1st successful mRNA vaccine Comirnaty by optimizing and up-scaling the LNP process as well as by supporting clinical and early market supply of the successful Covid-19 vaccine.
Dr Andreas Wagner studied Biotechnology at the University of Applied Life Sciences in Vienna, Austria and earned his Master and Ph.D. degrees in the field of biopharmaceutical technology. Dr Wagner is listed as inventor on multiple patents, like the liposome technology and some product patents of liposomal formulations. Furthermore, he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies

Tanja Weil

Prof. Dr. Tanja Weil has been one of the directors of the Max Planck Institute for Polymer Research since 2017 and heads the department “Synthesis of Macromolecules”. She studied chemistry (1993-1998) at the Technical University of Braunschweig (Germany) and the University of Bordeaux I (France) and obtained her PhD at the MPI for Polymer Research under the supervision of Klaus Müllen. In 2003, she was awarded the Otto Hahn Medal of the Max Planck Society. From 2002 to 2008, she held various management positions in Pharmaceutical Industry. In 2008, she accepted an appointment as Associate Professor at the National University of Singapore before she became Director of the Institute of Organic Chemistry III at the University of Ulm. She has received numerous competitive grants at national and international level, including a Synergy Grant from the European Research Council (ERC). Tanja is a member of the Senate of the German Research Foundation, the Senate of the Leibniz Association and she serves as an Associate Editor of the Journal of the American Chemical Society. Her scientific interests include the design of polymeric materials to control cellular function.

Peter Wick

Head of Particles-Biology Interaction, Empa

Peter Wick heads since 2010 the research laboratory for Particles-Biology Interactions at the Federal Laboratories on Materials Science and Technologies Empa in St. Gallen. He got his PhD degree in 2002 at the University of Fribourg, Switzerland. His general research interest is to study the interactions of nanomaterials with human tissues including barrier tissue *in vitro* and ex vivo with the purpose to obtain detailed mechanistic understanding about their uptake, accumulation, biotransformation, transport and effect on different types of cells or entire tissue. He has published more than 150 papers, was member of the advisory board of the Swiss Action Plan on Nanomaterials, member of the EDQM working group for NBCs, Editorial Board Member of Nanotoxicology, associated editor of the Journal NanoImpact, member of the Swiss National COVID-19 Science Task Force and coordinator of the Swiss National Contactpointnano.ch.

RECENT PUBLICATIONS

- Iranpour Anaraki N, Liebi M, Ong Q, Blanchet C, Maurya AK, Stellacci F, Salenting S, Wick P, Neels A, (2022) In-situ Investigations on Gold Nanoparticles Stabilization Mechanisms in Biological Environments Containing HSA, Adv Funct Materials 2110253
- Nikravesh N, Borchard G, Hofmann H, Philip E, Flühmann B, Wick P, (2020) Factors Influencing safety and efficacy of intravenous iron-carbohydrate nanomedicines: from production to clinical practive, Nanomed: Nanotechnol, Biol Med 26, 102178
- Jesus S, Marques AP, Duarte A, Soares E, Costa JP, Colaco MA, Schmutz M, Som C, Borchard G, Wick P, Borges O (2020) Chitosan nanoparticles: shedding light on immunotoxicity and hemacompatibility, Front Bioeng Biotechnol 8;100

Dominik Witzigmann

Chief Executive Officer, NanoVation Therapeutics

Dr. Dominik Witzigmann is an entrepreneurial scientist with a proven track record in translational nanomedicine research. With over 50 scientific articles and several patents, Dominik has made significant contributions to the field. Dominik holds a PhD in Pharmaceutical Technology from the University of Basel, where he focused on hepatocyte-specific drug and gene delivery via the asialoglycoprotein receptor (ASGPR). Dominik has led various research projects at renowned institutions worldwide. At the University College London his research focused on toxicological aspects; at the German Cancer Research Center he explored the use of RNA interference in cancer therapy (Dr. Sven Diederichs); at the University of Basel he worked on liver-specific nanoparticles for DNA delivery (Dr. Jörg Huwyler), at the University of Zurich he focused on RNA based genome ed-

iting approaches utilizing lipid nanoparticle (LNP) technology (Dr. Gerald Schwank), and at the University of British Columbia he developed LNP sytems for (extra)hepatic RNA delivery (Dr. Pieter Cullis). Dominik had leadership roles within the Gene Therapy Theme of the NanoMedicines Innovation Network (NMIN), a Canadian Centres of Excellence in Nanomedicine. He co-founded and led NMIN`s NanoCore to develop fit-for-purpose lipid-based nanomedicines for over 30 projects; and he served as a Board Member of the Controlled Release Society Focus Group "Gene Delivery and Genome Editing". Driven by a strong desire to bring innovative therapies to patients, Dominik has co-founded and leads NanoVation Therapeutics as Chief Executive Officer. NanoVation is a platform company developing next-generation LNP technologies to enable nucleic acid delivery to a variety of tissues for therapeutic as well as vaccine applications.

RECENT PUBLICATIONS

- Akinc, A.; […] Witzigmann D.; Kulkarni JA.; van der Meel R.; Cullis, PR. The Onpattro Story and the Clinical Translation of Nanomedicines Containing Nucleic Acid-Based Drugs. Nature Nanotechnology 2019, 14 (12), 1084–1087.
- Witzigmann, D.; Kulkarni, JA.; Leung, J.; Chen, S.; Cullis, PR; van der Meel, R. Lipid Nanoparticle Technology for Therapeutic Gene Regulation in the Liver. Advanced Drug Delivery Reviews 2020, 159, 344–363.
- Kulkarni, JA.; Witzigmann, D.; Thomson, S. B.; Chen, S.; Leavitt, BR.; Cullis, PR.; van der Meel, R. The Current Landscape of Nucleic Acid Therapeutics. Nature Nanotechnology 2021, 16 (6), 630–643.
- Schoenmaker, L.; Witzigmann, D.; Kulkarni, JA.; Verbeke, R.; Kersten, G.; Jiskoot, W.; Crommelin, D. J. mRNA-Lipid Nanoparticle COVID-19 Vaccines: Structure and Stability. Int. J. Pharm. 2021, 601, 120586.
- Rothgangl, T.; Dennis, ML.; […]; Witzigmann, D.; […]; Semple, SC.; Schwank, G.; In Vivo Adenine Base Editing of PCSK9 in Macaques Reduces LDL Cholesterol Levels. Nature Biotechnology 2021, 39 (8), 949–957.

Ada Yonath

Ada Yonath focuses on genetic code translation by ribosomes, on antibiotics paralyzing this process, on antibiotic resistance, on designing novel antibiotics and on origin of life. She graduated from Hebrew University, earned her PhD from Weizmann Institute (WIS) and completed postdoctoral studies at CMU and MIT, USA. In 1971 she established the first biological-crystallography laboratory in Israel, which was the only lab of this kind in the country for almost a decade. Since then, she has been a faculty member and the Director of Kimmelman Center for Biomolecular Structures at WIS. In 1978 she spent a Sabbatical in the Chicago University, and during 1980-2004 she headed the Max-Planck-Research-Unit for Ribosome Structure in Hamburg in parallel to her WIS activities.
Among others, she is a member of US-National-Academy-of-Sciences; Israel Academy of Sciences-and-Humanities; German Academy for Sciences (Leopoldina); European Molecular Biology Organization; Pontifical (Vatican) Academy of Sciences. She holds honorary doctorates from over 20 universities worldwide, in Israel, USA, Latin America, Europe and the Far East. Her awards include the Israel Prize; Linus Pauling Gold Medal; Albert Einstein World Award for Excellence; UNESCO-L'Oréal Award; Wolf Prize; Louisa Gross Horwitz Prize; Erice Peace Prize; Indian Prime-minister medal and the Nobel Prize for Chemistry.

RECENT PUBLICATIONS

- König G, Sokkar et al., (2021). Rational prioritization strategy allows the design of macrolide derivatives that overcome antibiotic resistance. Proc Natl Acad Sci USA 118(46):e2113632118; PMID: 34750269.
- Breiner-Goldstein, E., et al., (2021). Ribosome-binding and antimicrobial studies of the mycinamicins, 16-membered macrolide antibiotics from Micromonospora griseorubida. Nucleic Acids Res 49, 9560-9573. Nucleic Acids Res 49, 9560-9573.; PMID: 34417608
- Matzov, D., et al., (2020). Cryo-EM structure of the highly atypical cytoplasmic ribosome of Euglena gracilis. Nucleic Acids Res, 18; 48 (20): 11750-11761; PMID: 33091122.
- Halfon, Y., et al., (2019). Structure of Pseudomonas aeruginosa ribosomes from an aminoglycoside-resistant clinical isolate. Proc Natl Acad Sci U S A, 116, 22275-22281; PMID: 31611393.
- Shalev-Benami, M., et al, (2017). Atomic resolution snapshot of Leishmania ribosome inhibition by the aminoglycoside paromomycin. Nat Commun 8, 1589; PMID: 29150609
- Matzov, D., et al. (2017). The cryo-EM structure of hibernating 100S ribosome dimer from pathogenic Staphylococcus aureus. Nat Commun 8, 723; PMID: 28959035

Carolin Zhang

Professor

Dr. Liangfang Zhang is Joan and Irwin Jacobs Chancellor Professor of Nanoengineering and Bioengineering and Director of Chemical Engineering Program at UC San Diego. He received his B.E. and M.S. degrees in Chemical Engineering from Tsinghua University, and his Ph.D. in Chemical & Biomolecular Engineering from the University of Illinois at Urbana-Champaign in 2006 under the supervision of Prof. Steve Granick. He was a postdoctoral associate in the laboratory of Prof. Robert Langer at MIT during 2006-2008. He joined the Department of Nanoengineering at UC San Diego as an Assistant Professor in 2008 and was promoted to Professor in 2014. His research aims to create cutting-edge biomimetic nanotechnologies and exploit them for various biomedical applications with a particular focus on biomimetic nanodelivery and biological neutralization. He has published 260 peer-reviewed articles and was among the Clarivate Analytics list of "Highly Cited Researcher" during 2017-2022. He is an inventor of 120 patents and patent applications worldwide. Professionally, Dr. Zhang was elected to the Fellows of the American Institute for Medical and Biological Engineering (AIMBE) in 2015, the Fellows of the American Association for the Advancement of Science (AAAS) in 2018, and the Fellows of the National Academy of Inventors (NAI) in 2020.

RECENT PUBLICATIONS

- 1. Hu, C-M.; Fang, R.; Wang, K-C.; Luk, B.; Thamphiwatana, S.; Dehaini, D.; Nguyen, P.; Angsantikul, P.; Wen, C.; Kroll, A.; Carpenter, C.; Ramesh, M.; Qu, V.; Patel, S.; Zhu, J.; Shi, W.; Hofman, F.; Chen, T.; Gao, W; Zhang, K.; Chien, S.; Zhang, L.* "Nanoparticle biointerfacing by platelet membrane cloaking", Nature 2015, 526, 118-121.
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CURRICULA VITAE POSTERS

Wafa Al Jamal

Reader in Nanomedicine and Drug Delivery/ Queen's University Belfast

Dr Wafa Al-Jamal completed her PhD in Drug Delivery and Nanomedicine in 2008 from the School of Pharmacy, University of London (now known as UCL-School of Pharmacy). She is currently a Reader in Nanomedicine and Drug Delivery at School of Pharmacy, Queen's University Belfast. She is also a Prostate Cancer Fellow (2014-2019). Dr Al-Jamal joined School of Pharmacy, University of East Anglia, Norwich, as a Lecturer in Drug Delivery (2013-2017), after working as a Senior Research Fellow at University College London, and King's College London (2008-2013).

Dr Al-Jamal's main research interest focuses on engineering novel nanomaterials for biomedical applications. Her current research aims to design smart vectors to deliver a wide range of therapeutic agents and targeting moieties, and to fabricate multifunctional nanoparticles for combinatory therapy and theranostic applications. Her long-term research career is to facilitate the translation of nanoparticle-based therapeutics from the lab to the clinic.

Dr Al-Jamal is the GSK Emerging Scientist Award winner for 2015, and Gro Brundtland Award winner for 2017. Her multidisciplinary research has been funded by the Royal Society, Prostate Cancer UK, The Engineering and Physical Sciences Research Council (EPSRC), and Rosetrees Trust. She has published over 65 papers in high impact factor journals. Currently, she is a member the Prostate Cancer UK (PCUK) Research Advisory Committee, and a Visiting Professor at Guizhou Medical School, China.

RECENT PUBLICATION

- Pereira SGT, Ma G, and Al-Jamal WT*. 2021. Encapsulation of doxorubicin prodrug in heat-triggered liposomes overcomes off-target activation for advanced prostate cancer therapy. Acta Biomaterials, (accepted) [IF 8.9]
- Ma G, Severic M, Barker M, Pereira S, Ruiz A, Cheung C.C.L., and Al-Jamal WT*. 2021. Dually Targeted Bioinspired Nanovesicle Delays Advanced Prostate Cancer Tumour Growth In Vivo. Acta Biomaterials, S1742-7061(21)00452-9 [IF 8.9]
- Ma G, Kostevšek N, Monaco I, Ruiz A, Markelc B, Cheung CCL, Hudoklin S, Kreft ME, Hassan HAFM, Barker M, Marković K, Ščančar J, Franchini MC, and Al-Jamal WT*. 2021. PD1 blockade potentiates the therapeutic efficacy of photothermally-activated and MRI-guided low temperature-sensitive magnetoliposomes. J Control Release, 332: 419-433.[IF 9.7]
- Severic M, Ma G, Pereira S, Ruiz A, Cheung CCL, and Al-Jamal WT*. 2020. Genetically-Engineered Anti-PSMA Exosome Mimetics Targeting Advanced Prostate Cancer In Vitro and In Vivo. J Control Release, 330: 101-110. [IF 9.7]
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- Silva VL, Kaassis A, Dehsorkhi A, Koffi CR, Abdelhamid M, Nyimanu D, Morris CJ, Al-Jamal WT*. 2020. Enhanced selectivity, cellular uptake, and *in vitro* activity of an intrinsically fluorescent copper-tirapazamine nanocomplex for hypoxia targeted therapy in prostate cancer. Biomaterials Science, (9):2420-2433.[IF 6.8] (Journal cover)

Amy Barton Alston

PharmD, MS, CMPP, FASN, FCCP, FNKF
Medical Associate Director, Nanomedicine Science, CSL Vifor

Dr. Amy Barton Alston is a nephrology-trained clinical pharmacist. Prior to joining CSL Vifor as the Medical Associate Director for Nanomedicine, Dr. Alston had a 20+ year career as an academic researcher focused on translational investigations of intravenous iron-carbohydrate nanomedicines in the chronic kidney disease population. She was previously funded by the Food and Drug Administration to advance bioequivalence evaluation for intravenous iron-carbohydrate nanomedicines using *in vitro* and preclinical models. During her tenure as an academician, Dr. Alston maintained a clinical practice providing care to patients across the chronic kidney disease continuum with a focus on the in-center hemodialysis population. Dr. Alston holds a Master's degree in Healthcare Innovation and is a fellow of the American Society of Nephrology, National Kidney Foundation and the American College of Clinical Pharmacy.

Leila Arabi

Dr. Leila Arabi is an Assistant Professor of Pharmaceutical Nanotechnology, School of Pharmacy in Iran. She holds Doctor of Pharmacy and Ph.D. (summa cum laude) from Mashhad University of medical sciences, Iran. She had the one-year PhD internship during 2012-2013 at University Hospital Basel, Switzerland. Following her visit to several labs and meetings with academics in the US and Europe, she relocated back to Iran.

Her research is focused on developing nanoscale drug delivery systems with particular emphasis on developing liposomes for targeted cancer drug delivery, combination therapy, cancer immunotherapy, and gene therapy. Her researches have been recognized as highlight from the Controlled Release Society (CRS), and has led to several publications and research presentations in different nanomedicine conferences. She is currently the Communication chair of immune-Delivery focus group of CRS and serves as an ambassador in CRS Young Scientist Committee. Recently, she has been invited to join the Editorial Board of the Journal of Controlled Release (JCR).

As an Assistant Professor with a demonstrated history of working in the hospital & health care, her goal is to link the fields of Pharmaceutical Nanotechnology to cancer Biology and immunology to improve therapeutic efficacy of conventional cancer therapies. She is also interested in empowering the role of women in science and gender parity in STEM fields.

RECENT PUBLICATION

- S Shahvali, N Rahiman, MR Jaafari, L Arabi, Targeting fibroblast activation protein (FAP): advances in CAR-T cell, antibody, and vaccine in cancer immunotherapy. Drug Delivery and Translational Research, 2023
- V Jahani, M Yazdani, A Badiee, MR Jaafari, L Arabi, Liposomal celecoxib combined with dendritic cell therapy enhances antitumor efficacy in melanoma. Journal of Controlled Release, 2023
- M Mohammadi, L Arabi, M Alibolandi, Doxorubicin-loaded composite nanogels for cancer treatment, Journal of Controlled Release 2020
- L Arabi, A Badiee, F Mosaffa, MR Jaafari, Targeting CD44 expressing cancer cells with anti-CD44 monoclonal antibody improves cellular uptake and antitumor efficacy of liposomal doxorubicin, Journal of controlled release 2015

Andreas Åslund

Dr. Andreas Åslund is a Senior Research Scientist at SINTEF and has a background in organic chemistry with a PhD from Linköping University, Sweden. For the last decade he has worked exclusively on the development of targeted delivery and (nano)formulation of drugs. These activities include the establishment of ultrasound mediated blood-brain barrier opening in Norway and mechanistic studies on ultrasound mediated delivery of drugs and nanoformulations to the brain and other target tissue. Within formulation he has been part of the team that developed the lead formulation for NaDeNo and in addition he has extensive experience from other polymer nanoformulation technologies. He is at the bord of the European Technology Platform for Nanomedicine and Chair for the working group Nanotherapeutics and Targeted Delivery. At SINTEF he is the project leader for all activities related to NaDeNo.

RECENT PUBLICATION

- Hyldbakk, A.; Fleten, K. G.; Snipstad, S.; Åslund, A. K. O.; Davies, C. de L.; Flatmark, K.; Mørch, Y. Intraperitoneal Administration of Cabazitaxel-Loaded Nanoparticles in Peritoneal Metastasis Models. Nanomedicine: Nanotechnology, Biology and Medicine, 48, 102656 (2023) https://doi.org/10.1016/j.nano.2023.102656.
- Astrid Hyldbakk, Yrr Mørch, Sofie Snipstad, Andreas KO Åslund, Geir Klinkenberg, Vu To Nakstad, Ane-Marit Wågbø, Ruth Schmid, Peter P Molesworth Identification of novel cyanoacrylate monomers for use in nanoparticle drug delivery systems prepared by miniemulsion polymerisation–A multistep screening approach International Journal of Pharmaceutics 4, 100124 (2022)
- Andreas K.O. Åslund, Rob J. Vandebriel, Fanny Caputo, Wim H. de Jong, Christiaan Delmaar, Astrid Hyldbakk, Emilie Rustique, Ruth Schmid, Sofie Snipstad, Isabelle Texier, Kai Vernstad, Sven Even F. Borgos, A comparative biodistribution study of polymeric and lipid-based nanoparticles. Drug delivery and Translational Research DOI: 10.1007/s13346-022-01157-y (2022)
- A.K.O. Åslund, E. Sulheim, S. Snipstad, E. von Hartman, H. Baghirov W. R. Glomm, C. deL. Davies, Ý.Mørch, Quantification and qualitative effects of different PEGylations on PBCA nanoparticles, Molecular Pharmaceutics (ACS), 14 (8), 2560-2569 (2017)
- A.K.O. Åslund, S. Berg, S. Hak, Y. Mørch, S.H. Torp, A. Sandvig, M. Widerøe, R. Hansen, C.d.L. Davies, Nanoparticle delivery to the brain - By focused ultrasound and self-assembled nanoparticle-stabilized microbubbles, Journal of Controlled Release, 220, 287-294 (2015)

Vanesa Ayala-Nunez

I started my research career in Mexico, my country of origin, where I investigated the mode of action of silver nanoparticles against HIV-1 and antibiotic-resistant bacteria. I discovered that silver nanoparticles are effective antivirals against HIV-1 and are broad-spectrum antibacterial compounds that inhibit the growth of bacteria of clinical importance. Afterwards, I moved to the beautiful city of Groningen in The Netherlands, where I obtained my PhD degree after researching how virus (nano)particles enter and take over macrophages. I then proceeded my research journey as a postdoc in France (Strasbourg and Montpellier). During this time, I focused on the cell-delivery mechanism of virus particles (otherwise known as Trojan horse strategy) across the blood-brain barrier (BBB), in which cells from the immune system cross the BBB to invade the brain tissue. This story was published in Nature Communications (Ayala-Nunez et al., 2019) and was highlighted by Le Monde and some international science communication sites.

Currently, I work as a Senior Scientist at the Swiss Federal Laboratories for Materials Science and Technology (Empa) in St. Gallen, Switzerland. Here, I am focused on deciphering the mechanism of action of iron-carbohydrate nanomedicines in collaboration with CLS Vifor. This projects is mainly done with primary human macrophages, as they are one of the main targets of these nanoparticles post-IV injection in patients. Additionally, I am working to establish a human glioblastoma organoid model in collaboration with the Kantonsspital St. Gallen. This complex *in vitro* system will then be used to assess the effectivity of different nanoparticles with a potential anti-cancer activity.

Since July 2023, I am the Project Manager of Contact Point Nano, a Swiss platform that connects companies with experts in nanomaterials that will provide advice on nanosafety and regulatory matters. Besides, I am chair of the Postdocs and Scientists Community of the Materials Meet Life Department. This Community is a space for networking and strengthening social contacts of its members, and to promote career development beyond academia.

Last but not least, I am a very active science communicator. I coordinate the team´s social media account (Follow us! @nanointercell), I participated in Pint of Science 2023, and I am part of the tours Empa offers to the general public (topics: Nanomedicine and Organoids).

RECENT PUBLICATION

- Gaudin R, Brychka D, Sips G, and Ayala-Nunez NV. (2022) Targeting tight junctions to fight against viral neuroinvasion. Trends in Molecular Medicine. https://doi.org/10.1016/j.molmed.2021.10.007
- Ayala-Nunez NV and Gaudin R. (2020) A viral journey to the brain: current considerations and future developments. Plos Pathogens. https://doi.org/10.1371/journal.ppat.1008434
- Ayala-Nunez NV, et al. (2019) Zika virus enhances monocyte adhesion and transmigration favoring viral dissemination to neural cells. Nature Communications, 10, Article number: 4430.
- Ayala-Nunez NV, et al. (2016) How antibodies alter the cell entry pathway of dengue virus particles in living macrophages. Scientific Reports, 6: 28768.
- Ayala-Nunez NV, et al. (2013) Antibody-dependent enhancement of dengue virus infection is inhibited by SA-17, a doxorubicin derivative. Antiviral Research, 100(1):238-45.

Iris Batalha

Iris L. Batalha is currently a Junior Leader Research Fellow at the Institute of Bioengineering of Catalonia (IBEC) in Barcelona, a Panel Tutor in Nanotherapeutics at the University of Cambridge Institute of Continuing Education, a freelance Senior Innovation Consultant at Inspiralia (Spain and USA), a Co-founder, Director and Editor-in-Chief of the non-profit organisation Women Ahead of Their Time (WATT), and a Research Associate at Peterhouse College. From 2017 to 2020, she was a joint Research Associate at the Department of Engineering Nanoscience Centre and Department of Medicine Molecular Immunity Unit, University of Cambridge. From 2014 to 2017, she worked at the Department of Chemical Engineering and Biotechnology, University of Cambridge, and the biopharmaceutical company MedImmune/Astrazeneca, followed by a brief experience as a healthcare/pharmaceutical consultant. Her research interests and expertise lie in medical and pharmaceutical research and development, particularly in the fields of nanobiotechnology, bio-inspired materials, downstream processing, formulation and drug delivery.

Karina Benderski

PhD student
My name is Karina Benderski. I was born in Germany on 23.03.1995. I grew up bilingual, therefore learned German and Russian early on. I graduated from high school in 2013 and started my Bachelor of Science in Chemistry at Rheinisch Westfälische Technische Hochschule (RWTH) Aachen. 2016 I started my Master of Science in Chemistry and graduated in 2019. During my studies, I went abroad for half a year to do an Erasmus in Valencia, Spain, where I continued by master's program in Spanish language. My master thesis focused on the design and optimization of targeted drug delivery systems. In 2020 I started my PhD at the Institute of Experimental Molecular Imaging (ExMI), Uniklinik Aachen, under the supervision of Prof. Twan Lammers. My major research questions focus on monitoring the effect of the fibrotic tumor microenvironment on tumor-targeted drug delivery. My research activities involve the imaging-based characterization of the tumor microenvironment (TME) as well as the association of specific TME features. I try to understand and characterize cancer-associated multidrug resistance on cellular and tumor microenvironmental level. In collaboration with Universitästmedizin Mainz, I also work on the development and deep characterization of new (fibrotic) mouse models for hepatocellular carcinoma. In addition, I am developing synergistic nanotherapies for overcoming MDR and modulating the components of the TME.

RECENT PUBLICATION

- Effect of Cellular and Microenvironmental Multidrug Resistance on Tumor-Targeted Drug Delivery in Triple-Negative Breast cancer; O.Tezcan#, A.S. Elshafei#, K.Benderski#, Elena Rama, Maike Wagner, Diana Moeckel, Robert Pola, Michal Pechar, Tomas Etrych, Saskia von Stillfried, Fabian Kiessling, Ralf Weiskirchen, Steffen Meurer, Twan Lammers; Journal of Controlled Release, 2023
- Repurposing Tamoxifen for Tumor Microenvironment Priming and Enhanced Tumor-Targeted Drug Delivery; Ilaria Biancacci, Daneiel De Santis, Elena Rama, Karina Benderski, Jeffrey Momoh, Robert Pohlberger, Diana Möckel, Leonard Kaps, Christianne JF Rijcken, Jai Prakash, Marielle Thewissen, Fabian Kiessling, Yang Shi, Quim Pena, Alexandros Marios Sofias, Lorena Ceonselino, Twan Lammers; Advanced Therapeutics; 2023
- Monitoring EPR Effect Dynamics during Nanotaxane Treatment with Theranostic Polymeric Micelles; Ilaria Biancacci, Federica De Lorenzi, Benjamin Theek, Xiangyang Bai, Jan-Niklas May, Lorena Consolino, Maike Baues, Diana Moeckel, Felix Gremse, Saskia von Stillfried, Asmaa El Shafei, Karina Benderski, Armin Azadkhah Shalmani, Alec Wang, Jeffrey Momoh, Quim Peña, Eva Miriam Buhl, Johannes Buyel, Wim Hennink, Fabian Kiessling, Josbert Metselaar, Yang Shi, Twan Lammers; Advanced Science; 2022

Sven Borgos

Sven Even Borgos (born 1976) earned both his undergraduate and PhD degrees at the Norwegian University of Science and Technology in Trondheim, the main technical university of Norway. His undergraduate was in Biophysics and Medical Technology. His PhD, however, was in molecular biology, concerned with genetic engineering of the antibiotic-producing soil bacterium Streptomyces noursei in order to develop mutants producing derivatives of the clinically important antifungal antibiotic nystatin and related compounds, with improved pharmacological properties. He then did a post doc in systems biology, developing and validating a genome-scale metabolic model of the alginate-producing bacterium Pseudomonas fluorescens. Since 2006, he has been in SINTEF (Norway), which is one of the largest independent research institutes in Europe. Here, he has been working with advanced analytical chemistry, mainly based on mass spectrometry coupled to chromatography. The last ten years, he has been specializing in physicochemical characterisation of nanomaterials, with an emphasis on nanomedicines. He has been assay group leader for chemical characterization in the European Nanomedicine Characterisation Laboratory (EUNCL) H2020 project, and is involved in ongoing collaborations with e.g. Joint Research Centre (JRC) and National Institute of Standards and Technology (NIST) on standardization of nanomedicines analytical methods. Since 2017, the SINTEF group has been increasingly focused on RNA-based nanomedicines, and Borgos is SINTEF's PI several Norwegian and EU-funded projects on RNA therapeutics, both antisense oligonucleotides, siRNA and mRNA.

RECENT PUBLICATION

- Sonzini,S., Caputo,F., Mehn,D., Calzolai,L., Borgos,S.E., Hyldbakk,A., Treacher,K., Li,W., Jackman,M., Mahmoudi,N., et al. (2023) In depth characterization of physicochemical critical quality attributes of a clinical drug-dendrimer conjugate. International Journal of Pharmaceutics, 637, 122905.
- Simon,C.G., Borgos,S.E., Calzolai,L., Nelson,B.C., Parot,J., Petersen,E.J., Roesslein,M., Xu,X. and Caputo,F. (2023) Orthogonal and complementary measurements of properties of drug products containing nanomaterials. Journal of Controlled Release, 354, 120–127.
- Vervaeke,P., Borgos,S.E., Sanders,N.N. and Combes,F. (2022) Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics. Advanced Drug Delivery Reviews, 184, 114236.
- Åslund,A.K.O., Vandebriel,R.J., Caputo,F., de Jong,W.H., Delmaar,C., Hyldbakk,A., Rustique,E., Schmid,R., Snipstad,S., Texier,I., Vernstad, K., Borgos, S.E. (2022) A comparative biodistribution study of polymeric and lipid-based nanoparticles. Drug Deliv. and Transl. Res., 10.1007/s13346-022-01157-y.
- Fernandez,Y., Movellan,J., Foradada,L., Gimenez,V., Garcia-Aranda,N., Mancilla,S., Arminan,A., Borgos,S.E., Hyldbakk,A., Bogdanska,A., et al. (2021) In Vivo Antitumor and Antimetastatic Efficacy of a Polyacetal-Based Paclitaxel Conjugate for Prostate Cancer Therapy. Adv Healthc Mater, 10.1002/adhm.202101544.

Jonas Bossart

PhD student in computational biology at Empa
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I was born in 1995 in St. Gallen, Switzerland. After finishing elementary and high school in Herisau, a neighboring village, I completed my university entrance qualification in Trogen in the year 2015. My passion for the natural sciences and medicine is due to my emphasis on biology and chemistry. Even as a child, I enjoyed reading the package leaflets of medicines carefully. I was fascinated by the idea that these little pills and capsules could have such a positive effect on the human health.
I decided to follow this path and, after a clinical clerkship in two pharmacies and several months of civilian service in the refugee system, I moved to Zurich, where I completed my bachelor's degree in pharmaceutical sciences at the ETH Zurich from 2016 to 2019. In particular, I appreciated the broad curriculum with many branches of the natural sciences. In the subsequent master's program in pharmaceutical sciences, also at the ETH Zurich, I was able

to further strengthen my research skills in several research projects from 2019 to 2021. My time in the Computer-Assisted Drug De-sign laboratory was particularly formative. Thanks to the collaboration of my supervisors with the Laboratory of Biomolecular Research at the Paul Scherrer Institute, I was able to biotechnologically produce and experimentally test our AI generated protein sequences.
I still consider it a privilege that I was also able to complete my second civilian service in a meaning-ful way, which directly followed my university studies. This time, I even had the opportunity to apply and deepen my new skills in the field of data analysis. I spent the next six months at the Swiss MS Registry (University of Zurich), where I was able to publish my first scientific paper. I find it very ex-citing and fascinating how much hidden information can be extracted from large data sets.
At the end of 2021, I found the ideal opportunity at the Empa in St. Gallen to fulfill my desire to learn more about computational analysis of scientific data. Under the supervision of Dr. Marija Buljan (Empa) and Prof. Dr. Andrea Alimonti (ETH Zurich) I started a PhD in computational biology. In the group Multi-omics for Healthcare Materials, I mainly focus on the analysis of quantitative proteomics data from human immune cells to define their roles in disease development and treatment respons-es. Thanks to an industrial collaboration with CSL Vifor, I was given the opportunity to carry out the work submitted here regarding the response of macrophages to the treatment with iron-carbohydrate complexes, which, in addition to the scientific part, also gave me valuable insights into the activities of a large pharmaceutical company.

Roland Böttger

Roland Böttger obtained a PhD in Chemistry from Leipzig University in Germany and pursued a postdoc in Pharmaceutical Sciences at University of British Columbia in Canada. In 2020 he joined CureVac in Germany as a scientist and his current interests are focused on discovery of delivery systems for mRNA-based medicines.

Danielle Brain

Danielle was born in Stratford-upon-Avon, UK and has a background in pharmacology and immunology. She graduated with a first class B.Sc (Hons) degree in Pharmacology and her PhD in Pharmacology from the University of Liverpool. Her PhD research focused on investigating biocompatibility and immunological safety of Long-acting formulations. She is particularly interested in exploring the role of the inflammasome in mediating immunological responses to these long-acting formulations and also the role it plays in COVID-19 infection. Danielle has worked on a number of projects as a research associate looking at the immunocompatability of novel drug delivery systems. She is currently a postdoctoral research associate at the University of Liverpool, working on a project funded by Innovate UK titled intracellular drug delivery centre, which is working to help develop novel drug delivery technologies and support promising RNA vaccines and therapeutics. Selected publications: Brain D, Plant-Hately A, Heaton B, Arshad U, David C, Hedrich C, Owen A, Liptrott NJ. “Drug delivery systems as immunomodulators for therapy of infectious disease: Relevance to COVID-19.” Adv Drug Deliv Rev. 2021 Nov;178:113848.

Thomas Bruckdorfer

CSO & VP Business Development

I was born on April 1st in 1963 in the town Roth in Bavaria, Germany. To school i went in Schwabach and studied chemistry in Erlangen. All towns are around Nuremberg in Northen Bavaria in Germany. Aftern university I finished an MBA study and after a few positons at various companies I joind the fonder of Iris Biotech GmbH in 2002, in order to build and raise the company.
In 2013 I co-founded the company Iris Biotech Laboratories GmbH, located in Willstätt, Germany, which serves as a maunufacturing unit of Iris Biotech producing unusual building blocks and linkers.
In spring this year, in March 2023, I co-founded the company B4 PharmaTech GmbH, located in Berlin, which can produce biomolecules such as membrane enzymes, antibodies and antibody formats (e.g. nanobodies), including points of connectivity suitable for Linkerology.
I am member of the Brain-8-Network of the Biopark Regensburg, supporting with coaching and consulting within the topics chemistry, biotechnology, pharma, diagnostics, and analytics.
Co-autor of some 20 publications about peptide synthesis, PEGylation and related topics

Marion Casanova

Dr Marion Casanova is currently a postdoctoral researcher in the Interdisciplinary Center on Nanoscience in Marseille (CINaM) at the French National Scientific Research Center (CNRS) in France. She is working at the interface of chemistry and biology since the beginning of her academic career. Her research has been mainly focused on synthesizing, screening and biological evaluation of new therapeutic agents fighting against infectious diseases. She carried her PhD in medicinal chemistry under the supervision of Profs. Patrice Vanelle, Nadine Azas and Dr Julie Broggi at the Faculty of Pharmacy and the University Hospital Institute of Marseille. She has established biocompatible polymer-drug conjugates as nanomicelles to treat Plasmodium falciparum K1 infections. In particular, she designed a new, fast, and efficient synthetic approach of nanocarrier conjugates, and evaluated both their physico-chemical properties and mechanisms of action, as well as the antiplasmodial activity of original N-heterocyclic iminium drugs. Dr Marion Casanova is currently developing functional self-assembling supramolecular dendrimers for drug delivery in biomedical applications combating cancer and infectious diseases.

RECENT PUBLICATION

- M.C Casanova, M. Fil, Y. Zhao, N. Azas, S. Redon, P. Vanelle, J. Broggi, Synlett, 2023, 34, A-D.
- P. Lagardère, R. Mustière, N. Amanzougaghene, S. Hutter, M.C Casanova, JF Franetich, S. Tajeri, A. Malzert-Fréon, S. Corvaisier, N. Azas, P. Vanelle, P. Verhaeghe, N. Primas, D. Mazier, N. Masurier, V.Lisowski, Eur. J. Med. Chem., 2023, 5, 115115.
- P. Lagardère, R. Mustière, N. Amanzougaghene, S. Hutter, M.C Casanova, JF Franetich, S. Tajeri, A. Malzert-Fréon, S. Corvaisier, M. Since, N. Azas, P. Vanelle, P. Verhaeghe, N. Primas, D. Mazier, N. Masurier, V.Lisowski, Eur. J. Med., 2023, submitted.
- M.C Casanova, P. Vanelle, N. Azas, J. Broggi, a general review (in preparation).
- M.C Casanova, M. Rollet, S. Hutter, M. Fil, L. Charles, V. Monnier, N. Azas, P.Vanelle, J. Broggi (in preparation).

Noemi Stefania Csaba

Associate Professor

Graduated as Pharmacist (Ms.C) at the Univ. Semmelweis, Budapest, Hungary and obtained the Ph.D. degree at the University of Santiago de Compostela, School of Pharmacy in the field of nanomedicine and nanotechnology. Worked as a post-doctoral research fellow between 2005-2007 at the Swiss Federal Institute of Technology (ETH Zurich) at the Department of Chemistry and Applied Biosciences in the field of micro- and nanoparticle-based targeted delivery to dendritic cells, exploring surface modification and multilayer approaches for the formulation of mRNA, peptide and protein-based antigens.
Since 2008 I have been working at the University of Santiago de Compostela, Spain as grantee of competitive programs for excellence researchers, obtaining a permanent position as associate professor in 2016 at the School of Pharmacy. Since 2016 I am also the leader of the Natural Polymers and Biomimetics group at the Center for Research in Molecular Medicine and Chronic Disease – USC, Santiago de Compostela.
I have been the principal investigator of several international and national projects over the past ten years in the field of transmucosal delivery, anticancer therapy and nanomedicine. Authored > 60 original and review articles published in international peer-reviewed journals, 8 book chapters, one book as editor and several journal issues as guest editor. Have supervised of 15 PhD students (10 awarded, 5 in progress). I am also the co-inventor of 7 patent families extended as PCT, three of them licensed to biotech and pharma companies.

RECENT PUBLICATION

- L. N. Thwala et. al, Journal of Controlled Release, 291, 157-168, 2018
- C. Teijeiro-Valiño et. al. Journal of Controlled Release, 291, 157-168, 2018
- J. M. Ageitos et. al. Polymers 13 (13), 2094, 2021
- S. Robla et al., Drug Delivery and Translational Research, 11, 581–597, 2021
- P. Lundquist et. al. ACS Nano 2022, 16, 9, 14210–1422,

Anshuman Dasgupta

Google Scholar https://scholar.google.de/citations?user=JHZrvQQAAAAJ&hl=en

Education

2018- Ph.D., RWTH Aachen University, Germany
2014-2018 Masters of Science in Biomedical Engineering, RWTH Aachen University, Germany 2008-2012 Bachelor in Pharmacy, Birla Institute of Technology and Science, India

Research Experience

2018-2023 PhD, RWTH Aachen University, Germany. Prof. Twan Lammers 2017-2019 DAAD Fellow, Harvard University, USA. Prof. Samir Mitragotri

Awards

2023 Vevo Research Award
2022 World Molecular Imaging Conference Travel Stipend Award
2017 DAAD Fellowship, Harvard University
2017 RWTH Research Ambassador Scholarship

Oral Presentations

2023 European Molecular Imaging Meeting, Salzburg, Austria
2022 World Molecular Imaging Congress, Miami, USA
2021 Controlled Release Society, Zoom

Poster Presentations

More than 20 poster presentation at national and international conferences.

Flavia De Sousa

Flávia Sousa is a senior researcher at Adolphe Merkle Institute (AMI) in Fribourg, Switzerland. She completed her MSc in Pharmaceutical Sciences in 2013 and obtained her PhD in Biomedical Sciences at ICBAS, University of Porto in 2019. Her PhD was developed at I3S (Porto, Portugal) in collaboration with INL (International Iberian Nanotechnology Laboratory, Braga, Portugal), Aalborg University (Aalborg, Denmark) and Northeastern University (Boston, USA). After finishing her PhD studies, Flávia Sousa did her first postdoctoral studies at Imperial College London followed by Marie Curie MINDED fellowship at IIT (Istituto Italiano di Tecnologia). In 2022, she won an independent Women in Science (WINS) research fellowship awarded by the NCCR for Bio-Inspired Materials and she is the PI of the project.
Her research work has been pioneering regarding encapsulation of anti-angiogenic monoclonal antibodies and understanding the efficacy in the treatment of glioblastoma by normalizing the tumor vasculature and tumor microenvironment. Her research also showed that this bioengineered nanosystem was able to shuttle intratumorally and inhibit protein secretion, being an efficient alternative to deliver intracellularly monoclonal antibodies. She is currently developing immunostimulatory nanoparticles as a cancer nanovaccine for glioblastoma applying the same nanotechnology. Sousa's research work has shown promising outcomes in the treatment of brain cancer, being awarded with renowned international grants, such as, Fulbright, Marie-Sklodowska-Curie Fellowship, WINS fellowship from NCCR).
In the past 8 years, she has authored over 35 publications, received more than 10 scientific awards from different countries, acquiring over 1400 citations and yielding a H-index of 19. In 2023 she was nominated as Science Female Talents by Falling Walls Foundation, she was TEDx speaker at University of Twente in the Netherlands and she was selected as MIT Innovator Europe Under 35.

Sander De Weerd

I was born and raised in Vaassen, a village in the Netherlands where, when I was not attending middle school in Vaassen or high school in Apeldoorn, was working the fields in the weekends and holidays. When I turned 18 I made the decision to start studying pharmaceutical design and engineering in the Rijksuniversiteit Groningen. During my bachelors I worked a lot in sales companies, where, during the end of my bachelors I was asked to help grow another start-up by guiding trainees and doing the brunt of the sales work. In another start-up I helped set up and maintained a new channel to direct sales to a bigger target audience. After all this work not related to science, I decided to quit and focus on my masters which I attended in Groningen as well. Here, during the first research project I aided in the design, synthesis and characterization of novel molecules to target Asthma

and COPD where I had the opportunity to help in the preparation of 2 manuscripts.1,2 During the summer, me and my team won a gold medal in the iGEM competition. This competition for genetically modified machines gave me insights in biotechnology, management and interdisciplinary work. My second research project was in the lab of my now supervisor, Anna Salvati, which was cut short due to COVID-19. During the down-time I wrote a PhD proposal together with Wouter Roos, Jan-Jacob Schuringa and Anna Salvati and was awarded a scholarschip in Advanced Materials to develop the project to develop biomimetic nanoparticles for drug delivery in leukemia. During my PhD, I was able to present my work in a poster on several conferences and won the best PhD student Oral Presentation award at the annual Kollf institute for biomedical Engineering and Materials Science.

1. Zwinderman, M. R. H., De Weerd, S. & Dekker, F. J. Targeting HDAC Complexes in Asthma and COPD. Epigenomes 2019, Vol. 3, Page 19 3, 19 (2019).
2. Cao, F. et al. Induced protein degradation of histone deacetylases 3 (HDAC3) by proteolysis targeting chimera (PROTAC). Eur J Med Chem 208, 112800 (2020).

Mareike Deuker

Post doc

After graduating high school in 2013, I studied biomedical chemistry at the Johannes Gutenberg University of Mainz. During my studies, I spent four months at Durham University (UK) for a research stay in the group of Prof. Liam Hutchings. After completing my Master in 2019, with a master's project on the degradation of polyphosphoesters, I started to pursue my Ph.D in chemistry at the Max Planck Institute for Polymer Research in the group of Prof. Katharina Landfester. My thesis focused on the interaction of nanocarriers with anti-PEG antibodies and was completed in 2023.

Laura Dietz

I was born on December 1st in 1994 and grew up in Schwalmstadt Ziegenhain, Germany. I received my high school diploma from Schwalmgymnasium Treysa in 2013. Afterwards, I received a bachelor's degree in Molecular Biotechnology from the University of Heidelberg, Germany in June 2016 and a master's degree in Molecular Biotechnology from the University of Heidelberg in March 2020. During my master's degree, I became highly interested in novel nanomedicine applications like viral gene therapy and viral oncolytic therapy. Therefore, I wanted to explore the application of synthetic nanoparticles for drug delivery as an analogue to biologically derived nanoparticles during my PhD thesis. I started my PhD thesis in the group of Prof. Katharina Landfester in November 2020, where I work on extracellular vesicles and nanoparticles for drug delivery.

Philip Dreier

I studied chemistry at the University of Mainz from 2011 to 2018.
During my studies, I was a visiting researcher at the University of Warwick, England under supervision of Prof. Sebastien Perrier and Prof. Neil Cameron.
I obtained my doctorate in 2021 from the University of Mainz in polymer chemistry working on polyether-based materials for pharmaceutical applications and ion conductors under supervision of Prof. Holger Frey.
I am currently working as a post-doctoral researcher in the research group of Prof. Holger Frey and my work focuses on the future development of next-generation PEG-based materials.

Aroa Duro

Aroa joined Curapath in 2021 as R&D manager. She is a chemist by training and holds a PhD in Biochemistry and Biotechnology. She brings more than 10 years of experience in drug delivery and polymer science. Aroa did her PhD at the CIPF in Valencia at the Polymer Therapeutics Lab self-funded with a fellowship from the national scheme. Then, she moved to the Molecular Bionics lab in UCL London for a postdoctoral experience, with first the competitive Newton International fellowship from the Royal Society of UK, and later with a Marie Curie fellowship from the European Commission. Her phD and postdoc provided her with vast experience in materials science, polymer chemistry, bioconjugations, drug/biologics delivery as well as analytical techniques and biological methods to study drug delivery systems. She counts with more than 30 publications including articles, patents, research monographs and peer-review conferences. Aroa joined Curapath as a research awardee Torres Quevedo from the Spanish Ministry Program and since then, she has provided structure to the R&D Department to guarantee the continuous innovation of the company projects.

Sergio Esteban

Sergio joined Curapath in 2021 as R&D Product Developer. He is a pharmacist and holds a PhD. in Pharmacy. He brings more than 8 years' experience in drug delivery, hydrogel formulation, nanoparticulate characterisation and analytical development of polymeric structures. Sergio did his PhD at Complutense University Madrid at Pharmaceutical Technology department and performed a stage at Vornia Ltd. with a Marie Curie fellowship from the European Commission. His PhD. experience provided Sergio with vast experience in pharmaceutical technology, molecular biology, drug delivery and formulation characterisation. He counts with nine articles and patents, and several conferences presentations. Sergio progressed in Curapath until reaching his actual position as Analytical Development Lead, where he is providing a wide analytical implementation for formulation characterisation.

Nicolas Färber

I was born on 07.04.1996 in Augsburg, Germany, where I started my physics studies in 2015. During my studies I expanded my knowledge in biophysics through internships at Texas Christian University (Fort Worth, USA, 2017) and at the Institute Laue-Langevin (Grenoble, France, 2018). These internships were funded by governmental scholarships (German Academic Exchange Service and Erasmus+).
As part of my PhD thesis (completed in January 2023) in the biophysics group of Prof. Dr. Westerhausen at the University of Augsburg, I investigated the role of lipid molecules for cellular functions [1-4]. During these studies I developed a technique and a measuring system for the analysis of lipid-based compounds such as mRNA vaccines. After building a prototype, I set up a team and we successfully applied for the government funding "Exist Forschungstransfer", which will receive €1.1 million in funding. As part of this project, we will set up a company that will facilitate the development of lipid-based pharmaceutical compounds in the future.

RECENT PUBLICATIONS

- Färber N, Westerhausen C. Broad lipid phase transitions in mammalian cell membranes measured by Laurdan fluorescence spectroscopy. Biochim Biophys Acta - Biomembranes 2022;1864:183794
- Färber N, Reitler J, Kamenac A, Westerhausen C. Shear stress induced lipid order and permeability changes of giant unilamellar vesicles. Biochim Biophys Acta (BBA)-General Subj 2022;1866:130199.
- Färber N, Neidinger S V, Westerhausen C. Cell Membrane State, Permeability, and Elasticity Assessment for Single Cells and Cell Ensembles. Cell Viability Assays Methods Protoc., Springer; 2023, p. 225–36.
- Färber N, Reitler J, Schäfer J, Westerhausen C. Transport Across Cell Membranes is Modulated by Lipid Order. Adv Biol 2023:2200282.

Alexander Fuchs

I was born on 26.02.1998 in Frankfurt am Main, Germany. Currently I am working as a PhD student in the group of Prof. Dr. Lutz Nuhn at the Julius-Maximilians-Universität in Würzburg. Before starting the work on my PhD project in 2022 I studied Biomedizinische Chemie at the Johannes Gutenberg-Universität in Mainz where I completed both my B.Sc. (grade 1,3) and M.Sc. (grade 1,0).
My bachelor's thesis "Kationische Polycarbonat-basierte Nanogele zum Transport von immunstimulatorischen Oligonucleotiden" and master's thesis "Introducing Degradable Cationic Nanogels Carrying TLR Stimulating Oligonucleotides" were done at the Max-Planck-Institut für Polymerforschung in Mainz in the department of Prof. Dr. Tanja Weil and subgroup of Prof. Dr. Lutz Nuhn. These theses were both marked with a grade of 1,0 and I contributed to the project of Dr. Christian Czysch. (Czysch, C., Medina-Montano, C., Zhong, Z., Fuchs, A., Stickdorn, J., Winterwerber, P., Schmitt, S., Deswarte, K., Raabe, M., Scherger, M., Combes, F., De, J., Kasmi, S., Sandners, N. N., Lienenklaus, S., Koynov, K., -J. Räder, H., Lambrecht, B. N., David, S. A., Bros, M., Schild, H., Grabbe, S., De, B. G., Nuhn, L., Transient Lymph Node Immune Activation by Hydrolysable Polycarbonate Nanogels. Adv. Funct. Mater. 2022, 32, 2203490. https://doi.org/10.1002/adfm.202203490)

During my master's studies I spent half a year at the University of Toronto, Canada in the group of Prof. Dr. Molly Shoichet working on "Cationic Polymer Lipid Hybrid Nanoparticles (PLNPs) for RNA Delivery". This semester abroad was funded by an ISAP-DAAD scholarship. Currently I am receiving a Kekulé scholarship of the Fonds der Chemischen Industrie and during my studies in Mainz I was nominated for a scholarship of the Studienstiftung des Deutschen Volkes by my university.
Prior to my studies at university I went to the Albert-Einstein-Schule (Gymnasium) in Schwalbach where I received my high school diploma with a grade of 1,3. During my final three years of elementary school I attended an American school near Cincinnati, OH, USA while my first year was spent in Kronberg, Germany. While I was a student I did two short internships at Continental AG, Schwalbach and Braun GmbH, Kronberg.

Fabian Fuß

IBorn in 1997, I have been raised in Kirchheimbolanden, Germany. As I moved on along the standard route of education I developed a keen interest for chemistry, biology, and the advanced interdisciplinary topics of both those fields. Finishing my Abitur with a focus on natural sciences, my academic path started at the Johannes Gutenberg University of Mainz in 2016.
Pursuing the major of "Biomedical Chemistry" I was granted many insights into organic synthesis, biochemistry, pharmaceutical and toxicological research as well as polymer chemistry. During the time of my studies, I had the chance to experience the daily business of a pharmaceutical company first hand at Sanofi Germany for two months. In my master studies my field of interest shifted towards polymer research, especially for medical and pharmaceutical application. As the Covid-19 pandemic and the related vaccine research put nanomedical research into the spotlight, my preference of interdisciplinary research led me to writing my master thesis in the field of polymers for Nanomedicine in the group of Prof. Holger Frey, about a much-needed alternative for polyethylene glycol (PEG).
After graduating, the opportunity arose for me to spend seven months in Seoul, South Korea and join the research group of Prof. Yan Lee at Seoul National University. During my time at the SNU I contributed to the research project of Sungwhan Kim about ROS-sensitive Polyester materials which was published recently in Polymer Chemistry (DOI:10.1039/D3PY00239J).
I returned to Germany to begin my PhD research in June 2022 back in the group of Prof. Frey where I continue with the research of polyether-based PEG alternatives called rPEGs. These isomers of PEG aim at evading the recognition by anti-PEG Antibodies and preventing an accelerated blood clearance or severe immune reactions of PEGylated pharmaceuticals. Exceeding the evasion of the anti-PEG antibodies, the better understanding of the epitope of said antibodies by precision tailoring rPEGs is an additional topic of interest for my current research

Christina Galanakou

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Miss Christina Galanakou is a PhD candidate at the Centre Interdisciplinaire de Nanoscience de Marseille (CINaM) in France, affiliated with Aix-Marseille University and CNRS. In 2021, she obtained her integrated Master's in Pharmaceutical Sciences at the National and Kapodistrian University of Athens in Greece, a comprehensive program that combines both Bachelor's and Master's degrees for a total duration of 5 years. During 2020 and 2021, she conducted part of her undergraduate studies and an internship in the group of Dr. Ling PENG at CINaM, exploring the biomedical applications of amphiphilic dendrimers on Erasmus+ fellowships. Selected first for a French national PhD fellowship, she is now approaching the conclusion of her first year. Her PhD research centers on the development of fluorinated self-assembling dendrimers for theragnostic applications. Additionally, she actively investigates the potential of amphiphilic dendrimers in combating infections, leading to the publication of her first mini review titled: "Amphiphilic dendrimers against antibiotic resistance: light at the end of the tunnel?". Furthermore, she engages in research related to dendrimer-mediated hijacking of in situ secreted extracellular vesicles for cancer therapy.

Han Gao

I was born in China, I obtained my B.S in Public Health from North China University of Science and Technology in 2017. Within these five years' study, I got the knowledge of epidemiology, nutrition and medical statistics, as well as learning animal models. At the same year, I moved to Nanjing and started to pursue the master's degree on public health. In this journey, I further honored my abilities in epidemiology, molecular biology and underlying mechanisms on cancer progression. During these three years, I got my first scientific publication which encouraged me to further explore scientific phenomenon.

In 2020, after getting the scholarship from China Scholarship Council, I moved to Helsinki as a Ph.D. candidate in Department of Pharmacy, under the supervision of Prof. Hélder A. Santos. From 2020 to 2021, I was designing and developing polysaccharide-based biopolymers for targeted siRNA delivery. In addition, we also tried to check the application of developed nanomedicine on murine model with myocardial infarction. In 2022, due to the job mobilization of Prof. Hélder A. Santos, I came to Netherlands to continue my doctoral degree and research work. Currently, I'm a third-year Ph.D. student who is interested in developing siRNA nanomedicine for the treatment of cardiovascular diseases.

RECENT PUBLICATIONS

- H Gao, S Wang, Q Long, Z Liu, H A. Santos. Journal of Controlled Release. 2023, 357, 120-132.
- C Tapeinos, H Gao, T Bauleth-Ramos, HA Santos. Small. 2022, 18 (36), 2200291.
- H Gao, R Cheng, H A. Santos. View. 2021, 2 (3), 20200111.
- H Gao, Z Gao, F Yang, R Liu. Journal of cellular physiology. 2020, 235 (3), 2325-2335.

Barbara Graefen

Am Taubertsberg 2, 55122 Mainz,
Germany 06131-17-6560
bagraefe@uni-mainz.de

I was born on April 28th, 1989 in Daun, Germany. In March 2008 I graduated from high school in Gerolstein, Germany. In January 2011, I successfully graduated as a chemical laboratory assistant with an award in Trier, Germany with a focus on food consumer protection for the state investigation office of Rhineland-Palatinate. I stayed in this position until I went abroad to work in Philadelphia, USA as an AuPair, working for the Family of Mary Gay Scanlon (today U.S. Congresswoman). Within these two years, I was officially enrolled at the Delaware County Community College and was auditing classes at Swarthmore College. Moving back to Germany, I studied Biomedical Chemistry at Johannes Gutenberg University in Mainz. My bachelors thesis was done in the lab of Prof. Rudolph Zentel in Mainz, the former head of the SFB1066 with a focus on nanocarriers formed by block copolymers with functional groups for click-chemistry. During my master's studies, I organized a research internship for my master thesis in the lab of Prof. Berit Olofsson at Stockholm University, Sweden with a focus on hypervalent iodine transfer reagents for vinyl coupling reactions.

After finishing my master's degree in February 2019, I performed a three months orientation internship at Novartis Deutschland GmbH in Nuernberg, Germany at the business unit oncology (breast cancer) followed by the collection of 1.5 years of work experience as a sales representative for pain medicine on behalf of the company Gruenenthal GmbH. In April 2021 I followed my wish to carry out research closer to the patients and started as a PhD Student in the research group of Prof. Andrea Tuettenberg in the dermatology department in the University Medical Center of the Johannes Gutenberg University in Mainz. My current research interests include the influence of acidic extracellular pH on tumor cells and immune cells, including adjustment of the extracellular pH *in vitro* and *in vivo* with nanocarriers.

Sjoerd Hak

NARRATIVE CV SJOERD HAK

I obtained my MSc in Biomedical Engineering with great appreciation in 2007 at the Eindhoven University of Technology in the Netherlands in 2007. I did my PhD in Medical Technology at the Norwegian University of Science and Technology in Trondheim, Norway and defended in 2013.

My PhD and subsequent academic research is driven by a fascination for the complex interactions between man-made nanoparticles and biology. At the heart of my effort to understand these interactions lie various *in vivo* imaging modalities, like magnetic resonance imaging, whole animal optical imaging, and intravital microscopy. I have integrated state-of-the-art flow cytometry and mass spectrometry approaches into my research, to accurately map interactions of administered (nano)drugs with numerous cell types. This approach has provided new and exciting insights, which are pivotal for successful development of improved and novel (nano)therapeutic approaches.

In 2019 started at SINTEF, which is one of the largest independent contract research organizations in Europe. I am Research Scientist in the Department of Biotechnology and Nanomedicine where we are about 100 people. We are working in a wide variety of settings,

spanning from fundamental research in collaboration with universities to solving problems for industry customers. We have established state-of-the-art infrastructure and extensive and generic competence in polymeric and lipid nanoparticle design and synthesis, production, and physicochemical and *in vitro* characterization. We are internationally competitive in this arena, which is illustrated by our previous and current involvement in various EU projects, like for example EU-NCL, EXPERT and NANO-ENGINE. Our mass spectrometric analysis facility offers high-end infrastructure and expertise in liquid chromatography and field flow fractionation, as well as various mass spectrometry-based analytical tools. Our screening facility is a fully equipped lab with highly efficient and accurate robotic liquid handlers and high-throughput cell culturing and screening. We also have access to a well-equipped animal facility at NTNU/St. Olavs University Hospital in Trondheim.
I have published 28 papers in international peer reviewed scientific journals (Google scholar, 23/05/2023: h-index 18, total of 1507 citations, 6 as first author, 6 as last author, 4 reviews, one perspective with shared authorship) and one bookchapter (first author) since 2008. I delivered 4 oral presentations, of which two invited, and 10 poster presentations at international peer-reviewed conferences since 2010. Additionally, I gave more than 20 oral presentations at local and national seminars, workshops and conferences since 2008. 4 oral presentations where I was senior author have been delivered at international peer-reviewed conferences since 2018.

RECENT PUBLICATIONS

- Sofias, A.M., G. Bjørkøy, J. Ochando, L. Sønstevold, M. Hegvik, L. Davies Cde, O. Haraldseth, T. Lammers, W.J. Mulder, and S. Hak, Cyclic Arginine–Glycine–Aspartate-Decorated Lipid Nanoparticle Targeting toward Inflammatory Lesions Involves Hitchhiking with Phagocytes. Advanced Science, 2021. 8(13).
- Sofias, A.M., Y.C. Toner, A.E. Meerwaldt, M.M.T. van Leent, G. Soultanidis, M. Elschot, H. Gonai, K. Grendstad, A. Flobak, U. Neckmann, C. Wolowczyk, E.L. Fisher, T. Reiner, C.L. Davies, G. Bjorkoy, A.J.P. Teunissen, J. Ochando, C. Perez-Medina, W.J.M. Mulder, and S. Hak, Tumor Targeting by alphavbeta3-Integrin-Specific Lipid Nanoparticles Occurs via Phagocyte Hitchhiking. ACS Nano, 2020. 14(7): p. 7832-7846.
- Senders, M.L., A.E. Meerwaldt, M.M.T. van Leent, B.L. Sanchez-Gaytan, J.C. van de Voort, Y.C. Toner, A. Maier, E.D. Klein, N.A.T. Sullivan, A.M. Sofias, H. Groenen, C. Faries, R.S. Oosterwijk, E.M. van Leeuwen, F. Fay, E. Chepurko, T. Reiner, R. Duivenvoorden, L. Zangi, R.M. Dijkhuizen, S. Hak, F.K. Swirski, M. Nahrendorf, C. Perez-Medina, A.J.P. Teunissen, Z.A. Fayad, C. Calcagno, G.J. Strijkers, and W.J.M. Mulder, Probing myeloid cell dynamics in ischaemic heart disease by nanotracer hot-spot imaging. Nature Nanotechnology, 2020. 15(5): p. 398-405.
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Shunping Han

Final year PhD student, King's College London

Shunping is a PhD student in King's College London. She graduated from Zhejiang Chinese Medical University (China) with her bachelor's degree of pharmacy in 2014 and then continued her first MRes study in pharmaceutics from 2014 to 2017, under the supervision of Prof. Fanzhu Li. During this 3-year MRes study, she looked into the construction of the targeting drug delivery system, especially focussing on the modification of PAMAM dendrimers and mesoporous silica nanoparticles as drug carriers for glioma therapy. She then continued her second MRes study of drug discovery and development at the Chemistry Department, Imperial College London. Her research focussed on metal organic frameworks (MOFs) synthesis and modification as drug vehicles under the guidance from Dr. Rob Davies, Prof. Paul Lickiss and Prof. Jane Mitchell. Following the graduation with the degree of distinction, she has been awarded the King's-China Scholarship Council to continue her PhD under the supervision of Professor Khuloud Al-Jamal at Institute of Pharmaceutical Science, KCL. Her current project involves the investigation of the biodistribution of functionalized gold nanoparticles through intranasal administration route potentially applied for neurodegenerative disease treatment.

RECENT PUBLICATIONS

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- Han S and Al-Jamal K T. Combined facile synthesis, purification and surface functionalization approach yields monodispersed gold nanorods for drug delivery applications. Particle & Particle Systems Characterization, 2023 (Accepted).
- Helal D O, Rouatbi N, Han S, et al. A natural protein-based platform for the delivery of Temozolomide acid to glioma cells. European Journal of Pharmaceutics and Biopharmaceutics, 2021, 169: 297-308.
- Abdel-Bar H M, Walters A A, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, et al. An "eat me" combinatory nano-formulation for systemic immunotherapy of solid tumors. Theranostics, 2021, 11(18): 8738.
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Hajira Banu Haroon

Marie Sklodowska Curie ESR, Newcastle University, UK

Hajira Banu H has obtained her bachelor's degree in pharmacy (2013) and master's degree in pharmacy with specialization in Pharmacology (2015) from Rajiv Gandhi University of Health Sciences, India. After graduating as an M. Pharm, she was appointed as Assistant Professor of Pharmacology at M.S. Ramaiah University of Applied Sciences. Presently, She is pursuing her Ph.D as a Marie Sklodowska Curie ESR under the supervision of Prof. Moein Moghimi at Newcastle University, UK. Her PhD is focused on developing drug carriers for directing immune responses. Her research interests include nanotechnology-based drug delivery systems for immune modulation, and targeted therapeutics.

RECENT PUBLICATIONS

- Moghimi SM, Haroon HB, Yaghmur A, Hunter AC, Papini E, Farhangrazi ZS, Simberg D, Trohopoulos PN. Perspectives on complement and phagocytic cell responses to nanoparticles: From fundamentals to adverse reactions. J Control Release. 2023 Apr ; 356:115-129.
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Adrian Hauck

Since school days, chemistry has been of extraordinary interest to me. This resulted in the decision to choose chemistry as one of my two advanced courses in addition to mathematics. For the time after my school career, there was no question that I wanted to continue working in the chemical field. Since I had great interest in the working world, I decided to do a vocational training as a chemical technician at the Chemische Fabrik Budenheim KG, where I completed the training in a shortened procedure within 2.5 years. Afterwards I had the urgent ambition to dive deeper into the subject. Accordingly, after a short period of working as a trained chemical technician, I studied chemistry at the Johannes-Gutenberg University (JGU) in Mainz with an emphasis on organic chemistry and physical chemistry. I did my bachelor thesis in Prof. Siegfried R. Waldvogels group working on the electrochemical synthesis of fluorinated orthoesters from 1, 3-benzodioxoles. The work resulted in a publication in the journal ChemistryOpen.[1} After my bachelor's degree, I received a DAAD scholarship for a semester abroad at the University of Massachusetts (UMass) in Amherst. During this time, I participated the first semester of the graduated student program at the department of polymer science and engineering (PSE), followed by a two-month research internship in E. Bryan Coughlins group. During this time my interest in polymer chemistry was aroused and I was highly ambitioned to further pursue my interest during my progressing master study back in Germany at the JGU in Mainz. It was at this moment that the SARS-CoV-2 pandemic interrupted everybody's life and I was afraid, that this will delay my ongoing studies. Thanks to the university's excellent adaptation to the new situation, I was able to continue my master's degree in a hybrid study program. To extend my understanding in polymer chemistry I participated the lecture macromolecular chemistry, given by Prof. Lutz Nuhn. Next to general knowledge in the field of polymer chemistry, he gave insight into the work in his macromolecular therapeutics group, what sounded very appealing to me. Driven by that I applied for my master thesis in his group and got the chance to work on polycarbonates with acid-degradable ketal side groups for potential drug delivery systems. When I finalized my thesis, Prof. Lutz Nuhn was appointed as professor and chair of macromolecular chemistry at the Julius-Maximilians University (JMU) in Würzburg. Getting offered the opportunity, I decided to move with him to continue my research for my PhD in Würzburg.
During the first months at the JMU in Würzburg it was very exciting to support the moving process and setting up the new infrastructure. In parallel I continued my research project, improving the poly(benzyl ketal carbonate) nanocarriers from my master thesis and started the work on additional projects, including covalent cargo binding for more controlled drug release and introducing reductive responsive trigger mechanisms to the polymers.

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Beth Heaton

Beth Heaton is a third-year PhD student in the Immunocompatibility Group, based at the University of Liverpool under the supervision of Dr Neill Liptrott. She previously obtained her BSc (Hons) degree in Biochemistry and MRes in Biomedical Sciences and Translational medicine, exploring the impact of HIV antiretrovirals on glucose uptake and immune cell activation, profiling cellular bioenergetics, at the University of Liverpool. Currently, her PhD work is focused on investigating the interactions of super paramagnetic iron oxide nanoparticles (SPIONs) with components of the human immune and haematological systems, looking more specifically into the biocompatibility of these materials with the immune system. Her project is looking into the impact of how long these nano-enabled materials remain in place and how this affects their clinical utility. The project is part of the SAFE-N-MEDTECH OITB, funded by the European Commission.

RECENT PUBLICATIONS:

- Heaton BJ, Jensen RL, Line J, David CAW, Brain DE, Chadwick AE, Liptrott NJ. Exposure of human immune cells, to the antiretrovirals efavirenz and lopinavir, leads to lower glucose uptake and altered bioenergetic cell profiles through interactions with SLC2A1. Biomed Pharmacother. 2022 Jun;150:112999. doi: 10.1016/j.biopha.2022.112999. Epub 2022 Apr 20. PMID: 35461087.
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Manasa Manjunath Hegde

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I was born on 20th June 1994. I have done my Master of Science (M. Sc.) in Biochemistry, at University of Mysore, Karnataka, India in June 2017 with score of 73.3% and Bachelor of Science (B. Sc.) in Chemistry, Botany, Zoology, M M Arts & Science College, Sirsi, affiliated to Karnataka University, Dharwad, Karnataka, India with the score of 83.39%. After my Masters I have joined as PhD scholar in the Department of Radiation Biology and Toxicology, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India.
During my doctoral research, I focused on the development of targeted drug delivery systems for treating Glioblastoma Multiforme (GBM), a highly aggressive and lethal brain tumor. The objective was to engineer liposome-based nanoformulations capable of delivering therapeutic agents specifically to GBM cells while minimizing off-target effects. My research findings demonstrated that the dual drug loaded targeted liposomal delivery system exhibited superior therapeutic efficacy compared to traditional drug administration. The specific targeting also significantly reduced off-target effects, enhancing the overall safety of the treatment. Throughout

my PhD journey, I collaborated with fellow researchers, presented my findings at national and international conferences, and published research articles in reputable peer-reviewed journals.

RECENT PUBLICATIONS:

- Hegde MM, Sandbhor P, J. A, Gota V and Goda JS (2023) Insight into lipid-based nanoplatform-mediated drug and gene delivery in neuro-oncology and their clinical prospects. Front. Oncol. 13:1168454.
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Lifan Hu

PhD student

I studied Pharmaceutics at China Pharmaceutical University in Nanjing (China), and received my bachelor's degree in 2019. Then I continued to study at China Pharmaceutical University, and obtained my master's degree in 2022. My master's research focused on gene therapy to rare mitochondrial diseases. In November 2022, I started with my PhD in the group of Prof. Dr. Tanja Weil at the Max Planck Institute for Polymer Research.

RECENT PUBLICATIONS:

- Wang Y#, Hu LF#, Cui PF#, Qi LY, Xing L, Jiang HL. Pathologically Responsive Mitochondrial Gene Therapy in an Allotopic Expression-Independent Manner Cures Leber's Hereditary Optic Neuropathy. Adv Mater. 2021 Oct;33(41):e2103307.)

Nicole Martina Hutter

PhD student

I was born 1993 in Darmstadt, Germany, where I grew up and finished my secondary school education. In 2013 I started my Biomedical Chemistry studies at the Johannes Gutenberg-University of Mainz, Germany and finished with my bachelor thesis on the topic of discotic liquid crystals in the group of Prof. Heiner Detert in winter 2017. In 2019, I completed my master thesis in collaboration between the medicinal chemistry department of Merck KGaA Darmstadt, Germany, and Johannes Gutenberg University Mainz about the development of new anti-cancer agents under the supervision of Prof. Pol Besenius and Dr. Matthias Leiendecker (Merck KGaA).

Since spring 2020 I do my PhD studies at the Department of Chemistry of the Johannes Gutenberg-University Mainz, under the supervision of Prof. Pol Besenius in the research field of supramolecular chemistry.

Tore-Geir Iversen

Dr. Tore Geir Iversen is a biochemist by training and received his PhD at the Norwegian University of Science and Technology in Trondheim, Norway in 1995, in studies of microbial genetics. He joined the group of professor Sandvig in 1997, a group that has made significant contributions to our knowledge about endocytosis and intracellular transport focusing on different protein toxins as tools. Iversen is since 2006 a senior researcher and project group leader at the Department of Molecular Cell Biology, Institute for Cancer Research at Oslo University Hospital. He then turned his research activity into studying how nanoparticles are endocytosed and transported in cells. The Sandvig group has been heading a national competence building project in Norway entitled "Biodegradable Nanoparticles in Cancer Diagnosis and Therapy". Iversen was member of the project management group and coordinated the *in vitro* activities in the project, which had members from academia, university hospitals, research institutes and pharmaceutical industry. His research interests include drug targeting to cancer cells and tumors (by ligand binding; PDT/PCI effects of photosensitizers) and tumor-targeted combination therapies. His research is directed towards optimizing delivery of the NPs into cancer cells and understanding which cellular mechanisms that are involved in their uptake, depending on their size as well as which components add at the surface of the NPs. Studies of uptake and intracellular transport of NPs in different cancer cell lines are performed by confocal microscopy, super-resolution microscopy and live-cell imaging techniques. Furthermore, in addition to using cell-based toxicity assays, various cellular mechanisms (i.e. oxidative/ER stress, autophagy) that might be implicated in cell death are studied.

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- Fusser, M.; Overbye, A.; Pandya, A. D.; Morch, Y.;… Iversen, T.-G.; Sandvig, K.; Skotland, T.; Maelandsmo, G. M., Cabazitaxel-loaded Poly(2-ethylbutyl cyanoacrylate) nanoparticles improve treatment efficacy in a patient derived breast cancer xenograft. J Control Release 2019, 293, 183-192.
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- Szwed, M.; Torgersen, M. L.; Kumari, R. V.; Yadava, S. K.; Pust, S.; Iversen, T. -G.; Skotland, T.; Giri, J.; Sandvig, K., Biological response and cytotoxicity induced by lipid nanocapsules. J Nanobiotechnology 2020, 18, 5.
- Pandya, A. D., Iversen, T.-G.,…; Sandvig, K., Skotland, T., Mælandsmo, G. M., Biodistribution of Poly(alkyl cyanoacrylate) Nanoparticles in Mice and Effect on Tumor Infiltration of Macrophages into a Patient-Derived Breast Cancer Xenograft. Nanomaterials 2021, 11, 1140.

Nikita Jain

Senior Formulation Scientist

Nikita Jain (Born in India, 1988) received her B.Sc. in 2010 from St. Xavier's College (India) and her M.Sc. in 2012 from Indian Institute of Technology (IIT) Kanpur, where she worked as a bench chemist to synthesize biologically significant organic molecules like neurotransmitter analogs. This work sparked her interest to pursue her Ph.D. in Organic Chemistry. She immigrated to Vancouver to pursue her Ph.D. on synthesizing organic molecules for drug discovery at The University of British Columbia in the group of Prof. Marco A. Ciufolini. After Ph.D., she decided to utilize her expertise and experience in synthesizing complex organic molecules that has high commercial value. She joined SWITCH Materials Inc., Burnaby as a Research Scientist to generate library of photochromic organic compounds to be incorporated in automotive glass. During this time, she could not give up her love for drug discovery and joined Precision NanoSystems ULC (PNI) in 2018 as a Formulation Scientist for generating lipids that could be used in lipid nanoparticles. There, she designed novel ionizable lipids and built a team to grow PNI's lipid library and generate potency data. These ionizable lipids constitutes a crucial part of LNP formulations for applications like Gene Therapy, Gene editing, Vaccines, and Cell Therapy etc. Currently, she is working as a Senior Scientist in the R&D.

RECENT PUBLICATIONS

- Duhen, R.; Beymer, M.; Jensen, S. M.; Abbina, S.; Abraham, S.; Jain, N.; Thomas, A.; Geall, A. J.; Hu, H. M.; Fox, B. A.; Weinberg, A. D.; OX40 agonist stimulation increases and sustains humoral and cell-mediated responses to SARS-CoV-2 protein and saRNA vaccines, Front. Immunol 2022, 13, 1
- Roces, C. B.; Lou, G.; Jain, N.; Abraham, S.; Thomas, A.; Halbert, G. W.; Perrie, Y.; Manufacturing Considerations for the Development of Lipid Nanoparticles Using Microfluidics, Pharmaceutics 2020, 12 (11), 1095
- Blakney, A. K.; McKay, P. F.; Hu, K.; Samnuan, K.; Jain, N.; Brown, A.; Thomas, A.; Rogers, P.; Polra, K.; Sallah, H.; Yeow, J.; Zhu, Y.; Stevens, M. M.; Geall, A.; Shattock, R. J.; Polymeric and lipid nanoparticles for delivery of self-amplifying RNA vaccines, Journal of Controlled Release 2021, 338, 201

Ian Johnston

Senior Research Associate - Product Development

Ian Johnston completed his B.Sc. of Chemistry from the University of British Columbia. A formative experience for him was his first co-op placement in the Contrast Media Research Group of Bayer AG. There he got his first taste of nanomedicines by making a diverse set of metal oxide nanoparticles. After the completion of his undergraduate degree, he joined STEMCELL Technologies as a Research Technologist and investigated iron oxide nanoparticles for cell separation applications. Returning to academia, Ian completed a MASc. at the University of Toronto (U of T) in Dr. Frank Gu's group. While at U of T, he persued diverse interests and contributed to both mucosal drug projects and an investigation on the use of titanium dioxide nanoparticle for environmental remediation. Returning to biotech, he then joined Precision NanoSystems ULC (PNI) in 2021 as a Research Associate. There he and his expanding team test new hardware in development with an emphasis on PNI's commercial-scale systems. He also conducts fundimental research on microfluidic mixing. Currently, he works as a Senior Research Associate in R&D.

Carina Jung

I was born on May 2nd, 1997 in Karlsruhe, Germany and grew up in Baden-Baden for the first eighteen years of my life. After my graduation from Richard-Wagner-Gymnasium Baden-Baden, I decided to take a gap year and apply for different internships, as well as travel to Canada for a short-term stay. Four months of these internships were spent in a hospital, where I realized that the career path in medicine, which I had originally intended to follow, was not the right decision for me, and I decided on chemistry instead, which I started studying at University of Constance in Konstanz, Germany in 2015. I graduated with my Bachelor's degree in 2018 and finished my Master's degree in 2021. For both my Bachelor and my Master thesis, I followed my still very prominent interest in the medical research area, and worked in the group of Prof. Dr. Helmut Cölfen on "Polyoxazoline based, double hydrophilic block-copolymers for calcium absorption", which were intended for use in atherosclerosis therapy, as well as "Polysaccharide based and synthetic coatings for an improved bioactivity of PEEK bone implants". During my Master's studies, I spent a semester abroad at University of Toronto, Toronto, Canada, which I immensely enjoyed. After graduating from University of Constance, I decided to stay in the field of nanomedical research and therefore applied for a position in Prof. Dr. Katharina Landfester's group at the Max-Planck-Institute for Polymer Research in Mainz, Germany. Here, I am currently working on surface functionalization of nanocarriers for the purpose of targeted delivery and immunotherapy.mo, G. M., Biodistribution of Poly(alkyl cyanoacrylate) Nanoparticles in Mice and Effect on Tumor Infiltration of Macrophages into a Patient-Derived Breast Cancer Xenograft. Nanomaterials 2021, 11, 1140.

David Juriga

I graduated as a pharmaceutical-chemical engineer from the Budapest University of Technology and Economics, Hungary, and finished my Ph.D. in Pharmaceutical Sciences at Semmelweis University, Hungary. I have more than 10 years of experience in research from the basic to advanced level in the field of polymer chemistry, material sciences, drug delivery, nanoparticle characterization, and testing the cellular uptake and cytotoxicity of these systems on different cell lines. During this period, I joined several times to research groups around the world such as at the University of Groningen, RIKEN Institute Japan two times, Sungkyunkwan and Kyugnpook University in South Korea, and Istanbul Technical University in Turkey. I have published my research in several international journals and presented at several international conferences also as an invited speaker. Besides my research activities, I have been involved in teaching Medical Biophysics at Semmelweis University since 2014 and Physical and Colloid Chemistry since 2019.

In 2022, I successfully applied for the Eötvös research grant of the Hungarian state and spent 6 months in the research group of Prof. Anna Salvati at the Univeristy of Groningen. During this project, I developed different polyelectrolyte-based nanoparticles and polyelectrolyte-coated nanoparticle systems to investigate the effect of the chemical structure and physical parameters on the cellular uptake mechanism and uptake capacity. This research project deepened my interest in studying the cellular uptake of polyelectrolyte-based nanosized complexes which can reveal new concepts in the application of polyelectrolytes for targeted drug or nucleic acid delivery.

RECENT PUBLICATIONS

- Krisztina Toth et. al.: Implantable electrospun meshes based on different polymers combine prosperous features of prednisone and doxorubicin *in vitro*, Journal of Molecular Liquids, 2023, 381, 121854
- Krisztina Toth et. al.: Electrospinning of polysuccinimide-dopamine conjugates alters release kinetics and affects cell viability and uptake, Macromolecular Bioscience, 2200397, 2023
- David Juriga et. al.: Analysis of three-dimensional cell migration in dopamine modified poly(aspartic acid) based hydrogels, Gels: 8 (2), 65
- David Juriga et.al.: Kinetics of Dopamine release from poly(aspartamide)-based prodrugs, Acta Biomaterialia, 2018. 76, 225
- David Juriga et.al.: Biodegradation and osteosarcoma cell cultivation on poly(aspartic acid) based hydrogels, ACS Applied Materials and Interfaces, 2016. 8 (36), 23463

Jinhong Kang

Ph.D. candidate

Biography: My name is Jinhong Kang, I did my bachelor in the Yanbian University, China, studied pharmacy. After that, I went to South Korea and majored in drug ADME and Pharmacokinetics at Korea University. During my graduate period, I mainly studied the pharmacokinetics of drug candidates and use LC-MS/MS to evaluate drug concentration in plasma, tumors, and tissues. Based on the plasma concentration results from i.v. and p.o. In January 2022, I joined Volker Mailänder´s group at Department of Physical Chemistry of Polymers at the Max Planck Institute for Polymer Research (MPI-P). Measnwhile, I am working at Department of Dermatology, University Medical Center of the Johannes Gutenberg University Mainz. I am intereste in nanovaccine development of immunotherapy using dendritic cells, mainly focuse on the inflmatic disease like asthma and cancer.

RECENT PUBLICATIONS

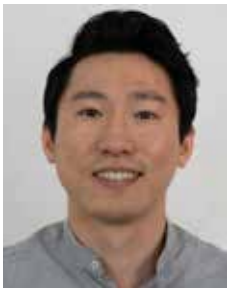
- Sang-Hyun Son,†, Jinhong Kang,†, Myunghwan Ahn, Soyeon Nam, Yong Woo Jung, Ki Yong Lee , Young Ho Jeon, Youngjoo Byun,* and Kiho Lee*; " Synthesis and Biochemical Evaluation of Baicalein Prodrugs" pharmaceutics, accepted
- Tao Tong, Ya-Jun Liu, Jinhong Kang, Cheng-Mei Zhang and Seong-Gook Kang*; " Antioxidant Activity and Main Chemical Components of a Novel Fermented Tea", Molecules, 2019,24, 2917.
- Zhou,†, Mun Hwan Oh,†, Yeon Joon Kim, Eun-yeong Kim, Jinhong Kang, Sung Chung; 2, Chung Ju, Won-Ki Kim and Kiho Lee*;"Metabolism and Pharmacokinetics of SP-8356, a Novel (1S)-(−)-Verbenone Derivative, in Rats and Dogs and Its Implications in Humans"

Laurine Kaul

I am a biomedical researcher with work and research stints in Germany, Switzerland, and Australia. I studied pharmacy as state examination at the University of Freiburg in Germany and became a registered pharmacist in 2016. As part of internships, I worked six months in a pharmacy and in Global Medical Affairs in the transplantation department at Novartis Pharma AG in Based (Switzerland). During a gap year travelling New Zealand and Australia, I was made aware of a Joint PhD program between the University of Adelaide (Australia) and the University of Freiburg (Germany). Based on a particular interest in microbiology and the development of novel formulations for the delivery of drugs, I embarked in a research project to develop a novel antibiofilm strategy for surgical site infections. Four years later and in September 2023, I will graduate as a PhD in medicine/applied microbiology from the University of Adelaide and a PhD in drug development from the University of Freiburg. Beginning on the 1st October 2023, I will start my own research group as part of the Department Pharmaceutics with the focus on developing novel drug delivery systems for small molecules and assessing the formulation in pre-clinical models.
I am an enthusiastic and driven scientist dedicated to convert promising therapeutics into successful therapies by developing drug delivery systems. While focusing on improving therapies against antibiotic-resistant bacteria, I developed an injectable hydrogel that contains antibacterial agents encapsulated into liposomes for the prevention and treatment of surgical site infections. This new treatment is now ready for animal studies, the first step in the transition from bench to bedside.
To date, I published 6 manuscripts (4 first author, 2 co-author) and 1 book chapter, contributed to 1 patent and established 2 international collaborations. At the University of Freiburg, I participated in teaching of 3 courses and mentored 3 students for research projects for up to 6 months.
My goal is to enhance bioavailability and targeting of drugs and ultimately to improve best medical care for patients.

Shiva Khorshid

Shiva Khorshid is a PhD Candidate between the University of Urbino (Prof. Casettari Lab – PharmaTechLab, Italy) and the RWTH Aachen University Hospital (Dr. Sofias Lab – ExMI, Germany Lab). In 2019, she obtained a Diploma in Pharmacy (PharmD) from the Zanjan University of Medical Science (Iran). Her research focuses on the development and evaluation of nanomedicine platforms via in-house 3D-printed microfluidic technologies (including liposomes, lipid nanoparticles, micelles, emulsions, and hydrogels). She utilizes her nanoparticles for diagnostic and therapeutic applications in oncological disorders (myeloproliferative neoplasms and triple-negative breast cancer), aiming to the identification of cell-specific targeting patterns.



Bumjun Kim

Postdoctoral scholar, Chemical and Biological Engineering Department, Princeton University

My research interests lie in designing and developing novel medicines/modalities for the treatment of complex human diseases. My undergraduate research experiences in Dr. Jaebeom Lee's lab and Dr. Robert Tranquillo's lab opened my eyes to biomaterials and drug delivery. During my PhD, I have designed and performed *in vitro* and *in vivo* studies to prove the efficacy of targeted lipid nanoemulsions that co-deliver chemotherapeutics to breast cancer under the guidance of Dr. Debra Auguste. I have learned to individually design, execute, and evaluate an anti-tumor efficacy experiments in addition to nanoparticle synthesis and characterization skills. Building on my previous training, the current postdoctoral training is focused on the development and application of FlashNanoPrecipitation (FNP) and inverse FlashNanoPrecipitation (iFNP) technology, which enable the scalable encapsulation of small molecules, peptides, oli-

gonucleotides, and proteins at high loading and encapsulation efficiency. Based on previous trainings, I focus on two main reserach projects. First, I am studying the relation between surface chemistry of nanoparticles and their distribution along the inflamed colons. Second, I am developing a targeted lipid nanoparticles (LNPs) and investigating the relationship between physiochemical properties and activity of LNPs.

PUBLICATIONS

- Kim B, Zhang D, Armstrong MS, Pelczer I, Prud'homme RK. Formulation of pH-responsive Methacrylate-based Polyelectrolyte Stabilized Nanoparticles for Applications in Drug Delivery. ACS Appl Nano Mater. 2022; 5(12): 18770-18778.
- Kim B, Liu D, Auguste DT. A lipid targeting, pH responsive nanoemulsion encapsulating a DNA intercalating agent and a HDAC inhibitor reduces TNBC tumor burden. Advanced Therapeutics. 2021; 4(3), 2000211.
- McManus S†, Zhang Y†, Kim B, Lee B, ElSayed ME, Prud'homme RK. Co-encapsulation by Flash NanoPrecipitation of Insulin, Trypsin Inhibitor and Caprate Permeabilization Enhancer for Oral Administration. Precis. Nanomed. 2020; 3(5): 710-723.
- Kim B, Pena CD, Auguste DT. Targeted lipid nanoemulsions encapsulating epigenetic drugs exhibit selective cytotoxicity on CDH1-/FOXM1+ triple negative breast cancer cells. Molecular Pharmaceutics. 2019; 16(5): 1813-1826.
- Tao Y, Li M, Kim B, Auguste DT. Incorporating gold nanoclusters and target-directed liposomes as a synergistic amplified colorimetric sensor for HER2-positive breast cancer cell detection. Theranostics. 2017; 7(4):899-911.

Jonas Koehler

My name is Jonas Koehler and I studied Pharmaceutical Sciences at the Albert-Ludwigs-University Freiburg from 2014 to 2020. I wrote my bachelor thesis in the research group of Prof. Dr. Regine Süss about the lyophilization of siRNA-loaded lipid-polymer-hybrid nanoparticles. I completed a research internship during my master's studies at the University of Toronto, Canada in the group of Prof. Dr. Rob Macgregor. My master's thesis was carried out in the research group of Prof. Dr. Heiko Heerklotz in collaboration with Prof. Dr. Ulrich Massing, where I developed a screening method for conditions of liposome preparation by means of dual centrifugation. Since October 2020, I am a Ph.D. student at the Department of Pharmaceutical Technology in the research group of Prof. Dr. Heiko Heerklotz and Prof. Dr. Ulrich Massing. My project is focused on the preparation of liposomes by Dual Centrifugation.

Zdeněk Kratochvíl

Zdeněk Kratochvíl is currently in the fourth year of his Ph.D. studies at the Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. At the same time, he is the head of the Laboratory of Nanomedicine in the Research group for Molecular Biology and Nanomedicine led by Assoc. Prof. Zbyněk Heger.

Within his research, he investigates the physicochemical and biological properties of pH-responsive nanoparticles. The main subject of his scientific explorations is the development of lipid nanoparticles to deliver therapeutic nucleic acids by means of a virus-like mechanism including their efficient release at the site of action in target cells, yet without any significant side effects, with aiming at the treatment of infectious diseases. To meet these requirements, it is necessary to adjust the appropriate surface modification of lipid nanoparticles, perform their subsequent comprehensive characterization and examine their biological effects. Additionally, during his Ph.D. studies, he completed two internships in the research group of Univ. Prof. Nicole Meisner-Kober at the Faculty of Natural and Life Sciences, Paris Lodron University Salzburg, Austria, where he focused on fluorescence detection of lipid nanoparticles at the single particle level and their interactions with different types of extracellular vesicles.

Besides this, Zdeněk Kratochvíl is the holder of numerous awards and fellowships, including Brno Ph.D. Talent funded by Brno City Municipality, fellowship Aktion Austria-Czech Republic for his research stay in Austria funded by OeAD-GmbH, the Jaroslav Koča Bridge Fund for the support of interdisciplinary research and the Best Presentation Award at the student conference MendelNet 2021 in the Applied Chemistry and Biochemistry and Animal Biology.

RECENT PUBLICATIONS

- Schürz, M., Danmayr, J., Jaritsch, M., Klinglmayr, E, Benirschke, H. M., Matea, C. T., Zimmerebner, P., Rauter, J., Wolf, M., Gomes, F. G., Kratochvil, Z., Heger, Z., Miller, A. D., Heuser, T., Stanojlovic, V., Kiefer, J., Plank, T., Johnson, L., Himly, M., Blöchl, C., Huber, C. G., Hintersteiner, M., Meisner-Kober, N. EVAnalyzer: High content imaging for rigorous characterisation of single extracellular vesicles using standard laboratory equipment and a new open-source ImageJ/Fiji plugin. Journal of Extracellular Vesicles, 2022, vol. 11(12), art no. 12282
- Krausova, K., Charousova, M., Kratochvil, Z., Takacsova, P., Tesarova, B., Sivak, L., Peskova M., Sukupova, M., Zivotska, H., Makovicky, P., Yamashita, I., Okamoto, N., Hynek, D., Haddad, Y., Pekarik, V., Rex, S., Heger, Z. Toward understanding the kinetics of disassembly of ferritins of varying origin and subunit composition. Applied Materials Today, 2022, vol. 28, art. no. 101535

Marcelo Kravicz

PhD

Dr Marcelo Kravicz is a Postdoc Research Scientist specializing in polymer and nanoparticle synthesis and characterization. He holds a PhD in Sciences from the University of Sao Paulo, where he focused on developing amphiphilic cationic polymers for RNA/DNA delivery. His doctoral research involved formulating polyplexes and evaluating their transfection efficiency in HeLa cells, contributing to cutting-edge advancements in targeted Gene Therapy.

With a Master of Sciences degree from the University of Sao Paulo, he conducted research on the preparation and characterization of membranes using silk fibroin and poly(vinyl alcohol). His expertise in this area demonstrated the potential of biomaterials in biomedical applications. Throughout his academic journey, Dr Kravicz gained international research exposure through a DAAD scholarship at the Institute of Chemistry and Biochemistry, Freie Universität Berlin, Germany. Additionally, he completed a short-term internship in Braga, Portugal, where he contributed to synthesizing di and tripeptides for in situ sol-gel applications.

As a Senior Postdoctoral Researcher at the University of Milan - Bicocca, Marcelo plays a role in formulating and characterizing nanoparticles. He is adept at working with various nanoparticle systems, including lipid-based, polymeric, and hybrid, demonstrating his versatility in targeted drug delivery.

Marcelo's expertise extends to assessing cellular viability and conducting studies using diverse cell lines, such as melanoma cells, hC-

MEC, HUVEC, and A549 cells. His research efforts have shed light on the uptake and transfection processes in different cell types, laying the groundwork for future advancements in nanoparticle-based therapies. His skills encompass Dynamic Light Scattering (DLS), Zeta Potential, and Nanoparticle Tracking Analysis (NTA), ensuring comprehensive evaluations of nanoparticle properties.

RECENT PUBLICATIONS

- Cancers 14 (2022), 2978 10.3390/cancers14122978
- International Journal of Molecular Sciences 23 (2021), 102 10.3390/ijms23010102
- Cancers 13 (2021), 4001 10.3390/cancers13164001
- Advanced Drug Delivery Reviews 153 (2020), 109–136 10.1016/j.addr.2020.02.005
- Macromolecular Bioscience 19 (2019), e1900117 10.1002/mabi.201900117

Joshua Krehan

Joshua Krehan completed his Masters and Bachelors degree in chemistry at the Johannes Gutenberg University Mainz. After completing his master thesis at the Max Planck Institute for Polymer Research with a focus on polymeric micelles for drug delivery applications, he joined the group of Prof. Dr. Andreas Walther as a PhD student in March 2021. His current research interests include the development of smart carrier systems with encapsulated pH-effects and pH-modulation systems for cancer immunotherapy. He is part of the collaborative research center 1066.

Adrian Kromer

I was born in 1998 in Stuttgart, Germany. I grew up in Saarland, where I graduated from high school in 2017. I moved to Munich to study Pharmaceutical Sciences at the Ludwig- Maximilians-University. I finished my bachelor's degree in 2020 with a thesis on the targeting of glioblastoma cells via a polymer-based siRNA delivery system. In the following two years, I completed my master's thesis on "Establishment of a novel LC-MS method for the analysis of rocaglate aerosols as pulmonary for drug delivery". Both studies were carried out in the laboratories of Prof. Olivia Merkel. Parallel to my studies, I worked in a biotech company named GNA Biosolutions (Martinsried, Germany) as a member of the regulatory affairs team. In 2022 I started my PhD in Prof. Merkel's lab with the goal to establish and optimize a polymeric RNA delivery system for inhalation-based therapy. Recently, I presented a poster titled "Design of Experiment methodology enables streamlined development of a siRNA based COVID-19 therapy" at the Controlled Release Society local chapter Germany summit in Würzburg, Bavaria.

Revadee Liam-Or

Final year PhD candidate

Revadee Liam-Or graduated from Mahidol University, Thailand, with a Doctor of Pharmacy degree. She worked as a pharmacist for the Government Pharmaceutical Organisation (Thailand) to maintain and improve Quality Standards of pharmaceutical products. She then continued studying for a master's degree in Pharmaceutical Technology at King's College London (KCL). During her master's, she joined Al-Jamal's lab working on exosomes to determine factors controlling their uptake in cells as drug delivery systems. Upon completing her master's, she was awarded the PGR International Scholarship to pursue her PhD studies in Pharmaceutical Science at KCL (success rate 1%). She has been working with exosomes more than 4 years. Her current project involves exploration of mesenchymal stem cell derived exosomes for drug delivery systems and regenerative medicine.

RECENT PUBLICATIONS

- Faruqu FN, Liam-Or R, Zhou S, Nip R, Al-Jamal KT. (2021) Defined serum-free three-dimensional culture of umbilical cord-derived mesenchymal stem cells yields exosomes that promote fibroblast proliferation and migration *in vitro*. FASEB J. 35(1):e21206. doi: 10.1096/fj.202001768RR.
- Xu L, Faruqu FN, Lim YM, Lim KY, Liam-Or R, Walters AA, Lavender P, Fear D, Wells CM, Tzu-Wen Wang J, Al-Jamal KT. (2020) Exosome-mediated RNAi of PAK4 prolongs survival of pancreatic cancer mouse model after loco-regional treatment. Biomaterials. 264:120369. doi: 10.1016/j.biomaterials.2020.120369.
- Xu L, Faruqu FN, Liam-Or R, Abu Abed O, Li D, Venner K, Errington RJ, Summers H, Wang JT, Al-Jamal KT. (2020) Design of experiment (DoE)-driven *in vitro* and *in vivo* uptake studies of exosomes for pancreatic cancer delivery enabled by copper-free click chemistry-based labelling. J Extracell Vesicles. 9(1):1779458. doi: 10.1080/20013078.2020.1779458.

Catia Lopes

Cátia D. F. Lopes graduated in Pathological Anatomy, Cytology and Thanatology from the School of Allied Sciences in Porto, Portugal, in 2010. In 2011 she was awarded with a competitive Ph.D. scholarship from the Fundação para a Ciência e a Tecnologia (FCT) – the Portuguese public agency to support science, technology, and innovation – and, in 2012 she started her Ph.D. works at the Instituto de Engenharia Biomédica (INEB) - University of Porto with the project ''Chitosan-based platform for targeted gene delivery to peripheral nervous system''. During her Ph.D. she developed novel neuron-targeted gene delivery nanovectors that were successful in promoting, *in vitro* and *in vivo*, a fast and efficient nerve regeneration after a peripheral and minimally invasive intramuscular administration. She finished her Ph.D. in Neurosciences in 2017, with the highest mention. Her PhD thesis resulted in 6 original research publications, 2 reviews and 1 editorial perspective.
In 2017 she joined the Neuroengineering and Computational Neuroscience Lab at Institute for Research and Innovation in Health (i3S – University of Porto), as a postdoctoral researcher. During this period, she studied in detail the complex process of neuronal communication, action potentials propagation and activity-dependent mechanisms regulating axonal trafficking – all important players in neurodegeneration and regeneration of the nervous system. This first postdoctoral position lasted until the beginning of 2021

and resulted in 5 original research publications, 3 Master thesis (as main supervisor), and 1 manuscript currently in the process of submission for peer-review. Along with her postdoctoral research activity, she also started her teaching activity at School of Allied Sciences – Polytechnic of Porto as an Invited Adjunct Professor. She was teaching "Human Physiology" and "Neuroscience in Occupational Therapy" to first-year undergraduate students.
Since August 2021, Cátia is working in the Molecular Bionics group at the Institute of Bioengineering of Catalunya (IBEC), Spain. In 2022 she was awarded a Marie Skłodowska-Curie Postdoctoral Fellowship, which has been allowing her to apply molecular engineering to create nanocarriers targeted to the blood-brain barrier (BBB) to mediate therapeutic genes delivery and create new disease-modifying tools for Alzheimer's Disease.
Cátia D. F. Lopes has participated as team member in 9 R&D financed projects. She has collaborated with +88 co-authors and co-organized 4 scientific events. She attended several highly recognized scientific meetings, and her abstracts were selected for 8 oral and +35 poster presentations. She has received 6 awards and/or honors. She has been actively involved in (co)supervision of Ph.D., M.Sc., and B.Sc. students as well as Mentoring programs (as a mentor). She has also contributed for the peer-review process of some scientific journals (e.g., Biomaterial, International Journal of Nanomedicine, Journal of Pain Research, etc), and has been active in different outreach activities.

Sandra López Cerdá

I am Spanish and I was born in Alicante in 1997. I performed my Bachelor's degree in Biotechnology at Universidad Politecnica de Valencia (Spain). During my bachelor's studies, I conducted two different research internships. First, I did an internship in which I learnt some basic techniques for the characterization of nanoparticles. Second, I did my bachelor's thesis in Hospital Clínico of Valencia, where I studied the influence of microRNAs on triple negative breast cancer. In 2019, I graduated from my Bachelor's degree with an average grade of 8.5/10. In the same year, I got a scholarship for the NANOMED for drug delivery Erasmus Mundus Master's degree, which was funding a monthly personal salary for two years of Master studies. During these two years, I had the chance to study at the University of Paris and University of Angers, both in France. I conducted my Master thesis at the University of Copenhagen (Denmark) in the topic of development of lipid-polymer hybrid nanoparticles for mRNA delivery to macrophages for cancer immunotherapy. I graduated from this Master's degree in 2021 with "very good" mention. After that, I obtained a Marie Skłodowska-Curie Doctoral scholarship to perform my PhD as part of the MSCA-ITN P4 FIT Innovative Training Network at the University of Helsinki under the supervision of Porf. Hélder A. Santos. As part of my PhD project, I have been working on the development of lipid-based nanosystems for gene delivery to macrophages and tendon cells in tissue injury conditions. This PhD has resulted into a publication that is now under submission. Besides, I gave an oral presentation in TERMIS 2022 (Krakow, Poland) and I participated in Faculty of Pharmacy Annual research seminar and summer workshops of the MSCA-ITN P4 FIT network.

Vacas Esther Loscertales

My name is Esther Loscertales, and I was born in Zaragoza, Spain. I am 26 years old. I have a degree in Physics and a master in Nuclear Physics. I started my research career three years ago on the development of novel nanocarriers for cancer treatment. Currently I am registered in the Physics Doctorate Program of Universidad Complutense de Madrid. I have been with a research contract at the Universidad Complutense de Madrid for the study of radiosensitization with gold nanoparticles and controlled release by ionizing radiation (X-rays and protons) of chemotherapy drugs using nanocarriers. Funded by the Community of Madrid. My Final Degree Project was titled- ''4D printing of liquid crystal polymers''-. 4D printing differs from 3D printing in that the printed structures are able to change their shape in response to stimuli such as heat or light. The main aim of the TFG was to carry out a comparative study between different 4D printing inks, studying their thermal properties, printability, structure formation, mechanical response and the reproducibility of these mechanical responses. My Master's Thesis was titled-'' Study of dosimetry with gold nanoparticles''. A detailed study was carried out on gold nanoparticles as radiosensitizing agents for protons and photons. The characterization of the dose enhancement in the nanoparticle environment was carried out thanks to the Monte Carlo simulation program , TOPAS-nBio, which allows to simulate the necessary experiments for those studies and provides an advanced understanding of the radiobiological effects at the cellular and subcellular scale. For the data analysis and processing, several programming languages were used, such as Matlab or Fortran. I also have the Advanced Certificate (University of Cambridge) and also the Goethe Certificate- Zertifikat A2. These three years gave me a wide experience in the synthesis of nanoparticles and knowledge of the techniques used for their characterization. Also, experience in acquisition and analysis of data obtained from experimental measurements with different programming languages. (Matlab, C++, Gnuplot, Monte Carlo Simulation (TOPAS), etc.).

Larissa Lubitz

My name is Larissa Lubitz and I am currently a PhD student at ABNOBA GmbH in Germany.
I was born in Wiesbaden (Germany) and raised in Magdeburg (Germany). From 2011 until 2016, I studied pharmacy in Frankfurt Main (Germany) and received my license as a pharmacist (approbation) in 2017. Then I worked for about 3 years in a public pharmacy. In July 2020, I moved to Pforzheim and started working as a PhD student for ABNOBA GmbH as part of the RELIEF project, which is funded by the European Union. I am doing my PhD in cooperation with the Karlsruhe Institute of Technology.
For my PhD, I am working on the optimization of fluorocarbon-in-water nano emulsions and its further processing to asymmetric liposomes, as well as the surface coatings of liposomal drug delivery systems for targeted tumor therapy and hemocompatibility.
I have already reached the fourth year of my PhD and plan to submit the thesis next spring or summer.

RECENT PUBLICATIONS

- A. Mellinger, L. Lubitz, C. Lépinoux-Chambaud, Gero Leneweit, Guillaume Bastiat, Joël Eyer; The use of NFL-TBS.40-63 peptide as a targeting agent to cross the blood-brain barrier and target glioblastoma cells. (Poster), SF Nano Annual Meeting, December

2022, Straßbourg

- M.-A. Jourdain, L. Lubitz, Gero Leneweit, Joël Eyer; Liposomes functionalized by GGGC-NFL-FAM peptide: a promising Drug Delivery System to target Glioblastoma (Poster), SF Nano Annual Meeting, December 2022, Straßbourg
- L. Lubitz, M. Haffner, Gero Leneweit; A small-molecule ligand on a liposomal drug delivery system for active targeting towards the blood-brain barrier and glioblastoma multiforme (Poster), Doktorandentagung des DPhG, March 2023, Bonn
- L. Lubitz, M. Haffner, Gero Leneweit; Investigation of surface modifications on liposomal drug delivery systems for active targeting towards the blood-brain barrier and glioblastoma multiforme (Poster), Transport- und Barriertage, May 2023, Bad Herrenalb
- A. Mellinger, L. Lubitz, C. Gazaille, Gero Leneweit, Guillaume Bastiat, C. Lépinoux-Chambaud, Joël Eyer; The use of liposomes functionalized with the NFL-TBS.40-63 peptide as a targeting agent to cross the *in vitro* blood-brain barrier and target glioblastoma cells. (Publication), International Journal of Pharmaceutics (under revision)

Anshika Maheshwari

I am Anshika Maheshwari, a doctoral student at Karolinska Institute in Stockholm, Sweden. I am working on the synthesis of biocompatible nanoparticles for vaccine delivery application. I was born in India on January 1st, 1996. I have always been a curious and hardworking person since an early age with a deep desire to contribute to society. Throughout my academic journey, I have pursued innovative approaches to understanding healthcare challenges, with a focus on creating tangible solutions that bridge the gap between science and society.

I started my scientific exploration with my bachelors at the prestigious Indian Institute of Technology (IIT), Roorkee. I cleared Indian Institute of Technology-Joint Entrance Exam (IIT-JEE) exam, the most competitive exam of India to join this pioneer technological institute of India. Guided by my vision of becoming a scientist, I chose to specialize in biotechnology. Over the course of four years, I actively sought internships and engaged in diverse projects spanning structural biology, microbiology, nano-biotechnology and bioinformatics. At the end of my 3rd year, I also got the opportunity to do summer internship in Germany at Helmholtz Centre for Infection Research. It was during my bachelor's thesis that I got the opportunity to work in the field of nano-biotechnology in a Drug Discovery lab. The objective of my project was to synthesize various composite antimicrobial agents of 'nano' dimension to combat antimicrobial resistance and use them for food packaging application. My work was appreciated by my department, and I was awarded an Institute medal for the Best Project in Engineering in the field of Biotechnology 2017 by the institute. During my bachelor's degree, I enthusiastically engaged in numerous extracurricular activities, which ultimately led me to receive the prestigious IIT Roorkee Heritage Annual Excellence Award for outstanding academic, co-curricular, and extracurricular achievements.

For my master's, I got accepted as a scholarship holder for Erasmus Mundus Joint Master's degree in Nanomedicine for Drug Delivery (EMJMD NANOMED). This international program allowed me to expand my knowledge and expertise in the field of nanomedicine by studying at University of Patras in Greece, Paris Descartes University and University of Angers in France. Moreover, I got the opportunity to explore the research culture in various labs as well as work and discuss with brilliant minds from all around the world. I carried out an internship in Paris Descartes university where I synthesized long lasting phosphorescence particles for diagnostic application. For my master's thesis, I joined Sotiriou lab (my current lab) at Karolinska institute in Stockholm, Sweden. The aim of my master's thesis was to synthesize phosphorescent nanoparticles for DNA sensing. During my project, I got the opportunity to learn a novel fabrication technique, flame spray pyrolysis, that is gaining traction in the field of biomedicine. The international and collaborative work environment persuaded me to continue in the same lab/group for my doctoral studies.

My PhD research aims to develop novel vaccine strategies using nanoparticles as delivery agents as well as adjuvants, offering promising solutions for combating infectious diseases. I have currently developed biocompatible calcium phosphate nanoparticles that can load antigen on the surface and have immunomodulatory properties according to the preliminary data. Apart from my research work, I am responsible for many instruments in the lab for which I have been continuously providing training to the new users. I have supervised bachelor's as well as a master's student, guiding them to learn various lab techniques as well as soft skills.

Throughout my academic journey, I have consistently demonstrated a strong commitment to research and a deep desire to contribute to the field of healthcare. My passion for science, combined with my relentless drive to solve problems, makes me a dedicated and enthusiastic researcher.

Zhengwei Mao

Dr. Zhengwei Mao is an Associate Professor (2010-2018) and then a Professor (since 2019) in the Department of Polymer Science and Engineering at Zhejiang University. He received a Ph.D. at Zhejiang University in the field of Materials Science and had a postdoc experience at Max Planck Institute of Colloids and interfaces, Germany. Dr. Mao's research is focused on polymeric biomaterials, and seeks to control microstructure of materials for the purpose to manipulating the responses of cells and tissues, with the application for cancer therapy and tissue regeneration. Dr. Mao has published more than 150 papers in peer-review journals such as Nat Nano, Nat Comm, Sci Adv, Adv Mater, J Am Chem Soc, Angew Chem Int Ed, Biomaterials, ACS Nano, Nano Letters and so on. The publications were cited for over 9,500 times and H index is 52. His research has received international attention and "young investigator award" from Chinese Association of Biomaterials. He now serves as one of the editors of Acta Biomaterialia.

RECENT PUBLICATIONS

- Fangfang Cao, Lulu Jin, Yong Gao, Yuan Ding, Hongyang Wen, Zhefeng Qian, Chenyin Zhang, Liangjie Hong, Huang Yang, Jiaojiao Zhang, Zongrui Tong, Weilin Wang*, Xiaoyuan Chen*, Zhengwei Mao*. Artificial Enzymes-Armed Bifidobacterium Longum Probiotics for Alleviating Intestinal Inflammation and Microbiota Dysbiosis. Nature Nanotechnology 2023, https://doi.org/10.1038/s41565-023-01346-x.
- Lulu Jin, Fangfang Cao, Yong Gao, Chenying Zhang, Zhefeng Qian, Jiaojiao Zhang, Zhengwei Mao*. Microenvironment Activated Nanozymes-Armed Bacteriophages Efficiently Combat Bacterial Infection. Advanced Materials, 2023, DOI: 10.1002/adma.202301349.
- Jiaojiao Zhang; Bingqiang Gao; Binglin Ye; Zhongquan Sun; Zhefeng Qian; Lisha Yu; Yanli Bi; Lie Ma; Yuan Ding*; Yang Du*; Weilin Wang*; Zhengwei Mao*. Mitochondrial-targeted Delivery of Polyphenol-Mediated Antioxidases Complexes against Pyroptosis and Inflammatory Diseases. Advanced Materials 2023, 35, 2208571.
- Yuan Ding, Zongrui Tong, Lulu Jin, Binglin Ye, Jiong Zhou*, Zhongquan Sun, Huang Yang, Liangjie Hong, Feihe Huang, Weilin Wang*, and Zhengwei Mao*. A NIR Discrete Metallacycle Constructed from Perylene Bisimide and Tetraphenylethylene Fluorophores for Imaging-Guided Cancer Radiochemotherapy. Advanced Materials 2022, Feb; 34(7): 2106388.
- Ren ZG, Sun SC, Sun RR, Cui GY, Hong LJ, Rao BC, Li A, Yu ZJ*, Kan QC*, Mao ZW*. A Metal-Polyphenol Coordinated Nanomedicine

for Synergistic Cascade Cancer Chemotherapy and Chemodynamic Therapy. Advanced Materials, 2020, 32: 1906024.

Florian Meier

Florian Meier holds a PhD in Analytical Chemistry earned from University of Ulm, Germany in 2013 and joined Postnova Analytics in 2014, where he is now Head of the Department Research & Applications.
Over the years at Postnova, Florian gained vast experience in the application of various Field-Flow Fractionation (FFF) techniques and related detection systems such as for example Multi-Angle Light Scattering, Dynamic Light Scattering or Inductively-Coupled Plasma Mass Spectrometry.
As a passionate researcher in an industrial environment, his research focuses on the characterization of samples in the nano- and micrometer size range (e.g., nanoparticulate drug delivery systems, vesicles, viruses, proteins, engineered nanomaterials, nanoplastics and many more), thereby exploiting and continuously pushing the limits of multi-detector FFF. In this respect, he was and is involved in several collaborative national and international research projects.
Being a designated member of the "Arbeitsausschuss Nanotechnologien" of the German Institute for Standardization (DIN), Florian also enjoys bringing in his FFF-expertise as an appointed expert to the ISO/TC 229 "Nanotechnologies" and the ASTM E56 committee "Nanotechnology".

List of national and international collaboration projects (excerpt)
1. NanoCELL, German BMBF, 2019-2022 (project coordinator)
https://www.nanopartikel.info/en/projects/current-projects/nanocell
2. SubµTrack, German BMBF, 2018-2021
https://bmbf-plastik.de/en/joint-project/submtrack
3. ACEnano, EU Horizon 2020 Programme, 2016-2021
http://www.acenano-project.eu/
4. NanoUmwelt, German BMBF, 2014-2017 (project coordinator)
https://www.nanopartikel.info/en/projects/completed-projects/nanoumwelt
5. SamrtNano, EU Framework 7 Programme, 2012-2016
https://www.linkedin.com/in/smartnano-project-47496763/

RECENT PUBLICATIONS

- J. Parot, D. Mehn, H. Jankevics, N. Markova, M. Carboni, C. Olaisen, A.D. Hoel, M.S. Sigfúsdóttir, F. Meier, R. Drexel, G. Vella, B. McDonagh, T. Hansen, H. Bui, G. Klinkenberg, T. Visnes, S. Gioria, P. Urban-Lopez, A. Prina-Mello, S.E. Borgos, F. Caputo, L. Calzolai, "Quality Attributes of LNP-RNA therapeutics – an incremental characterisation strategy", ACS Nano, 2023, under revision.
- I.K. Ventouri, W. Chang, F. Meier, R. Drexel, G.W. Somsen, P.J. Schoenmakers, B. de Spiegeleer, R. Haselberg and A. Astafanei, "Characterizing Non-covalent Protein Complexes Using Asymmetrical Flow Field-Flow Fractionation On-Line Coupled to Native Mass Spectrometry", Analytical Chemistry, 2023, 95(19), 7487–7494.
- Y. Kohl, M. Müller, R. Drexel, L. Kovar, S. Dähnhardt-Pfeiffer, D. Selzer, S. Wagner, T. Lehr, H. von Briesen and F. Meier, "Influence of Physicochemical Characteristics and Stability of Gold and Silver Nanoparticles on Biological Effects and Translocation across an Intestinal Barrier—A Case Study from In Vitro to In Silico", Nanomaterials, 2021, 11(6), 1358.
- R. Drexel, A. Siupa, P. Carnell-Morris, M. Carboni, J. Sullivan and F. Meier, "Fast and Purification-Free Characterization of Bio-Nanoparticles in Biological Media by Electrical Asymmetrical Flow Field-Flow Fractionation Hyphenated with Multi-Angle Light Scattering and Nanoparticle Tracking Analysis Detection", Molecules, 2020, 25(20), 4703.
- M. Hesler, L. Aengenheister, B. Ellinger, R. Drexel, S. Straskraba, C. Jost, S. Wagner, F. Meier, H. von Briesen, C. Büchel, P. Wick, T. Buerki-Turnherr, Y. Kohl, "Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models *in vitro*", Toxicology in Vitro, 2019, 61, 104610.

Sophie Luise Meiser

DPhD candidate, Pharmacist
Department of Biopharmacy and Pharmaceutical Technology,
Johannes Gutenberg University Mainz
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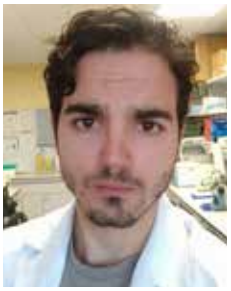
I was born on June 04, 1996 in Worms, Germany. As I was born and raised in Germany, I am native speaker in German. In 2015, I completed school by receiving my Abitur (high school diploma). Building upon this, I enrolled in pharmacy studies at Johannes Gutenberg University (JGU) Mainz in October 2015. I successfully completed my university studies in 2020, earning a degree in pharmaceutical sciences (2. Staatsexamen Pharmazie (state exam)) from JGU. During my studies and as part of my practical training to become a pharmacist, I worked in the Pharmacy of the University Medical Center Mainz, Germany. At the clinical pharmacy I was carrying out a project for improvement of patient safety by creation of training videos for professionals to use the software CATO (Computer Aided Therapy in Oncology). After the second part of the practical training, which I completed in a public pharmacy, I started working as a research assistant in the working group of Prof. Dr. Peter Langguth, Institute for Pharmaceutical and Biomedical Sciences, JGU Mainz, Germany until I obtained the final degree (third state exam in pharmacy).
From July 2021, I started my PhD studies in the same working group under supervision of Prof. Dr. Peter Langguth. My research focusses on formulation development for transcutaneous immunization. In detail, I am working on microneedle systems to deliver nano-scale drugs into the skin. My research is supported by the German Research Foundation grant CRC1066 TP B18, which is a highly interdisciplinary and collaborative research group. In addition to research, I am actively involved in teaching pharmacy students in 7th term, where I am responsible for the study content of semi-solid preparations, transcutaneous therapeutic systems as well as nanoscale systems like nanoparticles and liposomes.

RECENT PUBLICATIONS:

- Pielenhofer J, Meiser SL, Gogoll K, Ciciliani A-M, Klak M, Lang BM, Staubach P, Grabbe S, Schild H, Radsak MP, Spahn-Langguth H, Langguth P. Complaint management of a quality defect in a nanoparticulate Imiquimod formulation in an investigator initiated academic phase I/II clinical trial. Pharm. Ind. 85, Nr. 2 182-186(2023)
- Pielenhofer J, Meiser SL, Gogoll K, Ciciliani A-M, Denny M, Klak M, Lang BM, Staubach P, Grabbe S, Schild H, Radsak MP, Spahn-Langguth H, Langguth P. Quality by Design (QbD) Approach for a Nanoparticulate Imiquimod Formulation as an Investigational Medicinal Product. Pharmaceutics. 2023; 15(2):514. https://doi.org/10.3390/pharmaceutics15020514



Victor Mejias

PhD Student

Víctor Mejías Pérez graduated as a biochemist at the Universidad Autónoma de Madrid in 2018, doing his final degree thesis in the Alzheimer's Disease (AD) Pathogenic Mechanisms group led by Dr Maria Jesús Bullido at the Centro de Biología Molecular Severo-Ochoa (CBMSO). Within that group, he got funding from the EU Youth Guarantee Plan for a two-year project to study the involvement of the Herpes Simplex Virus type 1 as a risk factor in AD, which eventually resulted in a peer-reviewed scientific publication. During this time, in 2020-2021, he also pursued a Master's degree in Translational Medicine Research at Universidad Complutense de Madrid.
Currently, he is a PhD student at the Molecular Bionics group at the Institute for Bioengineering of Catalonia (IBEC) in Barcelona, under the supervision of Dr Iris Batalha and Prof Giuseppe Battaglia. His PhD project consists of the development of targeted nanotherapeutics for the treatment of bacterial infections, specifically tuberculosis.
In September 2022, Víctor was one of the 20 students selected worldwide to attend the EMBO Practical Course on Membrane Protein Expression, Purification, and Characterization (mPEPC2) in Hamburg, Germany. Later, in April 2023, he was also one of the Spanish students invited to participate in the workshop "FS2S: from Science to Society, translating today's research into tomorrow's solutions", held at the University of Cambridge Christ's Church College, UK, and funded by Fundación Rafael del Pino

RECENT PUBLICATIONS:

- Llorente P, Mejías V, Sastre I, Recuero M, Aldudo J, Bullido MJ. (2021) "Matrix metalloproteinase 14 regulates HSV-1 infection in neuroblastoma cells". Antiviral Research, 192, 105116.

Raphael Mietzner

Postdoctoral researcher at the department of Pharmaceutical Technology at the University of Regensburg, Germany.

I was born in 1990 in Villingen-Schwenningen, Germany. In 2011, I started studying pharmaceutical sciences at the University of Tübingen, Germany. I completed my studies in September 2015 with the second state examination. In November 2015, I joined the Pharmaceutical and Processing Development department of Hoffmann-La Roche (Basel, Switzerland) as part of the practical year for pharmacists, which follows the pharmacy studies as part of the training to become a pharmacist. From July 2016, I completed the second half of the practical year as intern in a public pharmcy in Mannheim, Germany and received my license to practice as a pharmacist in June 2017.
In October 2017, I went to Regensburg, Germany to start a position as a doctoral student at the Department of Pharmaceutical Technology at the University of Regensburg. Until April 2021, I successfully worked on nano- and microparticulate drug delivery devices for causative glaucoma therapy and received my PhD (Dr. rer. nat.) in 2021. I have decided to stay in this department as a postdoctoral fellow starting April 2021, working on the prolonged delivery of HIV-1 vaccine nanoparticles from hydrogels.

RECENT PUBLICATIONS:

- Ziegler, C. E.; Graf, M.; Nagaoka, M.; Groner, J.; Mietzner, R.; Breunig, M.; Goepferich, A. M., Molecular Pharmaceutics 2023, 20 (5), 2465-2476. DOI 10.1021/acs.molpharmaceut.2c01060.
- Mietzner R, Pawlak R, Tamm ER, Goepferich A, Fuchshofer R, Breunig M. Angiopoietin-1 Mimetic Nanoparticles for Restoring the Function of Endothelial Cells as Potential Therapeutic for Glaucoma. Pharmaceuticals (Basel). 2021;15(1):18. Published 2021 Dec 24. doi:10.3390/ph15010018
- Mietzner, R.; Kade, C.; Froemel, F.; Pauly, D.; Stamer, W.D.; Ohlmann, A.; Wegener, J.; Fuchshofer, R.; Breunig, M. Fasudil loaded PLGA microspheres as potential intravitreal depot formulation for glaucoma therapy. Pharmaceutics 2020, 12, doi:10.3390/pharmaceutics12080706
- Allmendinger, A.; Butt, Y.L.; Mietzner, R.; Schmidt, F.; Luemkemann, J.; Lema Martinez, C. Controlling Ice Nucleation during Lyophilization: Process Optimization of Vacuum-Induced Surface Freezing. Processes 2020, 8, 1263. https://doi.org/10.3390/pr8101263
- Mietzner, R.; Breunig, M. Causative glaucoma treatment: promising targets and delivery systems. Drug Discov. Today 2019, 24, 1606-1613, doi:10.1016/j.drudis.2019.03.017

Agata Mlynska

Immunologist | Cancer Researcher | Educator

I am a skilled immunologist and cancer researcher with extensive experience in tumor immunology. I hold a PhD in Biomedical Sciences, obtained in 2018 at Vilnius University (Lithuania), where I specialized in investigating the role of systemic and local immunity in tumor development and response to treatment. During my postdoc training in 2020-2022 at Lausanne University Hospital (Switzerland) I employed high throughput transcriptome and histology methods to analyze the combinatorial immunotherapy and targeted therapy studies in melanoma. I am now leading a project group at the National Cancer Institute (Lithuania)
I possess a strong background in scientific research, project management, and academic educating. My expertise spans across immune profiling of tumors, predictive immune biomarker discovery, and the development of dendritic cell vaccines for cancer immunotherapy. Currently, I am also delving into nanomedicine as a means of disrupting and reprogramming the tumor microenvironment.
I am an author of 11 indexed publications and a speaker at international immunology and cancer biology conferences.

RECENT PUBLICATIONS:

- Zilionyte K, Bagdzeviciute U, Mlynska A, Urbstate E, Paberale E, Dobrovolskiene N, Krasko JA, Pasukoniene V. Functional antigen processing and presentation mechanism as a prerequisite factor of response to treatment with dendritic cell vaccines and anti-PD-1 in preclinical murine LLC1 and GL261 tumor models. Cancer Immunology Immunotherapy. Cancer Immunol Immunother. 2022;71:2691-2700.
- Mlynska A, Vaisnore R, Rafanavicius V, Jocys S, Janeiko J, Petrauskyte M, Bijeikis S, Cimmperman P, Intaite B, Zilionyte K, Barakauskiene A, Meskauskas R, Paberale E, Pasukoniene V. A gene signature for immune subtyping of desest, excluded, and inflamed ovarian tumors. American Journal of Reproductive Immunology. 2020;84:e13244.
- Mlynska A, Salciuniene G, Zilionyte K, Garberyte S, Strioga M, Intaite B, Barakauskiene A, Lazzari G, Dobrovolskiene N, Krasko JA, Pasukoniene V. Chemokine profiling in ovarian cancer patients serum reveals candidate biomarkers for recurrence and immune infiltration. Oncology Reports. 2019;41:1238-52.
- Mlynska A, Povilaityte E, Zemleckaite I, Zilionyte K, Strioga M, Krasko J, Dobrovolskiene N, Peng MW, Intaite B, Pasukoniene V. Platinum sensitivity of ovarian cancer cells does not influence their ability to induce M2-type macrophage polarization. Ameri-

can Journal of Reproductive Immunology. 2018;80:e12996.
- Pasukoniene V, Mlynska A, Steponkiene S, Poderys V, Matulionyte M, Karabanovas V, Statkute U, Purviniene R, Krasko JA, Jagminas A, Kurtinaitiene M, Strioga M, Rotomskis R. Accumulation and biological effects of cobalt ferrite nanoparticles in human pancreatic and ovarian cancer cells. Medicina. 2014;50(4):237-44.

Marzieh Mohammadi

Marzieh was born in 1989 in Iran. In 2013, She received her Pharm.D. degree and the thesis title was "Targeted delivery of BCL9L siRNA to colon carcinoma stem cells using aptamer- conjugated carbon nanotubes" which was published in international journal of pharmaceutics. Then, she started her PhD in Pharmaceutical Nanotechnology in Mashhad University of Medical Sciences (MUMS), Iran and received her PhD degree in 2018. Her PhD thesis was entitled "Electrospun nanofibers containing BMP2-encapsulated liposomes to promote osteogenic differentiation" under the supervision of Prof. Mohammad Ramezani and Prof. Mahmoud Reza Jaafari. In 2017, she joined a short term visiting scholar program in Harvard-MIT division of health sciences and technology, USA under the supervision of Prof. Ali Khademhosseini.

Marzieh was ranked among top 10 in National medical students Olympiad, 2011 and she was the top graduated student (first rank) of Mashhad pharmacy school based on overall score (18.37 out of 20), 2013. Additionally, she ranked first among PhD candidates for PhD program in pharmaceutical nanotechnology in Iran, 2013.

In 2019, she started as an assistant professor of pharmaceutics at school of Pharmacy, MUMS, Iran. She teaches physical pharmacy, biopharmaceutics and pharmacokinetics to undergraduate students and her current research interest is focused on the design of drug delivery systems and their application in cancer therapeutics and regenerative medicine. The google scholar link to her publications is https://scholar.google.com/citations?user=EZIFni8AAAAJ&hl=en.

Patricia Mora-Raimundo

I was born in Madrid, Spain, where I performed all my undergraduate studies. I am a pharmacist by training (2011-2016) by the "Universidad Complutense de Madrid". I completed my 4th year of the degree (2014-2015) in "Universite Catholique de Louvain" in Brussels, Belgium, under the program ERAMUS. I was awarded both with the ERAMUS-plus scholarship as well as with the Official School of Pharmacy excellence award for outstanding international ERASMUS students. Once I finished my pharmaceutical degree I joined Prof. Maria Vallet-Regi group as doctoral researcher under an ERC project: "VERDI: polyvalent mEsopoRous nanosystem for bone Diseases". During my PhD studies, I published Nanoparticles to Knockdown Osteoporosis-Related Gene and Promote Osteogenic Marker Expression for Osteoporosis Treatment (Mora-Raimundo, P.; et al ACS Nano 2019, 13 (5), 5451–5464) (Citations: 77/99 (ISI/GS), Impact Factor: 18.027) and Osteoporosis Remission and New Bone Formation with Mesoporous Silica Nanoparticles (Mora-Raimundo, P.; et al Advanced Science. 2021, 2101107) (Citations: 23/33 (ISI/GS), Impact Factor: 17.521). My thesis dissertation titled "Mesoporous Silica Nanoparticles for the Potential Treatment of Osteoporosis", was defended by the end of 2020, receiving the maximum recognition Cum Laude and an International PhD distinction. During my PhD, I went to an internship at the Technion – Israel Institute of Technology, led by Prof. Avi Schroeder for a period of 6 months. During my stay, I took part in several research projects and publications (Kaduri, M.; Sela, M.; Kagan, S.; Poley, M.; Abumanhal-Masarweh, H.; Mora-Raimundo, P.; et al Sciences Advances. 2021, 7 (41):eabj5435) (Citations: 3/8 (ISI/GS), Impact Factor: 14.136). Once I finished my PhD (2021), I was offered to come back to Prof. Avi Schroeder lab as a postdoctoral researcher. I was awarded with the prestigious scholarship Azrieli International Postdoctoral Fellowship (564000 NIS, around 150000 $). Now I focused my research in the gender differences affecting nanotechnology and the design of nano delivery platforms for treating neurodegenerative diseases. Since I arrived, I published one research paper and a review related to how gender affects nanotechnology (Mora-Raimundo, P.‡; Poley, M.‡; et al ACS Nano. 2022, 16 (4) 5246-5257) (Citations: 7/5 (ISI/GS), Impact Factor: 18.027) (Poley, M.; Chen, G.; Sharaf, N.; Avital, A.; Kaduri, M.; Sela, M.; Mora-Raimundo, P.; et al Advanced NanoBiomed Research. 2022, 2200089) (Citations: 1/1 (ISI/GS), Impact Factor: NA). I have been invited by Prof. Maria Jose Alonso to give a seminar at CiMUS - University of Santiago de Compostela, Spain. I was awarded with the 2nd best lecture award at the Young Scientists Meeting of the control release society in Porto, Portugal (Feb 2023), and elected as part of the National Forum of BioInnovation of Teva 2023.

Fotios Mpekris

Post Doctoral Fellow and Lecturer,
Cancer Biophysics Laboratory, University of Cyprus

I earned a BS degree (with an excellent GPA) in Physics from the University of Cyprus in 2012 and the same year, I joined the Department of Mechanical and Manufacturing Engineering at the University of Cyprus and the Cancer Biophysics Laboratory in particular as a PhD student. I defended my PhD thesis in November 2016, and since then I have been a Postdoctoral fellow and now a Senior Research Fellow at the Cancer Biophysics Laboratory and a Part time Lecturer at the University of Cyprus. My research activity focuses on the study of the mechanical forces generated during tumor progression and how taming these forces can improve therapeutic outcomes in many cancer. During my research career, I was trained as a biomedical engineer and mathematical modeler, received experimental training on the biomechanical characterization of solid tumors and other biological tissues and polymers and I was extensively and successfully trained in murine tumor models, small laboratory animal handling and surgical procedures, as well as in anticancer drug treatments. Additionally, I have gained profound knowledge of tumor biology as well as ultrasound imaging techniques in small laboratory animals. Furthermore, I have expertise in bioluminescence and fluorescence imaging on animals and tissue specimens for preclinical research and for the *in vivo* detection of nano-drugs and metastasis in tumors. The implementation of my research has led to the publication of a remarkably large number of articles in high impact journals. I have co-authored 31 scientific articles in peer-reviewed journals (h-index=17, >1,700 citations, Google Scholar).

RECENT PUBLICATIONS:

- 1. Mpekris F., Panagi M., Michael C., Voutouri C., Tsuchiya M., Wagatsuma C., Kinoh H., Osada A., Akinaga S., Yoshida S., Martin J.D., Stylianopoulos T. (2023). Translational nanomedicine potentiates immunotherapy in sarcoma by normalizing the microenvironment, J Controlled Release, 353: 956-964. DOI: 10.1016/j.jconrel.2022.12.016
- Panagi M.*, Mpekris F.*, Chen P.*, Voutouri C., Nakagawa Y., Martin J.D., Hiroi T., Hashimoto H., Philippos D., Pierides C., Samuel

R., Fukushima S., Georgiou P., Papageorgis P., Papaphillipou P.C., Michael C., Koumas L., Costeas P., Ishii G., Kojima M., Kataoka K., Cabral H., Stylianopoulos T. (2022). Polymeric micelles increase tumor microenvironment reprogramming efficiency to potentiate nano-immunotherapy. Nature Communications 13: 7165. DOI: 10.1038/s41467-022—34744-1. * Equal contribution.

- Mpekris F., Panagi M., Voutouri C., Martin J.D., Samuel R., Takahashi S., Gotohda N., Suzuki T., Papageorgis P., Demetriou P., Pierides C., Koumas L., Costeas P., Kojima M., Ishii G., Constantinidou A., Kataoka K., Cabral H., Stylianopoulos T. (2021). Normalizing the microenvironment overcomes vessel compression and resistance to nano-immunotherapy in breast cancer lung metastasis. Advanced Science 8(3): e2001917. DOI:10.1002/advs.202001917
- Mpekris F., Voutouri C., Baish J.W, Duda D.G., Munn L.L., Stylianopoulos T., Jain R.K. (2020). Combining microenvironment normalization strategies to improve cancer immunotherapy. PNAS 117(7):3728-3737. DOI:10.1073/pnas.1919764117
- Mpekris F., Baish J.W., Stylianopoulos T., Jain R.K. (2017). Role of vascular normalization in benefit from metronomic chemotherapy. PNAS, 114(8):1994-1999. DOI:10.1073/pnas.17003401

Murgia Denise

Postdoctoral researcher at the department of Pharmaceutical Technology at the University of Regensburg, Germany.

I was born in 1990 in Villingen-Schwenningen, Germany. In 2011, I started studying pharmaceutical sciences at the University of Tübingen, Germany. I completed my studies in September 2015 with the second state examination. In November 2015, I joined the Pharmaceutical and Processing Development department of Hoffmann-La Roche (Basel, Switzerland) as part of the practical year for pharmacists, which follows the pharmacy studies as part of the training to become a pharmacist. From July 2016, I completed the second half of the practical year as intern in a public pharmcy in Mannheim, Germany and received my license to practice as a pharmacist in June 2017.
In October 2017, I went to Regensburg, Germany to start a position as a doctoral student at the Department of Pharmaceutical Technology at the University of Regensburg. Until April 2021, I successfully worked on nano- and microparticulate drug delivery devices for causative glaucoma therapy and received my PhD (Dr. rer. nat.) in 2021. I have decided to stay in this department as a postdoctoral fellow starting April 2021, working on the prolonged delivery of HIV-1 vaccine nanoparticles from hydrogels.

RECENT PUBLICATIONS:

- Angellotti, G.; Presentato, A.; Murgia, D.; Di Prima, G.; D'Agostino, F.; Scarpaci, A.G.; D'Oca, M.C.; Alduina, R.; Campisi, G.; De Caro, V. Lipid Nanocarriers-Loaded Nanocomposite as a Suitable Platform to Release Antibacterial and Antioxidant Agents for Immediate Dental Implant Placement Restorative Treatment. Pharmaceutics 2021, 13, 2072. I.F. 6.321.
- Mauceri, R.; Murgia, D.; Cicero, O.; Paternò, L.; Fiorillo, L.; De Caro, V.; Campisi, G. Leucocyte- and Platelet-Rich Fibrin Block: Its Use for the Treatment of a Large Cyst with Implant-Based Rehabilitation. Medicina 2021, 57, 180. I.F. 2.430.
- EzEldeen, M.; Toprakhisar, B.; Murgia, D.; Smisdom, N.; Deschaume, O.; Bartic, C.; Van Oosterwyck, H.; Vaz Sousa Pereira, R.; Opdenakker, G.; Lambrichts, I.; Bronckaers, A.; Jacobs, R.; Patterson J. Chlorite oxidized oxyamylose differentially influences the microstructure of fibrin and self-assembling peptide hydrogels as well as dental pulp stem cell behavior. Scientific Reports 2021, 11:5687. I.F. 4.379.
- EzEldeen, M.; Loos, J.; Nejad, Z.M.; Cristaldi, M.; Murgia, D.; Braem, A.; Jacobs, R. 3D-printing assisted fabrication of chitosan scaffolds from different sources and cross-linkers for dental tissue engineering. eCM Journal 2021, 41; 485-501. I.F. 3.942.
- Murgia, D.; Angellotti, G.; Conigliaro, A.; Carfi Pavia, F.; D'Agostino, F.; Contardi, M.; Mauceri, R.; Alessandro, R.; Campisi, G.; De Caro, V. Development of a Multifunctional Bioerodible Nanocomposite Containing Metronidazole and Curcumin to Apply on L-PRF Clot to Promote Tissue Regeneration in Dentistry. Biomedicines 2020, 8, 425. *Corresponding Author. I.F. 6.081.

Mariia Nesterkina

Dr. Mariia Nesterkina, Postdoctoral Researcher at Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) – was born in 1992 in Lviv, Ukraine. I graduated with honor in pharmaceutical chemistry from Odesa Mechnikov National University (Ukraine), and then completed my PhD in bioorganic chemistry at the age of 25 working on synthesis of novel terpenoid derivatives with anticonvulsant, analgesic and anti-inflammatory activity. In 2021, I received the title of Associate Professor in organic and pharmaceutical technologies. The scientific awards include Runner-Up Presentation by a Postdoctoral Researcher (Dublin, Ireland); Excellence Award from European College of Neuropsychopharmacology, ECNP (Lisbon, Portugal); the Galenus Support (Vienna, Austria); Scholarship of Cabinet of Ministers of Ukraine for young scientists (Kyiv, Ukraine).
In 2022, I joined Helmholtz Institute for Pharmaceutical Research Saarland as a postdoctoral researcher working simultaneously at Department for Drug design and Optimization (Prof. Anna Hirsch) and Department of Drug Delivery (Prof. Claus-Michael Lehr).
My primary research interests are focused on development and investigation of stimuli-responsive liquid crystals as nanostructures materials for skin drug delivery. As a fellow of Alexander von Humboldt Foundation, I currently involve additionally in two projects: "Molecular mechanisms of transdermal penetration of novel terpenoid prodrugs with anti-infective activity" and "Structure-based design of trypanothione reductase inhibitors".
Previously I was accepted as a member of American Chemical Society; currently – valid member of Controlled Release Society (CRS) and German-Ukrainian Academic Society.

RECENT PUBLICATIONS:

- Nesterkina M., Vashchenko O., Vashchenko P., Lisetski L., Kravchenko I., Hirsch A. K.H, C.-M. Lehr. Thermoresponsive cholesteric liquid-crystal systems doped with terpenoids as drug delivery systems for skin applications. European Journal of Pharmaceutics and Biopharmaceutics, 2023, in press.
- Nesterkina M., Kravchenko I., Hirsch A. K.H, C.-M. Lehr. Effect of Novel Thermoresponsive Liquid Crystal Systems on Fluidity of Phospholipid Membranes. 7th International Symposium on Phospholipids in Pharmaceutical Research, 12-13 September 2022, Heidelberg, Germany.
- Nesterkina M.; Smola S.; Rusakova N.; Kravchenko I. Terpenoid hydrazones as biomembrane penetration enhancers: FT-IR spectroscopy and fluorescence probe studies. Molecules 2022, 27, 206.

Emmanuel Okwelogu

chudi.ec@gmail.com
www.linkedin.com/in/emmanuel-okwelogu-34250714b
https://nanomedicinelab.com/people/emmanuel-okwelogu/

Emmanuel is a final year PhD student in Nanomedicine with a passion for exploring the unique properties of graphene oxide nanoparticles in biomarker discovery. My research revolves around the identification of proteins on the nanoparticle corona from the cancer cell secretome, leading to advancements in biomarker discovery.

My journey began with a Bachelor of Pharmacy degree at the University of Lagos, where he obtained a distinction for excellence in pharmaceutical sciences. He then worked as an Intern Clinical Pharmacist in the teaching hospital, delivering pharmaceutical care to patients, particularly in the oncology clinic.

Driven by a thirst for knowledge, Emmanuel pursued a Master's degree in Gene Therapy at Imperial College London and performed his master's research at the National Heart and Lung Institute. His project focused on the development of biodegradable polymeric nanoparticles for the delivery of nucleic acids to the Lung. During my master's research, I developed a great passion for nanomedicine and decided to pursue the profound challenge of a PhD in the Nanomedicine Lab at the University of Manchester.

Within the NanoOmics team, my doctoral research focused on the graphene oxide nanoparticle corona, and its ability to aid in biomarker discovery. I explored how the nanoparticle corona could provide an in-depth analysis of the cancer cell secretome.

Through my rigorous experiments and countless hours of analysis, I collaborated with multidisciplinary teams to integrate nanomedicine principles into real-world applications and contributed to many projects. Particularly, I contributed to the publication of the First in-human exposure to graphene oxide, where he analysed proteomic changes in the plasma of volunteers after control exposure to graphene oxide using the nanoparticle corona

My expertise extends beyond biomedical research to clinical pharmacy and data analysis, making me a well-rounded and versatile clinical scientist.

Myrofora Panagi

Dr. Myrofora Panagi is a postdoctoral fellow at the University of Cyprus in the Department of Mechanical and Manufacturing Engineering, working under the advisement of Dr. Triantafyllos Stylianopoulos. Dr. Panagi graduated from the University of Leicester with a bachelor's degree in Biological Sciences in 2011. She continued her studies at the University of Nottingham where she obtained an MSc in Oncology. She performed her doctorate studies at the University of Cyprus exploring the molecular links between stem cell mitosis and inflammation in colorectal cancer using the Drosophila as a model organism.

Her basic science research has focused on the modulation of the tumor microenvironment to enhance effectiveness of nanomedicine and immunotherapy using *in vivo* tumor models and employing range of experimental procedures including flow cytometry, immunohistochemistry, confocal microscopy and standard molecular techniques. Dr. Panagi has co-authored 13 peer reviewed articles (first co-author in 6 of them) in the fields of cancer research, tumor microenvironment, drug delivery and cancer immunotherapy including articles in Nature Communications, Advanced Therapeutics, Advanced Science, Theranostics and Development Journals (h-index=10, >400 citations, Google Scholar). Through competitive selection, she has presented her work in many international conferences including the American Association of Cancer Research (AACR), the European Association of Cancer Research (EACR), the Cold Spring Harbor Laboratory conference, the Summer Biomechanics, Bioengineering, and Biotransport Conference and attended the Annual Critical Issues in Tumor Microenvironment: Angiogenesis, Metastasis and Immunology course.

RECENT PUBLICATIONS:

- Mpekris F.*, Panagi M.*, Michael C., Voutouri C., Tsuchiya M., Wagatsuma C., Kinoh H., Osada A., Akinaga S., Yoshida S., Martin J.D., Stylianopoulos T. (2023). Translational nanomedicine potentiates immunotherapy in sarcoma by normalizing the microenvironment, J Controlled Release, 353: 956-964. * Equal contribution.
- Panagi M.*, Mpekris F.*, Chen P.*, Voutouri C., Nakagawa Y., Martin J.D., Hiroi T., Hashimoto H., Philippos D., Pierides C., Samuel R., Fukushima S., Georgiou P., Papageorgis P., Papaphillipou P.C., Michael C., Koumas L., Costeas P., Ishii G., Kojima M., Kataoka K., Cabral H., Stylianopoulos T. (2022). Polymeric micelles increase tumor microenvironment reprogramming efficiency to potentiate nano-immunotherapy. Nature Communications 13: 7165, * Equal contribution.
- Panagi M., Pilavaki P., Constantinidou A., Stylianopoulos T. (2022). Immunotherapy in soft tissue and bone sarcoma: unraveling the barriers to effectiveness. Theranostics 12(14):6106-6129.
- Mpekris F.*, Panagi M.*, Voutouri C.*, Martin J.D., Samuel R., Takahashi S., Gotohda N., Suzuki T., Papageorgis P., Demetriou P., Pierides C., Koumas L., Costeas P., Kojima M., Ishii G., Constantinidou A., Kataoka K., Cabral H., Stylianopoulos T. (2021). Normalizing the microenvironment overcomes vessel compression and resistance to nano-immunotherapy in breast cancer lung metastasis. Advanced Science 8(3): e2001917. * Equal contribution.
- Panagi M, Voutouri C., Mpekris F., Papageorgis P., Martin M.R., Martin J.D., Demetriou P., Pierides C., Polydorou C., Stylianou A., Louca M., Koumas L., Costeas P., Kataoka K., Cabral H., Stylianopoulos T. (2020). TGF-β inhibition combined with cytotoxic nanomedicine normalizes triple negative breast cancer microenvironment towards anti-tumor immunity. Theranostics 10(4):1910-1922.

Alexandra Paul

Dr Alexandra R. Paul graduated with a first-class chemistry BSc from the University of Kent, UK in 2018. During this time she also worked at Rothamsted Research UK on projects in the Biological Chemistry and Crop Protection Group.

She gained her PhD in chemistry, 'A Novel Strategy for the Synthetic Selection of Enhanced Therapeutic Aptamers' in 2022 from the University of Kent, UK. Her PhD project established a new method for synthesising and screening large drug candidate libraries, accelerating the drug discovery processes. It has presented a proof of concept by providing new aptamer drug candidates or carriers targeting the epidermal growth factor receptor to be used as cancer therapeutics. She was supervised by Dr Christopher J. Serpell, Prof Michelle D. Garrett and collaborated with Centauri Therapeutics. During her PhD she won a Royal Society of Chemistry Research Enablement Grant (£10k) in support of her project. Dr Paul started as a Postdoctoral Fellow in Nanomedicine working with Prof Khuloud T. Al-Jamal at King's College London, in May 2022. In October 2022, she was awarded the CW Maplethorpe Research and Teaching Fellowship to carry on her research 'Dual targeting lipid nanoparticles-assisted nucleic acid delivery for glioblastoma immunotherapy' and teaching on the Pharmacy, MPharm course. Dr Paul wishes to dedicate her future research career to designing and developing new cancer therapeutics and drug delivery processes.

Ling Peng

Research director

I'm currently a research director in the Interdisciplinary Center on Nanoscience in Marseille (CINaM) at the French National Scientific Research Center (CNRS) in France. I undertook my PhD program with Prof. Albert Eschenmoser at Swiss Federal Institute of Technology in Zurich, Switzerland, and my postdoctoral research with Prof. Maurice Goeldner at Louis Pasteur University of Strasbourg in France. I was recruited as a research scientist in CNRS in 1997, promoted as a research director since 2008.

I have been working actively at the interface of chemistry and biology, and in particular, developing functional dendrimers for biomedical applications. Our group has established bio-inspired structurally flexible dendrimers for nucleic acid delivery. Recently, we have inaugurated the concept of self-assembling supramolecular dendrimers for the delivery of anticancer drugs, nucleic acid therapeutics and imaging agents. Our team has been labelled by La Ligue contre Le Cancer in France since 2016, and myself was awarded with the Prize of Dr & Mme Henri Labbé of the French Academy of Sciences in 2017 and the Distinguished Member of French Chemical Society in 2020.

RECENT PUBLICATIONS:

- Jiang Y, Lyu Z, Ralahy B, Liu J, Roussel T, Ding L, Tang J, Kosta A, Giorgio S, Tomasini R, Liang X-J, Dusetti N, Iovanna J, Peng L, "Dendrimer nanosystems for adaptive tumour-assisted drug delivery via extracellular vesicle hijacking", Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2215308120.
- Chen J, Zhu D, Lian B, Shi K, Chen P, Li Y, Lin W, Ding L, Long Q, Wang Y, Laurini E, Lan W, Li Y, Tintaru A, Ju C, Zhang C, Pricl S, Iovanna J, Liu X, Peng L, "Cargo-selective and adaptive delivery of nucleic acids by bola-amphiphilic dendrimers", Proc. Natl. Acad. Sci. U.S.A. 2023, 120, e2220787120.
- Chen J, Zhu D, Liu X, Peng L, "Amphiphilic dendrimer vectors for RNA delivery: state-of-the-art and future perspective", Acc. Mater. Res. 2022, 3, 5, 484-497.
- Chen J, Ellert-Miklaszewska A, Garofalo S, Dey AK, Tang J, Jiang Y, Clément F, Marche PN, Liu X, Kaminska B, Santoni A, Limatola C, Rossi J, Zhou J, Peng L, "Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells", Nat. Protoc. 2021, 16, 327.
- Garrigue P, Tang J, Ding L, Bouhlel A, Tintaru A, Laurini E, Huang Y, Lyu Z, Zhang M, Fernandez S, Balasse L, Lan W, Mas E, Marson D, Weng Y, Liu X, Giorgio S, Iovanna J, Pricl S, Guillet B, Peng L, "Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors", Proc. Natl. Acad. Sci. USA, 2018, 115, 11454

Yannick Pilger

My name is Yannick Alexander Pilger and I was born and raised in the picturesque town of Bacharach near Mainz, Germany. My journey in science began at Stefan-George-Gymnasium Bingen, where my passion for life sciences ignited, when I chose chemistry and biology as two of my majors, leading me to pursue a higher education in the field of chemistry.

I commenced my academic pursuit by enrolling at Johannes Gutenberg-Universität in Mainz, a city close to my family, and a university that offered a specialized program in biomedical chemistry. Throughout my bachelor studies, I became increasingly captivated by macromolecular chemistry and its potential applications in medicine.

The culmination of my passion for macromolecular chemistry led me to conduct my bachelor's thesis in the workgroup of Prof. Dr. Holger Frey on ABA triblock copolymers. During that time, I also took a course on medically relevant biopolymers, which sparked my interest in targeted drug delivery. As my academic journey progressed in Mainz during my master studies, I elected further courses on biopolymers and polymers relevant for medicinal purposes, broadening my understanding of the field. During my master's program, I had the invaluable opportunity to work as a research intern in the esteemed group of Prof. Dr. Lutz Nuhn, who focuses on nano immunotherapy. This exposure kindled my fascination with nanomedicine, prompting me to pursue my master's thesis in the same group, delving deeper into this captivating subject.

To be able to continue working on those fascinating subjects I decided to join Prof. Nuhns' group in Würzburg at the Julius-Maximilians-Universität as a Ph.D. student. My research has revolved around macromolecular chemistry and targeted drug delivery, with a specific focus on nano immunotherapy for cancer treatment. This promising field holds tremendous potential for developing novel therapeutic strategies in cancer therapy, motivating me to contribute to its advancement.

I already had the privilege of presenting my research at a conference organized by the Controlled Release Society in Würzburg, where I shared my findings and engaged in insightful discussions with fellow researchers in the field of nanomedicine.

I look forward to sharing my insights, learning from esteemed researchers, and fostering collaborations to further elevate the field of clinical nanomedicine.

Corinne Portioli

PhD

I received my BSc in Biotechnology (2007) and MSc in Medical and Pharmaceutical Biotechnology (2009) from the University of Modena and Reggio Emilia, Italy. I then obtained a PhD in Neuroscience (2015) at the University of Verona, Italy (Bentivoglio Lab). I was entitled also Doctor Europaeus due to the Visiting PhD student period spent at the University of Manchester, UK (Kostarelos Lab). After 2 years, as a high school teacher and postdoctoral fellow at the University of Verona, I joined Italian Institute of Technology (IIT) (Cancedda & De Vivo Lab) in 2017 before moving to Baylor College of Medicine (BCM), US (Zhou Lab) to work on projects aimed at finding new cures for neurological diseases using a multidisciplinary approach based on structural biology, cell biology and drug discovery. In 2019 I was selected for a MSCA Individual Fellowship (GF), elucidating the structure-function relationship of NKCC1 co-transporter, a promising drug target for neurodevelopmental disorders. I spent the 1st year of outgoing phase at BCM and the 2nd year of incoming phase working at IIT. Since 2021, I am a MSCA COFUND Fellow (MINDED program) with a project that aims at the characterization of novel drug delivery nanosystems in neurodevelopmental disorders. My research field focuses on the use of an interdisciplinary approach to tackle the need of novel therapies. To complete my scientific profile, I also received the Prince2 certification in Project Management, which provided me key inputs to apply a process-based approach to manage on a tailored, and scalable method, all types of projects. I grew and enriched my experience in project management, redaction of business plans and patenting (Innovation & entrepreneurship trainings), also thanks to courses attended in Leading Project Teams, Cross-cultural Communication, and developing and Communicating Vision and Strategy. Since 2022, I have been selected for 2-year mandate as a member of the Marie Curie Alumni Association (MCAA) board, as ordinary member and secretary of

the Executive committee, with the aim of contributing to shaping science policy in EU, providing career development opportunities, and supporting the research community, chapters and working groups in integrating the association strategy.
I developed excellent organizational and planning skills: the experiences in academic and high school diverse environments, throughout tutoring, mentoring & teaching allowed me to develop didactic-educational skills, both from theoretical and practical point of view. The excellent written and oral communication skills acquired so far regard data presentation in internal and external meetings and conferences, and redaction of reports, publications, grant applications, communication to lay and scientific audience. I have experience in organizing events and conferences: I was part of the organization committee of the MCAA Annual Conference and General Assembly 03/2023, with ~300 participants. I am able to build strong collaborations, with the attitude to work within interdisciplinary teams, goal oriented, and with quick adaptation to new challenges. The work experiences in UK and US allowed developing confidence in managing daily situations and activities out of the comfort zone and a personal growing, in terms of scientific, cultural and interdisciplinary approaches.
So far, I contributed to 10 articles (H-index 9, Cit. 204), including 5 first author articles, 9 original experimental articles and 1 review article, in international peer-reviewed journals and to 26 conference abstracts in different scientific fields (computational chemistry and drug discovery, neuroscience, structural biology, nanomedicine), presenting data by means of oral talks (12 conferences) and poster presentations (14 conferences).
Among the scientific publications, I highlight 5 of them: 1. Shen et al., Extracellular domain of PepT1 interacts with TM1 to facilitate substrate transport. Structure. Jul 7;30(7):1035-1041.e3, 2022. 2. Portioli et al., Cation-coupled chloride cotransporters: chemical insights and disease implications. Trends in Chemistry 3, 832-849, 2021; 3. Portioli et al, Intracerebral injection of graphene oxide nanosheets mitigates microglial activation without inducing acute neurotoxicity: a pilot comparison to other nanomaterials. Small, Nov 10:e2004029, 2020; 4. Portioli et al, Novel functionalization strategies of polymeric nanoparticles as carriers for brain medications. J Biomed Mater Res Part A 105A, 847–858, 2017; 5. Portioli et al, Citrate-stabilized lanthanide-doped nanoparticles: brain penetration and interaction with immune cells and neurons. Nanomedicine (Lond) 11, 3039-3051, 2016.

Pottanam Chali

Postdoctoral researcher
Max Planck Institute for Polymer Research,
Mainz, Germany

Biography: Sharafudheen Pottanam Chali graduated (BS-MS) with distinction in 2018 from the Indian Institute of Science Education and Research (IISER) Pune, India and obtained his Ph.D. degree in Natural Science from the University of Muenster, Germany, in August 2021, within the CiM-IMPRS graduate program (Cells in Motion Interfaculty Centre-International Max Planck Research School). During his Ph.D. he worked on biodegradable supramolecular polymer nanocarriers for intracellular delivery applications under the guidance of Prof. Bart Jan Ravoo. In September 2021, he joined the Department of Physical Chemistry of Polymers at the Max Planck Institute for Polymer Research (MPI-P) as a postdoctoral researcher, working with Prof. Katharina Landfester. His research interest ranges from the design and synthesis of nanocapsules via miniemulsification, encapsulation of hydrophilic and hydrophobic therapeutic components, *in vivo* applications and nanocapsules as synthetic organelles.

RECENT PUBLICATIONS:

- Pottanam Chali S, Ravoo BJ, Polymer Nanocontainers for Intracellular Delivery. Angewandte Chemie International Edition. 2020, 59, 2962-2972. doi: 10.1002/anie.201907484.
- Pottanam Chali S, Ravoo BJ, Adamantane-Terminated Polypeptides:Synthesis by N-Carboxyanhydride Polymerization and Template-Based Self-Assembly of Responsive Nanocontainers via Host–Guest Complexation with β-Cyclodextrin. Macromolecular Rapid Communications. 2020, 18, 2000049. doi: 10.1002/marc.202000049
- Kudruk S,+ Pottanam Chali S,+ Matos A L L, Bourque C, Dunker C, Gatsogiannis C, Ravoo BJ,* Gerke V,* Biodegradable and Dual-Responsive Polypeptide-Shelled Cyclodextrin-Containers for Intracellular Delivery of Membrane-Impermeable Cargo. Advanced Science 2021, 2100694. doi:10.1002/advs.202100694 (+equal contribution)
- Pottanam Chali S, Hüwel S, Rentmeister A,* Ravoo BJ,* Self-Assembled Cationic Polypeptide Supramolecular Nanogels for Intracellular DNA Delivery. Chemistry–A European Journal 2021. doi: 10.1002/chem.202101924
- Belenguer-Sapiña C, Pellicer-Castell E., Pottanam Chali S, Ravoo BJ, Amorós P, Simó-Alfonso EF, Mauri-Aucejo AR,* Host-guest Interactions for Extracting Antibiotics with a γ-Cyclodextrin Poly(glycidyl-co-ethylene dimethacrylate) Hybrid Sorbent. Talanta 2021, 232, 122478. doi: 10.1016/j.talanta.2021.122478
- Pottanam Chali S, Azhdari S, Galstyan A, Gröschel A H, Ravoo BJ,* Biodegradable Supramolecular Micelles via Host-Guest Interaction of Cyclodextrin Terminated Polypeptides and Adamantane Terminated Polycaprolactones. Chemical communications 2021. Doi: 10.1039/D1CC03372G

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Liên Sabrina Reichel was born in Bac Ninh (Vietnam) in 1992. She studied pharmacy at Friedrich-Schiller-University Jena in Germany. During her study, she was an exchange student in the lab of Prof. Chun-Ching Lin at Kaohsiung Medical University in Taiwan. Prof. Lin's work focused on anti-liver cancer, anti-virus activity, and the mechanism of natural medicine. Furthermore, she was a member of the research group of Dr. Shakila Rizwan at the University of Otago in New Zealand, where she worked on the preservation methods to study lipid nanoparticles uptake into human cerebral microvascular endothelial cells (hCMEC/D3) for electron microscopy. After her graduation in 2017, she worked in community pharmacy until joined the research group of Prof. Ulrich S. Schubert, in the subgroup of Dr. Traeger at Friedrich-Schiller-University Jena as a PhD student. Her research focuses on biological investigations of different polymer-based nanomaterials for gene delivery *in vitro*.

Susanne Resch

Mag.a pharm. Susanne Resch, MSc. Scientific staff at BNN since 2015, expert in nanosafety and Safe-by-Design; involved in several national and international research projects, experienced in qualitative and quantitative safety assessments at industry partners in the frame of H2020 projects; nominated expert in ISO TC229 and CEN TC352; general coordinator of NanoMedicine-Austria.

Nadia Rouatbi

Final year PhD Student @Institute of Pharmaceutical Science, School of Cancer & Pharmaceutical Sciences, King's College London

Nadia obtained her BSc in Biomedical Engeineering from the University of Genoa, Italy. She undertook a two-year MSc in Bioengineering (Professors Laura Pastorino and Orietta Monticelli). Her MSc project focussed on the development of multicompartment hydrogels for the local delivery of chemotherapeutic drugs for the treatment of Glioblastoma. She spent 9 months as part of an ERASMUS+ internship at Professor Al Jamal's group, Institute of Pharmaceutical Science, King's College London during her MSc degree. Nadia's PhD project focuses on the development of lipid-based nanoparticles for *in vivo* gene-editing for brain cancer therapy.

RECENT PUBLICATIONS:

- Rouatbi, N., McGlynn, T., & Al-Jamal, K. T. (2022). Pre-clinical non-viral vectors exploited for *in vivo* CRISPR/Cas9 gene editing: An overview. Biomaterials Science. doi.org/10.1039/D1BM01452H
- Walters AA*, Santacana-Font G, Li J, Routabi N, Qin Y, Claes N, Bals S, Wang JT and Al-Jamal KT*. (2021) Nanoparticle Mediated in Situ Molecular Reprogramming of Immune Checkpoint Interactions for Cancer Immunotherapy. ACS Nano. doi.org/10.1021/acsnano.1c04456
- Helal DO, Rouatbi N, Han S, Tzu-Wen Wang J, Walters AA, Abdel-Mottaleb MMA, Kamel AO, Geneidi AS, Awad GAS, Al-Jamal KT*. (2021). A Natural Protein Based Platform for the Delivery of Temozolomide Acid to Glioma Cells. Eur J Pharm Biopharm. S0939-6411(21)00262-9. doi: 10.1016/j.ejpb.2021.10.007.
- Abdel-Bar HM, Walters AA, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, Osman R, Wang J, Al Jamal KT*. (2021) An "Eat me" Combinatory Nano-formulation for Systemic Immunotherapy of Solid Tumours. Theranostics. doi:10.7150/thno.56936; doi:10.7150/thno.56936.
- Rouatbi N, Lim YM, Grant V, Pollard S, Wang J and Al-Jamal KT*. (2019) CRISPR/Cas9 gene editing of brain cancer stem cells using lipid-based nano-delivery. Neuro-Oncology, Oxford University Press.21 (supplement 4): iv3-iv3.

Anna Ruppl

Anna Ruppl is a PhD student in the research group of PD Dr. Allmendinger at the Department of Pharmaceutical Technology and Biopharmacy at the University of Freiburg. Anna is working on formulation and process development of lyophilized mRNA lipid nanoparticles.

Previously, she received her license to practice as a pharmacist. Anna did her practical education at Boehringer Ingelheim, the pharmacy of the university hospital at Johannes-Gutenberg University Mainz, and a public pharmacy.

Lucia Sanjurjo Bouza

My early career was focused on the study of the basic mechanism of immunoregulation in the context of infectious and inflammatory diseases. During my Ph.D. Thesis in Biomedicine (University of Barcelona, UB, Spain), and thanks to my mentor, Dr. MR Sarrias, I received extensive scientific and technical training in Immunology. I was able to work on interdisciplinary projects in collaboration with academic groups, clinicians, and the private sector. Reflected in 8 high-impact publications. These studies were fundamental to proposing novel immunoregulatory players relevant to the tumor microenvironment.

As a postdoctoral researcher (Ph.D. graduation December 2014), I centered my studies on the tumor immunoregulation field. I had the opportunity to unravel the basic mechanisms of tumor immune escape (PMID: 29593730, 29465313), proposing the immune protein CD5L as a novel target in liver cancer. In parallel to our scientific production and keeping in mind our final goal, to contribute to better cancer patient health, we were also actively working on the transference of our results. We generated and protected a set of monoclonal antibodies against CD5L (anti-CD5L and uses thereof. WO2019086480A1). The therapeutic potential of the selected blocking antibody was assayed in syngeneic mouse models of liver and lung cancer showing promising results (PMID: 37054630). I envision direct applications of our products in cancer therapeutic settings, but also as non-invasive clinical diagnostic tools that may contribute to society in the future.

To broaden my postdoctoral training in the field of the tumor microenvironment and immunotherapy, I conducted international stays in leading Cancer Centers in The Netherlands (UMC-CCA, Amsterdam, 2018- 2019) and Portugal (i3S-IPATIMUP, Porto, 2019-2020). I receive advanced training in tumor microenvironment and in cancer glycobiology. During this stages, we described a novel immunomodulatory mechanism in which specific galectin-chemokine interactions tune the immune response in the tumoral microenvironment (PMID: 34931005, 36139125). We believe that this discovery is highly relevant for the design of more potent glyco-immunotherapeutics. My postdoctoral training abroad highly contributed to my proficiency as an independent researcher, expanded my European network, and also made me notice the potential of nanotechnology to solve the limitations of traditional immunotherapy.

With the strong belief that I could combine my background in (tumor)-immunology with new knowledge in nanotechnology and drug delivery, in June 2021 I joined Prof. MJ Alonso's group (IDIS, USC, Spain) a recognized world leader and pioneer in nanotechnology and nanomedicine. I am working as a postdoctoral researcher in the marc of the 2^2-INTRATARGET project (EURONANOMED III), focused on the development and evaluation of nanocarriers loaded with biological drugs (monoclonal antibodies and nanobodies) for cancer immunotherapy. Since my incorporation, I established in the

laboratory *in vitro* assays in cancer and immune cells to assay the toxicity, internalization, intracellular trafficking, and target engagement of nanocarriers loaded with immunotherapeutics. In parallel, I am receiving advanced training in nanocarrier formulation and characterization from a highly-experienced multidisciplinary team. Collectively, in the last ten years, our results lead to 17 PubMed-indexed publications (7 as first author), which counts with 590 citations h index 11 (source Scopus, June 2023). I had the opportunity to present our results at 22 conferences, where the capacity to effectively communicate our scientific results was highlighted by 2 awards for best oral and poster communications. I am genuinely interested in the supervision and mentoring of young fellows, I am a proud mentor of one PhD, two MSc, and two BSc.
As reflected by my multidisciplinary scientific path, I think that the combination of knowledge of general immunology (Ph.D.), cancer research (postdoctoral stages), and nanotechnology (actual position), could be relevant to the development of novel nanomedicines which might represent a breakthrough in the treatment of different pathologies. I am willing to establish myself in the raising field of cancer nano-immunotherapy. The interaction with leaders in the field and the possibility of presenting our results at the 14th European and Global Summit for Clinical Nanomedicine will be a great opportunity, for me and my research. Moreover, the complete program would help me to deepen my knowledge in the state of the art of clinical nanomedicine.

Maximilian Schaaf

PhD student

I obtained my bachelor's and master's degree in biochemistry at the University of Bayreuth (Germany). Parallel to my master, I also took part in the Biological Physics program within the Elite Network of Bavaria and did a seven month research internship at the GENYO institute in Granada (Spain). After finishing my masters in 2021, I spent eight months as a research assistant at the Technical University of Denmark. In May 2022, I joined the Landfester department at the Max Planck Institute for Polymer Research in Mainz (Germany) as a PhD student. Since then, I am working on the surface functionalization and drug loading of liposomes for biomedical applications.

Johanna K. Scheper

My name is Johanna K. Scheper. I got a strong educational and scientific background through the university degrees of Biology and Biochemistry (by the Universitat Autonoma de Barcelona (Spain) (2001), and later strengthened during my doctoral studies (under the supervision of Dr. Timothy Thomson Okatsu, at the IQAC-CSIC in Barcelona (Spain)) (2007) and a two-year postdoctoral experience at the CRBM-CNRS (France) (2007-2009). During these years I got a solid and deep understanding of all the concepts and principles related to the human health and the design of novel therapeutics and biosensors. After those years, in 2009 I returned to Spain recruited by the CIBER-BBN (Centro de Investigacion Biomedica en Red) as Project Management of the Nanomedicine Area of the centre. Thus, my professional career was at that time deepening even more into the field of the application of nanotechnology in the biomedical field, coordinating and participating in more than 20 R&D projects developing new (nano)materials and (nano)devices to create innovative treatments and/or diagnostic/medical devices, applied to different type of diseases. These projects were dealing with prototypes and/or technologies already at high TRLs and therefore the needs and challenges were even more complex and different than to what I was used to (i.e., licensing out of patents, translational issues, etc). I decided in 2010 to do a two-year Executive Master in Business Innovation. This ensured that my scientific knowledge on development of established and newly emerging (nano)materials, was complemented with other type of knowledge that provided me a deep understanding not only of the behaviour and physic-chemical properties of these advanced materials but also how to approach the challenges they face in their way to market.
This helped me to be of support in my daily work i.e., support other researchers and SMEs to push to higher TRLs their innovations through guidance on i) sector-specific regulations, ii) incorporation of safety and sustainability measures into their materials/products/processes, at an early-stage if possible, and iii) through the creation of new and circular business models. This helps to provide innovative solutions to the challenges that the European (and worldwide) society is facing, at the same time than securing no harm or negative impact on nature.
In August 2019 I moved to Graz (Austria) recruited by the BioNanoNet Forschungsgesellschaft mbH (BNN) as the head of Innovation and Scientific Support of the company. Since then my professional background has been also improved, opening to a broader spectrum of sectors (not only nanomedicine) where I am currently active. At BNN we currently participate in 20 EU-funded projects at some others funded at the national level where I actively participate with different roles, going from Innovation and Exploitation issues, implementing SSbD efficiently in real case studies, thus contributing to a better future by creating more sustainable products/processes/services, stakeholder engagement and link to regulatory and standardisation bodies and Scientific Communication.
Finally, during all these years I have published several scientific articles (https://orcid.org/0000-0003-2410-2936), as well as scientific and/or specific-field related roadmaps (e.g., nanofabrication) that can be found either through BNN's website and/or have been also shared with the community through Zenodo. I'm also main inventor of an International Patent (PCT/ES2007/000120 titled: "Compound with inhibitory effect on the interactions Ubc13-Uev, pharmaceutical compositions that include it and its therapeutics applications". Licensed-out to a pharma company) and since September 2009 I'm the CEO of a biotech company named GraceBio S.L with headquarters in Barcelona.

Julian Schmidt

I was born in 1996 in Bonn, Germany, and grew up with a strong curiosity for science, especially chemistry. After finishing high school in Oppenheim, Rhineland Palatinate, Germany, I decided to try something different and spent three months working on a farm in Norway. It was a unique experience that taught me to appreciate diverse cultures and lifestyles.
I pursued my passion for science by studying biomedical chemistry at Johannes Gutenberg Uni-versity Mainz. I wrote my thesis for my bachelor's degree at the Max Planck Institute for Poly-mer Research Mainz (MPI-P), focusing on nanoparticles for cancer treatment.
Fueled by my love for learning, I continued with a master's degree in biomedical chemistry in the group of Prof. Dr. Holger Frey. My master's thesis centered on a non-immunogenic alterna-tive to polyethylene glycol for medical applications.
Now, I'm in the exciting phase of my Ph.D. in the group of Prof. Frey,

where I combine macro-molecular chemistry with immunology. My research is concerned with the immunogenetics of polyethylene glycol and the biocompatibility of the new “rPEGs” (randomPEG). In a fruitful co-operation with the group of Prof. Dr. Grabbe and PD. Dr. Bros at JGU Medical Center, I am cur-rently conducting studies to understand the interactions of rPEGs with the human immune sys-tem. My investigations are also focused on analyzing the po-tential medical applications of rPEGs to enhance drug delivery and therapies. I am particularly thrilled by the idea that my research might directly impact clinical applications in the near future.
During my academic journey, I worked as a mentor for undergradu-ate students. Additionally, I had the opportunity to intern at the Fraunhofer Institute for Microengineering and Microsys-tems IMM, where I researched nanomaterials for cancer treatment.

Paul Schneider

PhD student

After successfully completing my bach-elor’s degree in biomedical chemistry (Jo-hannes Gutenberg University, 2020), I pro-ceeded to the corresponding consecutive master’s program. Here, my research focus changed from the synthesis of therapeuti-cally relevant polymers to their application in clinical research. Thus, I was able to successfully complete the master’s degree in biomedical chemistry in 2022. Since November 2022, I am a PhD candidate in the research group of Dr. Dr. Leonard Kaps, which is part of the SFB 1066 (B17). The main topic of my project, Hepatocellular Carcinoma, is mainly divided into three re-search fields: At first, a new clinically relevant murine tumor model will be developed. In this model, targets already known in the lit-erature will be validated so that they can be specifically knocked down with a combination of siRNA and nanoparticular transport vehicles. The target cells for siRNA therapy will be tumor-associat-ed fibroblasts (CAF).

Kathrin Schorr

I studied pharmaceutical sciences at the Julius-Maximilians-University Würzburg (Germany) with support of German Aca-demic Scholarship Foundation from 2015-2019. In 2021, after postgraduate educa-tion as a pharmacist at community and hospital pharmacy, followed by a short research stay at the Pharmaceutical Tech-nology and Biopharmacy group of Prof. Dagmar Fischer (Erlangen), I joint the group of Prof. Achim Goep-ferich, Chair of the Department of Pharmaceutical Technology at the University of Regensburg as a Ph.D. student. My thesis deals with the characterization of the surface interaction of polymeric core-shell nanoparticles with their target cells. A particular focus is on the quantification of these interactions. My further research interests are in the field of biodistribution and the pharmacokinet-ics of nanoparticles.

Dominik Schulz

Johannes Gutenberg-University Mainza

After finishing my Abitur at the Tile-mannschule in Germany – focused on natural sciences and math – I moved on to Johannes Gutenberg-University Mainz where I began academic studies in the field of chemistry.
I started my diploma thesis in March of 2020 in the research group of Prof. Dr. Holger Frey at the Depart-ment of Chemistry at the JGU Mainz, investigating novel initiators for the anionic ring opening polymerization of epoxides.
After successful conclusion of my diploma in chemistry in January of 2021, I joined the group of Prof. Frey as a staff member on an interim basis taking over duties and responsibilities in the supervi-sion of student lab courses and continuing research in the field of polymerization.
Ultimately, I joined the PhD program of the JGU Mainz and became a PhD student at the Frey group in July of 2021 which also allowed me to become a member of the SFB 1066 – an interdisciplinary promotion program for research in nano dimensional polymer therapeutics. Starting then, my research was focused on the inves-tigation of the synthesis and properties of novel polyethers for the purpose of biomedical applications.
During the past two years as a PhD student in addition to my re-search I was tasked with further supervision of lab courses and bachelor students and gained experience in poster presentations on several international conferences.

Jenny Schunke

M. Sc.

After successfully completing my bach-elor’s degree in Molecular Biology (2017), I started to study Biomedical Sciences. Dur-ing my master thesis (2019), I focused on the investigation of treatment resistances in penile carcinoma. Since October 2019, I am a Doctoral candidate of the AG Mailän-der at the University Medical Center in Mainz. My PhD project is settled in the field of nanoparticle-based immunotherapies. Spe-cifically, I am targeting dendritic cells (professional antigen pre-senting cells) with adjuvant-loaded protein-based nanocapsules for antigen-specific activation of T cells to generate an effective antitu-mor response in melanoma mouse models.

Armin Azadkhah Shalmani

Armin Azadkhah Shalmani is a PhD can-didate at the Institute for Experimental Molecular Imaging (ExMI), RWTH Aachen University, Germany. He received his PharmD from Tehran University of Medical Sciences (TUMS), Iran in 2018. His PharmD thesis focused on the therapeutic effect of monomethyl fumarate in sepsis. He start-ed his PhD in 2020 under the supervision of Prof. Twan Lammers. His projects mainly revolve around π electron-stabilized polymeric micelles. These include optimizing polymer synthesis via conven-tional free radical as well as RAFT polymerization, its large-scale production and preparation of formulations (co)loaded with differ-ent small molecules and ion pair complexes for combating highly fibrotic cancers.

RECENT PUBLICATIONS:

- Shalmani AA, Ghahremani MH, Jeivad F, Shadboorestan A, Hassanzadeh G, Beh-Pajooh A, Ganbari-Erdi M, Kasirzadeh S, Mojtahedzadeh M, Sabzevari O. Monomethyl fumarate alleviates sepsis-induced hepatic dysfunction by regulating TLR-4/NF-κB signalling pathway. Life sciences. 2018.
- Shalmani AA, Sarihi P, Raoufi M. Nanoparticles and biological environment interactions. Nanomaterials for Advanced Biological Applications. 2019.
- Shalmani AA, Ahmed Z, Sheybanifard M, Wang A, Weiler M, Buhl EM, Klinkenberg G, Schmid R, Hennink W, Kiessling F, Metselaar JM. Effect of Radical Polymerization Method on Pharmaceutical Properties of Π Electron-Stabilized HPMA-Based Polymeric Micelles. Biomacromolecules. 2023.

Isabelle Florence Silvestre

PhD student

Since September 2021 I am a PhD student in the group of Professor Tobias Bopp at the Institute of Immunology at the University Medical Centre Mainz, working on nanoscale glycopeptide vaccines in melanoma treatment, a SFB1066 project in the field of cancer immunology.
Prior to my PhD, I studied biomedical chemistry at the Johannes Gutenberg University in Mainz, Germany, where I completed my bachelor's thesis in 2019 on polymerosome-based DNA drug targeting in the group of Professor Rudolf Zentel and my master's thesis in 2021 on the establishment of a B16 cell-based preclinical melanoma model in the group of Professor Pol Besenius and Professor Edgar Schmitt. In the meantime, I have gained some experience in the global pharmaceutical company Roche Diagnostics Mannheim by working in R&D and Quality Control. Outside of science, I enjoy music and sport very much, singing in a gospel choir and playing tennis. Having grown up with both French and German nationality, I appreciate every multicultural experience when travelling.

Kai Speth

PhD student

Kai Speth, was born on 27th of June 1992 in Hadamar, a small town in Germany.
By training I am a (molecular) biologist, since i received both my Bachelor of Science in Molecular Biology and my Masters Degree in Biology in Mainz at the Johannes-Gutenberg University (JGU).
After that, I have had a short period of employment in industry. I worked for 2 years and 2 month at Merck KGaA in Darmstadt in the area of customer service and quality management.
In the longterm, I saw myself rather in research and wanted to complete my scientifc training with a PhD degree finally. Luckily I received in summer 2022 the opportunity to join the Department of Physical Chemistry of Polymers lead by Prof. Katharina Landfester at the Max-Planck Institute for Polymer Research in Mainz. Herein, i am working as a PhD student in the group of Prof. Volker Mailänder and study nanotechnology-biology (nano-bio) interactions, mostly on a cellular level. In particular, my research focuses on the intracellular trafficking and exocytosis of nanocarrier after their internalization in various biomedical relevant cell models. A special part is laying on the role of the protein corona, the layer of proteins that can adsorb upon the contact of a nanocarrier with bio-fluids, and its impact on the trafficking and exocytosis routes.

Rene Stein

René Stein studied Nanotechnology at the Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany. With the focus of working on the biomedical application of nanotechnology he successfully completed his bachelor's thesis at the Institute of Biomaterials led by Prof. Aldo R. Boccaccini in 2017 as well as his master's thesis in 2020 at the Section of Oncology and Nanomedicine (SEON) led by Prof. Christoph Alexiou at the Universitätsklinikum Erlangen. The main objective of both projects included the synthesis development and physicochemical characterization of various systems of nanoparticles tailored for biomedical applications.
In order to get an in-depth understanding of the challenges associated with the translation of medical research into an application as well as to further specialize in the development of nanoparticle systems especially for drug delivery systems, he started his PhD thesis at SEON in March 2020. The emphasis in his research is put on the chemical surface modification of superparamagnetic iron oxide nanoparticles in order to create reproducible systems which are able to transport drugs to deceased tissues.

Yael Suarez

My name is Yael del Carmen Suárez López, and I am a Ph.D. student at Uppsala University in Sweden, under the supervision of Prof. Alexandra Teleki (SciLifeLab and Uppsala University), Prof. Christel Bergström (Uppsala University), and Prof. Inge Herrmann (ETH Zürich). My project involves engineering iron oxide nanoparticles to develop a magnetic biosensor for the non-invasive diagnosis of Inflammatory Bowel Disease (IBD) in pediatric patients.
My academic journey started with a Bachelor's degree in Biotechnology Engineering from the Instituto Tecnológico y de Estudios Superiores de Monterrey in Mexico City. My specialization in Molecular Biology paved the way for my undergraduate thesis, which involved the development of a device for detecting glucose in saliva using an enzymatic reaction coupled with a redox reaction. As part of my undergraduate experience, I also had the chance to be a visiting student at the University College Cork in Ireland. I obtained my BSc. Biotechnology Engineering diploma on December 2018.
During my bachelor's and shortly after graduation, I gained some industry experience. I worked as a Quality Control Specialist at IQVIA, a Clinical Research Organization, I also interned at Degasa, a medical device innovation company, and I worked as a Clinical research intern at Sanofi, all of which were located in Mexico City.
Afterwards, I pursued a Master's degree in Nanomedicine for Drug Delivery (NANOMED) at the University of Paris in France, from 2019 to 2021. For my master's thesis, I had the opportunity to work on near-infrared luminescent nanoparticles for selective oligonucleotide detection under the supervision of Prof. George Sotiriou at the Karolinska Institute, Sweden. Additionally, I was a visiting student at the University of Pavia in Italy, where I completed a specialization in Nanomedicine production and biotechnology applications.

Prior to my Ph.D. studies and after graduating from my master's, I worked as a Research Assistant at the Karolinska Institute in Stockholm, Sweden. I led a project on bone tissue Engineering with bionanomaterials and stem cells at the SotiriouLab. The project's main objective was to investigate the properties of calcium phosphate nanoparticles that influence the proliferation and differentiation of stem cells toward osteoblasts (manuscript underwriting).
Throughout my educational and professional endeavors, I have been fortunate to receive several awards and scholarships. I achieved the highest ranking in my undergraduate program amongst all my peers. I was also awarded the Integral Student Award by the Instituto Tecnológico y de Estudios Superiores de Monterrey. I was also granted a full scholarship to the 46º International Summer Science Camp Dr. Bessie F. Lawrence at the Weizmann Institute in Israel, where I worked on a project exploring the role of MTCH2 in embryonic stem cells. I was also honored to be granted a fully funded European Union ERASMUS MUNDUS scholarship, which provided financial support for pursuing my Master's degree. Finally, I received the Best Poster Award at the American Institute of Chemical Engineering 2022 Meeting held in Phoenix, Arizona.
Regarding language skills, I am fluent in English, with a TOEFL score of 104. I also have proficiency in French, having attained a DELF B1 level. Finally, Spanish is my mother tongue.
As I continue my academic journey and delve deeper into the fascinating field of pharmaceutical nanotechnology, I am driven by a strong passion for innovation and the potential to make a meaningful impact in healthcare. I am excited about the opportunities that lie ahead and look forward to contributing to scientific advancements in the future.

Malin Svensson

PhD student

Ms. Svensson was born on 16th of June 1992 in Sweden, where she graduated from secondary school in June 2011. She started her bachelor studies in Molecular Biology at Uppsala University the year 2016 and finnished 2018 with her bachelor thesis at the institute of Microbiology and Immunology at Uppsala University. In the same year, she started her masters studies in Cell Biology at Uppsala University. During her master studies she worked on protein purification at the institute of Structure Biology at Uppsala University. During her masters she spent one semester abroad in Freiburg, Germany, where she studied mostly microbiology and immunology. After graduating her master in 2020 she moved to Mainz, Germany, where she started working in the group of Prof. Dr. med. Stephan Gehring at the Department of Pediatric Immunology and infectology in the University Medical Center Mainz.
In August 2021 she started her PhD in the group of Prof. Dr. med. Gehring, with her project focusing on antigen mRNA/adjuvant loaded nanovaccine for activation of non-parenchymal liver cells for tumor therapy.

RECENT PUBLICATIONS:

- Medina-Montano C, Cacicedo ML, Svensson M, Limeres MJ, Zeyn Y, Chaves-Giraldo JE, Röhrig N, Grabbe S, Gehring S, Bros M. Enrichment Methods for Murine Liver Non-Parenchymal Cells Differentially Affect Their Immunophenotype and Responsiveness towards Stimulation. Int J Mol Sci. 2022 Jun 11;23(12):6543. doi: 10.3390/ijms23126543. PMID: 35742987; PMCID: PMC9223567.
- Medina-Montano, C.; Rivero Berti, I.; Gambaro, R.C.; Limeres, M.J.; Svensson, M.; Padula, G.; Chain, C.Y.; Cisneros, J.S.; Castro, G.R.; Grabbe, S.; et al. Nanostructured Lipid Carriers Loaded with Dexamethasone Prevent Inflammatory Responses in Primary Non-Parenchymal Liver Cells. Pharmaceutics 2022, 14, 1611. https://doi.org/10.3390/pharmaceutics14081611

Nikolaos Tagaras

Particles-Biology Interactions, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

I was born in 1995 in Athens (GR), where I attended the primary school in Athens until 2006. Then my family and I moved to Luxembourg. When I graduated from high school in 2013, I moved back to Greece, specifically in Thessaloniki to do my B.Sc. (4-year program) in Biology at the Aristotle University of Thessaloniki (AUTh). During my third year, I decided to do a full year Erasmus+ Exchange program with full scholarship at the Freie Universität Berlin (FUB) in Germany in order to improve my English and German scientific language skills. During my last year of B.Sc. I undertook my thesis at the Department of Physiology with the title "Identification of miRNA and lncRNA expression profile in a genetic animal model of heart failure". After graduating I joined the Greek Army since the military service in Greece is mandatory. I spent 4 months in the borderland of Thrace as a nurse in the medical practice. For the next 5 months I was transferred to the General Military Hospital of Athens, where I pertained to the administrative group of the division and I was responsible for matters of an adminis-trative nature. After completing the military service in December 2018 I started applying for M.Sc. programs. During that period I moved to Kenya to teach Biology in two vocational training schools. In September 2019, I started my 2-year M.Sc. in Toxicology at the Karolinska Institutet in Stockholm, Sweden. During my studies I was member of various organizations which aimed to contribute to the improvement of local communities and student life. My M.Sc. thesis focused on the evaluation of pulmonary toxicity of micro- and nanoplastics using mono- and coculture models. Even though research in the lab was my passion, after graduation I decided to join the European Food Safety Authori-ty as a Regulatory Toxicologist Trainee. This position enabled me to get insights about the functions and processes of Regulatory Toxicology. Before completing my traineeship, I knew that I wanted to continue in academia and this is the reason I applied for my current PhD project at Empa St. Gallen with the title "intelligent single-atom nanozymes for effective and safe therapy of inflammatory dis-eases during pregnancy". During my first PhD year I decided to organize for the first time in St. Gallen the Pint of Science festival. This festival aims to bring researchers and scientists closer to the general public by presenting their research and scientific fields in a more casual and accessible setting, such as bars and pubs and to improve science communication.
I am looking forward to CLINAM 2023 to expand my knowledge in the field of nanomedicine and to interact with experts of the field.

Rifka Utami

2nd Year PhD Student @Institute of Pharmaceutical Science, School of Cancer & Pharmaceutical Sciences, King's College London

Rifka completed her BSc from the Faculty of Pharmacy, Hasanuddin University, Indonesia in 2015. She undertook a 1-year Pharmacist Professional programme at the same institution. In 2016, she was awarded a scholarship from the Indonesian Endowment Fund for Education (LPDP-RI) to complete an MRes degree at the University of Birmingham (UK) to investigate the formulation and toxicological aspects of organic nanomaterials

for nose-to-brain delivery. She received another scholarship from LPDP-RI to pursue her PhD in KCL. She joined Prof Al-Jamal's group in June 2021 and is currently working on developing nanoparticle formulations for peptide delivery to the brain to treat motor neuron disease (MND).

RECENT PUBLICATIONS:

- Han, S. Wang, J.T., Yavuz, E., Zam, A., Rouatbi, N., Utami, R.N., Liam-Or, R., Griffiths, A., Dickson, W., Sosabowski, J., & Al-Jamal, K.T. (2023) Spatiotemporal tracking of gold nanorods after intranasal administration for brain targeting. Journal of Controlled Release. Accepted.

Mireia Vilar-Hernandez

I am a 3rd year PhD student at LipoCoat in collaboration with the University of Twente. LipoCoat is a biotechnology company that designs bio-inspired coatings for different medical devices which improves safety, comfort and performance. My focus is to develop different phospholipid-based coatings for nanoparticles and investigate their interaction and impact on immune system activation. This coating can be tuned easily for different *in vivo* applications which will contribute to broaden the product portfolio of LipoCoat. Also, this study will anticipate the immunological responses when applied to LipoCoat products. My PhD project is part of the DIRNANO program funded by the EU - Marie Sklodowska-Curie Actions. In this program I have taken the opportunity to collaborate with different researchers and universities across different fields.

I started my bachelor's in Nanoscience and nanotechnology in 2015 at the Universitat Autònoma de Barcelona. This interdisciplinary program gave me the fundamentals of nanoscience, an understanding of physics and chemistry at the nanoscale and an overview of the different applications of nanotechnology with a special focus on the biomedical field.

During my bachelor's final year, I did my internship and thesis in the drug delivery and targeting (DDT) group at the Vall Hebron Institute of Research. There I actively contributed to ongoing projects related to drug delivery and targeting for cancer treatment, specifically in colorectal and breast cancer. For my bachelor's final thesis, I worked on a project focused on the development and validation of targeted polymeric micelles for cancer treatment. This involved designing and synthesizing polymeric micelles, performing characterization studies, and evaluating their effectiveness in delivering therapeutic agents to cancer cells. The research outcomes are part of the publication entitled "Polymeric Micelles Targeted against CD44v6 receptor increase Niclosamide efficacy against Colorectal Cancer Stem Cells and reduce Circulating Tumor Cells." [1]

To continue my academic formation in a more directed path towards research I enrolled on the Translational Biomedical Research at the Vall Hebron Institute of Research. I have gained comprehensive knowledge of various research methodologies, including experimental design, data analysis, and translational approaches. Together with a deeper understanding of different mechanisms of certain diseases, such as cancer, this knowledge has significantly contributed to my ability to design my research during my master thesis that focussed on the development of Solid Lipid Nanoparticles for cancer treatment. [2]

I would like to attend CLINAM since I am very interested in the nanomedical field and I want to pursue my career in it. I believe that CLINAM will bring me the opportunity to learn about the most recent advances in the field. Even though doing the PhD in a university spin-off is rewarding I would like to contribute to finding solutions for new healthcare treatments, especially in cancer. CLINAM will give me the opportunity to engage with various companies in the field which will broaden my options for my future career.

[1] F. Andrade et al., "Polymeric micelles targeted against CD44v6 receptor increase niclosamide efficacy against colorectal cancer stem cells and reduce circulating tumor cells *in vivo*," J. Control. Release, vol. 331, no. January, pp. 198–212, 2021, doi: 10.1016/j.jconrel.2021.01.022.

[2] J. German-Cortés, M. Vilar-Hernández, D. Rafael, I. Abasolo, and F. Andrade, "Solid Lipid Nanoparticles: Multitasking Nano-Carriers for Cancer Treatment," Pharm. 2023, Vol. 15, Page 831, vol. 15, no. 3, p. 831, Mar. 2023, doi: 10.3390/PHARMACEUTICS15030831.

Michaela Voljnikova

PhD Candidate

Michaela is a PhD candidate in Nanotechnology at the Central European Institute of Technology, Brno University of Technology, Czech Republic. Simultaneously, she engages as a research scientist in the Laboratory of Nanomedicine, part of the Research Group for Molecular Biology and Nanomedicine led by Assoc. Prof. Zbynek Heger at the Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. Her research is focused on developing theranostic nanomedicines based on bimodal lipid nanoparticles for magnetic resonance-guided focused ultrasound (MRgFUS) therapy. The primary objective of her work is to enhance early cancer detection and enable appropriate treatment without the risk of overdosing patients. In recognition of her research, Michaela received a prestigious Brno PhD Talent scholarship in 2021 to pursue this research. She was further honoured with the Director's Award for PhD Student of the Year in 2022. Prior to her PhD studies, Michaela earned both a Bachelor's and a Master's degree in Chemistry for Medical Applications from the Faculty of Chemistry, Brno University of Technology, Czech Republic. During her master's studies, she worked on the preparation of neural bioceramic scaffolds, which earned her the esteemed recognition of the best master's thesis in the Czech Republic from the Czech Ceramic Society. Michaela also won the Falling Walls Lab competition in 2021, showcasing her ability to communicate and present her research effectively. In addition to her academic pursuits, she serves as a dedicated supervisor, and her high-school student achieved an impressive 4th place at the International Science and Engineering Fair (ISEF2023) held in Dallas, Texas.

Shiqi Wang

Dr. Shiqi Wang received her Bachelor and Master degrees from Tsinghua University China in 2012 and 2014 respectively. Then she moved to Imperial College London as a Marie Curie Early Stage Researcher in the department of chemical engineering, where she got her Ph.D. degree in 2018. Afterwards, she joined the Faculty of Pharmacy, University of Helsinki as a postdoctoral researcher in Prof. Hélder Santos group. Recently she started her own research group at the same Faculty.

Dr. Wang's research interests include developing pH-responsive polymeric materials for intracellular drug delivery applications. She is a co-author of 58 peer-reviewed papers, with 3380 citations in

total (h-index=26). She has also received many awards and grants, including Weinberg Prize of Imperial College London for research of outstanding ingenuity, originality and elegance during PhD, Finnish Pharmaceutical Society most outstanding research article award, and Academy of Finland Fellowship grant.

RECENT PUBLICATIONS

- Gao, H., Wang, S., et.al. (2023). Rational Design of a Polysaccharide-based Viral Mimicry Nanocomplex for Potent Gene Silencing in Inflammatory Tissues. Journal of Controlled Release, 357, 120-132. https://doi.org/10.1016/j.jconrel.2023.03.037
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- Wang, S. et.al. (2020). Intracellular Delivery of Budesonide and Polydopamine Co-Loaded in Endosomolytic Poly(butyl methacrylate-co-methacrylic acid) Grafted Acetalated Dextran for Macrophage Phenotype Switch from M1 to M2. Advanced Therapeutics, 4 (1), 2000058. https://onlinelibrary.wiley.com/doi/full/10.1002/adtp.202000058
- Wang, S. et.al. (2020). Superfast and Controllable Microfluidic Inking of Anti-inflammatory Melanin-like Nanoparticles Inspired by Cephalopods. Materials Horizons, 7(6), 1573-1580. https://pubs.rsc.org/ko/content/articlehtml/2020/mh/d0mh00014k
- Wannasarit, S., Wang, S.* et.al. (2019). A Virus-Mimicking pH-Responsive Acetalated Dextran-Based Membrane-Active Polymeric Nanoparticle for Intracellular Delivery of Antitumor Therapeutics. Advanced Funtional Materials, 29, 1905352. https://onlinelibrary.wiley.com/doi/full/10.1002/adfm.201905352

Christoph Wilhelmy

PhD candidate and Pharmacist
Department of Biopharmacy and Pharmaceutical Technology- JGU Mainz
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I was born on December 30, 1994, in Mayen, Germany, and completed my Abitur (high school diploma) in 2013. In 2015, I enrolled in pharmacy studies at Johannes Gutenberg University (JGU) Mainz. I successfully completed my university studies in 2019, earning a degree in pharmaceutical sciences (2. Staatsexamen Pharmazie) from JGU. As part of my practical training to become a pharmacist, I conducted a research internship at the Department of Biopharmacy and Pharmaceutical Technology at JGU Mainz, starting in November 2019. During this internship, I focused on developing polymeric nanoparticles capable of controlled release under the influence of a magnetic field. Additionally, I conducted biopharmaceutical studies on surrogate buffers to enhance *in vitro* dissolution testing of solid dosage forms, aiming for better alignment with *in vivo* conditions. Following the research internship from May 2020 on, I completed the practical year of my pharmaceutical education at "Die Mainz Apotheke", where I gained valuable experience in pharmacy practice and patient care. In December 2020, I successfully conducted this practical part of my education, obtaining the final degree (3. Staatsexamen) and becoming a licensed pharmacist in Germany.
In January 2021, I commenced my PhD studies under the supervision of Prof. Dr. Peter Langguth in the Department of Biopharmaceutics and Pharmaceutical Technology at JGU Mainz. My research is carried out in the highly interdisciplinary collaborative research center CRC1066. In detail, my investigations revolve around the development and characterization of lipid nanoparticles (LNPs) tailored for mRNA delivery. To elucidate structural properties and critical quality parameters, I utilize small angle X-ray scattering (SAXS) techniques, to correlate the properties of the respective formulations with biological efficacy. In addition to research, I am actively involved in teaching activities, where I educate students on theoretical and practical aspects of sterile dosage form production.

RECENT PUBLICATIONS:

- Blechar JA, Al-Gousous J, Wilhelmy C, Postina AM, Getto M, Langguth P. Toward Mechanistic Design of Surrogate Buffers for Dissolution Testing of pH-Dependent Drug Delivery Systems. Pharmaceutics. 2020 Dec 10;12(12):1197.

Yu Xiaodong

I am Yu Xiaodong, a Ph.D. student at the Yong Loo Lin School of Medicine, National University of Singapore. Originally from China, I completed my Bachelor's Degree in Biology at Zhejiang University in 2019 with distinction.
In 2019, I embarked on an exciting Ph.D. journey, courtesy of the NUS Research Scholarship, under the guidance of Prof. Jiong-Wei Wang. Our lab is a dynamic and multidisciplinary team focused on translational research in cardio-metabolic diseases. Our mission was to discover pathogenic mechanisms, identify novel therapeutic targets, and develop viable nanomedicines for targeted therapies, with an emphasis on the heart, liver, and gut.
Throughout my academic pursuit, I developed a keen interest in the diagnosis and treatment of NASH using nanomedicine. This area of research held great promise, as nanomedicine could offer revolutionary solutions for NASH, a challenging condition.
In Prof. Wang's lab, I enthusiastically delved into exploring innovative nanocarriers for targeted drug delivery and investigating their impact on NASH diagnosis and treatment. The journey was demanding, but I remained driven by the potential to make a meaningful difference in patients' lives.
Reflecting on my journey so far, I am grateful for the support, mentorship, and invaluable experiences that have shaped me as a researcher. Looking ahead, I am eager to continue my research endeavors, contribute to scientific advancements, and make a positive impact in the field of medical science. Empowered by my education and fueled by curiosity, I am committed to pushing the boundaries of knowledge to create a healthier future for all.

RECENT PUBLICATIONS

- Xiaodong Yu, et al. Ceramide de novo synthesis in non-alcoholic fatty liver disease: Pathogenic mechanisms and therapeutic perspectives. Biochemical Pharmacology. 2022
- Jingjing Liu, Xiaodong Yu, et al. Myeloperoxidase Sensitive T1 and T2 Switchable MR Imaging for Diagnosis of Non-alcoholic Steatohepatitis. ACS Nano. 2023 (co-first author)
- Lingjun Tong, …, Xiaodong Yu, et al. Milk-derived extracellular vesicles protect intestinal barrier integrity in gut-liver axis. Science Advance. 2023

Meiling Yu

3rd PhD student, Institute of Pharmaceutical Science, King's College London

Meilling obtained her BSc in Pharmacy from Jiangsu University, China. She undertook a 3-year MSc in Pharmaceutics at Shenyang Pharmaceutical University, China. Her MSc project was the developement of intratumoral delivery of in situ gels containing chemotherapeutic drugs. Meiling started her PhD in 2020 and her PhD project focusses on the delivery system of gene-editing lipid particles for the treatment of brain disease at Al-Jamal's group.

RECENT PUBLICATIONS

- Yu, M., Zhang, C., Tang, Z., Tang, X., & Xu, H. (2019). Intratumoral injection of gels containing losartan microspheres and (PLG-g-mPEG)-cisplatin nanoparticles improves drug penetration, retention and anti-tumor activity. Cancer Letters. (IF 9.76).
- Yu, M., Yao, Q., Zhang, Y., Chen, H., He, H., Zhang, Y., & Xu, H. (2018). Core/shell PLGA microspheres with controllable *in vivo* release profile via rational core phase design. Artificial Cells, Nanomedicine, and Biotechnology. (IF 6.36)
- Zhang, Y., Fei, S., Yu, M., Guo, Y., He, H., Zhang, Y., Yin, T., Xu, H. and Tang, X. (2018). Injectable sustained release PLA microparticles prepared by solvent evaporation-media milling technology. Drug Development and Industrial Pharmacy. (IF 3.73)
- Chen, H. L., Cai, C. C., Ma, J. Y., Yu, M. L., Zhao, M. H., Guo, J. B., & Xu, H. (2018). Effect of the dispersion states of azone in hydroalcoholic gels on its transdermal permeation enhancement efficacy. Journal of Pharmaceutical Sciences. (IF 3.78)

Alaa Zam

2nd year PhD Student @Institute of Pharmaceutical Science, School of Cancer & Pharmaceutical Sciences, King's College London

Alaa Zam obtained her BSc Degree in pharmacy and pharmaceutical chemistry from Aleppo University, Syria, in 2014. She then completed her MSc studies in industrial pharmacy at Damascus University. Her MSc project focused on formulating propolis extracts in semi-solid dosage forms for burn treatment. She taught pharmaceutical technology, biopharmacy and pharmacokinetics subjects to undergraduate students. Alaa's PhD project is to develop lipid-based nano-carriers for *in vivo* delivery of nucleic acids for brain cancer immunotherapy. Her PhD study is funded by the Centre for Doctoral Studies, KCL and the Council for At-Risk Academics (CARA) foundation. She is a student representative at the International Students' House.

Yanira Zeyn

PhD Student

In October 2017, I started my masters studies in Biomedical Chemistry at the Johannes Gutenberg University Mainz where I was able to work on medicinal topics, e.g. during my master's thesis at the Max Planck Institute for Polymer Research in Mainz. There I focused on exploring bioactive peptides for promoting angiogenesis in artificial matrix mimicking materials. Besides, I spent a semester abroad in Trieste, Italy, during my master's, where I focused on self-assembling tripeptides and the effects of chirality changes. After my MSc graduation in April 2020, I was further able to gain useful working experiences as research associate at the Institute of Toxicology of the University Medical Center Mainz for one year. The topics I worked on included cell biological investigation of novel histone deacetylase inhibitors. In July 2021, I started my PhD in the group of Prof. Grabbe and PD Dr. Bros at the Department of Dermatology of the University Medical Center Mainz, where I focus on nanovaccines loaded with antigen-encoding mRNA / nucleic acid-based adjuvants to activate dendritic cells and liver non-parenchymal cells for tumor therapy.
Zeyn, Y., Harms, G., Tubbe, I., Montermann, E., Röhrig, N., Hartmann, M., Grabbe, S., & Bros, M. (2022). Inhibitors of the Actin-Bundling Protein Fascin-1 Developed for Tumor Therapy Attenuate the T-Cell Stimulatory Properties of Dendritic Cells. Cancers, 14(11), 2738.

RECENT PUBLICATIONS

- Medina-Montano, C., Cacicedo, M. L., Svensson, M., Limeres, M. J., Zeyn, Y., Chaves-Giraldo, J. E., Röhrig, N., Grabbe, S., Gehring, S., & Bros, M. (2022). Enrichment Methods for Murine Liver Non-Parenchymal Cells Differentially Affect Their Immunophenotype and Responsiveness towards Stimulation. International journal of molecular sciences, 23(12), 6543.
- Zeyen, P.*, Zeyn, Y.*, Herp, D., Mahmoudi, F., Yesiloglu, T. Z., Erdmann, F., Schmidt, M., Robaa, D., Romier, C., Ridinger, J., Herbst-Gervasoni, C. J., Christianson, D. W., Oehme, I., Jung, M., Krämer, O. H., & Sippl, W. (2022). Identification of histone deacetylase 10 (HDAC10) inhibitors that modulate autophagy in transformed cells. European journal of medicinal chemistry, 234, 114272.
- Pons, M., Zeyn, Y., Zahn, S., Mahendrarajah, N., Page, B. D. G., Gunning, P. T., Moriggl, R., Brenner, W., Butter, F., & Krämer, O. H. (2021). Oncogenic Kinase Cascades Induce Molecular Mechanisms That Protect Leukemic Cell Models from Lethal Effects of De Novo dNTP Synthesis Inhibition. Cancers, 13(14), 3464.
- Pons, M., Nagel, G., Zeyn, Y., Beyer, M., Laguna, T., Brachetti, T., Sellmer, A., Mahboobi, S., Conradi, R., Butter, F., & Krämer, O. H. (2019). Human platelet lysate as validated replacement for animal serum to assess chemosensitivity. ALTEX, 36(2), 277–288.

Bonan Zhao

Bonan Zhao studied Medicinal Chemistry at China Pharmaceutical University and graduated in 2021 (M.Sc.). He is currently a Ph.D. student under the supervision of Prof. Matthias Barz in the Division of Bio-Therapeutics of LACDR at Leiden University. He has published first-author papers in peer-reviewed journals, including the Journal of Nanobiotechnology. In the Barz lab, his research is focused on the synthesis and characterization of tailored polypept(o)ide-based cylindrical polymer brushes as multifunctional drug delivery platforms, which is pursued by the synthesis of various brush designs/morphologies, functionalization via *in vivo* click chemistry and immunoconjugation.

Ivan Zlotver

Ivan Zlotver earned his bachelor's degree in Pharmacy from The School of Pharmacy at the Hebrew University in Israel. He then pursued a master's degree in medicinal chemistry at the same institution, working in the laboratory of Prof. Galia Blum. His research focused on developing a targeted inhibitor of M2 macrophages for the treatment of drug-resistant epilepsy. Currently, Ivan is a Ph.D. student at the Laboratory of Pharmaceutical Nanomaterials Science in the Faculty of Materials Science and Engineering at the Technion-Israel Institute of Technology. His research interests are centered around inorganic nanomaterials that are combined or functionalized with organic matter and their applications in treating diseases. His main Ph.D. project is the development of Glycosylated Hybrid TiO2/Polymer Nanomaterials for Sonodynamic Therapy of Pediatric Tumors. Ivan has extensive experience in a variety of fields, including organic chemistry, nanoparticle synthesis and modification, biochemical methods utilizing both 2D and 3D cell culture models, and rodent in-vivo experimentation.

RECENT PUBLICATIONS

- D. Tsvirkun, Y. Ben-Nun, E. Merquiol, I. Zlotver, K. Meir, T. Weiss-Sadan, I. Matok, R. Popovtzer, G. Blum, J Am Chem Soc 2018, 140, 12010.
- A. Pariente, E. Peled, I. Zlotver, A. Sosnik, Mater Today Chem 2021, 22, 100613.
- . Dobrynin, I. Polishchuk, L. Portal, I. Zlotver, A. Sosnik, B. Pokroy, Mater Today Bio 2022, 14, 100265.
- H. Moshe Halamish, I. Zlotver, A. Sosnik, J Colloid Interface Sci 2022, 626, 916.

László Dézsi

Research Associate Professor

He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999). Meanwhile he received fellowships at Albert Ludwigs Universität, Freiburg, Germany working in the field of local regulation of blood flow in skeletal and cardiac muscle studying nitric oxide; and at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center working in the field of cerebral blood flow and metabolism as well as cerebral ischemia and reperfusion in animal stroke models. He was head of laboratory, CRO monitor, research project manager in vascular and safety pharmacology at Gedeon Richter (GR) Pharmaceutical Plc. (1999-2012), and manager of Analgesic Research Laboratory (2006-2012), a joint venture of GR and University of Pécs, Department of Pharmacology. He participated in curriculum development and he had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000). Now invited member of the MSc BE Committee at Technical University, Budapest. He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor (PD) of physiology in 2006. He became staff member of Semmelweis University in 2020. He established his own teaching course in 2008 entitled "Cardiorespiratoric and neurophysiological measuring techniques" at the Institute of Translational Medicine. He participates in postgradual education in nanomedicine. He is a physiology and pathophysiology teacher at Semmelweis University. Currently he is working at the Institute of Translational Medicine, Nanomedicine Research and Education Center (2012-) investigating cardiopulmonary and immunological effects of nanoparticles, incl. LNP-mRNS vaccines in various *in vivo* models of complement activation related pseudoallergy (CARPA) and participates in the development of new models. He was a member of the EU FP7 "NanoAthero" Consortium (2013-2018), now he is a member of „EXPERT" project EU's Horizon 2020 research and innovation programme (2019-) and holding a Semmelweis Univ. Grant "STIA-KFI-2022" (2023-).

RECENT PUBLICATIONS

- Harald Unterweger, Christina Janko, Tamara Folk, Iwona Cicha, Noémi Kovács, Gyula Gyebnár, Ildikó Horváth, Domokos Máthé, Kang H Zheng, Bram F Coolen, Erik Stroes, János Szebeni, Christoph Alexiou, László Dézsi, Stefan Lyer (2023) Comparative *in vitro* and *in vivo* Evaluation of Different Iron Oxide-Based Contrast Agents to Promote Clinical Translation in Compliance with Patient Safety. International Journal of Nanomedicine, 18:2071-2086, DOI: 10.2147/IJN.S402320
- László Dézsi, Tamás Mészáros, Gergely Kozma, Mária H-Velkei, Csaba Zs. Oláh, Miklós Szabó, Zsófia Patkó, Tamás Fülöp, Mark Hennies, Miklós Szebeni, Bálint András Barta, Béla Merkely, Tamás Radovits, János Szebeni (2022) A naturally hypersensitive porcine model may help understand the mechanism of COVID-19 mRNA vaccine-induced rare (pseudo) allergic reactions: complement activation as a possible contributing factor. GeroScience, 44:597-618, DOI: 10.1007/s11357-021-00495-y.
- Ákos Pethő, Dorothea Piecha, Tamás Mészáros, Rudolf Urbanics, Christoph Moore, Bernard Canaud, László Rosivall, Tom Eirik Mollnes, Sonja Steppan, Gábor Szénási, János Szebeni, László Dézsi (2021) A porcine model of hemodialyzer reactions: roles of complement activation and rinsing back of extracorporeal blood, Renal Failure, 43:1609-1620, DOI: 10.1080/0886022X.2021.2007127
- Gergely Milosevits, Tamás Mészáros, Erik Őrfi, Tamás Bakos, Miklós Garami, Gábor Kovács, László Dézsi, Péter Hamar, Balázs Győrffy, András Szabó, Gábor Szénási, János Szebeni (2021) Complement-mediated hypersensitivity reactions to an amphotericin B-containing lipid complex (Abelcet) in pediatric patients and anesthetized rats: Benefits of slow infusion, Nanomedicine: Nanotechnology, Biology and Medicine, 34, 102366, DOI: 10.1016/j.nano.2021.102366

Rahaf Mihyar

MSc

Rahaf obtained her bachelor's degree in pharmacy from the University of Jordan (Jordan) in 2020. Afterwards, she has been awarded an EMJMD scholarship to pursue the master's program in Nanomedicine for Drug Delivery between Université Paris Cité (France), University of Patras (Greece), Università di Pavia (Italy), and Université d'Angers (France). As part of her program, she did her thesis at Institute for Experimental Molecular Imaging (ExMI) under the supervision of Prof. Dr. Dr. Twan Lammers, obtaining a master's degree in Nanomedicine in 2022. In the same year, she joined ExMI as a PhD student, as part of the Research Training Group (RTG 2375) "Tumor-Targeted Drug Delivery". During her master's program, Rahaf has undertaken two internships; First, at the laboratory of pharmaceutical technology at the University of Patras (Greece), where she focused on protein-encapsulating liposomal formulations using microfluidic mixing and evaluated their properties after freeze-drying. Secondly, she joined ExMI at RWTH University Clinic (Germany) to work on the formulation development of polymeric micelles and explore strategies to enhance their long-term stability. Additionally, during her undergraduate studies, she has worked on novel protein-based drug delivery systems for controlled per oral drug release.

Simona Steponkiene

PhD

I was born in Lithuania, Raseiniai city. I received my master's degree in Biophysics and finished PhD studies in 2014 (Biomedical sciences, Biophysics). Since 2010 I work in the Biomedical Physics Laboratory, National Cancer Institute (Engineer (1 year), Young researcher (4 years), Researcher (1 year)), and Senior Researcher (3 years) in the field of cancer diagnostics and therapy by nanoparticles and nanostructures (quantum dots, gold nanoclusters, magnetic nanoparticles, quantum dot – photosensitizer complex, upconverting nanoparticles). My PostDoc practice took place in a private Pharmaceutical Company Valentis as a Scientific Manager of the R&D department (2015-2018), project leader. I successfully finished and applied the liposomal technology to the production of food supplements (holds author rights to a patent).

Currently, I am a Senior Researcher at the National Cancer Institute and a Principal Investigator of the research project – "Mesenchymal stem cells as vehicles for targeted delivery of theranostic nanoparticles into the aggressive type of cancer cells", grant no. S-MIP-22-31

Maternity leave:

In 2018-2020 I went on Maternity Leave and my working carrier was stopped for 2 years. Currently, I have a full-time job and a wonderful 5-year-old boy.

RECENT PUBLICATIONS

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Katharina Beck

I was born in Waldshut-Tiengen, Germany, on 21st February 1994. In my hometown, I finished school in 2012. Afterwards, I studied Pharmacy at the Eberhard Karls University in Tübingen graduating as a licensed pharmacist in 2019. I spent my practical year working at the Nordring Apotheke in Tübingen and started my additionally Master's degree in Pharmaceutical Sciences and Technologies. Under the supervision of Prof. Daniels (Insitute for Pharmaceutical Technology, Tübingen), I performed my Master's thesis about formulation concepts of metastable emulsions and their foamability.

In June 2020 I started my PhD joining Prof. Heerklotz's research group at the University of Freiburg, Department of Pharmaceutical Technology and Biopharmacy. Under the supervision of Dr. Maria Hoernke, I am currently focusing on antimicrobial membrane active peptides and suitable model systems. Most recently, we published a paper on "Membrane permeabilization can be crucially biased by a fusogenic lipid composition – leaky fusion caused by antimicrobial peptides in model membranes" (K. Beck et al. Soft Matter 19.16 (2023): 2919-2931.)

In October 2023 I will join a team of young scientist at the University of Augsburg to develop and offer a measuring system to analyze lipid-based pharmaceutical compounds.

Sara Elsafy

Sara Elsafy completed her Bachelor and Masters' of Science in Pharmacy from the German University in Cairo (GUC). Her Masters' was a joint collaboration between GUC in Egypt and Bonn University in Germany, and was generously funded by the DAAD. During her Master's thesis, Sara focused on the development of polymeric nanoparticles for the delivery of therapeutic proteins to treat liver fibrosis. In 2022, Sara joined the Institute of Experimental and Molecular Imaging in 2022 as a PhD student and a member of the Tumor-Targeted Drug Delivery Research Training Group (RTG 2375). Her current research focuses on the large-scale production of lipid-based nanoparticles, with a keen interest in elucidating the key critical process parameters that influence nanoparticles' in-vivo behaviour.

Asmaa Said Sayed Elshafei

Mrs. Asmaa Elshafei joined the Institute of Experimental Molecular Imaging as a PhD student after obtaining a prestigious DAAD-Egypt scholarship from the German Academic Exchange Service (DAAD reference No. 91664480).

Mrs. Elshafei conducts research in the fields of functional and molecular imaging, hypoxia, and multidrug resistance, with a particular focus on vascular promotion and microenvironment modification as a novel conceptual approach to improving nanomedicine-based drug delivery and immunotherapy. Asmaa has developed a unique, unconditional drug delivery approach that can be applied to all categories of drug delivery systems. This approach not only enhances chemotherapy delivery but also has the potential to enhance immunotherapy and radiotherapy. Her achievements have been impressive, and she has gained recognition for her work. She was awarded the Women in Molecular Imaging Network (WIMIN) Scholar Award at the World Molecular Imaging Congress (WMIC) 2023 in Prague, in addition to the Young Investigator Award at the European Molecular Imaging Meeting (EMIM) 2023 in Salzburg.

Furthermore, she has demonstrated outstanding presentation skills and has given talks at several prestigious international meetings, winning the Best Presentation Award at the Controlled Release Society (CRS) Europe Meeting in 2022 and the Poster Award at the 2022 International Graduate Symposium on Biopharmaceutics. Moreover, Asmaa has recently published an impressive research paper this year regarding the effect of cellular and microenvironmental multidrug resistance on tumor-targeted drug delivery in triple-negative breast cancer using multimodal molecular imaging in the Journal of Controlled Release. Asmaa has been involved in various projects and has also co-authored several manuscripts in leading journals.

Mrs. Elshafei has collaborated with Prof. Twan Lammers and Prof. Fabian Kiessling. Throughout these years, she has demonstrated a strong desire for knowledge acquisition and the ability to tackle challenges and solve problems associated with day-to-day experiments. Despite having a full-time job as a mother with two children, she courageously traveled from Egypt to pursue her doctoral research with us, displaying a high level of commitment to achieving her scientific aspirations. She is truly remarkable—a highly dedicated, talented, and motivated student—and possesses all the qualities necessary for success in academia and industry.

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CLINAM

European Foundation for Clinical Nanomedicine

14/2023

Basel, October 8 – 11

ABSTRACTS SPEAKERS

IONIZABLE LIPID NANOPARTICLES IN ACTION & BEYOND DELIVERY

KHULOUD AL-JAMAL[1], Yue Qin[1], Nadia Rouatbi[1], Rafal Baker[1], Julie Tzu-Wen Wang[1], James Spicer[2], Adam A Walters[1]
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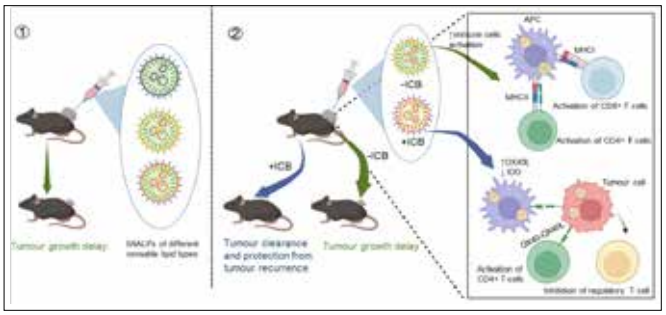
Immunotherapy is currently the standard of care in the treatment of many malignancies. However, unexpected side effects caused by systemic administration of highly immunostimulatory molecules have been a serious concern within this field. Nucleic acid-based approaches, such as the delivery of plasmid DNA (pDNA) and small interfering RNA (siRNA) to express and silence immunogenic and immunoinhibitory molecules, respectively, could represent the next generation of cancer immunotherapy. In particular, pDNA has the potential to be a highly potent candidate due to its inherent immunogenicity. In this study, we employed stable nucleic acid lipid particles (SNALPs) to co-deliver nonspecific pDNA and siRNA or constructs specific to two prominent immunotherapeutic targets (OX40L and indoleamine 2,3-dioxygenase-1 (IDO)) in situ. Using the B16F10 model it was demonstrated that intratumoural delivery of SNALP formulated nonspecific pDNA and siRNA led to strong local immune activation and tumour growth inhibition even at low doses. These effects could be attributed to the immunogenic nature of pDNA. When non-specific constructs were substituted with pOX40L and siIDO, significant immune activation, as demonstrated by increased immune cell infiltration in tumours and tumour draining lymph nodes (TDLN), and increased CD69+ (T cell activation marker) and decreased FOXP3+ (T-regulatory cell marker) in TDLN were observed. Consistent with this, pOX40L alone or in a combination of siIDO treatment could prolong overall survival, resulting in complete tumour regression and the formation of immunological memory in tumour rechallenge models. This was not the case with non-specific constructs. Our results suggest in situ administration of plasmid DNA and siRNA combinations using lipid nanoparticles is a promising approach for cancer immunotherapy.

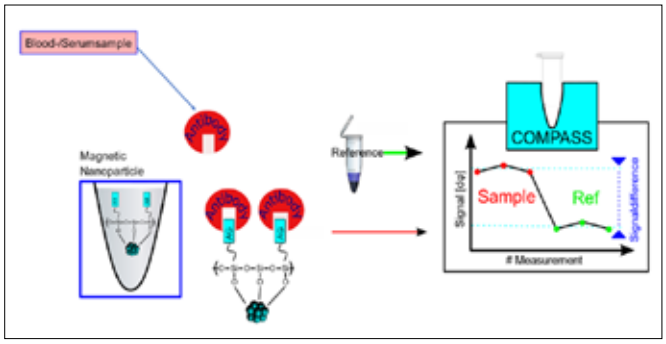


An overview of the work hypothesis and main findings are shown in the above scheme.

NEW POTENTIAL OF SPIONS FOR DIAGNOSTIC PURPOSES

CHRISTOPH ALEXIOU, Department of Otorhinolaryngology, Head and Neck Surgery, Section of Experimental Oncology and Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung Professorship, Universitätsklinikum Erlangen, 91054 Erlangen, Germany.

A precise diagnostic is of indispensable importance for a successful guidance of the therapist. Screening of blood components and detection of corresponding disease markers is routinely used in high-throughput laboratories. Current bioassays for detection of antibodies or antigens such as ELISA (Enzyme-linked Immunosorbent Assay) or Chemo luminescent Immuno Assays (CLIA) are relatively inflexible, expensive, time-consuming and sample preparation requires many purification steps to suppress matrix interference.
Taking advantage of the magnetic properties of iron oxide nanoparticles offers new possibilities. Upcoming methods, such as ACS (AC susceptometry) or MPS (Magnetic Particle Spectroscopy), exploit the magnetization response of functionalized magnetic nanoparticles (MNP) ensembles to assess specific information about the MNP mobility as well as conjugations of chemical or biological compounds on their surface. Both methods have shown promising results in the past but cannot reach the sensitivity of above-mentioned immune-chemical methods.
Recently, in close collaboration with physicists from Würzburg University, we have developed a new method based on a modified MPS being sensitive to minimal changes in mobility of MNP ensembles. It is based on the superposition of a permanent magnetic field while at the same time an alternating magnetic field is applied to the particle ensemble. This results in critical points at the higher harmonics which are extremely sensitive with phase jumps to minimal changes at the particle surface. This facilitates robust and easy-to-handle measurements of analytical targets after linking bait molecules on particle surfaces. As an example, we detected SARS-CoV-2 antibodies binding to the S1 antigen on the surface of functionalized MNPs. Without any purification or incubation, we could show a sensitivity of less than 7pM of SARS-CoV-2 antibodies in samples.



DESIGN AND DEVELOPMENT OF A DRUG DENDRIMER CONJUGATE

MARIANNE ASHFORD, Advanced Drug Delivery, Pharmaceutical Sciences, AstraZeneca, R & D, UK.

We designed a drug dendrimer conjugate to overcome the tolerability issues and improve the therapeutic index of a potent dual Bcl-2/Bcl-xL inhibitor. This class of drugs is expected to deliver therapeutic benefit in many hematological and solid tumors, but their clinical application has been limited by tolerability issues. AZD4320, a potent dual Bcl-2/Bcl-xL inhibitor was chemically linked to Starpharma's DEP® dendrimer platform, a 5th generation PEGylated poly-lysine dendrimer via a hydrolytically labile linker (Patterson et al, 21). This resulted in an improved therapeutic index pre-clinically and enabled progression of this Bcl-2/Bcl-xL inhibitor into clinical development.
This presentation will briefly summarise the design of the drug dendrimer conjugate, some its physicochemical properties and characterization as well as some of its early pharmaceutical development. It will describe some of the considerations as to the positioning of a drug dendrimer conjugate as a complex API and how we approached understanding the molecule and assessing its likely critical quality attributes. It will also show how this differs from some other nanomedicines and how this should help both later development and commercialization of this type of nanomedicine.
Patterson, C.M., Balachander, S.B., Grant, I. et al. Design and optimisation of dendrimer-conjugated Bcl-2/xL inhibitor, AZD0466, with improved therapeutic index for cancer therapy. Commun Biol 4, 112 (2021). https://doi.org/10.1038/s42003-020-01631-8

THE WORLDWIDE IMPACT OF NANOMEDICINES FOR RARE AND NEGLECTED DISEASES

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Rare (orphan) diseases are chronic and progressive severe conditions affecting a small number of patients. On the other hand, neglected tropical disease (NTD) is a group of 20 deadliest parasitic and bacterial diseases endemic to the developing countries of Latin America, Africa and Asia, and affects more than one billion people. Rare and neglected diseases present a significant public health challenge. Some of the challenges that militate against treating these diseases are lack of approved therapy, late diagnosis, high toxicity and low bioavailability of available treatment options. The financial difficulties encountered in new drug research and development for a limited patient population have deterred pharmaceutical industries from venturing into this area. However, the advent of nanomedicine, a rapidly evolving and emerging branch of medicine with diagnostic, therapeutic and theranostic applications, has revolutionalized the outlook of various diseases, including rare and neglected diseases. There is a paradigm shift from developing newer drugs to using nanocarriers in delivering old and potent drugs, thereby saving costs. The great potential of nanomaterials and nanodevices in diagnosing and treating these diseases has been reported. This summary provides an overview of rare and neglected diseases and highlights the impacts of nanomedicine in diagnosing and treating these diseases. It also focuses on the applications of different nanosystems, the preclinical and clinical studies, challenges and future perspectives for rare and neglected diseases. Such delivery systems as nanoemulsions, lipid nanoparticles, liposomes, polymeric nanoparticles, organic and inorganic nanoparticles etc, which provide the platform for the oral, topical and parenteral delivery of actives for treating rare and NTD will be highlighted.

Keywords: rare diseases, neglected diseases, nanotechnology, nanomedicines

SCIENCE, PSEUDO-SCIENCE, FALSE, AND FAKE "SCIENCE"

LOU BALOGH

Scientific communication has numerous problems today. Some are due to external factors, such as the computer revolution and the internet. Others are caused by conflicts between the interests of science, authors, institutions, and business, not to mention the influence of politics. Publications are not science; they are yesterday's information and knowledge, organized, stored, and shared on various media, and not everything is true just because it appears in print. In addition to existing challenges, recently conversational AI appeared and changed our communication forever. It is important for authors to understand these issues, be aware of their solutions, and learn what publishers do for publications, and consider what they can do.

NANO-LIPOSOMAL-CANDESARTAN EFFECT ON TUMOR MICROENVIRONMENT ENABLED THE EFFICACY OF IMMUNE CHECKPOINT INHIBITORS IN MICE 4T1 MODEL

YECHEZKEL BARENHOLZ[1], Ahuva Cern[1,] Zhanna Yehtina[1], Atara Hod[1], Daniel Zilbersheid[1], Emmanuel Loeb[2],

1 Laboratory of Membrane and Liposome Research, Department of Biochemistry, The Hebrew University of Jerusalem, Israel

2 Patho-Logica, Ness Ziona, Israel

The accepted paradigm of nanomedicine," enhanced permeability and retention" (EPR) effect assumes that anticancer drugs can be delivered selectively to tumors using nanomedicines which should increase efficacy and minimize adverse effects. However, in "real-life" while indeed side effects were reduced, the improvements in the patients' survival is only modest. By contrast, immune-checkpoint inhibition (ICI) has provided unprecedented improvements in survival outcomes of a subset of patients. However, ICI is currently estimated to benefit <13% of patients with cancer. These disappointing findings may be related to the nature of tumor microenvironment (TME) which prevent the drugs and nano-drugs distribution in the tumor. Modulating ("normalization") the TME structure and pathophysiology may therefore improve nano-drugs and ICI distribution in the tumor and improve efficacy. One proven approach to affect TME is by Angiotensin Receptor Blockers (ARBs). ARBs are a family of drugs used to treat hypertension. ARBs are also known for their ability to manipulate the TME and lead to improved response to cancer treatment. This activity was demonstrated in several cancer models. In addition, data from a series of retrospective studies involving patients with different cancer types as well as a prospective phase II trial involving patients with locally advanced pancreatic ductal adenocarcinoma, showed that ARBs have the potential to extend patients' survival. However, ARBs lower blood pressure limiting their use in cancer therapy. Our computational approach suggest that several ARBs are excellent candidates for nano-liposomal delivery. Therefore, we suggest overcoming the reduction in systemic blood pressure using PEGylated nano-liposomal loaded with candesartan, a potent ARB. Our nano-liposomal candesartan formulation demonstrated no release in serum, and it didn't affect mice mean blood pressure we demonstrated substantial candesartan concentrations in tumors. In vitro studies further showed release of candesartan from the liposomes in the presence of tumors. Finally, efficacy studies in 4T1 model showed that nano-liposomal candesartan by itself inhibit tumor growth and it also enabled the activity of ICI in this model (ICI alone were inactive in this model). Histopathology of the tumors confirmed that nano-liposomal candesartan normalized the TME compared to control mice resulting in the efficacy of ICI in this model.

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MICROTISSUE APPROACH TO UNRAVEL MECHANOBIOLOGY AND ECM-CELL DYNAMICS DRIVING TISSUE GROWTH: PAVING THE PATH FOR FUTURE NANOMEDICINE INTEGRATION

MARIO BENN

Tissue growth is essential for multicellular life and delicately organized across length-scales by spatio-temporally controlled cell proliferation and differentiation until homeostasis is reached. As the cells are embedded in extracellular matrix (ECM), which they synthesize and remodel, tissue growth and remodeling processes can only be understood if the intimate crosstalk between cells and their environment is revealed. Little is known how ECM gradients are formed, or remodelled, and how these gradients steer cell phenotype transitions during tissue growth. Myofibroblastic phenotypes play central roles in tissue growth, but are also associated with many inflammatory or fibrotic diseases. How myofibroblasts orchestrate tissue growth processes, and how the ECM vice versa facilitates their disappearance or drives persistent myofibroblast activation and disease progression is still elusive. As 2D cell culture approaches do not resemble the biophysical characteristics of tissues, we developed a 3D µTissue platform that allows to investigate tissue growth and maturation processes at high spatiotemporal resolution. We asked how tissue growth and maturation is regulated by the tensional state of the ECM fibers which they produce, cell phenotype transitions and certain transiently expressed ECM components. Using de novo grown µTissues, we identified crucial sequential events that steer tissue growth and maturation, the latter being associated with a disappearance of myofibroblasts. Understanding how ECM gradients regulate tissue growth and the cell phenotype transition is crucial to developing novel treatment strategies to optimize wound healing, and counter fibrosis and cancer progression.

RECENT DEVELOPMENTS AT THE EUROPEAN PHARMACOPEIA RELATED TO NANOMEDICINES

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gerrit.borchard@unige.ch

The European Directorate for the Quality of Medicine & Healthcare (EDQM) contributes to the access to high quality medicines and healthcare by elaborating of official standards for the manufacture and quality control of medicines in the form of the European Pharmacopoeia (Ph. Eur.). In this context, EDQM organized a symposium on existing and potential future activities in the field of nanomedicines in June 2022. Activities of EDQM with respect to iron carbohydrates were presented, and challenges in the area of mRNA vaccines discussed.

One of the outcomes of the symposium was the renaming of the existing working party on non-biologic complexes, which has been elaborating a monograph on iron sucrose concentrated solution, to "Nano working party", indicating to broaden the scope of the working party to other nanomedicines. A second consequence was the suggestion to establish a working party on mRNA vaccines for human use, which was accepted by the Pharmacopoeia commission soon after. Following a call for experts, a working party of now 45 global experts from regulatory authorities, academia, and industry was established. Upon internal discussions, the working party decided to first focus on the drafting of three texts concerning the DNA template for the creation of the mRNA, the drug substance, and the drug product of such medicines. Holding several meetings both online and face to face, the working party has already made significant progress in the revision of these texts, which will be published for public comment soon.

The mRNA vaccine working party at EDQM represents a perfect example for the coordinated global effort to assure the quality of these so-called "information medicines" going forward.

BEYOND ACADEMIC PUBLICATIONS: DEMOCS GAMES AND PUBLIC COMMUNICATION

DONALD BRUCE, Managing Director, Edinethics Ltd., Scotland (UK).

Are we communicating enough? With increased emphasis by funders on dissemination and "impact", the primary system of papers written in academic journals leaves a large gap. Papers are read by a select few in the same research field, but remain unseen (and incomprehensible) for most other people. In animal research, the EU and UK legislations require the publication of a "Non-technical Summary" of the project, capable of being understood by a lay person. But these summaries have been widely criticised as of poor quality and much too technical in style and content, and seen by relatively few people who have an active interest.

Popular science journals like the New Scientist serve a role in bringing the more important studies to a much wider audience, but still to a largely scientifically-literate readership. Press releases reach the general population if newspapers or other media pick up the story, but are always translated into a "media-speak model", where the imperative is to grab headlines and capture attention. Even the best newspapers or programmes are apt to give a more sensational (positive or negative) presentation than perhaps the work justifies and at worst can just mislead people. For example, if asked what the famous cloned sheep Dolly died of, the common answer is premature ageing, not a respiratory disease caught from another sheep. Cloning indeed showed that a lot of people's understandings of science are much influenced by world of film, where scientific dissemination is seen through the lens of the artistic conceptions of the screenplay writer and director. A well researched TV documentary can be an effective communication tool, but few researchers get such an opportunity, and it is somewhat subject to the angle the producer decides to take. An imaginative dissemination via social media may succeed in getting people talking, but now anyone is the expert and misinformation can go viral. So as soon as it is mediated, pure science ceases to be pure, because other humans transmit it.

Many of these media are linear communications to people who just receive the data transmitted to them. The crisis of the sudden rejection of genetically modified food in early 1999, highlighted the need for an interactive public engagement, in which people get to air their views and discuss them with scientists and policy makers. In some fields this has become de rigeur.

Nanotechnology was seen as the first new engagement opportunity, aiming to avoid it becoming "the next GM". It turned out to be hard to do, because, despite being now so widely used, nanotechnologies still suffer from being unfamiliar to publics, and the "nano" concept is difficult to grasp beyond just being about something very small. Like genetic modification, it raised the concerns of "do you really know what you are doing when you manipulate matter at such fundamental levels?" and "do we really need this?" Attitudes may also depend on the application. Nanomaterials in inanimate engineering applications do not usually raise public outcry. In nanomedicine the public desire to cure disease works in favour of (conditional) acceptance, but in food, people may be much more wary. Sick people need a treatment, but most of our food seems to be OK, so "if it ain't broke don't try and fix it".

Public engagement exercises usually reach relatively a few people who happen to be asked to answer a survey or are invited to take part in a focus group exercise. People are busy and the issue under discussion may not be high on their agenda, and these exercises are expensive, so their reach in the population is not wide. They are primarily used as representative samplings.

In contrast, the Democs card game concept seeks to address engaging publics in a different way. It asks in what natural contexts are people meeting in small groups, and can we invite them to play a card-based discussion together to explore an issue like, say, using nanotechnology to detect and maybe prevent atherosclerosis? Most people are aware of the possibility of sudden heart attacks or strokes among family or friends, so the topic is highly relevant, but what is this nano stuff about? We have found that discussing an

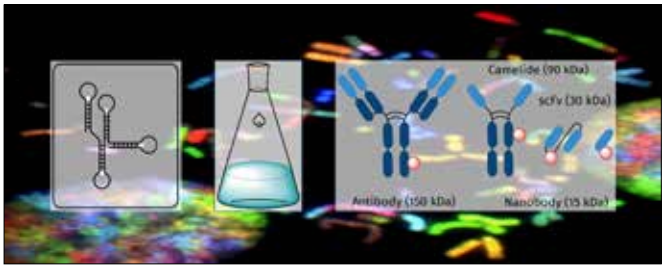
unfamiliar technology in a small group of 6-8 people is much more effective than singly, because shared insights of others adds fresh colours to our ideas.
The Democs idea is to help people to develop informed opinions on a new subject by discussing together the content and issues raised by a set of cards produced by experts, but written in lay language. Story cards, information cards and issue cards are chosen by the group members from a bank of available cards and debated and discussed. The group is asked to express its views on the issues in the form of cluster cards, and each individual is offered the chance to give their opinions in writing on different applications, policies or questions. The primary purpose of the game is to get a group of people to discuss the subject, but the data can also be gathered and analysed along with other groups' outputs, seeking patterns from the qualitative information and insights from the players. Unless a large number of games are played to even out biases in the data, the outputs must be treated with caution, but the groups in a Democs exercise are arguably more truly realistic than an artificially created "representative" focus group of the population.
Democs has for many years been in producing effective engagement with wider publics on emerging scientific and technical developments. Its small scale means it suffers the generic problem of all public engagement exercises. To reach large numbers of the population would require a large public awareness campaign to publicise the existence of a game, and to grab people's imagination. But our experience shows that where people hear about Democs games and play them, it works.

LINKEROLOGY® - PREPARING CARGOS FOR CONJUGATION: A SURVEY FROM BIOENGINEERING TO SURFACE MODIFICATION

THOMAS BRUCKDORFER, CSO & VP Business Development, thomas.bruckdorfer@iris-biotech.de, Iris Biotech GmbH, Germany, www.iris-biotech.de

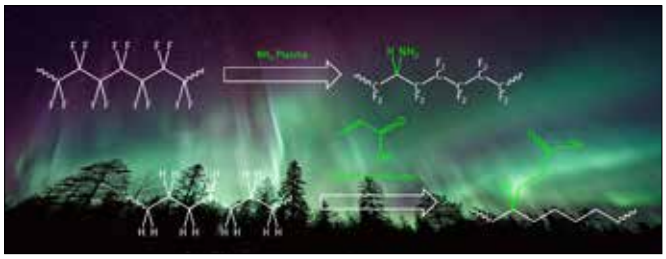
At Iris Biotech, we are experts in the field of **linker technologies** (= Linkerology®), i.e. in the conjugation of any type of cargo molecule to any type of carrier by permanent or cleavable/self-immolative linkers. The demand for **sophisticated conjugation** continues to grow, especially due to the advancement of antibody-drug conjugates in clinics. And there are more applications to be discovered! In our presentation, we spotlight two extremes: cell-free *de novo* synthesis of modified proteins and functionalization of inert surfaces by plasma treatment.

Example 1: cell-free production of antibodies, antibody fragments and proteins:



In cell-free synthesis, microsomal vesicles natively present in CHO cell-free lysate are enriched. De novo protein synthesis is then performed in coupled transcription/translation reactions. The unique advantages of this open system are easy handling, scalability, high yields, and HTS-compatibility. It allows rapid synthesis of all kinds of antibody formats, challenging membrane proteins, toxic proteins, and protein-conjugates. Mutations and non-canonical amino acids can easily be introduced allowing for subsequent introduction of linkers and cargo molecules.

Example 2: surface preparation of plastic polymers and decoration with linkers and biomolecules:



From inert to interactive – polymers are normally inert to chemical reactions and any covalent attachments. With the help of plasma technology specific functional groups, such as amine or carboxylic acid can be implemented on formerly inert materials, like poly(tetrafluoroethylene) or poly(ethylene). In a subsequent step any compound from small molecules, to peptides, proteins, nano- or antibodies, can covalently bound to the surface and prepared for cell growth. Specific bio-coating, bio-decoration, bio-masking of formerly inert and bio-incompatible surfaces can be achieved. With the aid of XP spectroscopy, a detailed analysis of down to 10 nm of a surface layer including mapping across the area can be performed.
We offer our expertise as CRO in cargo preparation and Linkerology® for any appropriate project.

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CHALLENGES IN CLINICAL DEVELOPMENT OF NANOMEDICINES

BASTIAAN BUDDINGH

The field of nanomedicines is rapidly evolving due to the unique pharmacokinetic and therapeutic effects nanoparticles offer. These include enhanced dissolution rate and oral bioavailability, targeted delivery, enhanced efficacy and reduced toxicity. In addition to the evaluation of nanomedicines for treatment of diseases such as cancer and autoimmune disorders, nanoparticles are also explored for imaging purposes. However, the control of materials in the nanometer size range requires scientifically demanding chemistry, analysis and manufacturing techniques. Therefore, these aspects often pose significant challenges in the clinical development of nanomedicines and require a carefully planned development scheme executed by scientists experienced in these typical nanoparticle-

specific challenges. Ardena is an organization that recognizes these challenges and is at the forefront of the nanomedicine landscape. We provide our expertise as a contract development and manufacturing organization to navigate nanomedicine products into the clinic.

ANATOMICAL AND CELLULAR BARRIERS FOR TARGETING PATHOGENS IN RODENT AND HUMAN TISSUES

DIRK BUMANN, Biozentrum, University of Basel (CH)

Bacterial infections are a major threat to human health worldwide. Increasing antimicrobial resistance of major pathogens severely limits treatment options and endangers many aspects of modern medicine including surgery, cancer treatment, and intensive care. Novel approaches to control bacterial infections are urgently needed. Non-conventional strategies based on monoclonal antibodies, bacteriophages, phage-derived endolysins, and nanoparticles present fascinating new opportunities. However, a key requirement for these as well as more conventional antibiotics is the ability to reach the bacteria in the infected tissues. This can be challenging because of anatomical barriers. Moreover, many pathogens can reside in infected human cells restricting their accessibility. It is thus vital to determine the localization of bacterial pathogens in the infected tissue.

Although microscopy has been used to detect pathogens in tissue sections for more than 100 years, these observations are usually only anecdotal because only a few bacteria from individual patients are visualized. A more representative overview is challenging because it requires detection of micrometer-sized bacteria in centimeter-sized tissues with complex three-dimensional structures.

In our lab, we have developed novel methods to localize thousands of Salmonella with single-cell resolution and sensitivity in whole-organ tomograms of spleen, liver, and lymph nodes in a mouse model of typhoid fever. All these bacteria reside inside host cells. Some Salmonella migrate to a special compartment of the spleen, the so-called white pulp, where they are inaccessible to macromolecules in the blood circulation. Even conventional antibiotic therapy fails against this Salmonella subset in the white pulp because of lacking support by local inflammation.

We recently extended our research to Staphylococcus aureus-infected human patients. In collaboration with surgeons and infectiologists at the University Hospital Basel we acquired and analyzed dozens of biopsies from deep-seated infections. These infections are particularly relevant because of frequent treatment failures. We again developed novel imaging techniques to detect individual bacteria in centimeter-sized biopsies. Our results from ~12,000 localized S. aureus cells in biopsies from 22 patients show that the staphylococci reside largely intracellularly in human monocytes.

These comprehensive in-vivo datasets reveal relevant anatomical and cellular barriers that need to be overcome to develop urgently needed novel and efficacious control strategies for bacterial infections.

CHARACTERIZATION OF QUALITY ATTRIBUTES OF NANOVACCINES FOR COVID-19

LUIGI CALZOLAI, European Commission, Joint Research Centre (JRC), Ispra, Italy

Several vaccines against COVID-19 use nanoparticles to protect the antigen cargo (either proteins or nucleic acids), increase the immunogenicity and ultimately the efficacy. The characterization of these nanomedicines is challenging due to their intrinsic complexity and requires the use of multidisciplinary techniques and competencies. We have recently proposed a general strategy for their accurate pre-clinical characterization based on combination of physicochemical, immunological and toxicological assays [1].

In this presentation I will focus on mRNA vaccines and on the identification of key quality attributes, the available analytical techniques for their measurement and on some open questions. The availability of accurate methods for the characterization of these complex nanomedicines will help to address some challenges in the field and will guide the rapid development of safe and effective vaccines for current and future health crises.

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INFLAMMATION AND PAIN: NOVEL NANOTHERAPIES

PATRICK COUVREUR, J. Feng, F. Dormont, M. Varna, S. Lepêtre
Institut Galien Paris-Sud, UMR CNRS 8612, Université Paris-Saclay, Orsay Cedex, France.

If nanotechnologies have significantly improved the delivery of drugs, the currently available nanomedicines have not yet been able to apply to many drugs and diseases, due to the poor drug loading and the often uncontrolled “burst release” of the loaded drug after administration. And this explains the relatively low number of nanomedicines on the market, although the use of lipid nanoparticles for mRNA COVID-19 vaccination has recently boosted the field. In this context, we discovered the so-called “squalenoylation” concept, based on the surprising observation that the chemical conjugation of a drug to the squalene, a natural and biocompatible lipid, resulted in amphiphilic molecules with spontaneous self-assembling properties under the form of discrete nanoparticles (100 nm), without the use of any other excipient. This was attributed to the unique folded conformation of the squalene in aqueous media, allowing specific supramolecular chemistry organizations. This prominent nanotechnology is clearly going beyond the state-of-the-art by shifting from the commonly used “physical” drug encapsulation process into nanoparticles, to the “chemical” encapsulation, which enable to dramatically increase the nanoparticle drug payload and avoiding the drug “burst” release, the two major aforementioned limitations in the nanomedicine field. Due to its flexibility and generic character, this breakthrough nanomedicine approach has found applications for the pre-clinical treatment of many severe diseases, including cancers[1,2], resistant intracellular infectious diseases[3], and neurological disorders, like stroke and spinal cord injury[4].

In this presentation, we will also show that the rapidly metabolized Leu-enkephalin neuropeptide may become pharmacologically efficient owing to a simple conjugation with the lipid squalene . The Leu-enkephalin neuropeptide was included into the nanoparticles by conjugation to squalene using three different chemical linkers (i.e., dioxycarbonyl, diglycolate, and amide bond). These new squalene-based nanoformulations prevented rapid plasma degradation of Leu-enkephalin and conferred to the released neuropeptide a significant anti-nociceptif effect in a carrageenan-induced paw oedema model in rats (Hargreaves test) which lasted longer than after treatment with morphine.

Pretreatment with opioid receptor antagonists such as naloxone (brain-permeant) and naloxone methiodide (brain-impermeant) reversed the nanoparticles induced anti-hyperalgesia, indicating that the Leu-enkephalin-squalene nanoparticles acted through peripherally located opioid receptors. Moreover, the biodistribution of fluorescently labelled Leu-enkephalin nanoparticles showed a strong accumulation of the fluorescence within the inflamed paw, while no signal could be detected in the brain, confirming the peripheral effect of the nanoparticles. Thus, the Leu-enkephalin-

Squalene nanoparticles demonstrated the ability to use the enhanced permeability and retention (EPR) effect to specifically deliver the neuropeptide into inflamed tissues for pain control without tolerance and addiction as observed with morphine and opioid derivatives.

By following the same way of reasoning, a novel multifunctional nanoparticle formulation simultaneously delivering adenosine and tocopherol has been developed to treat uncontrolled inflammation and sepsis in mice[6]. In fact, out-of-control inflammation is often caused by feedback loops between pro-inflammatory signalling and an imbalance of free radicals and antioxidants. By taking advantage of the EPR effect due to the inflammatory process, delivering the drugs in tandem to targeted sites, was expected to bolster each drug's effectiveness. Indeed, adenosine has been shown to fight inflammation effectively, but not without serious side effects when administered systemically at high doses (which is needed due to the very short half-life of this molecule, ie., 10 sec). To reduce side effects and build on adenosine's potential, the multidrug nanoparticles have been prepared by conjugating adenosine with squalene and encapsulating the pair with alpha-tocopherol as an anti-oxidant. The efficacy of those "multidrug" nanoparticles have been evaluated in mice injected with lipopolysaccharide (LPS), triggering a cascade of inflammation, and resulting in a potentially lethal "cytokine storm". Mice that received the multidrug nanoparticle injections showed a significant decrease in inflammatory tumour necrosis factor alpha and an increase in anti-inflammatory interleukin-10. The measure of pro-inflammatory cytokines in the mice's organs demonstrated that the lungs, the liver, and the kidneys displayed significantly reduced levels, 4h after treatment. While nanoparticles with only squalene-conjugated adenosine or tocopherol free were poorly effective at warding off inflammatory cytokines, the multidrug treatment proved most powerful. Noteworthy, only the group of mice treated with the multidrug nanoparticles allowed 100% mice survival, which was not the case with all the other control treatments (drug free cocktails, one drug only treatments or untreated animals).

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IN USE STABILITY OF COVID-19 VACCINES

DAAN CROMMELIN

The present mRNA-vaccines against COVID-19 must be stored at subzero conditions. Upon thawing these products should be handled by a health care professional to prepare them for intramuscular injection. Insert texts describe these preparatory steps in detail (e.g. 1). The question is whether the proper products are administered to the patient. Little information on this subject is available in the public domain. But, the publications (e.g. 2,3) that have appeared indicate that the components of mRNA-lipid nanoparticles may be chemically stable within the boundaries of the different storage conditions, but that the physical stability is readily jeopardized, i.e. aggregation and/or mRNA loss from the LNP occur(s). No data is available on the consequences of these drug product changes to the mRNA-LNP performance *in vivo*.

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THE STORAGE AND IN-USE STABILITY OF MRNA VACCINES AND THERAPEUTICS: NOT A COLD CASE

DAAN CROMMELIN

A drawback of the current mRNA-lipid nanoparticle (LNP) COVID-19 vaccines is that they must be stored at (ultra)low temperatures. Understanding the root cause of the instability of these vaccines may help to rationally improve mRNA-LNP product stability and thereby ease the temperature conditions for storage.

In this presentation the proposed structures of mRNA-LNPs, factors that impact mRNA-LNP stability and strategies to optimize mRNA-LNP product stability will be discussed. Analysis of mRNA-LNP structures reveals that mRNA, the ionizable cationic lipid and water are present in the LNP core. The neutral helper lipids are mainly positioned in the outer, encapsulating, wall. mRNA hydrolysis is the determining factor for mRNA-LNP instability. It is currently unclear how water in the LNP core interacts with the mRNA and to what extent the degradation prone sites of mRNA are protected through a coat of ionizable cationic lipids.

To improve the stability of mRNA-LNP vaccines, the mRNA nucleotide composition and structure should be carefully designed. Secondly, a better understanding of the milieu the mRNA is exposed to in the core of LNPs may help to rationalize adjustments to the LNP structure to preserve mRNA integrity. Moreover, drying techniques, such as lyophilization, are promising options to be further explored.

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DESIGN OF LIPID NANOPARTICLES THAT ENABLE GENE THERAPIES

PIETER CULLIS, University of British Columbia

Gene therapies employing genetic drugs such as small interfering RNA (siRNA) for gene silencing and messenger RNA (mRNA) for gene expression have the potential to cure most diseases. However, sophisticated delivery systems are required to enable the therapeutic use of nucleic acid polymers as they are quickly broken down in biological fluids, do not accumulate at sites of disease and cannot penetrate into target cells even if they arrive at target tissues. Lipid nanoparticle (LNP) technology is increasingly enabling the clinical potential of genetic drugs by packaging the nucleic acid in well-defined nanoparticles that protect the payload following systemic *in vivo* administration and facilitate intracellular delivery following uptake into target cells by endocytosis. The first clinical validation of this approach was achieved by the approval of Onpattro by the US FDA in 2018 to treat the disease transthyretin-induced amyloidosis (hATTR. Onpattro consists of LNPs containing siRNA that silences production of transthyretin in the liver (in hepatocytes) following intravenous administration. In this talk I will describe the historical development of LNP systems leading to the development of Onpattro and how related LNP delivery technology is being employed to enable many mRNA-based gene therapy drugs. A notable example of the success of this approach is the development of Comirnaty, the Pfizer/BioNTech COVID-19 mRNA vaccine, which has played a leading role in alleviating the Covid-19 pandemic.

WHAT CAN BE LEARNED FROM BLANK LIPID NANOPARTICLES: A CLOSER LOOK

RAMIN DARVARI

Lipid nanoparticles (LNPs) are well recognized as vehicles for cytosolic delivery of RNA and have earned their place in the commercial space in the recent years. While understanding of the RNA interaction with the lipid components is critical in design and engineering of effective LNPs, there are other critical aspects of the LNP dispersions that are independent of the RNA payload. This presentation will take a closer look at the use of blank LNPs in expanding our understanding of LNP drug products.

INTRODUCTION AND OVERVIEW OF 10YRS NBCD DISCUSSIONS

JON DE VLIEGER

In the past decades, novel approved therapeutics have become increasingly complex. Next to the well-known class of biologicals, we have also witnessed the advance of synthetic non-biological complex drugs (NBCDs) for many therapeutic areas. The complexity and variety of NBCDs are providing a real challenge for regulatory systems worldwide. Some 10 years ago, the NBCD Working Group was established to identify and discuss regulatory challenges, to offer insights and suggestions on how to deal with these challenges and to stimulate worldwide alignment on regulatory guidance and practice. This presentation gives a brief overview of the achievements of the NBCD Working Group and provides a brief interpretation of the newly proposed EU Pharma Legislation sections relevant to complex drug products.

HIERARCHICALLY ORGANIZED DELIVERY SYSTEMS FOR BRAIN DISEASES

PAOLO DECUZZI, Senior Researcher and Professor
Director, Laboratory of Nanotechnology for Precision Medicine, Italian Institute of Technology – Genova, Italy

Despite tremendous advancements in targeted therapies and image-guided surgical interventions and radiation therapies, the standard of care for high-grade gliomas has not changed over the past 20 years. Since the introduction of the Stupp protocol in 2005, high-grade gliomas are still treated by maximal safe resection followed by adjuvant radiotherapy and chemotherapy, mostly with temozolomide. This complex and expensive treatment plan provides only a modest improvement in life expectancy (a few months) and various degrees of therapy-induced complications, including the deterioration of physical, emotional, and social functions. Key challenges in the treatment of high-grade gliomas, and brain tumors in general, are related to overcoming unique anatomical barriers, such as the blood-brain barrier and dense tumor-associated extracellular matrix, and biological complexity, including the interplay among neurons, immune and tumor cells. In this talk, a compartmentalized polymeric micro-implant, named microMESH, will be presented for the sustained and localized delivery of a variety of therapeutic molecules, including chemotherapeutic drugs, small inhibitors, antibodies, and nanomedicines, and their combination. Multiple microMESH with different therapeutic configurations will be discussed in terms of fabrication, physico-chemical and mechanical characterization, and pharmacological behavior on tridimensional cancer spheroids. Finally, the preclinical therapeutic efficacy of microMESH will be presented in orthotopic models of glioblastoma obtained in immunodeficient and immunocompetent mice. microMESH has the potential to deliver complex chemo-immunotherapies safely and effectively for the eradication of high-grade gliomas.

FYARRO® – NANOPARTICLE ALBUMIN BOUND SIROLIMUS – A NEWLY APPROVED NEXT GENERATION MTOR INHIBITOR

NEIL DESAI, Founder, Executive Chairman and former CEO, Aadi Bioscience Inc, Pacific Palisades, California, USA

FYARRO® (*nab*-sirolimus) is an albumin-bound nanoparticle version of sirolimus (an mTOR inhibitor) with a nanoparticle size of approximately 100 nm, that can target various tissues based on mechanisms of albumin transport. FYARRO is the latest of the drugs approved utilizing 'nanoparticle albumin bound' technology platform first utilized in ABRAXANE®. FYARRO was approved in late 2021 for an extremely rare, aggressive sarcoma known as Advanced Malignant PEComa in which a significant proportion of patients harbor relevant mutations in the mTOR pathway which are considered as drivers for this disease. The AMPECT registrational trial was the first prospective study in advanced malignant PEComa. FYARRO is also being studied in other cancer indications and is the subject of a new registrational trial (PRECISION 1) for a tumor agnostic indication in *TSC1* and *TSC2* alterations in the mTOR pathway. The results of the AMPECT registrational trial and strategies in other indications will be discussed.

THE NANOPRIMER: A SIGNIFICANT OPPORTUNITY TO BOOST THE EFFICACY OF CANCER VACCINES.

JULIE DEVALLIÈRE

Nanotechnologies are among the most promising novel immune delivery system and possess great potential as therapeutic agents in cancer vaccines, immunotherapy, gene therapy, and personalized medicine. However, their use is still limited by nanoparticle accumulation and sequestration in the liver. Hepatic clearance applies particularly to intravenously administered therapeutics whose efficacy is greatly impeded by low delivery, especially in the case of extra-hepatic targets such as tumors. Moreover, the unintended liver distribution could cause harmful side effects.

Curadigm's innovative Nanoprimer technology seeks to shift the balance of therapeutics bioavailability and toxicity. The platform is designed to decrease liver trapping, increase systemic bioavailability for optimal accumulation in target tissues and reduce potential toxicity [(1)]. This technology has the potential to redefine the benefit/risk ratio of therapeutics, improving their clinical outcomes and treatment value. Since a large panel of therapeutics is cleared by the liver, the Nanoprimer can be widely applied and adapted to work with a variety of therapeutic classes. The Nanoprimer is developed as a platform to be easily combined with different therapeutics (e. g. DNA or RNA encapsulated in viral or non-viral vectors, encapsulated small molecules, or genome editing therapies) at varying stages of development, from highly innovative or novel pre-clinical assets to marketed products.

The Nanoprimer is an engineered, biocompatible nanoparticle that transiently and specifically occupies the liver clearance pathways responsible for sub-optimal therapeutics bioavailability. The Nanoprimer is administered just before the therapeutic and does not contain any active principal ingredient. Based solely on its specific physico-chemical properties, the Nanoprimer transiently occupies cells of the mononuclear phagocytic system, enabling temporary reduction of drug clearance and elimination. The administration is performed sequentially: First, the Nanoprimer is injected, occupying primarily the Kupffer cells and liver sinusoidal endothelial cells, which are responsible for the clearance of many therapeutics (e. g. almost all nanomedicines); then, the therapeutic is injected (figure 1).

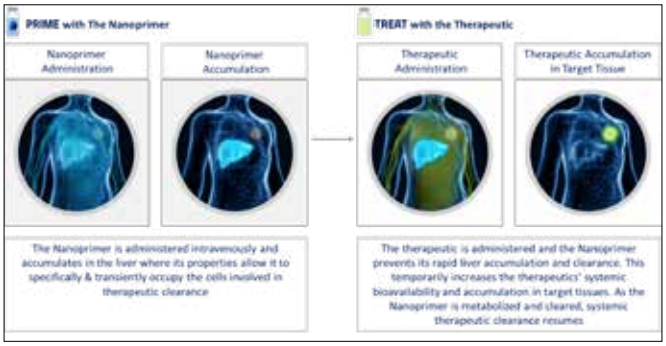

Figure 1: Curadigm's technology: priming the body to receive the treatment.

Proofs-of-concept were realized by combining the Nanoprimer with different therapeutic agents such as irinotecan-loaded liposomes and nucleic acid-loaded lipid nanoparticles (LNP) [(3)]. One example is a study conducted in collaboration with the Langer Lab at MIT that shows that the Nanoprimer increases mRNA- and siRNA-based therapeutics efficiency by 32% and 49% respectively[(2)]. Efficacy studies in triple-negative breast cancer mouse model reveal that the Nanoprimer increases by 2-fold the anti-tumor efficacy of siRNA LNP therapy. Data demonstrate the safety of the Nanoprimer, along with its ability to maximize the inhibition of primary tumor growth and pulmonary metastasis by systemic siRNA LNP treatment.

Finally, in a collaborative study with the NCL (Nanotechnology Characterization Laboratory), the Nanoprimer was shown to stimulate immune response in model vaccine ovalbumin-coated gold nanoparticles (GNP-OVA). Administration of the Nanoprimer before immunization of mice by intravenous injection of GNP-OVA, results in a dramatic increase in anti-OVA antibody levels with IgGs detected as early as 7 days. Furthermore, IgGs and IgMs antibodies remain elevated at day 28 showing a robust and sustainable immune response. Nanoprimer ability to enhance immune response shows its great potential, especially for cancer vaccines.

Altogether, these data demonstrate that the ubiquitous nature of the Nanoprimer mode of action allows its application to a broad spectrum of therapeutics, from nucleic acid-loaded LNP to vaccines. Efficacy studies conducted in various mouse models highlight the potential for the Nanoprimer to empower nanotechnology treatments for a large panel of diseases.

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EMPLOYING MRNA AGAINST CANCER

MUSTAFA DIKEN

Thanks to its favorable characteristics, mRNA is a unique tool for antigen-specific activation of the immune system. Vaccination with antigen-coding mRNA has been shown to be capable of efficiently inducing T and B cell responses paving the way for the first mRNA-based vaccine against SARS-CoV-2. mRNA-based vaccines are currently being tested in several clinical trials with promising results for various indications. This lecture will summarize the evolution of this versatile platform and its use against cancer.

PEPTIDE-BASED NUCLEIC ACID NANOMEDICINES FOR GENE MODULATION IN CANCER – TARGETING KRAS ONCOGENE

GILLES DIVITA & NEIL DESAI, Aadigen, LLC, Pacific Palisades, CA (USA) and Divincell SAS, Nimes (F)

RNA is a class of promising nucleic acid therapeutics to treat a variety of diseases, including genetic diseases. Therapeutic mRNAs constitute a new generation of safer, albeit temporary, treatments to restore functional version of mutated/missing proteins in patients and to facilitate CRISPR based gene editing. However, effective *in vivo* delivery of functional mRNAs and CRISPR machinery to tissues other than the liver remains significant limitation in the gene modulation therapeutic field and requires new improved drug delivery systems

We have developed the ADGN strategy that combines a RNA with a tumor selective peptide nanocarrier, to impair cancer proliferation by either directly editing an oncogene (e.g. *KRAS*) or by rescuing tumor suppressor function (e.g. *P53*) as potential therapeutic approaches in cancers. ADGN-technology is based on short amphipathic targetable peptides that form stable nanoparticles of ap-

proximately 100 nm with nucleic acids through non-covalent electrostatic and hydrophobic interactions. The molecular composition and sequences of ADGN-nanoparticles have been designed to overcome hepatic accumulation and to target tumors or certain organs. ADGN-CRISPR technology was successfully applied to impair cancer cell proliferation by directly targeting G12V, G12D or G12C mutations of the *KRAS* oncogene. Activating mutations in KRAS play potent roles in cancer initiation, propagation, and maintenance. ADGN-nanoparticles efficiently and selectively silenced $KRAS^{G12V,}$ $KRAS^{G12D}$, or $KRAS^{G12C}$ mutated colorectal, pancreatic and lung cancer cells. To date, only treatment targeting $KRAS^{G12C}$ mutation have been successful in the clinic and approved. However, it is hampered by adaptive resistance, mainly associated with the emergence of other KRAS mutations, activation of feedback pathway and high amplification of $KRAS^{G12C}$ allele. ADGN-CRISPR mediated silencing of the KRAS mutations strongly impaired cell proliferation and the phosphorylation of the downstream effector pathways ERK and AKT. We demonstrated that only two intravenous administrations of the $KRAS^{G12D}$ inhibitor ADGN-121 containing CRISPR/gRNAG12D or of the $KRAS^{G12V}$ inhibitor ADGN-122 containing CRISPR/$gRNA^{G12V}$ abolished Panc1 (pancreatic cancer) or SW403 (colorectal cancer) tumor growth in a dose dependent manner, respectively. In contrast, no effect on tumor growth was observed with ADGN-NP containing non-specific gRNA. The combinations of ADGN-121 and ADGN-122 with drugs currently used in the clinic (nab-paclitaxel and capecitabine) showed strong synergy in these models. We showed, that the $KRAS^{G12C}$ inhibitor ADGN-123 containing CRISPR/gRNAG12C can effectively strongly impair the cell proliferation and inhibit the phosphorylation of ERK and AKT in cancer cells with acquired resistance to sotorasib or adagrasib ($KRAS^{G12C}$ inhibitors) and that no resistance occurs after ADGN-123 treatment. The treatments are well tolerated, no sign of clinical toxicity, inflammatory response, off target or emergence of other KRAS mutations was detected after single or repeated administrations.
ADGN-NP can be applied to target driver mutations of cancers *in vivo* and permanently disrupt the oncogenic alleles or to restore tumor suppressor function. These approaches could be used as single agent therapy or in combination together with standard therapies for potent combinatorial cancer treatment.

ASSESSING NANOPARTICLES IMMUNOTOXICITY IN THE 21ST CENTURY: CELLS, ANIMALS AND BEYOND

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Immunotoxicity of drug products includes adverse effects on the structure and function of the immune system and may also affect other organs as a result of immune dysfunction. The current evaluation of immunotoxicity is based on a traditional framework of *in vitro* and *in vivo* methods aimed at identifying target cells and organs and uncovering the underlying causes and mechanisms. While being instrumental in identifying immunotoxic drugs, the traditional framework has some limitations. The increasing complexity of modern drug products further compounds this issue. Therefore, assessing the immunotoxicity of nanotechnology-based products in the 21st century requires unprecedented approaches and interdisciplinary efforts, which in turn, rejuvenate the old concept of methods harmonization and standardization. In this presentation, I will discuss case studies from the experience of the Nanotechnology Characterization Laboratory covering the application of both traditional (*in vitro* and *in vivo*) methods and novel approaches, including tissue co-cultures, machine learning and artificial intelligence.

INTRODUCTION PHOSPHOLIPID RESEARCH CENTER

SIMON DRESCHER

This talk shortly introduces the Phospholipid Research Center (PRC) Heidelberg, Germany, summarizes its vision and mission, and promotes and provides a foundation for the use of phospholipids in pharmaceutics. The different PhD and postdoc projects funded by the Phospholipid Research Center cover all facets of phospholipid research: from basic to applied research, including the use of phospholipids in various forms of application such as liposomes, mixed micelles, emulsions, extrudates, self-emulsifying systems, and lipid nanoparticles up to industrial application-oriented research. The projects also include all route of administration, namely topical, oral, and parenteral. PRC—Connecting the world of phospholipids.

THE TRIPLE EFFECT OF NEOADJUVANT IMMUNOTHERAPY: MORE CURES, SHORTER TREATMENTS, LESS SURGERY

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The Neoadjuvant Immunotherapy Revolution: One of the most important current developments , that will profoundly change patient management in multiple tumor types, is the application of anti-PD1 based neoadjuvant immunotherapy or immuno-chemotherapy approaches.
Landmark developments in Cutaneous Melanoma (CM): In the landmark paper by Blank and coworkers in Nature Medicine in 2018, they demonstrated that patients with macroscopic stage III melanoma treated with neoadjuvant combination immunotherapy with ipilimumab (I) + nivolumab(N) increased T-cell clone diversity as well as T-cell clones amplitudes compared with patients treated adjuvantly after lymph node dissection. Neoadjuvant I+N in the Neo-Opacin trial demonstrated 70% pathologic CR (pCR) / near pathologic CR (npCR) rates and < 5% relapse rates in these stage III patients. Moreover the subsequent PRADO trial showed that pCR/npCR patients did *not need a therapeutic lymph node dissection*, and *no further adjuvant therapy*. Thus neoadjuvant I+N in macroscopic stage III melanoma is “recipe for cure” : more cures, fewer treatment cycles, less sugery. Pooled analyses of multiple neoadjuvant immunotherapy trials in melanoma have confirmed the above observations. **Non-Melanoma Skin Cancers**: Similarly impressive activity of neoadjuvant therapy with cemiplimab (anti-PD1) has been reported in patients with >50% pCR rates **in** stage II-IV locally advanced cutaneous squamous cell cancers in the head and neck area. **Lung Cancer:** In 358 randomized patients in the Checkmate-819 trial the neoadjuvant combination of nivolumab with platimum-based chemotherapy was superior compared with chemotherapy only: pCRs were observed in 24.2% (Immuno-Chemo) vs 2.2% (Chemo), with a significant impact on Eventfree Survival with 31.6 mts (Immuno-Chemo) vs 20.8 mts (Chemo), Hazard Ratio (HR) 0.63; p = 0.005, and a significant improvement of Overall Survival, HR 0.57, P=0.008. Similarly impressive results were recently published regarding the the KEYNOTE 671 trial in early stage NSCLC comparing neoadjuvant pembrolizumab+chemotherapy vs chemotherapy followed by post resection adjuvant pembrolizumab or placebo. **Esophageal-GEJ and Gastric Cancers:** In 677 surgically treated patients in 21 studies, a 52% major pathological response and a 29.5% pCR was reported. in MSI DNA-repair deficient gastric/gastric-esophageal junction cancers (dMMR), pathologic responses were observed in 58.6% of resected patients. Moreover an addi-

tional 10% of patients refused surgery because of endoscopic biopsy proven pCRs. **Muscle Invasive Bladder Cancer (MIBC):** In a meta-analysis including 843 patients in 22 studies a 24% pCR rate for anti-PD1 monotherapy and 32% for anti-PD1+anti-CTLA4. Immunochemotherapy resulted in 42.6% pCR rates. **MSI-Colorectal Cancer: C**halabi et al.reported extraordinary results of neoadjuvant I+N in this setting: close to 100% major pathologic response rates in T3-T4 MSI colorectal/rectal cancers with an extraordinary pCR in 69% and zero relapse rates at 13 months follow up. A recipe for cure and the end of programming rectal cancer resections in these patients. Cercek et al. reported a 100% pCR rate in 12 consecutive patients with locally advanced MSI rectal cancers with no relapses at a minimal follow up of 12 months.**Triple Negative Breast Cancer (TNBC):** The KEYNOTE-522 trial demonstrated significantly improved pCR rates (64.8% vs 51.2%, p<0.001) and significantly improved Event-free Survival (EFS) rates (84.5% vs 76.8%, HR:0.63, p<0.001) with pembrolizumab containing neoadjuvant immune-chemotherapy compared with neoadjuvant chemotherapy
Conclusion Neoadjuvant Immunotherapy Revolution: Across multiple tumor types neoadjuvant immunotherapy strategies that are anti-PD1 based, improved by anti-CTLA4 or anti-LAG3 and in various tumors to be combined with chemotherapy are currently yielding spectacular progress, defined by more cures, less surgery and shorter treatments, and are thus redefining patient management across multiple tumor types.

PRECISION ARTERIAL DOXORUBICIN DRUG DELIVERY AND TREATING SOFT TISSUE TUMORS: LONG-TERM FOLLOW-UP

ELDAD ELNEKAVE

Desmoid fibromatoses (DFs) are locally aggressive mesenchymal tumors composed of monoclonal fibroblasts within an abundant extracellular matrix. Systemic doxorubicin treatment is effective, but toxic. We investigated arterial doxorubicin eluting embolization (DEE), an approach characterized by high drug concentrations in the tumor alongside limited systemic drug exposure. The primary and secondary endpoints were radiological response using MRI and RECIST 1.1, respectively. The study included 24 patients (median age, 24; interquartile range, 16–34 years). Data were collected prospectively for 9 patients and retrospectively for 15 patients. The most frequent tumor locations were chest/abdomen wall and neck/shoulder/axilla (29% each). Of 24 patients, 7 (24%) were treatment naïve, and 17 (71%) had received one or two prior treatments. Patients underwent a median of two treatments (range, 1–4), with a median of 49 mg (range, 8–75) doxorubicin/treatment. Efficacy outcomes were available for 23 patients. With a median follow-up of 8 months (interquartile range, 3–13), median tumor volumes decreased by 59% (interquartile range, 40–71%) and T2 signal intensity decreased by 36% (interquartile range, 19–55%). Of 23 patients, 9 (39%), 12 (52%), and 2 (9%) had a partial response, stable disease, and progressive disease, respectively. DEE was safe and well tolerated, with one reported grade 3–4 adverse event (cord injury). In conclusion, DEE was safe and achieved rapid clinical/volumetric responses in DFs.

PROBING IMMUNOLOGICAL INTERACTIONS OF TWO-DIMENSIONAL (2D) MATERIALS: GRAPHENE AND BEYOND

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Understanding biological interactions of graphene-based materials and other 2D materials is crucial for assessing their hazard potential following intentional or inadvertent exposure. In particular, interactions between 2D materials and the immune system are of great importance. The immune system is comprised not only of dedicated hematopoietic cells but also includes non-hematopoietic cells such as the epithelial cells in the lungs and gastro-intestinal tract which act not only as barriers but also as sentinels of the "expanded" immune system. Here, a snapshot of studies conducted in the frame of the EU-funded Graphene Flagship are presented. Using RNA-sequencing, we could show that graphene oxide (GO) triggered size-dependent transcriptional effects in non-transformed human lung cells and distinct pathways were activated in cells following short-term *versus* long-term exposure to GO. Furthermore, we have found that GO can trigger type 2 immune responses in the gut, and this occurs *via* an aryl hydrocarbon (Ah) receptor dependent mechanism. We could also show that certain transition metal dichalcogenides trigger trained immunity in macrophages, and evidence was provided for epigenetic signaling as well as metabolic rewiring of the cells exposed to the TMDs.

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FURTHER READING:

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3D PRINTED MEDICAL DEVICES: ISSUES FOR PATIENT SAFETY

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CITATION Ilise L Feitshans "3D Printing of Medical Devices: Issues of Patient Safety" Medicine and Law Journal World Association for Medical Law, Vol 41 March 2022 p53-65, André Dias Pereira Editor.

One consequence of the Covid19 pandemic in 2020 was disrupted supply chains for medical devices. In response, many providers began onsite 3d printing medical devices, without special training or custom tailored instructions. Traditional laws protecting patient safety and tort liability regarding possible malfunction of medical devices are not prepared to address the manufacture of medical devices by enduser health care facilities. The USA Food and Drug Administration (FDA) is one regulatory body that has expressed its desire to validate these uses of 3d printing in emergencies, despite concern about these home-grown devices from the standpoint of patient safety. This article explores the uncharted legal landscape concerning the growing need for national or international regulation addressing 3D printed medical devices in Health care facilities (HCFs) and point of care (POCs) venues. New laws may be needed to protect the integrity of medical products within an overarching duty to protect patient safety.

TARGETING NANOCARRIERS IN VIVO AND MAXIMIZING TUMOR THERAPY EFFECTS WITH ANTIGEN/ADJUVANT COMBINATIONS IN PROTEIN NANOCAPSULES

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Insufficient efficacy of tumor vaccines still represents a major challenge due to poor adjuvant potency and inefficient dendritic cell targeting. Combining antigen and adjuvants of different classes bears the potential to induce a broad spectrum of anti-tumor immune responses. Here we demonstrate a novel nanocarrier (NC)-based vaccine combining the type I interferon-triggering STING agonist diamidobenzimidazole (diABZI) compound 3 and the well-established TLR7/8 agonist resiquimod (R848). Encapsulation of both adjuvants into polymeric nanocapsules enables the simultaneous transport of immunostimulatory molecules with tumor antigens. Thereby achieved co-delivery further improved DC stimulation and subsequent anti-tumor immune responses.

Combined encapsulation of R848 and diABZI enhanced DC activation and induced stronger antigen-specific T cell responses compared to the single adjuvant NC treatment or using soluble forms of antigens and adjuvants *in vitro* and *in vivo*. This was determined by the vigorous expression of CD80, CD83, and CD86. Furthermore, the dual adjuvant therapy initiated the highest secretion levels of different pro-inflammatory cytokines and chemokines.

Moreover, a substantial antigen-specific T cell proliferation led to robust tumor remission in a murine B16 melanoma model. Subcutaneous administration of R848/diABZI-loaded NCs induced enhanced infiltration of CD4+ and CD8+ T cells as well as neutrophils in tumor-draining lymph nodes (LN) and tumor tissue. Encapsulating the melanoma-specific antigenic peptide of TRP-2 into the adjuvant-loaded NCs reduced the growth of B16 melanoma and prolonged the overall survival. The herein presented novel anti-tumor vaccination strategy avoids the use of structural compounds, increases the antigen load of dendritic cells, uses a fixed combination of antigen and two potent adjuvants and bears the potential to overcome the immunosuppressive tumor microenvironment inducing vigorous antigen-specific anti-cancer immunity.

RPEGS - NON-IMMUNOGENIC, UNIVERSAL PEG ALTERNATIVES OBTAINED BY ISOMERIZING THE PEG STRUCTURE

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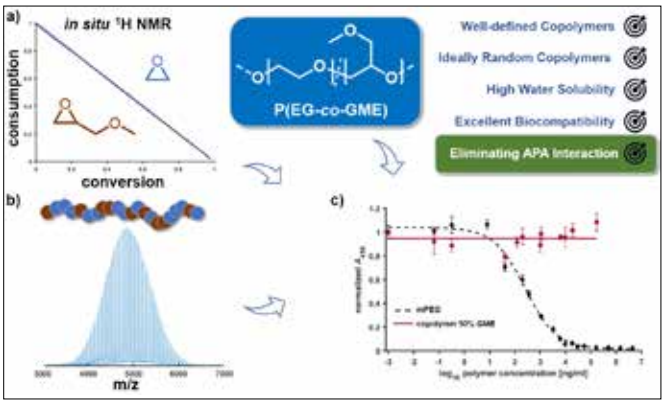
Currently, we are witnessing the triumph of nanomedicine, a field that developed rapidly in the 2000s, culminating in the development of the mRNA vaccines against COVID-19, which is enabled by lipid nanoparticles (LNP). Poly(ethylene glycol) (PEG) is at the heart of nanomedicine as a highly biocompatible polymer structure, which plays a key role in conjunction with many nanotherapeutics due to its high water solubility and excellent biocompatibility, both for the stabilization of LNPs as well as for the widely used "PEGylation" biotherapeutic molecules or by surface coverage of medical nanocarriers. Whereas it was initially believed that PEG is immunologically inert, it has become obvious in recent decades that an increasing number of individuals shows hypersensitivity to PEG and 11PEGylated pharmaceuticals,[1] ranging from mild symptoms to life threatening anaphylaxis reactions.[2] These adverse reactions are attributed to anti-PEG antibodies (APAs). Consequently, various potential polymer alternatives based on different polymer classes or proteins were investigated as substituents for PEG in the last couple of years, e.g., hydrophilic poly(2-oxazoline)s or polysarcosine.

We introduce a fundamentally different approach to avoid adverse recognition of PEGylated therapeutics and nanomedicines by anti-PEG antibodies. Mimicking nature, the well-defined incorporation of statistically distributed "point mutations" in PEG chains is achieved by anionic random copolymerization of ethylene oxide (EO) with hydrophilic glycidyl ethers, such as glycidyl methyl ether (GME), disabling antibody recognition. These structures are designated "rPEGs". We believe that this concept is universally applicable to generate non-immunogenic polymer-conjugated nanotherapeutics. It should be emphasized that any random copolymer of EO and GME is a structural isomer of PEG. Figure 1 shows the structure of copolymers the respective P(EG-co-GME) copolymers formed by anionic ROP copolymerization of EO and GME as well as a) in situ NMR data, evidencing ideally random copolymerization; b) a typical MALDI-TOF spectrum of a P(EG-co-GME) copolymer; c) competitive ELISA data comparing mPEG and 50 mol% GME-containing copolymer with respect to APA interaction, demonstrating that recognition by APA is avoided.



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LYSO-PHOSPHATIDYLCHOLINE FOR THE STABILIZATION OF PHARMACEUTICAL PROTEINS AGAINST ADSORPTION AND AGGREGATION

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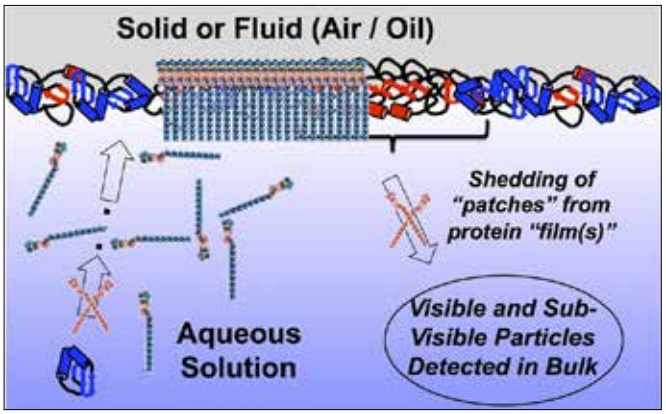

Therapeutic proteins outnumber small molecules in the top ten list of the best-selling drugs. These proteins, formulated as aqueous solutions for injection, often face physical and chemical instability. Surfactants like polysorbate 20 and 80 as well as poloxamer 188 are commonly used to protect against interfacial stress, but hydrolysis and oxidation of PS in these formulations can lead to quality issues and adverse effects in patients. Therefore, it is crucial to identify alternative surfactants. In this context, we assess lyso-phosphatidylcholines (LPCs) as stabilizers for protein formulations.
LPCs disrupt erythrocyte membranes already at low µM concentrations. Therefore, we initially tested the hemolytic activity of several LPC variants in whole blood. Hemolytic activity decreases by several orders of magnitude when tested in biologically relevant plasma. PC 18:2/0 and PC 14:0/0 displayed HC50 values of 7 and 2 mg/ml, respectively. Thus, hemolytic activity is not critical in the presence of serum and at the concentrations used in protein formulation. Overall, the hemolytic activity depends on chain length and level of saturation.

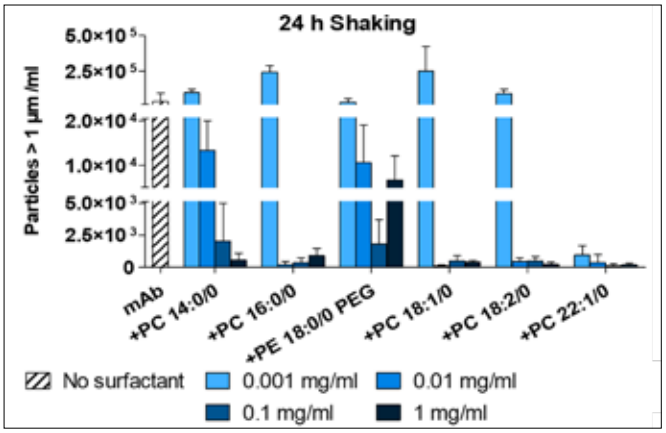

Figure 1. Particle formation upon shaking of a mab solution in absence and presence of different LPC variants at different concentration.

Monoclonal antibody (mab) Formulations were subjected to interfacial stress via reciprocal shaking and analyzed for aggregation propensity using sub-visible and visible particle analysis, as well as turbidity. LPC shows the same interfacial protection as PS 80 and PX 188 at concentrations > 0.01 mg/ml, LPC-PEG-variant containing formulations display slightly higher particle counts. All formulations containing > 0.01 mg/ml of surfactant remain within the regulations set by Ph.Eur. and USP.
Although LPC 18:2/0 and PE 18:0/0-PEG effectively stabilize proteins, they are highly susceptible to oxidation. Unfortunately, the observed LPC product loss undermines the previously observed protein stabilization effects. In contrast, mono-unsaturated and saturated LPCs were shown to be chemically stable. All LPCs exhibit greater resistance to hydrolysis caused by esterase activity of the protein drug substance compared to polysorbate. The stability of lipids in liquid and lyophilized states, including the impact of PC 14:0/0 on mAb stabilization during storage, is currently monitored over a period of 1 year. In addition the stabilizing effect of LPCs on proteins against freeze-thaw stress could be shown.
Grazing incidence off-specular x-ray scattering and Total reflection x-ray fluorescence provided evidence of mab presence at the interface at low LPC concentrations of 0.01 mg/ml. At higher LPC concentrations the interface appears to be completely covered by surfactant molecules as protein molecules could not be detected there. This corresponds to the fact that increasing the LPC concentration enhances the protein protection against interfacial stress.
Together these findings indicate the potential of lyso-lecithins to become alternatives to the currently used, but controversial surfactants in therapeutic protein formulation.

RADIOLIGAND THERAPY: HARNESSING THE POWER OF RADIOACTIVE ISOTOPES TO TREAT PATIENTS

LORENZA FUGAZZA

Radioligand therapy (RLT) is an emerging treatment approach which may offer an alternative treatment option for cancers patients.
RLT uses targeted drugs containing radioactive particles to deliver precision-targeted radiation to cancer cells anywhere in the body, with the goal of limiting damage to
surrounding tissue. In addition, precision imaging radioligands allow to localize cancer cells, choose personalized therapies, and track treatment progress.
Different isotopes and ligands can be combined to diagnose, monitor, and/or potentially treat a variety of cancers. This makes Radioligand therapy a technology platform – a foundational tool that can be tailored for different needs
Unique properties of this new cancer treatment platform create unique opportunities but also bring challenges associated to the physical characteristics of radioactive isotopes. So, while the concept and mechanism behind RLT is elegantly simple, producing and delivering these therapies is fairly complicated. Radioligand therapies are generally produced as single patient doses and have a limited shelf-life, often only a few days, which demands robust manufacturing capabilities and supply chain to ensure on-time delivery to patients.
This session will review the peculiarities of RLT products driving their development, manufacturing, distribution and ultimately their availability to patients.

DRUG CO-ENCAPSULATION IN LIPID NANOPARTICLES FOR A MULTIMODALITY APPROACH TO CANCER THERAPY

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Introduction: Encapsulation of doxorubicin (DOX), a potent cytotoxic agent and immunogenic cell death- inducer in pegylated

(Stealth) liposomes is well known to have major pharmacologic advantages over treatment with free doxorubicin [1]. Reformulation of alendronate (ALD), a potent amino-bisphosphonate, by encapsulation in pegylated liposomes results in significant immuno-modulatory effects through interaction with tumor-associated macrophages and activation of gamma delta T lymphocytes [2]. Co-encapsulation of multiple drugs in the same nanocarrier is a unique tool of nanomedicine offering multiple pharmacologic advantages such as co-delivery in space and time of two or more agents maximizing their additive or synergistic effects in cancer therapy or other fields of medical therapy [3, 4]. We have developed a liposome formulation with 2 active ingredients, doxorubicin (DOX) and alendronate (ALD) that display very different mechanisms of action and have no overlapping toxicity [5].

Results: Recent findings of our research work with a formulation of DOX and ALD co-encapsulated in pegylated liposomes (PLAD) and explore further aspects of its biological performance *in vitro* and *in vivo*, with emphasis on the enhanced permeability and retention (EPR) effect. Results of biodistribution, radionuclide imaging, characterization of tumor-infiltrating immune cells, and therapeutic studies suggest that PLAD is a unique product with distinct tumor microenvironmental interactions. The pharmacological properties of PLAD vs free doxorubicin and the clinical formulation of pegylated liposomal DOX will be discussed.

Conclusions: Co-encapsulation of ALD and DOX in pegylated liposomes leads to a unique multi-modality platform with non-overlapping toxicity and with a unique mechanism of activity that blends chemotherapeutic and immune-boosting properties and may have a profound impact in cancer therapy particularly in combination with immune checkpoint inhibitors.

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DEVELOPMENT OF PROMITIL®, A LIPIDIC PRODRUG OF MITOMYCIN C IN PEGYLATED LIPOSOMES: FROM BENCH TO BEDSIDE

ALBERTO GABIZON

Several liposome products have been approved for the treatment of cancer. In all of them, the active agents are encapsulated in the liposome water phase passively or by transmembrane ion gradients. An alternative approach in liposomal drug delivery consists of chemically modifying drugs to form lipophilic prodrugs with strong association to the liposomal bilayer. Based on this approach, we synthesized a mitomycin c-derived lipidic prodrug (MLP) which is entrapped in the bilayer of PEGylated liposomes (PL-MLP, Promitil®), and activated by thiolytic cleavage. PL-MLP is stable in plasma with thiolytic activation of MLP occurring exclusively in tissues and is more effective and less toxic than conventional chemotherapy in various tumor models. PL-MLP has completed phase I clinical development where it has shown a favorable safety profile and a 3-fold reduction in toxicity as compared to free mitomycin c. Clinical and pharmacokinetic studies in patients with advanced colorectal carcinoma have indicated a significant rate of disease stabilization (39%) in this chemo-refractory population and significant prolongation of median survival in patients attaining stable disease (13.9 months) versus progressive disease patients (6.35 months). The pharmacokinetics of MLP was typically stealth with long T½ (~1 day), slow clearance and small volume of distribution. Interestingly, a longer T½, and slower clearance were both correlated with disease stabilization and longer survival. This association of pharmacokinetic parameters with patient outcome suggests that arrest of tumor growth is related to the enhanced tumor localization of long-circulating liposomes and highlights the importance of personalized pharmacokinetic evaluation in the clinical use of nanomedicines. Another important area where PL-MLP may have an added value is in chemoradiotherapy, where it has shown a strong radiosensitizing effect in animal models based on a unique mechanism of enhanced prodrug activation and encouraging results in early human testing.

GRANAGARD: A NANO-FORMULATION OF POMEGRANATE SEED OIL A SMART FOOD SUPPLEMENT FOR THE PREVENTION OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases, such as Alzheimer's (AD), Creutzfeldt-Jacob (CJD), Parkinson and others, are late onset fatal brain disorders that affect large numbers of individuals in our society. Since diagnosis usually is performed after considerable death of brain cells over long periods of time, there is a clear unmet need for prevention/delay of disease onset in subjects at risk, which constitute most of the population. Prevention of these diseases requires administration of candidate compounds for many years to healthy individuals, implying such reagents must to be safe and compatible with "maintenance" drugs. To this effect, and since oxidative stress, aggregation of individual key disease proteins and mitochondrial dysfunction are the common denominators in the pathogenesis of all neurodegenerative conditions, we developed a nano-formulation of pomegranate seed oil (PSO), which main component is Punicic Acid (PA), one of the strongest natural antioxidants. While PA, as well as its main metabolite, Conjugated Linoleic acid (CLA) cannot reach the brain following administration of natural PSO, Nano-PSO (Granagard) targets CLA to the brain in significant levels. Granagard administration to transgenic models of CJD, AD, MS and brain injury demonstrate a strong neuro protective effect on neurological, cognitive and pathological markers of disease. This includes reduced neuronal death, delay of disease onset, reduction of key disease protein accumulation, restoration of mitochondrial activity and induction of neurogenesis. All these are also anti-aging hallmarks. No side effects in mice as well as in ongoing human trials have been reported. We conclude that long term administration of GranaGard is safe and may be effective for the prevention/delay of aging and neurodegenerative diseases.

REDEFINING CANCER WITH INTEGRATIVE TUMOR IMMUNOLOGY

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We have previously shown that tumors from human colorectal cancer with a high-density of infiltrating memory and effector-memory T-cells are less likely to disseminate to lympho-vascular and perineural structures and to regional lymph-nodes. We also demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We found that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients' prognosis. We defined these parameters as the "immune contexture". We characterized the immune landscape within human tumors, and showed the importance of several adaptive immune cells. Analyses revealed a large inter- and intra-metastatic tumor cell and immune heterogeneity. We further demonstrated the significant role of Immunoscore and immunoediting in affecting metastatic clonal dissemination. We hence proposed a "parallel immune selection model" of tumor evolution incorporating the effects of the immune system in shaping and driving metastatic spread. We proposed a continuum of cancer immunosurveillance from pre-cancer to metastasis, and novel concepts underpinning tumor evolution at the pre-cancerous stages will be advocated.

REGULATORY SAFETY EVALUATION OF NANOMEDICAL PRODUCTS: KEY ISSUES TO REFINE

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Nanotechnologies enable great opportunities for the development and use of innovative medical products. As is common for scientific and technical developments, regulatory safety evaluation presents challenges. The REFINE project on a regulatory science framework for nanomedical products has provided a highly valuable body of knowledge needed to address regulatory challenges and gaps in currently available testing methods. In order to better understand whether the identified regulatory needs are sector-specific for health products or might also hinder the progress in other domains, the REFINE consortium reached out to communities representing other sectors that also exploit the potential of nanotechnology, i.e. industrial chemicals, food and cosmetics. Through a series of transsectorial workshops, REFINE partners identified common as well as sector-specific challenges and discussed possible ways forward. Potential solutions lie in a more strengthened collaboration between regulatory and research communities resulting in a targeted production and exploitation of academic data for the regulatory decision-making. Furthermore, a coordinated use of knowledge sharing platforms and databases, trans-sectorial standardisation activities and harmonisation of regulatory activities between geographical regions are possible ways forward, in line with European political initiatives such as the Chemical Strategy for Sustainability (CSS).

IS THERE A VALID BUSINESS MODEL IN AMR IN TODAY'S MARKET ENVIRONMENT?

MARC GITZINGER

- Describe the issue around the AMR business model
- What needs to be done to fix the economic problem?
- Where do we see still a business opportunity today?
- BioVersys pipeline and innovation.

GENERATING ARTIFICIAL TARGETS TO DELIVER THERAPIES SPECIFICALLY TO THE BRAIN

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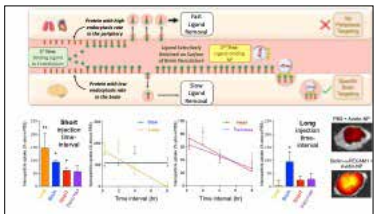

Figure 1. Generation of artificial brain targets by exploiting the low endocytic rate of brain endothelial cells.

Treatment of neurological disorders such as Alzheimer's disease is hindered by the presence of the blood-brain barrier (BBB), a protective barrier composed of the specialized endothelial cells lining the brain vasculature. To overcome the BBB, current brain-delivery strategies bind nanoparticles to target proteins on the brain vasculature. However, such strategies have inherent brain-specificity limitations, as the target proteins are also found in the peripheral vasculature, leading to off-target nanoparticle delivery to organs like the lungs and liver.

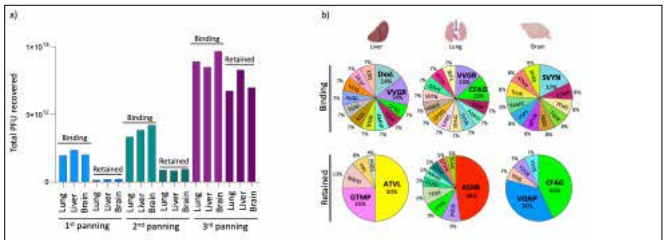

Figure 2. Identification of peptides binding to, or retained on the surface of liver, lung or brain endothelial cells.

Here, we present a novel delivery strategy[1] which exploits the specialization of the BBB to generate 'artificial' targets selectively on brain endothelial cells (BEC) (figure 1), thereby boosting brain specificity. We demonstrate the low-endocytic rate of BEC *vs.* peripheral EC[2] may be harnessed to selectively retain free ligands on the surface of the brain vasculature, thereby acting as targets to direct nanoparticles towards the brain with no increased accumulation in peripheral organs.
In addition, we outline a novel ligand selection paradigm to identify brain-targeting peptides based on probing the endocytic internalization rates of individual cell-membrane components across different endothelial phenotypes. We identify peptides selectively retained on the surface of BEC (fig. 2) to generate artificial targets for the delivery of proteins to the brain. Hence, this selection paradigm identifies peptides for brain-targeting which would have been overlooked by conventional screening procedures, thereby increasing the repertoire of cell-membrane components able to be exploited for targeting nanoparticles to the brain.

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„ENGINEERING OF IMPROVED RNA NANOPARTICLE SYSTEMS BY CONTROLLED SELF-ASSEMBLY"

HEINRICH HAAS

Development of RNA therapeutics for novel applications requires tailored delivery systems, where RNA cargo, application route and intended therapeutic intervention need to be taken into consideration. So far, certain lipid-based nanoparticles have demonstrated to be most successful for delivery of different nucleotide formats. Polymers can be an interesting alternative to lipid nanoparticles because they allow to extend the design space and thus to achieve particle properties, which are difficult to obtain with lipids.

Here, an approach for controlled self-assembly between negatively charged RNA and positively charged polymers is presented. Very small nanoparticles, consisting of single polymer-complexed RNA copies, are obtained, which can be of interest for vaccination and therapy. With their high degree of compaction, these novel non-viral RNA delivery systems provide genetic information at very high density and may come close to the ideal 'magic bullets' described by Paul Ehrlich more than 100 years ago.

BREAKING BARRIERS WITH NANOMEDICINES: PHASE 2 APPLICATIONS IN ONCOLOGY AND NEUROLOGY FROM A SCIENCE-ENTREPRENEUR PERSPECTIVE

STEFAN HALBHERR, CEO/Country Manager Switzerland, InnoMedica

InnoMedica is currently developing two nanomedicine-based products (Talidox and Talineuren) in clinical trials.

Talidox (TLD-1) makes use of three major design tweaks that positively interact with each other. First of all, the liposome size was scaled down to the curvature limit for cholesterol-rich phospholipid membranes of approx. 30 nanometers. A small nanoparticle size brings along multiple desirable features such as a greater capacity to accumulate in solid tumors, but also a faster transcytotic and endocytotic process. The small particle size also comes with faster drug release upon trigger, while minimizing pre-mature drug release in circulation. In a second step, drug loading per liposome was reduced. The altered lipid-to-drug-ratio provides another desirable effect, namely a reduction of the drug load in clearance organs such as the liver and spleen. As a third element, the liposome's nanosurface was more densely decorated with PEG2000 and an outward-only orientation of the PEG molecule was ensured, as inward facing PEG2000 was regarded as non-functional and thus should be avoided. The high surface density of PEG2000 again further reduced the drug load in clearance organs and greatly prolonged serum half-life to previously unseen degrees. It is subject of ongoing research how prolonged pharmacokinetics connect with tumor tissue uptake over time, especially in human tumors. In a phase I/IIa safety study with a total of 43 patients with solid tumors it was found that the PK parameters of Talidox were significantly different from currently marketed Doxil/Caelyx, showing e.g. longer half-life and larger AUC for Talidox. Taking into account the largely reduced particle diameter, this was seen as a potentially positive drug characteristic, as the availability of the nanodrug in blood constitutes the total available amount of the nanomedicament to interact with tumor vasculature and adjacent tumor tissue. At the same time, unwanted adverse drug effects could be reduced due to the increased timeframe that the body is given to metabolize the drug load, thus avoiding the well-known toxicity spikes caused by cytotoxic molecules such as doxorubicin. Looking at efficacy, while taking into account the late stage of disease and many prior lines of treatment the patient population had, the clinical activity profile of Talidox was noteworthy, generating a clinical benefit ratio of 75% (SD + PR + CR according to RECIST 1.1) in the metastatic breast cancer subgroup. In sum, Talidox is pivoting a new approach aiming to make nano drug delivery systems more modular and technologically upgradable. This should enable a broader, easier, and safer use of nanomedicine in oncology through the platform character, as a myriad of different molecules currently used in clinical routine could be significantly improved using state-of-the-art nano drug delivery.

Talineuren (TLN-1), InnoMedica's second product, is a lipid nanoparticle with a unique composition that crosses the blood-brain barrier. It carries the active substance GM1 ganglioside, which has neuroprotective and neuroregenerative properties. Talineuren has recently shown preliminary clinical efficacy in pushing back Parkinson's disease and was well tolerated by patients. We were intrigued to find that after only 8 weeks of treatment, patients' UPDRS total scores improved by 12.1 points and remained stable on those ameliorated levels even after 24 and 36 weeks of treatment. Talineuren is used as an add-on therapy with the goal of slowing or halting disease progression. It is injected in addition to standard symptom-relieving treatments already prescribed by physicians. A confirmatory, randomized, double-blinded, placebo-controlled phase IIb/III study (LIBRA) is in planning. Additionally, the application of Talineuren in ALS models has also generated highly promising preclinical data, urgently calling for a clinical trial with Talineuren in ALS patients. In sum, Talineuren offers a first-in-class novel treatment for Parkinson's disease and possibly a number of other neurodegenerative conditions with an entirely new mechanism of action compared to current approaches.

THE LYSOLIPID PARADOX – WHY LYSOLIPID-BASED THERMORESPONSIVE LIPOSOMES REMAIN TIGHT IN THE GEL PHASE

HEIKO HEERKLOTZ

Lysolipids play multiple key roles in biology and pharmaceutical applications. One application has been the design of low-temperature thermosensitive liposomes (LTSL) as in Thermodox, a formulation designed to overcome the shortcomings of drug release from the classic Doxil. In my view, the recent stage-3 failure of this drug candidate should not be taken as an argument to give up on fundamental research on such systems. On the opposite – the deeper, quantitative and mechanistic biophysical understanding of potential drug delivery systems may help preventing such disappointments in the future.

In fact, the understanding of LTSL had been quite limited in spite of decades of development. The activity of the lysolipid to induce liposome leakage at the transition to the fluid phase had been explained as a detergent effect – but typical detergents do just the opposite! At concentrations that are still well tolerated within a tight fluid bilayer, they make gel phases break apart as they accumulate in defects between quasi-crystalline gel clusters that do not accommodate "contaminants" such as lysolipid. This is what we call the lysolipid paradox – why is the vulnerability of the gel and fluid phase of membranes to detergent-induced leakage reversed for lysolipids as compared to other surfactants? How can these large amounts of lysolipids be accommodated in the gel phase without breaking it?

The answer lies in a phenomenon that had been described before but never been recognized to play a role in LTSL. Lysolipids can, unlike other surfactants, form an interdigitated gel phase in 1:1 mixtures with saturated-chain phospholipids. This way, a large amount of lysolipid can be "safely stored" in the gel phase that becomes leakage-active only as the interdigitated phase melts.

Interactions of lysolipids with membranes are very unusual compared to other surfactants also in other respects. Added to membranes from the outside, they insert only into the outer (cis) leaflet but do not translocate to the inner (trans) one over hours or days. This applies to other impermeant surfactants as well but whereas these others finally crack in, i.e., transiently permeabilize the membrane at a given threshold of asymmetric insertion, lysolipids don't.

First, they cause budding of small daughter vesicles. This is limited to "using up" excess area and stops as the mother vesicles become spherical. Then, some more lysolipid enters the outer leaflet (building up stress) until it simply stops inserting and, ultimately, forms micelles interacting only very little with the liposomes. This may hold for many days even though the amount of lysolipid may suffice to solubilize all lipid into mixed micelles once equilibrated between the leaflets.
In summary, lysolipid has a detergent-like structure and forms micelles but shows a much more complex behavior than typical technical detergents. Understanding these effects in detail is very helpful for pharmaceutical applications.

STABILITY OF POLYMERIC MICELLES

WIM HENNINK

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Amphiphilic block copolymers spontaneously form polymeric micelles in aqueous solutions above the critical micelle concentration. Generally speaking, polymeric micelles are characterized by a core-shell structure with sizes that range from 10-100 nm. The hydrophilic shell ensures their colloidal stability whereas the hydrophobic core can be used for the loading of particularly hydrophobic drugs. Because of these attractive features, polymeric micelles are under investigation as drug delivery system and some formulations have reached clinical evaluations [1,2]. It should be noticed, however, that polymeric micelles are dynamic systems which means that destabilization might occur when their concentration drops below the CMC. Further, the loaded drugs can be rapidly released in biological media due to the presence of proteins that act as solubilizers of the drugs. (e.g., albumin, lipoproteins). To enhance the stability of polymeric micelles, strategies to chemically crosslink polymeric micelles and also to covalently link the drug to the core have been exploited. To ensure biodegradability, crosslinking/coupling methods are used in which bonds are present that degrade under physiological conditions (e.g., ester and disulfide bonds) [3, 4]. To avoid the use of the chemical methods, physical strategies have also been investigated to increase the stability of polymeric micelles [5]. In our research programme, we have employed π–π stacking to increase the stability of polymeric micelles as well as the retention of loaded drugs using block copolymers of PEG and pHPMA-Bz (poly(N-2-benzoyloxypropyl methacrylamide). It was shown that the anticancer drugs (paclitaxel and docetaxel) were well retained in the micelles, even in the circulation, and good therapeutic efficacy was seen in different animal models [6, 7]. In another study, we solubilized the pharmacological active compound curcumin in the same polymeric micelles. Although the micelles showed good stability in the circulation, the loaded curcumin was rapidly extracted from the micelles [8]. Although curcumin, just as paclitaxel and docetaxel, is a compound with a high log P and also contains aromatic groups, the reasons for its fast release in blood are not fully understood yet. Recently we developed polymeric micelles composed of amphiphilic block copolymers of pHPMA and pHPMA-Bz with and without a biotin terminus [9,10]. These biotinylated micelles loaded with different hydrophobic drugs were incubated in buffer, plasma and even full blood to study their release characteristics. Due to the biotin decoration, these micelles could be removed from the release medium using streptavidin-coated magnetic beads, allowing quantification of both the released drug and the amount of drug still retained in the micelles [11]. This method confirms our previous findings that paclitaxel was better retained in the micelles than curcumin.
In conclusion, polymeric micelles are attractive systems for solubilization and delivery of hydrophobic drugs. However, more research has to be done to understand their physicochemical and pharmaceutical characteristics to fully exploit their potential as tumor-targeted nanomedicines.

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ACCELERATING BIOCONJUGATES DEVELOPMENT: A HOLISTIC APPROACH FOR TECHNOLOGY SELECTION AND MANUFACTURING INTEGRATION

NINA HENTZEN

In recent years, bioconjugates have experienced remarkable success, as evidenced by the FDA's approval of eight antibody-drug conjugate products between 2019 and 2022. However, the continuous evolution of protein modalities, payloads and linkers leads to increased complexity in the development and manufacturing of bioconjugates. The presentation explores approaches to navigate the complex technology landscape and accelerate the path from initial bioconjugate design to the clinic. Special emphasis will be placed on next-generation bioconjugation technologies that enable the development of fully integrated manufacturing processes.

INORGANIC ANTIBIOTICS – CATALYTICALLY ACTIVE METAL OXIDES AS ANTIMICROBIALS AND ANTIMICROBIAL COAT-INGS

INGE HERRMANN, ETH Zurich and Empa

Antimicrobial infections pose global health concerns, and the emergence of bacterial resistance is poised to exacerbate this issue in the upcoming years. Several bacterial strains have developed the ability to evade antibiotic treatment by concealing themselves within cells. Traditional antimicrobial agents struggle to permeate or remain within infected mammalian cells. Recent strategies aimed at surmounting these challenges have centered on load-carrier systems, necessitating controlled re-lease mechanisms that involve intricate kinetics. Achieving a balance between potent antimicrobial activity and high compatibility with mammalian cells is essential for effective intracellular function. This balance has proven elusive within conventional inorganic systems, like silver-based nanoparti-cles, despite their well-established nature.

Within this presentation, I will introduce an innovative approach where load and carrier functionalities are seamlessly integrated into a singular functional inorganic nanoparticle system. This system har-monizes antimicrobial efficacy with compatibility for mammalian cells. Synthesized in a single step, these multifaceted nanohybrids, based on cerium oxide, overcome the complexities of material inte-gration. These nanoparticles assemble into structures akin in size and surface charge to bacteria, thereby facilitating their uptake into the same subcellular compartments. Once situated, these nano-hybrids unleash their antibacterial potency. The inherent antibacterial qualities of these nanohybrids remarkably diminish bacterial survival within macrophages without causing harm to the host cells. Furthermore, insights into the mechanism of action are gleaned from the inhibition of nanoparticle en-docytosis and the application of subcellular electron microscopy. Additionally, these nanohybrids can also be applied to implant surfaces as antimicrobial coatings. This demonstration of ceria-based na-noparticle antibacterial activity within mammalian cells and as surface coatings outlines a pathway towards straightforward and resilient antibacterial agents, independent of payload delivery or biologi-cal constituents.

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CHALLENGES IN ORAL NANOPARTICLES BY PHYSIOLOGICALLY BASED BIOPHARMACEUTICS MODELLING (PBBM)

MARTIN HINGLE, Associate Director, Early Phase Product Development, Technical Research and Development, Novartis Pharma AG, Basel (CH)

The objective of this presentation is to provide an insight into the challenges of physiologically based biopharmaceutics modelling (PBBM) of oral nanoparticles. The use of PBBM models during drug development has increased significantly over the last decade as evidenced by an increased interest within industry, academia, and regulators. A popular approach to increase the bioavailability of poorly soluble drugs is to reduce the size of the drug particles. However, there are challenges in the simulation of human PK predictions for oral drug product nanoparticles particularly in terms of their absorption behaviour. The main challenges affecting the absorption behaviour of oral formulated nanoparticles are solubility (intrinsic, kinetic and thermodynamic) particle size distribution (nanoparticle drug substance and formulated nanoparticle drug product), wettability, dissolution, supersaturation, precipitation and permeation. In these areas, the development of appropriate biorelevant and biopredictive methodologies will provide a better understanding of drug absorption and improve PK predictions for nanoparticles using PBBM.

NANOMEDICINE: FROM SCIENCE FICTION TO AN INDISPENSIBLE CONTRIBUTION TO MEDICINE: ACHIEVEMENTS, CHALLENGES AND OUTLOOK.

PATRICK HUNZIKER

Nanomedicine has progressed from science fiction and futuristic promises to tangible implementation in research and industry. The label “nano” has become less of importance as the nanotechnologies have now evolved into core components of current diagnostic and pharmaceutic technologies. This talk delineates the scope of nanomedicine as a very interdisciplinary field and highlights recent achievements that result in real-world benefit. It points towards unmet medical needs and explores avenues to further progress that can be achieved by the interdisciplinary translation of the nanosciences in concert with key enabling technologies, aiming at the “**benefit of patients and of society**”, the fundamental goal of CLINAM.

NEW STRATEGIES FOR IN VIVO EVALUATION OF GENE DELIVERY TECHNOLOGIES

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Evaluation and optimization of gene delivery technologies remain a challenge due to the difficulty to extrapolate from *in vitro* cell-culture based test systems to the situation in higher vertebrates. Using the example of a lipid nanoparticle-based gene delivery system (LNP), a test strategy will be presented comprising an in-depth physico-chemical characterization of the particulate gene delivery system, *in vitro* studies using cell culture models, and zebrafish (Danio rerio) larvae as an *in vivo* vertebrate screening model.

To validate this novel approach, monodisperse preparations of fluorescent labeled LNPs with similar size and zeta potential were injected into transgenic zebrafish lines expressing green fluorescent protein in their vasculature. Their circulation behavior and vascular distribution pattern were evaluated qualitatively and semi-quantitatively using image analysis. Expression of a transgene, encoded by mRNA, was monitored qualitatively and quantitatively in terms of expression efficiency and potency. Finally, expression of a fluorescent marker protein in wildtype mice was visualized by fluorescence imaging.

Our findings indicate that the zebrafish model is a useful vertebrate screening tool for nanoparticulate drug delivery systems to predict their *in vivo* performance with respect to systemic circulation time, exposure, and gene expression. When combined with *in vitro* screening models, mechanistic insights are obtained accelerating the process of formulation optimization and a further evaluation in mice. This approach allowed us to optimize the lipid composition and thus the performance of new generations of LNPs.

DEVELOPMENT OF EFFECTIVE AND SAFE RNA-LNP MEDICINES FOR THE CLINIC

LLOYD JEFFS

Precision NanoSystems is a global leader in nanoparticle technologies and solutions with the goal of empowering our clients to develop genomic medicines, including mRNA vaccines and therapeutics, that define the future of medicine. We have developed a Genomic Medicine Toolbox for end-to-end development of RNA-lipid nanoparticles (RNA-LNP). This toolbox comprises an RNA drug substance platform, a nanoparticle delivery platform, and a microfluidics-based nanoparticle manufacturing platform. In this presentation, we provide examples of how these platform technologies are enabling research scientists to rapidly discover new RNA-LNP based vaccines, gene therapies and cell therapies. Furthermore, we will show how PNI's BioPharma Services Team can de-risk and accelerate the development of promising RNA-LNP drug candidates for clinical evaluation and successful commercialization.

NUCLEIC ACID BASED LIPID NANOPARTICLE VACCINES FOR LYME DISEASE

MICHAEL JOHNSTON

The emergence of COVID-19 and the success of lipid nanoparticle based mRNA vaccines to blunt the pandemic suggest that LNP systems will become a new modality in the prevention of numerous diseases of public health concern. For instance in North America and other regions, the increase in the incidence of Lyme disease (LD) and the current lack of a human vaccine suggests that LNP vaccines to protect against LD will be developed in short order. To prepare the regulatory challenges of these systems, research laboratories within Health Canada's Centre for Oncology, Radiopharmaceuticals and Research are developing functional model LNP vaccine systems, with multiple antigens, as a regulatory research tool. It is anticipated these tools will aid in the development of physicochemical, *in vitro* and *in vivo* assays for LNP vaccines with progress on this project presented and discussed.

INVESTIGATIONS INTO MRNA LNP SHELF-LIFE STABILITY UNDER NON-FROZEN CONDITIONS

MICHAEL KELLER

mRNA LNPs are prone to lose activity over short timespans (weeks to months) and as a consequence require frozen storage and transport conditions to maintain full functionality when used. Current commercially approved mRNA LNP vaccines require frozen storage and supply chain. This is a major inconvenience to overcome in the future to eliminate unnecessary costs and challenges for storage and transport. In this study we aimed to shed light on the time span for non-frozen storage and transport conditions of mRNA LNPs without loss of activity. To this end, we carried out a stability and *in vitro* cell culture delivery study with five mRNA LNPs composed of a standard formulation composition as used in the three currently three commercially available LNP formulations. We selected the five structurally diverse ionizable lipids C12-200, CKK-E12, DLin-MC3-DMA, SM-102 and lipid 23 from the literature and used them in a standard LNP formulation with all other components identical. LNPs containing mRNA payload were manufactured using microfluidics mixing technology. Shelf-life stability was measured over nine weeks at 2–8 oC, 25 and 40 ºC, respectively, using a broad range of analytical techniques. We found that the hydrodynamic diameter, zeta potential, encapsulation efficiency and polydispersity of all LNPs were little impacted by the different temperatures over the investigated period. By means of RiboGreen-> assay, no notable loss of mRNA was measured. However, we noticed significant differences of the EGFP protein expression *in vitro* cell culture (HEK293 cells) for the five LNPs. Only LNP 1 (C12-200) and LNP 4 (SM-102) LNPs exhibited high EGFP expression levels at T0 with more than 90 % of HEK293 cells transfected and mean fluorescence intensity (MFI) levels >1. Intriguingly, over 11 weeks, LNP 1 (C12-200) showed largely unchanged levels of *in vitro* activity at both 2–8 oC and 25 oC storage temperatures. Conversely, LNP 4 (SM-102) kept its functionality when stored at 2–8 oC over 11 weeks, but gradually lost *in vitro* activity over the same time span when stored at room temperature. Noteworthy, we observed differential LNP architectures for the five LNPs by means of cryo-EM imaging, emphasizing the need of a better understanding about structure-activity relationships of such complex nanoparticle structures to overcome storage and stability limitations, which will greatly help to apply this technology in other areas than vaccines.

THE ROLE OF PET AND RADIONUCLIDE THERAPY IN CANCER IMMUNOTHERAPY

ANDREAS KJAER, PhD, DMSc, Professor, chief physician, Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, University of Copenhagen (DK)

Cancer immunotherapy has been a game changer in the treatment of cancer. However, closer non-invasive monitoring of the relevant targets for checkpoint inhibitors, e.g. PD-L1/PD-1, as well as the lymphocyte infiltration of tumors by T cells using PET imaging, may in the future improve treatment planning and the success rate of cancer immunotherapy.
In addition, the recent breakthrough in the use of targeted radionuclide therapies, considered by many to be the radiotherapy of tomorrow, makes it possible to locally induce a cellular immune response that may pave the way for subsequent use of e.g. checkpoint inhibitors. In this way, targeted radiopharmaceuticals can transform "cold" tumors into "hot" tumors, making them eligible for immunotherapies.
We believe that the use of targeted radionuclide therapies in combination with immunotherapies holds great promise for improving the prognosis and lives of many cancer patients.

CLINICAL TRANSLATION OF GRAPHENE & LESSONS LEARNT

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The use of nanomaterials in medicine has been growing at an unprecedented rate for a variety of therapeutic, diagnostic or combinatory applications. The clinical translation of advanced materials that are being discovered or synthetically engineered is considered by many the critical factor to determine the ´success´ or ´failure´ in nanomedicine. In this talk, our decade-long experience in the clinical translation of graphene two-dimensional nanosheets will be

discussed. Recent progress in the development of clinically used graphene oxide will be described, with emphasis on two very different first-in-human clinical investigation studies undertaken recently using graphene-based technologies. Emphasis will be placed on common lessons learnt and whether graphene can serve as a case study on the early-stage clinical translation of advanced nanomater

LECITHIN AND MONOACYL LECITHIN AS INTERACTING EXCIPIENTS IN ORAL FORMULATIONS OF POORLY WATER-SOLUBLE DRUGS

MARTIN KUENTZ, University of Applied Sciences and Arts, Northwestern Switzerland HLS- Institute of Pharma Technology

Drug candidates from modern discovery often exhibit a poor aqueous solubility, which generally comes with biopharmaceutical challenges of incomplete dissolution and absorption of the dose in the gastro-intestinal tract. To cope with these challenges, oral formulations with lipids and phospholipids have a long tradition and there has been a recent interest in monoacyl lecithin (or monoacyl phosphatidylcholine) as an excipient for drug delivery. In the present work, a series of drugs were formulated as solid dispersions using monoacyl phosphatidylcholine and absence of drug crystallinity was demonstrated by means of differential scanning calorimetry as well as X-ray powder diffraction analysis. A simple rule of thumb based on a drug's enthalpy of fusion and partition coefficient is proposed to predict which drugs would likely result in a successful amorphization at an equimolar ratio of active compound and excipient. In a second step, a Monte Carlo – Molecular Dynamics simulations are presented to gain molecular insights into preferred molecular configurations using a series of structurally diverse drugs (Fig. 1). A key finding was that there were different molecular configurations evidenced for a given drug rather than a well-defined binding mode of drug and monoacyl phosphatidylcholine so care is needed with the view of a molecular drug-excipient complex.

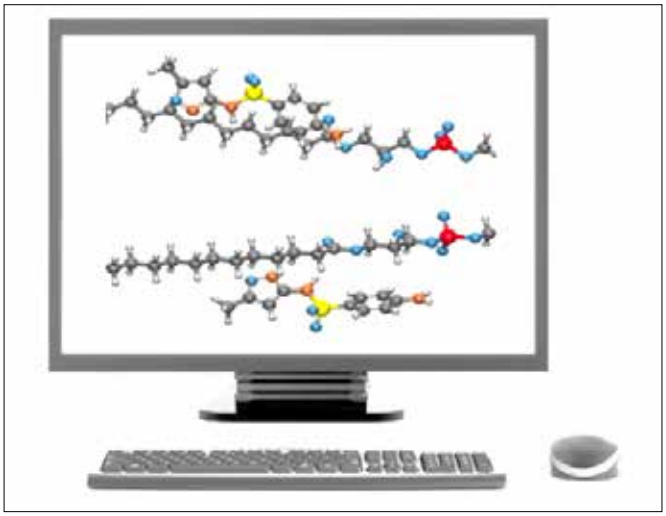

Fig. 1 – Example of a computational modeling approach (in this case a Monte Carlo – Molecular Dynamics simulation) to study preferred molecular association of monoacyl phosphatidylcholine with a series of drugs (here with the example of sulfamethoxazole)

The release behavior of various drugs in mixtures with phosphatidylcholine was further studied in a biorelevant medium by UV imaging. As a result, the more hydrophilic excipient monoacyl phosphatidylcholine showed compared to the diacyl phosphatidylcholine a generally higher drug release rate and solubilization. This beneficial effect on drug release was attributed to the colloids formed in aqueous medium and the given drug partitioning behavior. A more recent computational modeling approach is presented to estimate such drug partitioning and finally, comments are made regarding a translation of such formulation research to the development of viable drug products in clinical testing or even on the market.

CHEMICAL EVOLUTION OF AMPHIPHILIC XENOPEPTIDES FOR CAS9 RIBONUCLEOPROTEIN DELIVERY

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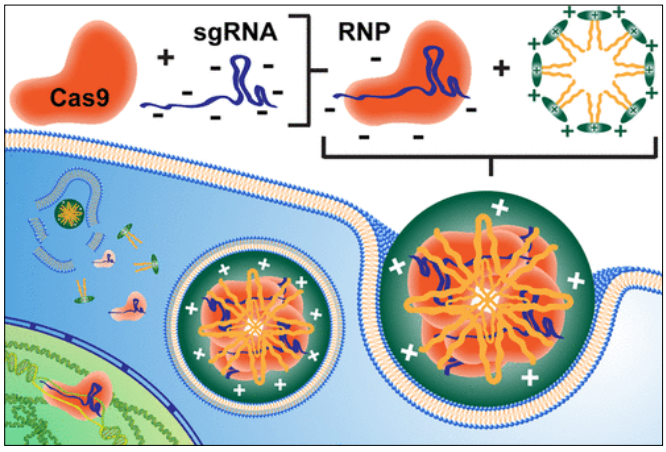
The CRISPR/Cas9 system has become a fundamental gene editing technology in modern biomedical research.[1-2] Introduction of the system into cells in form of Cas9/sgRNA ribonucleoproteins (RNP) is an efficient, straight-forward strategy: RNP are immediately functional once delivered into cells, but also rapidly eliminated, which decreases the exposure time to genomic DNA and the risk of off-target effects. [3]
We have previously identified an amphiphilic xenopeptide generated by solid-phase synthesis as an efficient RNP delivery vehicle. [4] In that approach, an artificial oligoamino acid serves as ionizable unit to encapsulate negatively charged Cas9 RNP into nanoparticles and facilitate cellular delivery (Figure 1A).

A



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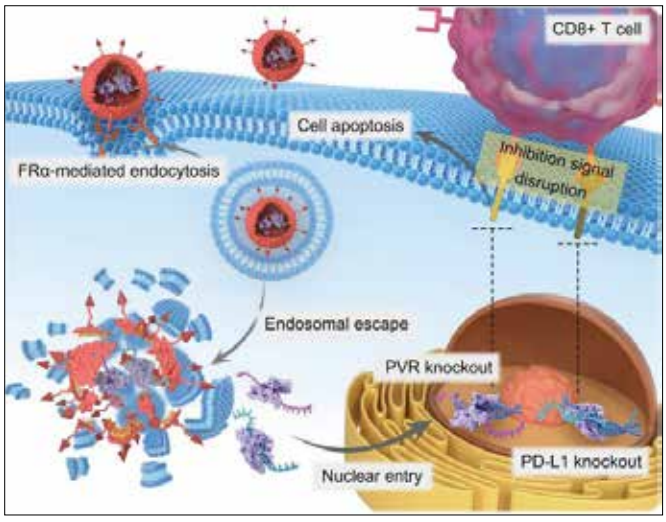

Figure 1. (A) Schematic illustration of Cas9 RNP nanocarrier assembly. (B) Schematic illustration of Cas9 RNP based cancer immunotherapy via dual immune checkpoint disruption.

Here, the extension of the delivery platform towards the next generation of Cas9 RNP nanoformulations is presented. A strong enhancement of potency was achieved by structural variation of the lead structure in two complementary approaches: (1) conjugation of targeting ligands to utilize receptor-mediated delivery, and (2) fine-tuning of physicochemical properties of the carrier molecules. With conjugation of folic acid (FolA)-PEG ligands, folate receptor α (FRα)-mediated delivery and gene editing was realized in cancer cells.[5] In an immunotherapy approach, the FolA-modified nanocarriers were used to knockout two immune checkpoints, PD-L1 and PVR, in cancer cells simultaneously (Figure 1B), which resulted in significant *in vivo* tumor growth inhibition in a murine colon carcinoma model.

In the second approach, the previous lead structure was varied systematically to modulate the physicochemical properties of Cas9 RNP carriers (Figure 2). A library of 78 carrier molecules was generated and biological evaluations revealed that hydrophobic characteristics play a decisive role for potent gene editing, especially at low concentrations. A correlation between the xenopeptide octanol-water distribution coefficient ($logD_{7.4}$) and genome editing potency was found. The optimized carriers enable eGFP gene knockout at subnanomolar concentrations and up to 40 % gene knockin via homology-directed repair (HDR) in eGFP/BFP switchable HeLa cells.

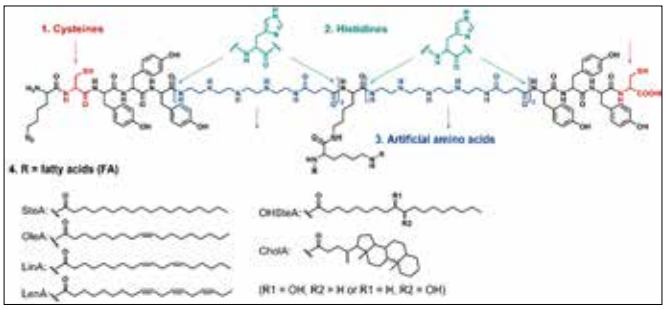

Figure 2. Exemplary chemical structures of artificial xenopeptides for Cas9 RNP delivery.

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MONITORING POLYMERIC MICELLE TUMOR TARGETING

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Polymeric micelles are extensively used to improve the efficacy and reduce the toxicity of chemotherapeutic drugs. Polymeric micelles and other cancer nanomedicines traditionally rely on the EPR effect for target site accumulation, which is highly variable, both in animal models and in patients[1]. To tackle heterogeneity in target site accumulation, and to improve the performance and translation of polymeric micelles, we are working on materials and methods to monitor and modulate tumor-targeted drug delivery[2]. In the present lecture, recent progress on several of these efforts will be summarized, including (1) imaging-based analysis of EPR effect dynamics during treatment with theranostic polymeric micelles[3]; (2) correlation analysis of polymeric micelle tumor targeting with therapeutic outcome[3]; (3) imaging-based analysis of polymeric micelle targeting to metastases in mouse models and cancer patients; and (4) proof-of-concept that histopathological assessment of tumor tissue (biopsy) biomarkers can be used for cancer nanomedicine patient stratification. The insights obtained provide a rational basis for promoting polymeric micelle (and cancer nanomedicine) clinical translation.

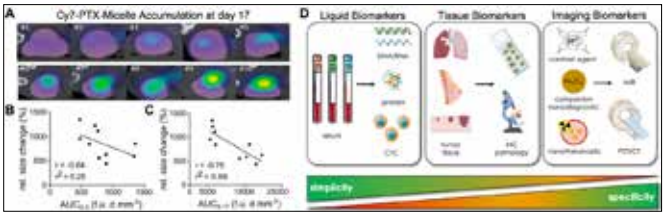

Figure 1: Imaging and biopsy biomarkers for cancer nanomedicine patient stratification. *A: The tumor accumulation of polymeric micelles loaded with paclitaxel and labeled with Cy7 was monitored multiple times during 3 weeks of treatment, showing that higher levels of micelle accumulation correlate with better treatment outcome[3]. B-C: Monitoring micelle tumor accumulation once (B: on day 3, AUC_{0-3}, in fluorescence units per day per cubic millimeter) less accurately predicts tumor response than monitoring micelle accumulation multiple times during 17 days (C: AUC_{0-17})[3]. D: Schematic depiction of the specificity vs. simplicity of biomarkers that can be used for cancer nanomedicine patient stratification[2].*

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INTRATHECAL EXOSOMES BRAIN IMAGING FOR CEREBROPSPINAL FLUID (CSF)-LYMPHATIC EFFLUX AND NEUROIMMUE INTERFACE

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On behalf of KW Kim, JY Park, MS Suh, Y-S Lee, YS Gho

Neuroimmune interface have begun to be understood on the correct anatomy (brain and spinal cord have lymphatics) and the imaging methodology for visualization (iDISCO for ex vivo, MRI and PET for *in vivo*).

Cerebrospinal fluid space works as immune border between systemic immune and central nervous systems and also the drainage route of the wastes of the brain/spinal cord via lymphatics of

dura of brain/spinal cord. Segmental drainage of the wastes from the CSF was visualized by ex vivo fluorescence imaging and *in vivo* contrast MRI and most recently Cu-64 PET. Skull/vertebrae bone marrow (BM) immunocytes, either myeloid or lymphoid, visit the brain/spinal cord from skull/vertebrae slowly-but-definitely via BM-dura-connecting veins-gaps within arachnoid barrier-CSF and are also probably coming back from parencyma brain/spinal cord to skull/vertebrae.

[Cu-64] Cu-albumin, stable in CSF after intrathecal administration in mice, were found to be drained via lymphatics and stay in para-vertebral lymph nodes (cervical and sacral) and pass to reach liver to be metabolized and finally probable [Cu-64] Cu-NOTA-labelled fragments were excreted via kidneys to the bladder. No intestinal excretion was found. Aging-related decline of CSF-lymphatic drainage of [Cu-64] Cu-albumin was disclosed.

Cu-64 was labelled to extracellular vesicle (EV, exosomes)-mimetic nanovesicles (ENV) using the same DBCO-N3 click method to yield 96%< labeling efficiency. [Cu-64] Cu ENV, average 30 nm, intrathecally administered through lumbar intervertebral space in mice, showed deep cervical, superficial cervical, sacral and iliac lymph nodes almost similarly at 1-hour PET imaging. After showing para(pre)-vertebral lymph nodes, systemic (mostly liver) radioacvitiy increased slowly to the next day, while lymph nodes' activity sustained. [Cu-64] Cu ENV in body outside CSF/CNS space were excreted through kidneys to bladder, probably in the form of [Cu-64] Cu-NOTA labelled fragments.

With the above observation, we assume that intrathecal exosomes in CSF be drained via (dural) lymphatics of brain/spinal cord, probably segmentally, to the pre/paravertebral lymph nodes. I propose that sentinel lymph nodes watch out the central nervous system probably segmentally.

FROM SUPRAMOLECULAR TOWARDS ADAPTIVE (NANO)CHEMISTRY BIOORGANIC AND BIOMEDICAL ASPECTS

JEAN-MARIE LEHN, ISIS, Université de Strasbourg

Supramolecular chemistry is intrinsically a *dynamic chemistry* in view of the lability of the non-covalent interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their components. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibility, so as to allow a continuous modification in constitution by reorganization and exchange of building blocks. These features define a *Constitutional Dynamic Chemistry* (CDC) on both the molecular and supramolecular levels.

CDC operates on dynamic constitutional diversity and performs component *selection* to achieve *adaptation*, in response to either internal or external factors, thus opening towards an *Adaptive Chemistry*. Bioorganic, biomaterial and bio(nano)-chemical aspects will be presented.

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HIGH-CAPACITY POLYMERIC MICELLES

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Polymeric micelles have been around for quite a while for solubilization/formulation/delivery of poorly soluble active pharmaceutical ingredients (API). However, scanning the literature space, it is apparent that the amount of API that can be stably dispersed is often rather limited, typically below 20 wt.% and below 10 g/L. But is this physico-chemical limitation relevant for their application? Arguments have been put forward for and against benefit of high-drug loading.[1,2]

This open question notwithstanding, it is an interesting and important question as to how highly drug loaded micelles can be realized. There are only very few micellar systems, for which drug loading significantly higher than 30 wt.% have been reported. We have previously reported on a polymer platform based on so-called poly(2-oxazoline)s and poly(2-oxazine)s which is allows to formulate unusually large amounts of the extremely water insoluble paclitaxel [3-5] and many other APIs and API combinations [6-8] with up to 50 wt.% incorporated in the polymer micelles. In some cases, higher drug loading was found to be beneficial for therapeutic efficacy [5]. We have identified pronounced effects of small structural variations in the polymers on the formulation capacity for a variety of different drugs [9-12]. In addition, we have found that the hydrophilic shell of the polymer micelles is strongly involved in the drug interactions [13-15], which, depending on the polymer structure, can severely compromise drug loading, stability and dissolution rates [14]. Using large scale all-atom simulation, we have recently investigated molecular interactions between drug molecules, polymers and water in these drug loaded micelles at different drug loadings. The results suggest that difference in the number and life-time of hydrogen bonds between all the different components correlate will with the observed maximum drug loading, and therefore might explain the observed, highly unusually high drug loading.[16] Current studies are testing this hypothesis further and hopefully lead to a much improved molecular understanding of drug loaded polymer micelles.

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STABILIZATION OF LYSOPC-LEVELS IN PATIENTS WITH CANCER

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Metastatic cancer cells consume/degrade systemic Lyso-Phosphatidylcholine (LysoPC) very rapidly, leaving behind cancer patients with very low LysoPC-levels. As shown by studies with patients suffering from sepsis, strongly reduced LysoPC-level contribute to organ failure and death, by impairing the lung function (surfactant production), the immune system or the fatty acid/energy delivery to muscles and heart, to give a few examples. Thus, stabilizing LysoPC in cancer (and sepsis patients) is expected to support patients' health in many ways which might contribute to a better anticancer therapy and survival, including cancer immune therapy. However, simple infusion of high amounts of LysoPC to stabilize or increase LysoPC levels in cancer patients appears not possible due to its lytic effects to erythrocytes.
We could show by studies in humans, rats, and mice that LysoPC is distributed within the whole body, with only 5 % of the total LysoPC in plasma. Exchange of LysoPC between plasma and deeper compartments is very fast. Total LysoPC amount in healthy humans is in the multiple gram range and the LysoPC turnover is high (about 120 g/day), which explains its essential role in supporting and fueling various important body functions.
Benefiting from the fact that the distribution of LysoPC between plasma and deeper compartments is very fast, and that LysoPC will bind to albumin plasma in the first place, we could show that a continuous infusion of LysoPC instead of bolus injection completely avoided hemolysis. LysoPC will immediately bind to albumen and, in the next seconds, distributed to deeper compartments, keeping its plasma concentration clearly in a sub-hemolytic concentration. Altogether, LysoPC turned out to be a very important, but somehow forgotten player in lipid turnover which can easily be manipulated by simple infusion of LysoPC to the benefit of heavily ill cancer patients.

NANOPARTICLES: AUTOMATING YOUR RESEARCH FOR ISOLATION AND ACCURATE SINGLE PARTICLE CHARACTERISATION

STEPHANE MAZLAN, Izon Science

Efficient purification and accurate quantification and size determination are imperative in nano biological studies involving lipid nanoparticles (LNPs). The progression from bench-to-bedside, a hallmark of translational research and application, renders both quality and accuracy of LNP purification and measurement increasingly critical. Indeed, advancement in purification and measurement technology has evolved to meet this need.
In terms of LNP measurement, high resolution is an important aspect. It has been shown that optical techniques lack the resolution necessary to for accurate and precise nanoparticle measurements especially when it comes to multimodal samples. This is highly important especially in measuring complex nanoparticles where size heterogeneity is an aspect. The level of detail and certainty that Tunable Resistive Pulse Sensing (TRPS) offers is indeed beneficial in the LNP field. This technology has demonstrated precision in both size and concentration determination where subpopulations in multimodal samples can be accurately portrayed and distinguished.

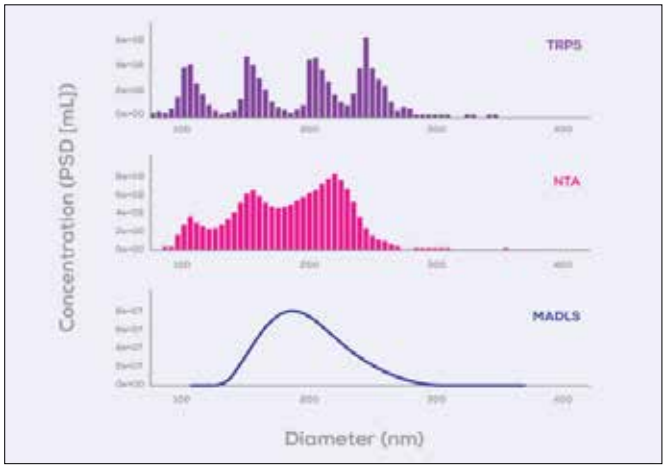

Figure 1: Tunable Resistive Pulse Sensing (TRPS), Nanoparticle Tracking Analysis (NTA) and Multi-angle Dynamic Light Scattering (MADLS) measurements of quadrimodal sample C (CPN100/CPN150/CPN200/CPN240 at 25/25/25/25). TRPS, NTA and MADLS measurements were averaged over 3 runs. TRPS identifies all four sub populations clearly. NTA was able to identify that multiple sub populations were present. MADLS was not about to identify any sub populations. Modified from Vogel et al, 2021.

The current TRPS instrument, the Exoid is also able to discern differences not only in size and concentration but also zeta potential of loaded and unloaded negatively charged LNPs.

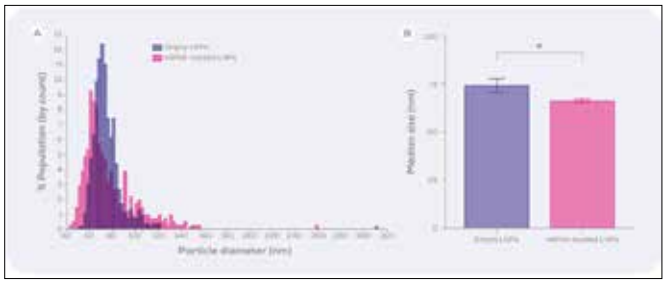

*Figure 2: Size of empty LNPs and mRNA-loaded LNPs. (A) Representative size distribution graph generated using the Exoid. (B) Median size ± interquartile range of empty LNPs (n=4) and mRNA-loaded LNPs (n=4), compared using the Mann-Whitney U test, *p<0.*

In Figure 2A depicts a representative size distribution chart comparing the empty and mRNA-loaded LNPs. The median size of the empty LNPs was 74 nm, while the median size of the mRNA-loaded LNPs was 66 nm, across 4 measurements of each particle type. Fig-

ure 2B shows how this small change in size was replicable between measurements and statistically significant ($p<0.05$), demonstrating just how capable the Exoid is of consistently detecting small size differences between samples.

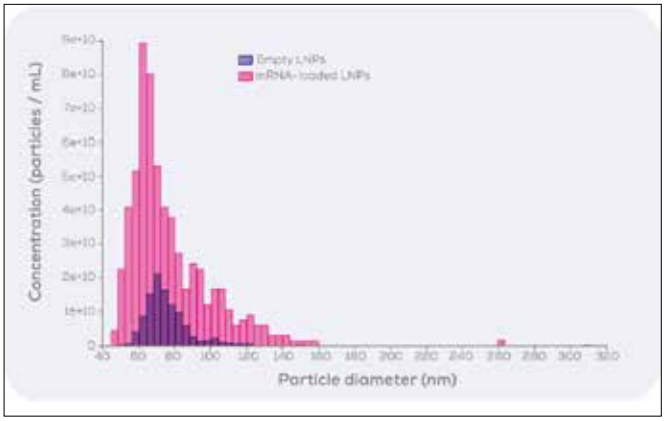

Figure 3: Size and concentration of empty and mRNA-loaded LNPs.

While the size difference is less easy to see by eye, the difference in concentration (shown on the y-axis) is completely evident. The mRNA-loaded LNPs were 5.9x more concentrated than empty LNPs.

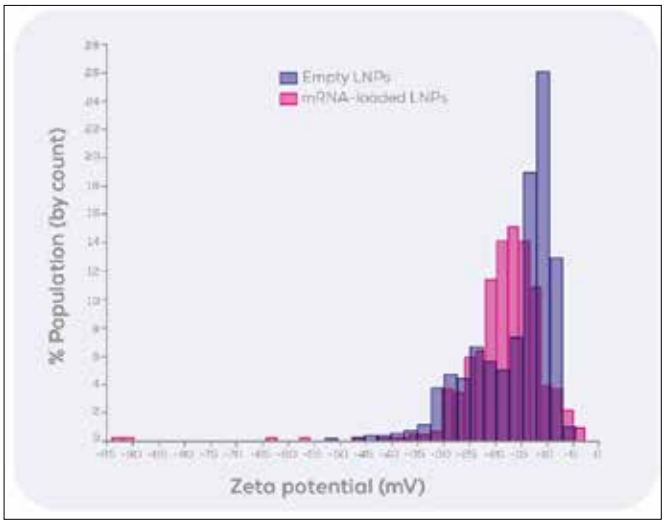

Figure 4: Zeta potential of empty and mRNA-loaded LNPs.

In Fig 4, it can be observed that the zeta potential or surface charge differs between the two particle types; the empty LNPs had a zeta potential (mode ± SD) of -10 ± 1.1 mV, whilst the mRNA-containing LNPs had a lower zeta potential of -17 ± 1.1 mV, potentially reflecting the loading of negatively charged mRNA.

Unlike other technologies, TRPS measures particles individually, which reduces the chances of overlooking small yet potentially crucial differences between samples. In the field of nanomedicine, this is incredibly important as these differences could reflect quality issues that could cause harm and derail your therapeutic advances.

In terms of LNP purification, our qEV columns that employ size exclusion chromatography (SEC) can aid in separating liposomes from bioactives which did not load or incorporate. As the columns contain resin beads with pores, small particles enter the pores and are thus delayed allowing large particles to flow through. This leads to a separation of large particle-rich isolate and if needed, a small particle-rich isolate. To cater to differing volumes of samples and to aid in scalability, our qEV columns currently cater to sample volumes of 150 µL to 100 mL. In tandem, the Automatic Fraction Collector (AFC) provides an element of automation for improved throughput and reproducibility and aided customized separation.

The nanomedicine community is beginning to make advances in developing LNPS as a new class of biological therapeutics. As the potential for LNP therapeutics grows, so does the need for purification and measurement of these complex nano biological particles – a challenge that Izon strives to achieve.

WHEN JOURNALS BECOME DETECTIVES

SPENCER MCGRATH

Topic: How journals respond to possible research misconduct

Abstract: The responsibility for the content a journal publishes rests with the Editor-in-Chief. They are ultimately responsible for the rigor of peer review undertaken at the journal, and the values upheld by the team of handling editors, editorial board members, editorial staff, and invited reviewers. As a result, when allegations of research misconduct, plagiarism, fabrication, or falsification are made, the Editor-in-Chief should always have the final decision about retracting published work. However, in many cases, Editors, learned societies, and publishers do not have the resources to investigate such allegations. And certainly not in a manner that will produce clear evidence of research misconduct. The Committee on Publication Ethics (COPE) Retraction Guidelines state, "Retractions are not usually appropriate if an editor has inconclusive evidence to support retractions or is awaiting additional information such as from an institutional investigation." It is in this context that editors and the journals they lead must rely on the authors and institutions to provide conclusive evidence for retraction. Because when journals become detectives, we are no longer focused on our core responsibility of rigorous, unbiased peer review of all submissions.

ARE LNPS DRUG SUBSTANCE OR DRUG PRODUCTS? A RECENT REVIEW OF REGULATORY APPROVALS"

SCOTT MCNEIL, Professor of Nanopharmaceutical and Regulatory Science, University of Basel (CH)

Nanotechnology-based drug delivery systems (i.e., nanomedicines) offer novel therapies for a wide variety of clinical indications. Compared to legacy formulations, nanomedicines give the resulting drug products (DPs) different physical and chemical properties. Simultaneously, these DPs display a high level of structural complexity, which may raise regulatory challenges. The active substance, for example, may include components that were previously considered as excipients, due to their ability to influence pharmacological activity. Recently, the COVID-19 pandemic has thrown the spotlight on Lipid Nanoparticles (LNPs) as nanocarriers for messenger ribonucleic acid (mRNA)-based vaccines. This talk will provide a case study on three approved RNA-LNP nanomedicines. We demonstrate how similar LNPs have been classified in regulatory dossiers, either as "starting materials" for the active substance, or as excipients. We will discuss how the differing classification of the DP components directly impacts the structure/data requirements for regulatory dossiers, and show the need for regulatory consensus on the classification of nanomedicines.

DESIGN OF TRANSCYTOTIC CANCER NANOCARRIER: AN ALTERNATIVE APPROACH TO REACH SOLID TUMOR BEYOND THE CLASSIC EPR EFFECT

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While it was popular to use enhanced permeability retention (EPR) theory to interpret the enrichment of intravenously injected nanocarriers in solid tumors, the effectiveness and validity of EPR is still controversial. Due to limited analytical methodology at nano/bio

interface, the basis for “enhanced permeability” is not completely understood. By developing various imageable nanoparticle probes, we now know that for certain tumor types with a dysplastic stroma (e.g., pancreatic cancer and triple-negative breast cancer), the collapsed blood vessels are frequently not “leaky” and adhered by pericytes on top of endothelial cells. Ultrastructural TEM imaging revealed that stroma-rich tumor could utilize an intracellular vesicular transport mechanism, a.k.a., “transcytosis”, allowing the nanoparticle access without the need of blood vessel leakiness. I will introduce the silicasome drug delivery platform, which finds its tumor access primarily via transcytosis in pancreatic cancer. I will then talk about the transcytotic delivery of chemotherapeutic agent in the FOLFIRINOX regimen (i.e., irinotecan or IRIN) in Kras orthotopic pancreatic cancer. Our data demonstrated that IRIN-laden silicasomes can improve the PK and drug content over free drug and Onivyde® liposome at the tumor site. Moreover, the improved delivery was accompanied by improved efficacy and bone marrow and gastrointestinal toxicity reduction compared to the free drug and the liposome. We also demonstrated the expanded use of this platform in an orthotopic colon cancer model. In addition to IRIN, it was also possible to design transcytotic nanocarrier for the delivery of platinum-based drug, drug pair, and more recently mRNA. In the end, I will briefly mention the leveraged use of transcytotic delivery in non-carcinoma diseases.

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COMBINATION OF TUMOR THERMAL ABLATION, CYTOKINES AND LIPIDIC ADJUVANT PROVIDE A DISTAL IMMUNE RESPONSE

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CONTEXT

Cancer immunotherapy in combination with nanomedicine is of high interest to achieve a systemic immune response. Additional physical methods can bring the specificity related to immunogenic cell death. In the literature, interleukin-12 (IL-12) encoding plasmid associated with sonoporation has been shown to enhance antitumoral activity. Moreover, non-viral carriers and high-frequency ultrasound have both been shown to promote immune response activation. Finally, in situ activation of dendritic cells post-radiofrequency was shown to enhance the immune response.

MATERIALS AND METHODS

Cytokines or IL-12 encoding pFAR4-plasmid have been used in these studies. Local hydrogel or non-viral carrier stimulating the immune response were locally delivered. Thermal ablation by radiofrequency or focused ultrasound were applied to release tumor antigens. The concept was evaluated on cloned colorectal mice tumor, which tumor growth was followed by bioluminescence imaging.

RESULTS

Conditions of thermal deposition using HIFU were optimized. Lipid-mediated cell transfection and intratumoral injection into CT26 tumor mice using pFAR4-IL-12 led to the secretion of the IL-12 cytokine into cell supernatant and mice sera, respectively. The plasmid encoding pFAR4-IL-12 or TLR2 agonist alone had no impact on tumor growth compared with control mice, whereas the complete treatment consisting of pFAR4-IL-12, TLR2 lipid agonist, and HIFU limited tumor growth.

CONCLUSION

HIFU conditions can be modulated to stop tumor growth. The combined therapy was the most efficient in terms of IL-12 and IFN-γ production and mice survival. The study showed the interest of the combination of physical methods and nanocarriers to induce a potent immune response.

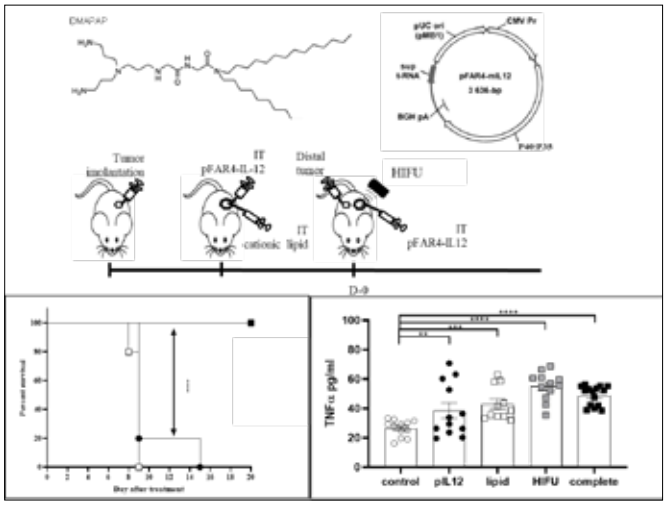

Figure : Lipid used as an adjuvant, Plasmid pFAR4 used to produce cytokines, Experimental protocol in mice. Results presented as survival rate and TNF α production.

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NEXT-LEVEL CHEMICAL TOOLS FOR BIOORTHOGONAL CLICK-TO-RELEASE

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The toolbox of bioorthogonal reactions has expanded substantially in the past decade, providing chemists with highly selective methods to achieve efficient ligation in complex biological environments, even in living systems [1]. In parallel, the concept of bioorthogonal bond-cleavage has further expanded the repertoire of *in vivo* chemical methods, enabling controlled molecular disassembly under physiological conditions [2]. Among these chemistries, the '*click-to-release*' reaction of tetrazines (Tz) and cleavable *trans*-cyclooctenes (TCO) stands out due to favorable reaction kinetics and adaptability [3,4]. This bioorthogonal elimination has enabled strategies for targeted drug delivery [4] and prodrug activation, leading to the first bioorthogonal approach tested in clinical trials [5], which marked the beginning of the era of clinical translation of *in vivo* chemistry.

To date, however, the concept of bioorthogonal bond-cleavage has been limited as existing tools have fundamentally lacked the performance characteristics (i.e., click kinetics, cleavage kinetics, cleavage yield) needed to make more advanced strategies plausible. For instance, efficient TCO-release has yet been restricted to Tz with relatively low click reactivity, while highly reactive Tz (e.g., bis(2-pyridyl)tetrazines) achieve only poor release (<10%). Based on our rigorous pursuit of mechanistic understanding [6,7], we have recently developed chemical tools with exceptional performance and unique capabilities. In this talk, I will present our key molecular designs and the challenges we faced on the way to boost bioorthogonal Tz/TCO bond-cleavage reactions.

To outmaneuver the yet uncontrollable orientation of the initial click reaction, we have developed a C_2symmetric TCO-linker (C_2TCO) that can be cleaved nearly instantaneously, enabling the concept of bioorthogonal turn-off [7, 8]. To address the drawback of C_2TCO in turn-on applications (i.e., no regiocontrol of release), hydroxypyridyl-substituted Tz have been designed that achieve complete and up to 20-fold accelerated TCO-release under physiological conditions. Finally, a universal *trans*-cyclooctene, iTCO, will be presented that can be cleaved independent from the structure of the Tz via an unexpected reaction mechanism based on intramolecularly controlled post-click tautomerization and subsequent elimination. Thereby, highly reactive Tz can be applied as molecular scissors, finally to '*exit the release cube*' [6] and enable bioorthogonal bond-cleavage with unprecedented click kinetics and release efficiency.

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NANOTECHNOLOGIES FOR COVID-19 AND BEYOND

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Besides millions of death and environmental challenges, Coronavirus disease 2019 (COVID-19) pandemic put an unprecedented strain on health infrastructure, economic status and wealth, and mental health across the globe. COVID-19 also constituted an opportunity; lessons learned may not only be successful in preparing for another pandemic, but also has opened up new research priorities. For instance, development and success of mRNA-loaded lipid nanoparticle-based SARS-CoV-2 vaccines has showcased the potential of mRNA technology not only for the development of next-generation vaccines for other viral and bacterial infections as well as complex diseases such as certain tumors, but also for the therapy of difficult diseases and disorders in adults and pediatric patients. Nevertheless, several challenges still remain and include identification of individual cancer neoantigens for mRNA vaccine design, validation of the most feasible vaccine administration route, identification of the most effective and safe transport routes for nucleic acid delivery across biological barriers, and overcoming adverse responses to nanoparticle-based technologies. This presentation will address these challenges through systems approaches and introduces work in progress towards development of biomarker detection technologies, "pseudovirus-like" nanoparticles for mechanistic studies, more effective and safer vehicles and therapeutic nanoplatforms than lipid nanoparticles for nucleic acid delivery across the biological barriers, and global solutions to nanoparticle-mediated adverse reactions.

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LYOTROPIC NONLAMELLAR LIQUID CRYSTALLINE NANOPARTICLES FOR IMMUNOMODULATION

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Non-lamellar lyotropic liquid crystalline (LLC) nanoparticles comprising cubosomes, micellar cubosomes and hexosomes are a di-

verse group of versatile self-assembled drug carrier platforms with unique structural, mechanical and surface properties that offer many advantages over liposomes and lipid nanoparticles for encapsulation of amphiphilic, hydrophobic and hydrophilic drugs. Our laboratories have produced a wide range of colloidally stable LLC nanoparticles from different lipids with and without stabilizers, and characterised them through integrated approaches including synchrotron small-angle X-ray scattering, cryogenic transmission electron microscopy and nanoparticle tracking analysis. This presentation will comment on diverse LLC morphology and inner architectural arrangements, dynamic LLC changes and transformations in biological fluids and microenvironments, composition-based control of immune responses such as complement activation, and biomedical and health-related applications based on LLC clearance mechanisms following intravenous and subcutaneous injection routes.

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ENGINEERING MICROBUBBLES FOR MECHANICAL MODULATION OF BIOLOGICAL BARRIERS UPON COMBINATION WITH ULTRASOUND

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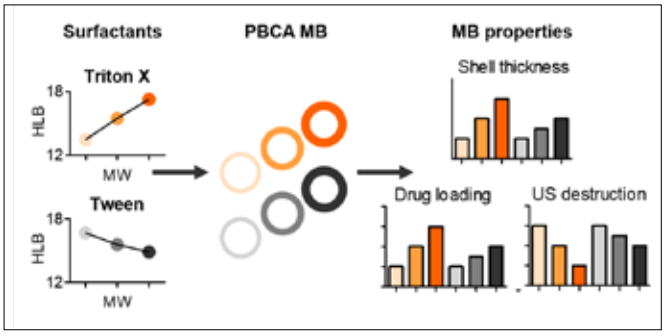

Figure 1. Manipulation of PBCA MB characteristics through chemical means

Gas-filled microbubbles (MB) are routinely used in the clinic as ultrasound contrast agents. Beyond their use for diagnostics, MB are increasingly explored as drug delivery vehicles, based on their tailorable acoustic responses, high drug loading capacity, and surface functionalization capabilities. Moreover, in recent years, MB are also being exploited to enhance perfusion of drugs in specific tissues (sonoporation) by opening biological barriers, such as the endothelial wall in tumors or the blood-brain barrier.

Surfactants, lipids, proteins, and polymers can stabilize the MB shell. Although soft-shell lipid MB tend to be clinically preferred for ultrasound imaging because of their excellent oscillation profile, hard polymeric MB can outperform their lipid counterparts for drug delivery applications, since their thicker shell can be loaded with higher amounts of drugs and still display very good US contrast capabilities. For instance, poly(butyl cyanoacrylate) (PBCA) is a polymer commonly used for MB synthesis, since it is biodegradable and used in an FDA-approved surgical glue for wound closure. PBCA MB performance as diagnostic and therapeutic agents is dictated by the shell physicochemical characteristics, which affect MB blood circulation, stimuli response and drug loading capacity, among others.

In this presentation, we will discuss different (bio)chemical approaches to manipulate the MB characteristics from highly elastic to highly brittle and how these affect MB application to ultrasound imaging, controlled drug release and sonoporation of biological barriers.

NC6300 NANOMEDICINE MODULATES THE TUMOR MICROENVIRONMENT AND IMPROVES THE EFFICACY OF IMMUNOTHERAPY

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INTRODUCTION

Anthracyclines are a class of chemotherapy used to treat cancers, including sarcomas, with immunomodulatory properties that could prime tumors for immune checkpoint inhibition therapy [1]. Doxorubicin is the standard first line treatment in patients with soft tissue sarcoma. Epirubicin, even though it induces less cardiac toxicity, is not FDA approved for the treatment of soft tissue sarcoma yet. The efficacy of both drugs is limited, and various severe rate-limiting toxicities remain, so more effective and safer anthracyclines are desired [2, 3]. NC-6300 is a formulation of epirubicin in a polymeric micelle nanocarrier. To form this nanocarrier, a salt of epirubicin is bound to a block-copolymer with a poly (ethylene glycol; PEG)-poly(L-aspartic acid) framework via an acid-labile hydrazine linkage, which enables selective release in the acidic tumor microenvironment (TME) and late endosomes of cancer cells. NC-6300 (epirubicin micelle) demonstrates anti-tumor activity in sarcoma patients, but whether it is combinable with immune checkpoint inhibition is unclear. Recently, we conducted a phase Ib dose escalation clinical trial of NC-6300 in patients with sarcoma (NCT03168061) and initiated an expansion cohort based on encouraging toxicity and efficacy data. Nonetheless, to what extent NC-6300 is combinable with immune checkpoint inhibitors is unclear. To this end, we tested in the current study [4] the efficacy of NC-6300 combined with anti-PD-L1 antibody treatment and investigated the effects of this combination on anti-tumor immune response and TME normalization.

RESULTS AND DISCUSSION

Fibrosarcoma tumors were generated by inoculating 6-week old C57BL/6 female mice with 2.5×105 MCA205 cells in 50 µL of serum-free medium into the flank. Osteosarcoma tumors were generating by implanting subcutaneously 1 mm3 dissected tumor chunks from K7M2-WT tumors into 6-week old BALB/c female mice. We tested the tumor growth delay induced by NC-6300 at 1 mg/kg, 3 mg/kg, and 10 mg/kg combined with anti-PD-L1 antibody in MCA205 fibrosarcoma (Figure 1A). While anti-PD-L1 antibody monotherapy did not induce a statistically significant reduction in tumor volume compared to control, 10 mg/kg of NC-6300 did (Figure 1B). Furthermore, at any dose of NC-6300, the combination with anti-PD-L1 antibody induced tumor growth inhibition (Figure 1B). Finally, we found that the combination using 3 mg/kg and 10 mg/kg NC-6300 induced significant tumor growth inhibition compared to anti-PD-L1 antibody alone (Figure 1B).

Then, we tested whether administering T cell depletive antibodies (anti-CD8 and anti-CD4 antibodies) reduces the effect on tumor growth and response to therapy of the combination of NC-6300 and anti-PD-L1 antibody (Figure 1C). Indeed, only the combination of NC-6300 and anti-PD-L1 antibody without depletion of T cells reduced tumor growth in K7M2, while the T cell-depleted combination therapy groups did not reduce tumor volume significantly compared to controls (Figure 1D). Furthermore, the combination therapy group had three responders (was defined as a stable or reduced tumor volume on the day of study completion compared to tumor volume at initiation) out of eight mice bearing K7M2 tumors and the combination group with anti-CD4 antibody also had one responding mouse (Figure 1E).

Furthermore, we tested whether NC-6300 can induce vascular and extracellular matix (ECM) normalization. Both NC-6300 alone and the combination with anti-PD-L1 antibody increased the fraction of pericytes associated with tumor blood vessels (Figure 2A,B) and reduced density of CAFs (Figure 2C) but only the combination reduced the fraction of proliferating CAFs (Figure 2D). CAFs produce ECM components, and these components together collaborate to compress tumor blood vessels. Both NC-6300 alone and its combination with anti-PD-L1 antibody reduced collagen I (Figure 2E, F).

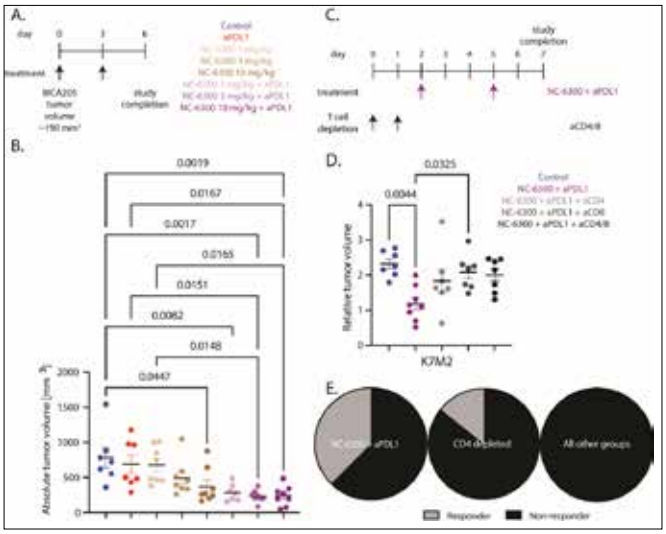

Figure 1. (A) Experiment schedule on MCA205 fibrosarcoma. (B) Tumor volume on day 6 of the study. (C) Experiment schedule of T cell depletion experiments in K7M2 tumors. (D) K7M2 tumor volumes at day 7 relative to treatment initiation. (E) Fraction of mice bearing K7M2 tumors responding to treatment. P-values less than 0.05 are denoted on the graphs.

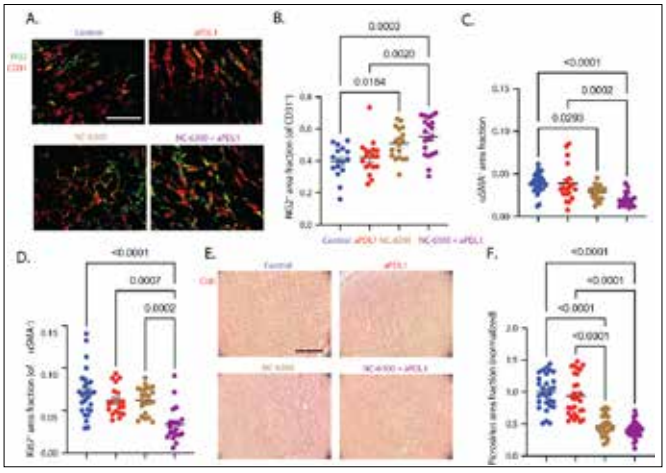

Figure 2. (A) Representative immunofluorescence images of NG2+ pericytes (green), CD31+ endothelial cells (red), and their colocalization (yellow). White scale bar indicates 0.1 mm. (B) Graph of the area fraction of the pericyte marker NG2 colocalized with the endothelial cell marker CD31 over the total CD31 area in immunofluorescence images. (C) Graph of the area fraction of the cancer-associated fibroblast marker αSMA in immunofluorescence images. (D) Graph of the area fraction of the proliferation marker Ki67 colocalized with αSMA over the total αSMA area in immunofluorescence images. (E) Representative light microscope images of Picrosirius red (red color). Black scale bar indicates 0.2 mm. (F) Graph of the area fraction of Picrosirius red indicating collagen fibers in tissue sections normalized to the average of value in control tumors. All groups were compared using one-way ANOVA with Tukey's correction. P-values less than 0.05 are denoted on the graphs.

Given the observation that NC-6300 normalizes the tumor microenvironment, we hypothesized that it could enhance the effects of anti-PD-L1 antibody on the migration and proliferation of T cells in tumors [5]. Indeed, both anti-PD-L1 and its combination with NC-6300 increased the amount of CD3+ T cells in the tumor (Figure 3A). However, only the combination increased CD8+ T cell density compared to every other treatment (Figure 3B). The proliferation of these T cells was increased by the combination compared to every other treatment group (Figure 3C). Thus, NC-6300 potentiated the ability of anti-PD-L1 antibody to induce migration and proliferation of CD8+ T cells in tumors.

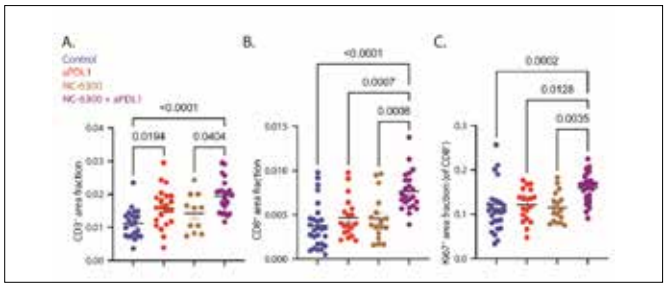

Figure 3. (A) Graph of the area fraction of the T cell marker CD3 in immunofluorescence images. (B) Graph of the area fraction of the T cell marker CD8 in immunofluorescence images. (C) Graph of the area fraction of the proliferation marker Ki67 colocalized with CD8 over the total CD8 area in immunofluorescence images. All groups were compared using one-way ANOVA with Tukey's correction. P-values less than 0.05 are denoted on the graphs.

CONCLUSION

We demonstrated in preclinical sarcoma models that the combination of NC-6300 and anti-PD-L1 antibody induces therapeutic responses not observed with either monotherapy. Additionally, NC-6300 causes tumor microenvironment normalization alone and in combination with anti-PD-L1. As a result, the combination is superior to anti-PD-L1 antibody monotherapy in increasing T cell distribution and proliferation in tumors.

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TRANSLATING INNATE IMMUNOTHERAPY TO THE CLINIC

WILLEM MULDER

Immunotherapy is revolutionizing the treatment of diseases. Most immunotherapy strategies engage the adaptive immune system. In recent years, emerging evidence has shown that the innate immune reprogramming induces long-term changes through metabolic and epigenetic programming of myeloid cells, including monocytes, neutrophils and macrophages. Therefore, targeting myeloid cells and their progenitors is a powerful 'innate immunity-regulating framework' to govern the delicate balance of immune homeostasis, priming/training and tolerance. This Presentation will not only showcase how nanomedicine-based immunotherapies can achieve long-term therapeutic benefits in immune-mediated diseases, it will also lay out the entrepreneurial activities we established for clinical translation.

BERNOULLI PRINCIPLE: FORCES ACTING ON LIPID BILAYERS IN THE CARDIOVASCULAR SYSTEM

BERT MÜLLER

The disease arteriosclerosis generally leads to plaque formation and related constrictions in arteries. By the age of 65, almost all people are affected to certain degree. It is regarded as the most frequent cause of death and disability in the developed world [1]. The economic impact cannot be underestimated. For example, just in U.S. during 2011, coronary atherosclerosis resulted in aggregate inpatient hospital costs of $10.4 billion [2]. By means of endogenous molecules or drugs termed vasodilators, the vessels can be widened. Their systemic application, however, has serious limits, because a critical stenosis of about 1 cm length is just 10-10 of the entire human blood vessel system. The question arose whether the blood flow, modified by the constriction, could be employed as a pure physical trigger to release a vasodilator from a suitable container [3]. A biomimetic approach for this container would be a lipid bilayer, well known from the cell membrane, termed liposome. The mechanical stimuli acting on spherical liposomes with diameters below 1 µm, however, are too small to induce a cargo release. Non-spherical, facetted liposomes are a valid approach for targeted vasodilator release [4].

The combination of microfluidics and spatially resolved small-angle X-ray scattering allows for the detection of flow-induced structural changes in nanometer-sized liposomes [5]. Modifications in the projected liposome size and in the bilayer thickness are detectable [5].

It has been demonstrated that the predicted pressure-gradient force [6] at stenosis changes the bilayer thickness of the non-spherical liposomes close to the inlet [5]. This phenomenon is much stronger than the impact of the shear stress within the constriction for the flow conditions selected. The measured bilayer thickness increase of about 30% fits the transition from interdigitated lipids to non-interdigitated ones associated with a substantial stiffness reduction. This stiffness reduction changes the liposome shape to the equilibrium states.

Non-spherical liposomes can be fabricated from artificial lipids. By interdigitation, they form a stiff bilayer, known from paper, which can only be bent in one direction. The defects of the non-spherical liposomes allow for structural changes by pressure-gradient forces known from the Bernoulli equation. These structural transitions are associated with cargo release and can be exploited for a vasodilator release at constricted arteries within the human body [4].

This work has been based on ideas developed during scientific discussions between Dr. med. Till Saxer (Geneva) and the author. It was largely funded by the Swiss National Science Foundation (project 126090) via the National Research Program 'Smart Materials'. The author thanks the former PhD-students Marzia Buscema, Margaret Holme, and Sofiya Matviykiv for their substantial contributions. The essential support of the scientists Hans Deyhle, Marianne Liebi, Viviane Lutz Bueno, Thomas Pfohl, and the research team of Andreas Zumbuehl is gratefully acknowledged.

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ELUCIDATING THE USE OF LYSOPHOSPHOLIPIDS IN SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS

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Self-nanoemulsifying drug delivery systems (SNEDDS) for oral drug administration have garnered significant attention as versatile platforms for enhancing the absorption of both poorly water-soluble drugs and peptides. SNEDDS are isotropic mixtures of oil, lipophilic surfactants (co-surfactants), hydrophilic surfactants and co-solvents that form a (nano)emulsion upon dispersion in an aqueous medium. Poorly soluble drugs are often dissolved in the SNEDDS, whereas peptides are often added as a lipophilic complex.

Lysophospholipids have emerged as potential co-surfactant in SNEDDS due to their unique physicochemical properties and biological compatibility. Lysophospholipids are derived from phospholipids by the removal of one fatty acid chain, which renders them less lipophilic and they thus have better amphiphilic properties than phospholipids. Lysophospholipids can form micelles and have a relatively low critical micelle concentration, whereas phospholipids form vesicles in aqueous media.

Addition of lysophospholipid to SNEDDS increases viscosity and therefore only up to around 30% can be added. Lysophospholipid decrease the size of the nanoemulsion formed by dispersion of the SNEDDS in aqueous media. This is especially the case for SNEDDS based on medium chain lipids. Upon *in vitro* digestion, simulating the conditions in the small intestine, the presence of lysophospholipid in SNEDDS can abolish the formation of multilamellar vesicles, otherwise observed upon digestion of SNEDDS. This ability to change the properties of SNEDDS can potentially change drug absorption from lysophospholipid containing SNEDDS, compared the SNEDDS without lysophospholipid.

Another property of lysophospholipid is that it can also work as a permeation enhancer, by catalyzing the opening of the tight junction between the cells in the intestinal membrane. Inclusion of a permeation enhancer in the formulation is a prerequisite for oral peptide administration. In addition, lysophospholipids can be used for making a lipophilic complex of peptides prior to loading into SNEDDS. This has led to increased peptide incorporation in SNEDDS and also increased absorption.

For some poorly soluble drugs with low lipophilicity, a lipophilic complex can also increase drug solubility in SNEDDS, thus leading to a higher dose. This approach has also shown to increase the absorption of a poorly soluble drug.

In conclusion, the integration of lysophospholipids into SNEDDS potentially represents a significant advancement in the field of oral drug delivery. The unique attributes of lysophospholipids offer the potential to enhance the formulation of SNEDDS, leading to improved drug solubilization and absorption. Further research and development are needed to fully understand the mechanisms underlying the interactions between lysophospholipids, drug compounds, and physiological environments, thereby enabling the translation of this innovative approach into practical applications for improved therapeutic efficacy.

BIOMEDICAL APPLICATIONS OF SYNTHETIC BIOLOGY

LIOR NISSIM

One of the significant challenges in the biotech industry is expressing the right transgene in a specific cell state, at the exact timing and the exact concentrations. However, precise gene regulation is complex, leading to insufficient safety, poor efficacy, and cost overhangs. To address this challenge, the Nissim lab developed several proprietary synthetic biology platforms that enable tightly regulated gene expression. These platforms have been implemented in

multiple companies, including anti-viral vaccines, cancer immunotherapy, cultivated meat, and adoptive cell therapies.

NEXT-GENERATION ANTI-VIRAL VACCINES:

Most current vaccines mediate either extracellular exposure to viral particles (traditional vaccines) or intracellular expression of viral antigens (nucleic-acid based vaccines), thus ineffectively activating the immune system and limiting the efficacy and duration of immunization. The Nissim lab and Prof. Chezy Barenholz developed a formulation that generates both intracellular viral protein expression and the release of attenuated viral particles into the extracellular matrix. This approach would enable robust, safe, and cost-effective nucleic-acid based anti-viral vaccines.

SYNTHETIC PROMOTERS WITH ENHANCED CELL-STATE SPECIFICITY (SPECS):

SPECS consist of artificial sequences that regulate gene expression with superior specificity over native regulatory sequences. Thus, SPECS constitute key enabling technology for targeted gene expression. We developed a novel platform to identify SPECS for virtually any cell, which can be executed in a fraction of the time, cells, and cost compared to the previous methodologies.

SYNTHETIC GENE CIRCUITS THAT EXECUTE BOOLEAN LOGIC IN LIVING CELLS:

The Nissim lab developed several synthetic gene circuits that integrate SPECS activity via Boolean logical gates, such as AND, XOR, or NAND, and express output genes only when the logic conditions are met. Integrating the activity of multiple SPECS via logical gates provides enhanced cell-state specificity, as well as tunability, minimal activation threshold, and input amplification that enhances both the safety and efficacy of targeted therapies.

DISPELLING THE MYTH – LOOKING AT BENEFIT/RISK

MARISA PAPALUCA AMATI

Nanomaterials from fabric dyes to mobile phones are part of modern life. Nanomaterials allow new ways of dealing to physical and chemical challenges, manufacturing extraordinarily resistant materials, accelerating miniaturisation of electronic goods, progressing labs on chips and much more in a variety of Industries. The damage induced by certain nanoparticles in living organisms, sparkled pivotal thinking and research on structure-related *in vitro* and *in vivo* behaviours and risk mitigation approaches. Three decades of learning process on “nano” risks taught us a lot on the clinical significance of the interaction between nanoparticles and living organisms depending on size, chemistry, surface, charge, shape etc. We learned also how to master those properties to produce “nanomedicines”, unique therapeutics and vaccines, New Active molecular Systems. From the early liposomes, to more recent Lipid Nano-Particles and nano-theranostics, we fostered unique desirable pharmacokinetics properties, multiple functions, preferential delivery of otherwise too toxic or too fragile payload at a desired target. After many years of focus on risks and uncertainties, we now have a rising wave of true therapeutic innovation and modern tools to accelerate our actual learnings of the evolving benefit/risk balance.

DEVELOPING AN EFFECTIVE MRNA-LNP VACCINE AGAINST A HIGHLY LETHAL BACTERIUM

DAN PEER [1,2,3,4,‡,*], Edo Kon[1,2,3,4,†], Yinon Levy[5,†], Uri Elia[1,2,3,4,5,†], Hila Cohen[5], Inbal Hazan-Halevy[1,2,3,4], Moshe Aftalion[5], Assaf Ezra[1,2,3,4], Erez Bar-Haim[5], Gonna Somu Naidu[1,2,3,4], Yael Diesendruck[1,2,3,4], Shahar Rotem[5], Nitay Ad-El[1,2,3,4], Meir Goldsmith[1,2,3,4], Emanuelle Mamroud[5,‡], Ofer Cohen[5, ‡y]

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Lipid nanoparticle (LNP) mRNA vaccines have emerged as an effective vaccination strategy. Although currently applied towards viral pathogens, data concerning the platform's effectiveness against bacterial pathogens are limited. Herein, we developed an effective mRNA-LNP vaccine against a lethal bacterial pathogen by optimizing mRNA payload GC content and antigen design. We designed a nucleoside-modified mRNA-LNP vaccine based on the bacterial F1-capsule antigen, a major protective component of Yersinia pestis, the etiological agent of plague. Plague is a rapidly deteriorating contagious disease that has killed millions of people during the history of humankind. Currently, the disease is treated effectively with antibiotics, however, in the case of a multiple-antibiotic-resistant strain outbreak, alternative countermeasures are required. Our mRNA-LNP vaccine elicited humoral and cellular immunological responses in C57BL/6 mice and conferred rapid, full protection against lethal Yersinia pestis infection after a single-dose. These data open avenues for urgently needed effective anti-bacterial vaccines.

MODULAR AND ADAPTIVE SELF-ASSEMBLING DENDRIMERS FOR NANOMEDICINE

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The application of nanotechnology is widely expected to bring breakthrough in medicine for disease treatment and diagnosis. Dendrimers are ideal materials for elaborating nanomedicine by virtue of their well-defined structure, multivalent cooperativity and nanosize *per se*. We have recently established modular and adaptive self-assembling dendrimer nanosystems[1] for the delivery of imaging agents,[2] anticancer drugs[3] and nucleic acid therapeutics[4] for cancer detection and treatment (Figure 1). Remarkably, these dendrimer nanosystems are able to exploit the *in situ* tumor-secreted extracellular vesicles for intercellular delivery and deep penetration in tumor tissue, overcoming tumor heterogeneity and dynamic evolution (Figure 2).[3] Also, selective delivery of nucleic acid cargoes can be achieved using amphiphilic dendrimers of different generations yet benefiting the delivery advantages of both lipid and polymer vectors (Figure 3).[4] Our findings offer a fresh perspective for exploiting the advantageous features of supramolecular dendrimers to reach the ultimate goal of smart nanomedicine.

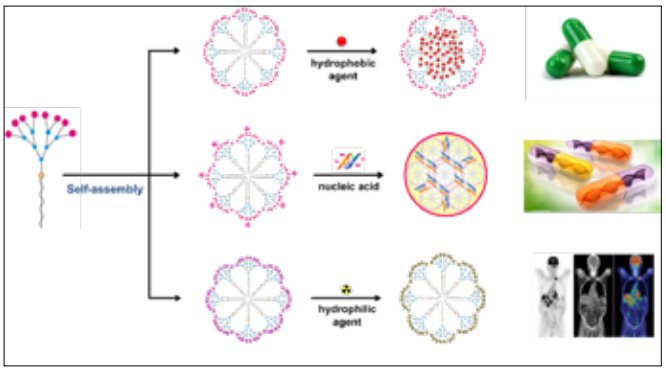

Figure 1: *Cartoon representations of the self-assembly of amphiphilic dendrimers into supramolecular dendrimer nanosystems for the delivery of hydrophobic anticancer drugs and hydrophilic imaging agents as well as negatively charged nucleic acid therapeutics.*

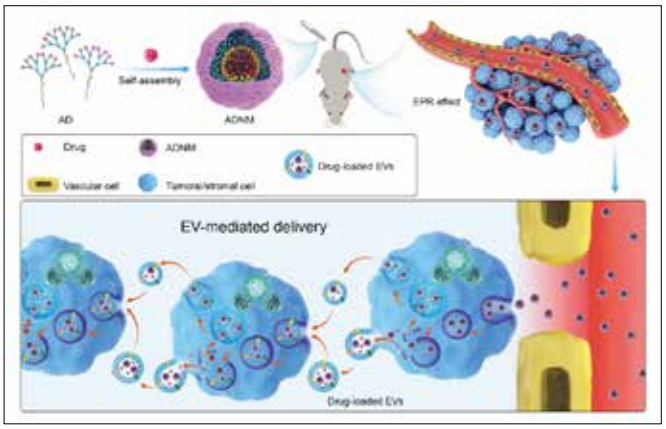

Figure 2: *Amphiphilic dendrimer nanomicelles (ADNMs) encapsulate the anticancer drug and induce tumor-assisted drug delivery via extracellular versicle (EV)-mediated intercellular transport for overcoming tumor heterogeneity and dynamic evolution.*

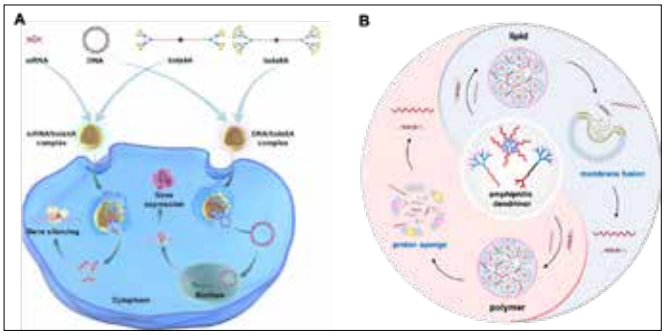

Figure 3: *(A) Selective delivery of nucleic acid cargos mediated by bola-amphiphilic dendrimers of different generations. (B) Amphiphilic dendrimers for nucleic acid delivery by making use of the delivery advantages of both lipid and polymer vectors*

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NANOPARTICLE DELIVERY ENHANCES THE ANTI-NOCICEPTION EFFECT AND DURATION OF A CGRP RECEPTOR ANTAGONIST IN ORAL CANCER PAIN MODELS

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PROBLEM

Affecting 30,000 Americans annually, oral cancers are difficult to treat with a modest 50% 5-year survival rate1. Oral cancers are extremely painful with increasing pain as the disease progresses2. It is well established that the majority of cancer pain is initiated at the tumor site3. Pressingly, the therapeutic options to treat cancer pain are limited to opioids, which have profound drawbacks, including sedation, respiratory depression, nausea, constipation, tolerance and dependence4, 5. As an alternative to opioids, we target the calcitonin gene-related peptide (CGRP) receptors on cancer cells, which we recently demonstrated are implicated in cancer pain signaling.6 Due to the leaky vasculature and enhanced lymphatic drainage characteristic of oral cancers, maintaining drug concentrations in the tumor microenvironment (TME) is challenging7. This limits the duration of efficacy of free small molecule CGRP antagonists. Our goal is to enhance the duration and intensity of analgesia by using CGRP antagonist loaded nanoparticles, which (1) are better retained in the TME due to their large size (100 nm) compared to small molecule drugs (10 Å) and (2) can be designed for sustained drug release.8

METHODS

The CGRP antagonists olcegepant was encapsulated into PLA-PEG nanoparticles using the Flash NanoPrecipitation (FNP) process, in which nanoparticles are assembled via diffusion limited self-assembly driven by hydrophobic interactions.9, 10 The hydrophobic ion pairing (HIP) nanoformulation approach was used to control the olcegepant release rate. Nanoparticles were characterized by standard physicochemical characterization techniques. To look at the NP uptake, a co-culture model with human Schwann cells and human squamous cell carcinoma (HSC-3) cancer cells was used. To test antinociception *in vivo*, an HSC-3 cancer xenograft paw and tongue model were used. After the mice developed cancer nociception, the mice were injected with either with free olcegepant (500 nM) or olcegepant-loaded nanoparticle (500 nM). Saline and empty NPs served as controls. To measure cancer nociception development in paw or tongue and to determine the treatment efficacy, we used the von Frey filament and Hargreaves assays for mechanical and thermal allodynia, respectively.

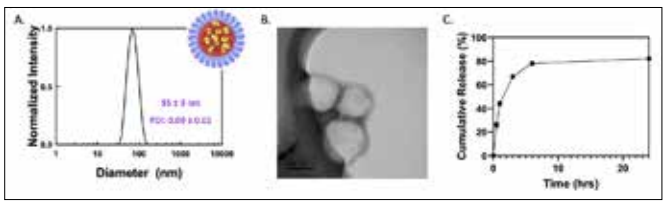

Figure 1. A. A representative DLS trace of the olcegepant NPs showing a narrow size distribution and an intensity average diameter of 95

nm. B. TEM image of the olcegepant-loaded NPs. C. Olcegepant nanoparticle release kinetics.

RESULTS

Olcegepant was successfully encapsulated into 100 nm PLA-PEG nanoparticles using the HIP nanoformulation approach with pamoic acid as the counter ion. Nanoparticles had an olcegepant drug loading of 5 wt%. The olcegepant FNP encapsulation efficiency was 60%. The nanoparticles exhibited a slow drug release rate with 80% drug release after 24 hours. (Figure 1.)

In an *in vitro* co-culture model, NPs were observed to be taken-up equally both by HSC-3 cancer cells and Schwann cells. In the cancer paw model, free olcegepant reduced 75% mechanical and 50% thermal cancer nociception for 6 hours post-administration. The olcegepant-loaded NPs-loaded reduced 98% mechanical and 100% thermal cancer nociception for 12 hours post-administration with a persistent reduction in pain lasting up to 24 hours. The NPs outperformed free drug both in the intensity and duration of analgesic effect. Importantly, NPs show near complete reversal of cancer pain. (Figure 2.)

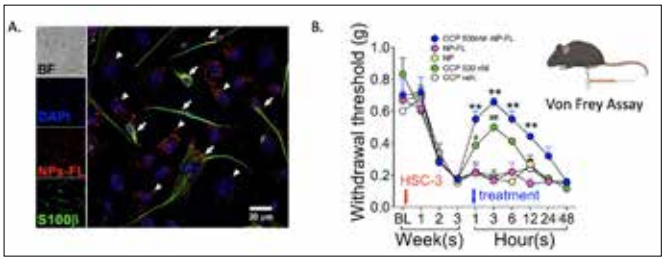

Figure 2. A. HSC-3 (arrowheads) and mouse Schwann cells (arrows) showed rubrene-labeled OCP-NPs uptake. B. OCP-NPs reduced mechanical nociception for 9 hours longer and >2 times greater than OCP (AUC 1.4 ± 0.18 vs 0.5 ± 0.1).

IMPLICATIONS

Inhibiting CGRP receptor signaling is a promising approach for the treatment of cancer pain. By using a nanoparticle drug delivery approach to deliver olcegepant in the TME, the duration and intensity of antinociception can be dramatically increased. This opens a new avenue beyond opioids for cancer pain therapeutics.

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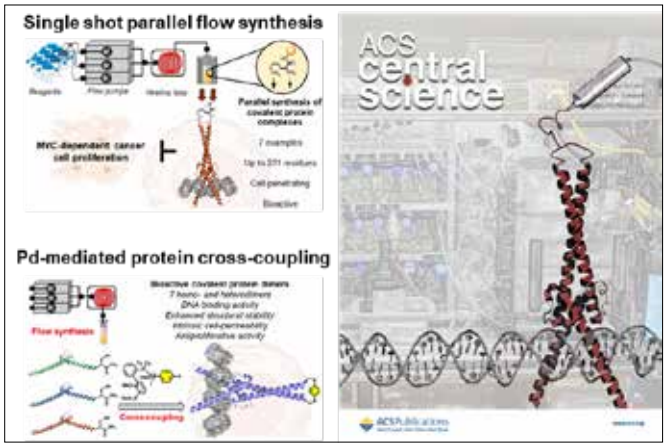
ENGINEERING BIOACTIVE DIMERIC TRANSCRIPTION FACTOR ANALOGS TO INHIBIT MYC DEPENDENT GENE PROGRAMS IN CANCER

SEBASTIAN POMPLUN

Dysregulation of the transcription factor MYC is involved in the majority of human cancers. The dimeric transcription factor complexes MYC/MAX and MAX/MAX bind to the same enhancer box (E-Box) DNA. MYC/MAX activates, and MAX/MAX inhibits gene transcription. Inspired by the MAX/MAX activity, we engineered covalently linked, synthetic homo- and heterodimeric analogs of MYC, MAX, and Omomyc, to inhibit MYC-dependent gene transcription. We prepared the dimers (167-231 residues) in a single shot via chemical flow-synthesis or via palladium mediated protein cross-coupling. All variants displayed correct folding, DNA binding activity, and enhanced structural stability compared to their non-covalent counterparts. The dimers are intrinsically cell-penetrating and inhibit cancer cell proliferation in low micromolar concentrations. Via RNA sequencing and RT-qPCR we showed that the synthetic dimers specifically downregulate MYC-dependent transcription. This work shows the intriguing potential synthetic protein based modalities for artificial gene regulation.



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(+ = equal contribution)

NANO GOES ICU: HEPATOCELLULAR TARGETING OF PI3K-SIGNALING IN SEPSIS TO RESTORE LIVER FUNCTION

ADRIAN PRESS

Sepsis affects a substantial number of adults in the world wide, leading to a significant number of in-hospital deaths each year. The current understanding of sepsis has shifted from an emphasis on systemic inflammatory responses to the intricate interplay between the host's defense mechanisms and the abreaction affecting both directly involved and remote organs. In this context, metabolic adaptation plays a crucial role. Identifying successful therapeutic approaches requires a deeper comprehension of the clinical and biological interactions that define disease phenotypes. Among these phenotypes, impaired hepatic excretory function has been associated with the most unfavorable outcomes.

Previous research has shown that mice lacking PI3Kγ are protected against hepatic excretory dysfunction during sepsis, yet they remain vulnerable to peritonitis due to a severe immune defect. This indicates conflicting roles of PI3Kγ in liver parenchyma and immune cells, and raises the possibility of selectively targeting PI3Kγ to hepatocytes without compromising immune function. Achieving such selective targeting while avoiding potential deleterious effects on immunity would represent a fundamental improvement in sepsis therapeutics, as previous adjunct therapies have failed to yield satisfactory results.

Nanomedicine holds great promise in addressing the challenges of sepsis treatment, but the design of therapeutics with optimal characteristics, such as low toxicity, simplicity, and efficacy, is critical. The pharmacological and toxicological properties of nanocarriers depend on factors like size, composition, surface charge, and shape, which in turn determine uptake, immune cell recognition, and circulation time. However, the presence of the enhanced permeability and retention (EPR) effect poses a significant obstacle in conditions like sepsis, where barrier functions are universally disrupted. To overcome this challenge, modifying nanocarriers with dyes known to act as ligands for organic anion transporters can enable substantial enrichment of the drug cargo into hepatocytes, while minimizing uptake by neutrophils or tissue macrophages lacking these transporters.

In this study, we aimed to restore liver excretory function by selectively targeting PI3Kγ in hepatocytes. Our hypothesis posited that such targeted intervention could attenuate liver failure in sepsis-induced peritonitis, thereby minimizing side effects on immune function and ensuring an unimpaired peritoneal host response. To test this postulate, we employed dye-functionalized liposomes to deliver AS605240, a PI3Kγ inhibitor, specifically to hepatic parenchymal cells in a severe polymicrobial peritonitis model. This model was chosen as it allowed us to examine the effects of selective PI3Kγ targeting in a context where compromising immunity would have detrimental consequences.

The findings of this investigation have the potential to advance our understanding of sepsis therapy by exploring the intersection of clinical nanomedicine and targeted interventions. By selectively modulating PI3Kγ activity in liver cells, we may be able to improve liver function while preserving crucial immune responses. Moreover, the use of dye-functionalized liposomes offers enhanced drug delivery to hepatocytes while reducing uptake by immune cells lacking specific transporters.

In summary, this research explores a novel approach in the field of clinical nanomedicine for sepsis treatment. The results provide valuable insights into the potential of selective hepatocellular targeting of PI3Kγ as a therapeutic strategy, with implications for improving liver function and patient outcomes in sepsis. Furthermore, this study contributes to the development of targeted therapies, addressing the long-standing challenges in sepsis management where conventional adjunct therapies have fallen short.

CLDN6 CAR-T CELL THERAPY OF RELAPSED/ REFRACTORY SOLID TUMORS

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BACKGROUND

We are evaluating a chimeric antigen receptor (CAR)-T cell therapy targeting the oncofetal antigen claudin 6 (CLDN6) as either a monotherapy or in combination with an mRNA vaccine (CARVac) designed to stimulate CLDN6 CAR-T cells *in vivo*.

METHODS

The ongoing first-in-human trial BNT211-01 assesses CLDN6 CAR-T cell therapy ± CARVac after lymphodepleting chemotherapy in patients with relapsed/refractory CLDN6-positive solid tumors. Key endpoints include safety, tolerability, and anti-tumor efficacy.

RESULTS

Twenty-two patients, 13 with testicular germ cell tumors (GCT), 4 with epithelial ovarian car-cinoma, and 5 with other solid tumors received 1x107 or 1x108 CLDN6 CAR-T cells ± CAR-Vac. Robust engraftment and expansion were observed, and both treatments were well tol-erated. Dose-limiting toxicities occurred in 2 patients at the higher dose level, resolving with-out sequelae. Cytokine release syndrome was reported in 10 patients (grade 3 in 1 and ≤ grade 2 in 9 patients). Grade 1 neurotoxicity occurred in 1 patient. Most treatment-emergent adverse events ≥ Grade 3 were related to chemotherapy or were asymptomatic transami-nase/lipase elevations.

The overall response rate (ORR) was 33%, with one complete and 6 partial responses in 21 evaluable patients. Seven patients had stable disease (disease control rate (DCR) of 67%). The highest response rate was observed in GCT patients at the higher dose level (ORR 57%, DCR 85%), with 42% progression-free survival at 6 months.

CONCLUSION

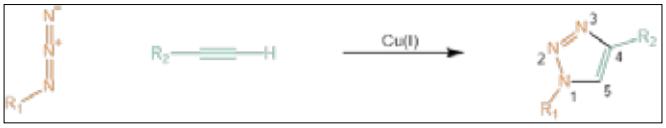
In this multicenter study, CLDN6 CAR-T cells ± CARVac had a manageable safety profile and induced encouraging responses in heavily pretreated patients with CLDN6-positive tumors. (Funded by BioNTech Cell & Gene Therapies GmbH; NCT04503278).

CLICR® – AN INNOVATIVE CLASS OF METAL FREE CLICK REAGENTS TO ENABLE A BROAD DIVERSITY OF BIOCONJUGATIONS

CRISTIANNE RIJCKEN

Cristal Therapeutics focuses on its proprietary CliCr® technology, an innovative class of cyclic alkynes for metal-free strain-promoted click chemistry. CliCr® has a higher stability, enables faster reactions, and generates optimal bioconjugate products. Our mission is to offer our CliCr® technology to partners under license agreements in different diagnostic and therapeutic areas.

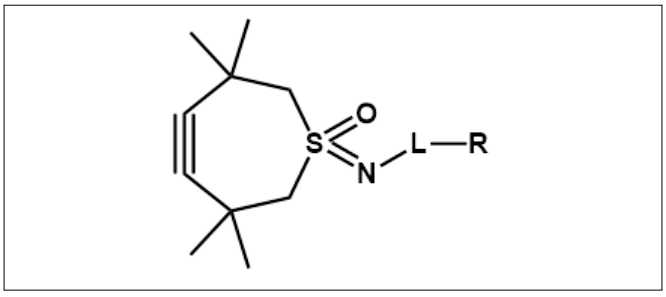
BACKGROUND



Schematic illustration of the click reaction between an azide and an alkyne.

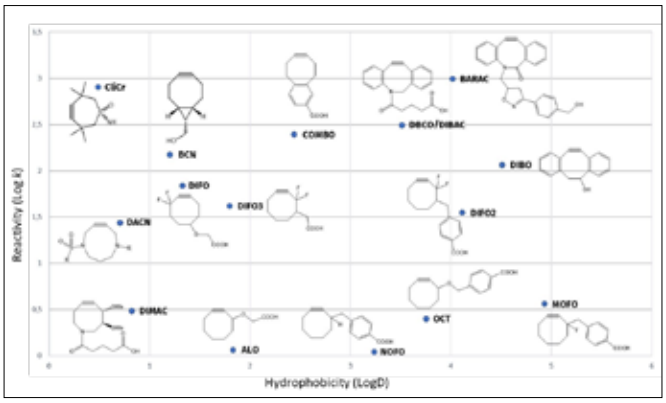
Alkynes and azides can undergo a Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) to afford the 1,4-disubstituted triazoles. This type of chemical transformation was dubbed "Click Chemistry" and awarded with the 2022 Nobel Prize for Chemistry.

The reactivity of an alkyne strongly depends on the electronic and steric characteristics of neighbouring substituents as well as structural strain. In comparison to linear acyclic alkynes, strained cyclic alkynes show remarkably high reactivity without addition of a catalyst, e.g. copper, as typically used for click chemistry. Thus, the metal-free strain-promoted azide-alkyne cycloaddition (SPAAC) is a valuable tool especially for future *in vivo* applications. Many SPAAC reagents are hydrophobic, show slow conversion, are incompatible with biomolecules or are too unstable. Herein, we present a novel SPAAC alkyne reagent, which overcomes the aforementioned drawbacks.



Chemical structure of the CliCr® base compound and its derivatization possibilities.

The CliCr® reagent meets with all demands as required in the life science industry by its innovative TMTHSI moiety. The 7-membered ring can be conveniently functionalized with a variety of linkers, e.g. via acylation, sulfonylation, N-alkylation, or carbamoylation.



aReactivity and hydrophilicity of CliCr® compared to other strained alkynes.

The versatility of CliCr-reagents to conjugating other moieties has been illustrated by the reaction of TMTHSI-derivatives with small molecules, exemplified by the click-reaction with a dye and a folic acid derivative, as well as peptide, proteins (including ADC), nucleic acid biologics and nanoparticles. Besides, a plethora of additional important applications is envisioned, such as surface plasmon resonance (SPR) applications and conjugation of chelator moieties for radioactive isotope incorporation in theragnostic applications. In comparison to other strained alkynes, CliCr® creates faster, more hydrophilic bioconjugates with superior profiles and more attractive costs of goods.

CliCr® is shown to be > 5 times more reactive than BCN. The reaction progress of 5 mM CliCr® or BCN-OH, respectively, with 1.3 eq. of benzylazide in CDCl3 at room temperature was monitored by MS. The reaction conversion was measured based on the increase of the triazole signals (see https://doi.org/10.1039/d0sc03477k).

Within our portfolio, we offer both CliCr® as a base compound and a selection of CliCr® derivatives. The CliCr® derivatives can be functionalized with a moiety of your own preference and subsequently clicked to azide compounds with high efficiency and excellent stability.

In sum, the key advantages of this technology are:

- shorter reaction times than all other marketed copper-free click reagents
- more hydrophilic than other strained alkynes
- highly selective, site-specific conjugation
- Stable bond formed between protein and linker
- High variability of linkers and payloads can easily be attached to the reagent

Currently, Cristal has collaborations with Synaffix on ADC application, with Iris Biotech on catalogues sales and with Lonza as they have incorporated CliCr® in their bioconjugation toolbox. Meanwhile, we are actively discussing with various other biotech and pharma companies on other applications and product developments.

CliCr® is patent by Cristal Therapeutics. For information on acquiring a license to this technology, please contact Cristal Therapeutics, Oxfordlaan 55, 6229 EV Maastricht, The Netherlands info@cristal-therapeutics.com.

LNP FORMULATION SCREENING, A CDMO PERSPECTIVE

UMBERTO ROMEO

Nanoparticles have been extensively investigated for drug delivery for decades. Lipid-based nanoparticles such as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have demonstrated tremendous clinical success in delivering both hydrophobic and hydrophilic therapeutics.

Particularly, lipid nanoparticles (LNPs) have been recognized as an ideal carrier for nucleic acids like DNA, mRNA, and different synthetic oligonucleotides such as siRNA, saRNA, miRNA and other regulatory RNAs, due to their outstanding biocompatibility, biodegradability, and entrapment efficiency. ONPATTRO (Patisiran) is the first approved double-stranded small interfering RNA delivering LNP (2018). [2]

RNA therapeutics have shown potential in various medical applications, including virus vaccines, cancer immunotherapy, and gene editing. The need of drug delivery vehicles including lipid nanoparticles (LNP) for RNA therapeutics have been developed because of the instability of RNA. Currently, ionizable lipids are considered as crucial components of LNP-based RNA therapeutics because they are positively charged at a low pH to enhance the encapsulation of negatively charged RNA, and the charge becomes less positive or almost neutral at physiological pH (~7.4), to reduce the toxicity 5. In

addition, various mRNA engineering methods have been developed to enhance the stability and the translation efficacy of mRNA therapeutics, such as a selection of untranslated regions (UTRs), addition of a poly-A tail, capping, and nucleoside modification 6. Based on these advanced techniques, two LNP-based mRNA vaccines (BNT 162b2, Pfizer-BioNTech; and mRNA-1273, Moderna) were successfully developed and obtained authorization from regulatory agencies in 2020 in multiple countries 7. Additionally, multiple types of LNP-based RNA therapeutics are under active investigation to treat various infectious diseases and cancers 8. [3]

CordenPharma played an important role during the Covid-19 pandemic response, ramping up its lipid manufacturing capacity across multiple sites to support the global demand of GMP grade lipids needed for the manufacture of Moderna's Spikevax™ vaccine.

Acknowledging the importance of LNPs as non-viral based delivery systems with the potential to revolutionize the current standard of medical therapy for a multitude of disease as well as enabling new therapeutic strategies for unmet clinical needs, CordenPharma initiated in late 2021 a strategic investment program at our Sterile Injectable site CordenPharma Caponago (Italy) to establish LNP formulation development and cGMP manufacturing capabilities to support an promote fastrack Nanoparticles-based drug development for our partners globally.

Exploiting our state of the art technologies we demonstrated through the planning and execution of a pilot study to be able to reproduce commercially viable LNP formulations such as the ones used for the COVID-19 vaccines, but as well novel LNP formulations usable in a pre-clinical and eventually clinical setting through the assessment of the efficacy in terms of encapsulation efficiency (EE%) and transfection capability in different cell lines.

The present discussion will be focused on the evaluation of the data generated through the aforementioned pilot study.

In the wake of the obtained results, we've already started to create much more combinations of lipidic mixtures to formulate new LNPs that will be fully characterized and tested as well, with the aim of creating a wide library of LNPs with specific physical and biological characteristics to be eventually progressed in pre-clinical and clinical development in in collaboration with our partners.

POLOXAMERS: INDIVIDUAL, VERSATILE AND SAFE

MEIKE ROSKAMP

Poloxamers, a family of triblock copolymers composed of polyethylene oxide (PEO) and polypropylene oxide (PPO), are used in various pharmaceutical applications. Their physicochemical properties, including surface activity and gel point, highly depend on molecular weight and PEO/PPO ratio. Most applications tolerate small variations in chemical composition and are therefore less affected by batch to batch variations of commercially available poloxamers, but some applications benefit from a more precise chemical composition to achieve reproducible performance. To address the needs of these applications, we are developing new poloxamer grades that not only address batch to batch variability but also provide an improved safety profile.

THE QUEST FOR NEXT GENERATION LIPID NANOPARTICLES FOR CELL-SPECIFIC DELIVERY OF MRNA.

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The field of nanomedicine is moving from an age of renaissance towards industrial revolution, in part, due to the ability of lipid nanoparticles (LNP) to package and deliver antigenic mRNA against SARS-CoV2, leading to the development of a powerful vaccine against COVID. The next horizon for our field remains tissue/cell-specific mRNA delivery that can lead to treatment of several diseases. Our lab has worked extensively in LNP design, synthesis, structure, and its impact on intracellular delivery of mRNA. I will talk about our labs pursuit onto understanding the journey of an LNP-mRNA within the cellular interior. These fundamental insights led us to design nanoparticles that can deliver mRNA to different tissues for the treatment of cystic fibrosis, retinal degeneration, and as COVID-19 therapeutics. We have developed novel LNPs that target the lungs after inhalation or IV injection for gene delivery and editing. We have shown that novel peptide guided LNPs can deliver mRNA selectively in the retina of non-human primates. Evolution of a cell-selective carrier for mRNA delivery through approaches will be discussed. Such platform technologies with capability to precisely deliver cargo to manipulate cells, correct diseases, with minimal off-target effects, will transform modern medicine.

INTERACTIONS OF CORONA PROTEINS WITH CELL RECEPTORS AND NANOPARTICLE UPTAKE MECHANISMS FOR TARGETING

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Increasing evidence has shown that the biomolecules adsorbing on nanomedicines upon administration can mediate specific interactions with cell receptors and may be used for targeting nanoparticles (for instance as reported for Onpattro). We previously showed that by modulating cell receptor interactions, the corona proteins also affect the mechanism cells use for nanoparticle internalization.[1] This may have important implications for transfection and intracellular RNA delivery as well. Based on similar observation, several efforts have been focused in correlating corona composition and cell uptake in order to identify corona proteins promoting or reducing uptake and, based on this, potential nanoparticle receptors.[3,4] The identified proteins can then be used to create artificial single-protein corona: as an example of this, we showed that a histidine-rich glycoprotein (HRG) corona could be used to strongly reduce nanoparticle uptake into cells.[3]

However, this type of correlation studies only allows to make hypotheses on potential nanoparticle receptors. More recently, instead, we optimized a new method based on reversible biotinylation of cell membrane proteins to directly identify the cell receptors mediating nanoparticle uptake in live cells.[4] Cell receptors are first biotinylated, then the cells are exposed to nanoparticles and the biotinylated receptors internalized with the nanoparticles are purified and identified by proteomics. By using nanoparticles with different uptake efficiency, the receptors mediating more efficient uptake can be distinguished, as well as those mediating uptake specifically in the targeted cells. As an example of this, we found that integrin receptors mediate uptake in liver endothelial cells and by creating an artificial corona with their endogenous ligand, vitronectin, nanoparticle uptake could be increased 30 times in respect to what observed for the natural corona in serum.[4]

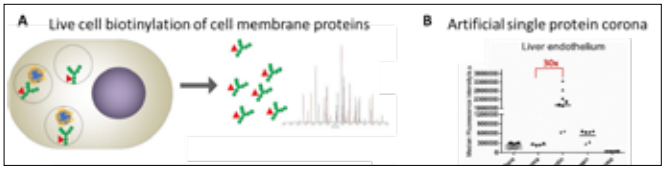

Figure 1. Reversible biotinylation of cell membrane proteins to identify cell receptors mediating nanoparticle uptake. Adapted from Aliyandi et al.[4]

Still, the results also showed that nanoparticles with a human plasma corona are internalized by cells via multiple receptors, thus a more selective corona would be required in order to exploit similar interactions for targeting. Additionally, we found that even when interacting with specific receptors, nanoparticles can be internalized by cells via different mechanisms than what it is usually observed for their endogenous ligands. For instance, nanoparticles interacting with the LDL receptor (LDLR) via their corona are internalized by cells via a mechanism that is not clathrin-mediated, as usually it is observed for LDLR endogenous ligands.5 Instead, we found that several curvature-sensing proteins are involved in the uptake and their involvment changes depending on nanoparticle curvature.[5]

These findings highlight the importance of understanding how nanoparticles are modified by biological environments and how cells interact with and process nano-sized materials, in order to be able to control nanomedicine design for targeted drug delivery.

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EXTRACELLULAR VESICLES: MECHANISM OF FORMATION, CHARACTERIZATION AND POSSIBLE CLINICAL USE

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Extracellular vesicles (EVs) can be formed by budding of vesicles from the plasma membrane, and they are then called microvesicles or ectosomes. EVs can be also be released after fusion of intracellular organelles such as multivesicular bodies (MVBs), amphisomes (the fusion product of autophagosomes and MVBs) and lysosomes. The focus will here be on exosomes, which are defined as the vesicles released after fusion of MVBs with the plasma membrane. A challenge in the field is however the separation of different types of vesicles, and there is evidence that even one MVB has a heterogenous content of vesicles which may have been produced by more than one mechanism by budding from the endosomal membrane [1]. There is now evidence for formation of internal vesicles in the MVBs by both ESCRT-dependent and independent mechanisms, and in some cell types one may inhibit the formation/release of exosomes by inhibition or knockdown of neutral sphingomyelinase which converts sphingomyelin (SM) to ceramide which has a smaller head group [2]. Importantly, in other cell types this is not the case [3]. It was originally proposed that SM giving rise to ceramide involved in exosome formation was found on the cytosolic side of the endosome, a localization which is different from that normally seen for SM. Recently, it has been reported that that ceramide is brought to the maturing endosome by ceramide transport protein (CERT) [4], previously known to transport ceramide from the ER to the trans-Golgi apparatus. A protein involved in MVB-vesicle formation is ALIX which is reported to bind the unusual lipid BMP (bis(monoacylglycerol)phosphate), earlier called lysobisphosphatidic acid (LBPA) [5]. Clearly the lipid composition is essential for the formation and properties of exosomes.

To characterize exosomes we have performed lipidomic studies and investigated the lipid species in these structures. Compared to cells there is an upconcentration of cholesterol, sphingolipids (SM and glycosphingolipids) and phosphatidylserine (PS), whereas there is less of phosphatidylcholine (PC) and phosphatidylinositol (PI). Furthermore, there is an increase in the fraction of phosphatidylethanol (PE) -ether lipids [6]. The lipid content makes the exosomes raft-like, and this may contribute to their survival in the extracellular environment. If exosomes are reported to contain a large fraction of cholesterol-esters or triacylglycerol, this could be an indication of a contribution from lipid droplets [1].

In agreement with an important role for lipids in the formation/release of exosomes is the finding that the ether lipid precursor hexadecylglycerol not only increases the level of ether lipids but also stimulates the release and changes the protein composition of exosomes derived from PC3 cells [7]. Interestingly, this is associated with a downregulation of glycolipids, revealing an unknown relationship between production/stability of these lipid classes. By using data from quantitative lipid analyses and molecular dynamic simulation it could be shown that there is a cholesterol-dependent interdigitation between the inner and outer leaflet of exosomes, facilitated by the long-chain SM in the outer leaflet and PS in the inner leaflet [8, 9]. Such an interaction is likely to be important for specific functions of the exosomes. Not surprisingly, since the membrane composition of different cell types differ, something which is also the case for the apical and basolateral side of polarized cells, the mechanisms of exosome formation and release may be governed by different mechanisms. However, one should be aware of that the lipid composition of cells as well as their endocytic capacity is cell-density dependent [10], so even a change in the time cells are growing, reaching different levels of confluency, is likely to change the fate of MVBs and exosomes.

Although we today know a number of the molecular players when it comes to formation and release of exosomes, screening and subsequent verification is ongoing and our recent studies have revealed that more SNARE molecules than previously described are involved.

A complete characterization of newly secreted as well as stored EVs is essential for optimal exploitation of these structures in the clinic. EVs contain molecules that originate from their mother cell, and they are present in all types of biological fluids (e.g. urine, semen, blood and saliva). Thus, several research groups are trying to use EVs to find biomarkers for disease such as cancer, and to use them to monitor response to treatment. Furthermore, drug-filled EVs may be exploited to administer drug, with the hope of getting less side effects. As EVs in some cases may have a negative effect on disease also the possibility of inhibiting their release is being evaluated. It should be noted that it is a challenge to obtain market approval for human use since one then has to document reproducibility of EV-production, and these vesicles contain thousands of different molecules [1].

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MODULATION OF PHARMACOKINETICS OF DUAL-TARGETING NANOMEDICINES FOR BRAIN DISORDERS CROSSING THE BLOOD-BRAIN BARRIER

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Brain diseases represent a substantial social and economic burden, currently affecting one in six individuals worldwide. Brain research has been focused of great attention in order to unravel the pathogenesis and complexity of brain diseases at the cellular, molecular and microenvironmental levels.
Due to the intrinsic nature of the brain, the presence of the highly restrictive blood-brain barrier (BBB) and the pathophysiology of most diseases, therapies can hardly be considered successful by the simple administration of drugs to a patient. Apart from improving pharmacokinetic parameters, tailoring biodistribution and reducing the number of side effects, nanomedicines are able to actively co-target the therapeutics to the brain, as well as to achieve the delivery of multiple cargos with therapeutic, diagnostic and theragnostic properties. Dual-targeting nanomedicines can be personalized according to the disease needs, with capacity to achieve enhanced therapeutic responses across the fields of brain regeneration and neuroprotection, vascularization-related therapies, brain tumors and immunotherapies.
In the present talk, two particular examples of multifunctional nanomedicines exhibiting capacity to cross the BBB are described, for two emergent, clinical unmet brain diseases, as glioblastoma (GBM) and multiple sclerosis.
Glioblastoma (GBM) is the most common and lethal type of primary brain tumor. The 5-year survival of GBM patients is still limited to a dismal 5%, highlighting the need to advance more effective GBM therapies. GBM tissue presents an abnormal expression of the L-type amino acid transporter 1 (LAT1), for which histidine (His) is an inexpensive and powerful targeting ligand. Although His is expected to provide higher accumulation of drug nanoparticles (NPs) into GBM cells via LAT1 binding, consequently enhancing the anti-tumor response, it has been poorly explored in GBM-targeted therapies.
Driven stimuli-responsive NPs for docetaxel (DTX) delivery to GBM are reported, with multifunctional features that circumvent insufficient BBB trafficking and lack of GBM targeting. NPs are dual-surface tailored with a (i) brain-targeted acid-responsive Angiopep-2 moiety that triggers NP structural rearrangement within BBB endosomal vesicles, and (ii) L-Histidine moiety that provides NP preferential accumulation into GBM cells post-BBB crossing. In tumor invasive margin patient cells, the stimuli-responsive multifunctional NPs target GBM cells, enhance cell uptake by 12-fold, and induce 3-times higher cytotoxicity in 2D and 3D cell models. Innovative 3D glioblastoma microtissue-forming unit containing cancer cells, macrophages and endothelial cells were established to validate the effect of L-Histidine decorated NPs on macrophage polarization and cell metabolic activity. MCTMs cell uptake and anti-proliferative effect was 8- and 3-times higher for H-NPs, respectively, compared to the non-targeted NPs and to free DTX. H-NPs showed a particular biomolecular signature through reduced secretion of an array of medium cytokines (IFN-γ, IL-1β, IL-1Ra, IL-6, IL-8, TGF-β). Moreover, the *in vitro* BBB permeability is increased by 3-fold. In vivo studies in a mice model revealed a clearly higher brain accumulation for this multifunctional NPs, compared to the other NP controls. Lastly, following intravenous treatment at a low DTX dose, the multifunctional NPs increased the median survival of mice by around 20 days and long-term survivors by 50%. Taken together, these results revealed the key role of both acid-sensitive bioresponsive properties, and BBB and GBM dual-ligand targeting, to achieve an effective multistage anti-GBM therapy.
Demyelinating disorders, with a particular focus on multiple sclerosis (MS), have a multitude of detrimental cognitive and physical effects on the patients. Current treatment options that involve substances promoting remyelination fail in the clinics due to difficulties in reaching the central nervous system (CNS). Here, the dual encapsulation of retinoic acid (RA) into lipid nanocapsules, coupled with super paramagnetic iron oxide nanoparticles (SPIONs) was accomplished, and joined by an external functionalization process with a transferrin-receptor binding peptide. This nanosystem showed a 3-fold improved internalization by endothelial cells compared to the free drug, ability to interact with oligodendrocyte progenitor cells and microglia, and improvements in the permeability through the blood-brain barrier by 5-fold. The lipid nanocapsules also induced the differentiation of oligodendrocyte progenitor cells into more mature, myelin producing oligodendrocytes, as evaluated by high-throughput image screening, by 3-5-fold. Furthermore, the ability to tame the inflammatory response was verified in lipopolysaccharide-stimulated microglia, suppressing the production of pro-inflammatory cytokines by 50-70%.
Overall, the results show that lipid nanocapsules surface-functionalized with a peptide moiety to transferrin receptor can act in both the inflammatory microenvironment present at the CNS of affected patients, but also stimulate the differentiation of new oligodendrocytes, paving the way for a promising platform in the therapy of MS.

MODULATING THE BRAIN IMMUNE SYSTEM TO FIGHT CANCER IN 3 DIMENSIONS

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Despite the remarkable efficiency of immune checkpoint modulators against primary and metastatic brain neoplasms, there is a low percentage of responders, and clinical trials report severe immune-mediated side effects and disease relapse. An accumulated body of evidence shows that non-tumor cells within the tumor microenvironment (TME), specifically the brain microenvironment (BME), including tumor vasculature and immune stromal cells, such as astrocytes, microglia, and pericytes, dictate the overall therapeutic efficacy. We synthesized a biodegradable, off-the-shelf, and cost-effective nano-sized polymeric platform that combines a cancer vaccine with the targeted inhibition of molecular and/or cellular immune suppressive players. These precision nano-sized

medicines aim to re-educate and harness patient T-cell response against tumors, leading to an immunological memory able to control tumor relapse without any follow-up treatment. The design of these advanced peptide and RNA immunotherapeutics is guided by the identification of lead immune suppressor factors and tumor-specific antigens and proinflammatory cytokines using novel 3D bio-printed brain tumor-immune models developed in our lab. Using this unique model platform, we identified P-selectin as a novel immune checkpoint in the brain regulating cancer cell-microglia-tumor-associated macrophages (TAMs) interactions. Combining a P-selectin inhibitor with our first nano-immunotherapy candidates sensitized brain malignancies-bearing mice to immune-checkpoint modulators, dramatically increasing disease-free survival rates. We are now testing a P-selectin inhibitor in a 30-patient (with glioblastoma or melanoma brain metastases) clinical trial at Sheba Medical Center. Furthermore, we are currently validating our 3D-bioprinting platform in an 80-patient "basket" clinical trial running at Sheba Medical Center.

EXTRACELLULAR VESICLES VERSUS LIPID NANOPARTICLES

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Lipid nanoparticles have taken center-stage in the current revolution in nucleic acid therapeutics. They couple a good delivery efficiency with manufacturability in a GMP process. The success of the COVID-vaccines has been followed up by an avalanche of clinical activity on mRNA therapeutics and inspired the first clinical gene editing approaches based on these systems.
Still, for many applications expression is insufficient or the tropism of the lipid nanoparticles is not suited for the application. In our lab we have investigated extracellular vesicles as a biological alternative to synthetic lipid nanoparticles. Although these vesicles face challenges in loading efficiency, characterization and scalable and GMP manufacture, they possess intriguing capabilities. Stoichiometric comparisons between vesicles and nanoparticles revealed that vesicles are more potent in delivery than clinically used lipid nanoparticle formulations. This could be related to different internalization pathways and specific interactions with endosomal components.

MECHANO-GENOMICS OF CELL-STATE TRANSITIONS

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Extracellular mechano-chemical signals regulate gene expression programs and cell behavior, although the underlying mechanisms are still unclear. In this talk, I will first highlight the tight coupling between extracellular signals, 3D chromosome organization, and gene expression. I will then discuss how sustained mechanical signals, through cell-geometric confinement, can induce cell-state transitions and provide avenues to reprogram and rejuvenate aging cells. Furthermore, I will show that the spatio-temporal alterations in chromatin organization during cell-state transitions within tissue microenvironment, identified using fluorescence imaging combined with machine learning, can serve as robust biomarkers for ageing-related diseases including cancer and neurodegeneration. Collectively, our results may have important applications in regenerative medicine and early disease diagnostics.

"DEVELOPMENT OF SELECTIVE ORGAN TARGETING (SORT) LIPID NANOPARTICLES (LNPS) FOR THE CORRECTION OF DISEASE CAUSING MUTATIONS"

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Gene editing and messenger RNA-based protein replacement therapy hold tremendous potential to effectively treat disease-causing mutations with diverse cellular origin.[1, 2] However, it is currently challenging to rationally design nanoparticles that selectively target specific tissues.[2] I will describe a strategy termed selective organ targeting (SORT) wherein multiple classes of lipid nanoparticles (LNPs) are systematically engineered to edit extrahepatic tissues via addition of a supplemental SORT molecule.[3-6] Lung-, spleen-, and liver-targeting SORT LNPs were designed to selectively edit therapeutically relevant cell types including epithelial cells, endothelial cells, B cells, T cells, and hepatocytes. Mechanistically, the chemical nature of the added SORT molecule controls biodistribution, global/apparent pKa, and serum protein interactions of SORT nanoparticles. SORT LNPs operate using an endogenous targeting mechanism whereby organ targeting occurs via desorption of poly(ethylene glycol) lipids from the LNP surface, binding of distinct proteins to the nanoparticle surface because of recognition of exposed SORT molecules, and subsequent interactions between surface-bound proteins and cognate receptors highly expressed in specific tissues.[7] These findings establish a crucial link between the molecular composition of SORT nanoparticles and their unique and precise organ-targeting properties and suggest that the recruitment of specific proteins to a nanoparticle's surface can enable drug delivery beyond the liver. SORT is compatible with multiple gene editing techniques, including mRNA, CRISPR/Cas9, base editing, and prime editing. SORT LNPs have enabled CRISPR/Cas based knockout of serum and protein levels of PCSK9, a therapeutically attractive target for treatment of cardiovascular disease. Successful correction of mutations by Homology Directed Repair (HDR) and base editing (BE) will also be discussed if time allows.[8-11] As SORT LNPs have recently been used in a human clinical trial for mRNA delivery to treat primary ciliary dyskinesia (PCD), it is envisioned that further development of SORT LNPs may yield various protein replacement and gene correction therapeutics in targeted tissues.

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MECHANISMS OF ACCUMULATION OF NANOCARRIERS IN THE SKIN: RELEVANCE TO TOXICITIES

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Introduction: Skin constitutes the largest organ in the body with important immune functions, and the accumulation of drug delivery systems could have significant implications for skin toxicity. Thus, PEGylated liposomal doxorubicin (PLD) induces serious skin toxicity in the palms and feet called palmar-plantar erythrodysesthesia (PPE). However, there has been little research into the mechanisms of skin accumulation of liposomes. We followed the dynamics of accumulation of membrane-labeled and internally labeled fluorescent liposomes and PLD in the mouse skin. The liposomes were injected i.v. into BALB/c mice, and the accumulation was studied by longitudinal fiber optical near-infrared spectroscopy, ex vivo imaging and confocal microscopy.
Results: Non-PEGylated liposomes showed short circulation half-life and immediate aggregation in the blood, with some aggregates lodging in skin microvasculature soon after the injection. PEGylated liposomes showed long circulation half-life (22 h) and no aggregation in the blood. PEGylated liposomes started to accumulate in the skin microvasculature as soon as 5 min after the injection. Within 1 h post-injection, PEGylated liposomes were very widespread in extravascular space in the dermis and sub-dermis. Liposomes were present in the skin for at least 7 days post-injection. All skin areas showed accumulation, but there was enhanced extravasation in the areas of pressure (foot pads). High resolution imaging in the foot skin showed that liposomes up to 500 nm size extravasated intact, likely through gaps in the vasculature, and did not require active transport for extravasation. Following extravasation, fluorescent lipids exhibited migration and spreading. Dendritic cells, endothelium, CD45+ immune cells and fibroblasts were found to be positive in the dermis. Also, all liposomal components efficiently accumulated in sweat glands, which alludes to the PPE pathology following injection of PLD. Lastly, liposomes showed enhanced accumulation after accumulation of Doxil, suggesting self-amplification mechanism.
Conclusions: This research shows that liposomes quickly extravasate and migrate in the skin, in particular in the areas of pressure, mostly passively. Further understanding of the mechanisms of this important phenomenon can improve the safety of nanocarriers.
Acknowledgements: The study was supported by the NIH grant CA194058 and Skaggs School of Pharmacy seed grant program (DS);

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BIODISTRIBUTION, PHARMACOKINETICS AND EXCRETION STUDIES OF INTRAVENOUSLY INJECTED NANOPARTICLES AND EXTRACELLULAR VESICLES

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There are huge expectations for the use of nanoparticles (NPs) or extracellular vesicles (EVs) to deliver therapeutics and for imaging of different diseases, such as cancer. To obtain regulatory approval for human use of such products, a large number of studies has to be performed, including description of manufacturing, physiochemical characterization, and preclinical and clinical studies in order to document safety and efficacy [1]. Knowledge of biodistribution, pharmacokinetics and excretion are important parts of such documentation often referred to as ADME (Administration, Distribution, Metabolism and Excretion) studies. If particles made of non-endogenous substances are degraded or excreted very slowly one needs to perform more long-term and costly investigations to document safety than for particles made of endogenous substances or those that are rapidly degraded and excreted [1].
In the early research phase such studies are most often performed in small animals like mice. Advantages and disadvantages by using various imaging modalities (such as PET, SPECT, MRI, CT, ultrasound or optical imaging) for whole body biodistribution studies will be discussed. In order to perform such studies with NPs or EVs, these particles must in most cases be labelled e.g. with a radioactive isotope or a fluorescent molecule. Such labelling techniques may, however, change the surface properties of the labelled particles such that they will show a biodistribution different from the unlabelled particles one wants to investigate. It is also important to critically consider if the labels are associated with the particles at the time of analyses [2].
With a long experience from ADME studies in in pharmaceutical R&D, I will discuss possibilities, challenges and pitfalls when performing ADME studies with NPs or EVs. Although major improvements concerning instrumentation and methodology have been obtained in this field during recent years, we still have much to learn and we do presently not have available an ideal way of performing ADME studies with most NPs or EVs. Frequently various imaging methods are used and one can often see statements like "Seeing is believing". Regarding the subjects I am going to discuss

it might be beneficial to add: What do we actually see, the particles or just a released label? For a complete discussion of these topics, see the review article [2].

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NANOMEDICINE HITCHHIKING WITH IMMUNE CELLS

ALEXANDROS MARIOS SOFIAS

In recent years, we described nanomedicine hitchhiking with immune cells as a mechanistic procedure in which systemically administered nanoparticles target circulating immune cells that eventually extravasate in cancerous and inflammatory lesions, actively transporting such nanomedicines into the tissue-target. The substantial engagement between targeted nanomedicines and immune cells can further be utilized not only for immune cell-mediated transportation, but also for immune cell modulation in hematopoietic organs.

By designing passive and active targeting nanomedicines (liposomes, nanoemulsions, micelles, lipid nanoparticles), and by building a highly complementary multimodal and multiscale imaging approach composed of whole-body imaging (PET/CT, FLT/CT), state-of-the-art intravital microscopy, together with supplementary ex vivo fluorescence reflectance imaging, gamma counting, flow cytometry, and histology, we investigated the real-time interactions between various nanomedicine formulations and myeloid immune cells *in vivo*. In order to expand our understanding on such interactions, we have studied nanoparticle-immune cell engagement in various diseases, such as triple negative breast cancer, wound-healing inflammation, sepsis, and myeloproliferative neoplasms. Among others, we identified active targeting nanomedicines to be able to improve targeting in hematopoietic organs (e.g., bone marrow, spleen), engage with circulating and tissue resident myeloid immune cells, and ultimately being actively transported in malignant lesions by circulating neutrophils; cells that are characterized by high motility and high tumor infiltrating propensity.

Considering advances in immunology and immuno-oncology, we focused on targeting, engaging, and modulating inflammation and cancer-associated immune cells in the diseased microenvironment, circulation, and immune cell-enriched tissues. In the long run, we envision that nanomedicines will assist in restoring the immunological equilibrium at the whole-body level; a strategy which holds potential for the treatment of various disorders in oncology and beyond.

NANOENGINEERING GONE VIRAL: PLANT VIRUSES AGAINST CANCER

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Nanoscale engineering is revolutionizing the way we detect, prevent and treat diseases. Viruses are playing a special role in these developments because they can function as prefabricated nanoparticles. We utilize and build-upon the high-precision assemblies of the viral capsids and utilize them as platform technologies, engineered and repurposed for a desired function. More specifically, we turned toward plant viruses as a platform nanotechnology. We have developed a library of plant virus-based nanoparticles and through structure-function studies we are beginning to understand how to tailor these materials appropriately for applications targeting human, veterinary and plant health. A particular exciting avenue is the development of plant virus-like particle platforms for cancer immunotherapy. The idea pursued is an 'in situ vaccination' to stimulate local and systemic anti-tumor immune responses to treat established disease, and most importantly to induce immune memory to protect patients from outgrowth of metastasis and recurrence of the disease. In this presentation, I will highlight engineering design principles employed to synthesize the next-generation cancer nanotherapeutics using plant virus-based platform technologies, and I will discuss the evaluation of such in preclinical mouse models and canine patients.

INTRAVENOUS ADMINISTRATION OF LIPOSOMAL DHA HALTS ATHEROSCLEROSIS PROGRESSION AND ENHANCES PLAQUE STABILITY

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Background: Atherosclerosis is the main cause underlying cardiovascular disease (CVD), including ischemic heart disease and stroke. Docosahexaenoic acid (DHA, 22:6n-3) is a hydrophobic polyunsaturated fatty acid that exerts anti-inflammatory and antioxidant activities. However, the beneficial effects of DHA on CVD have been controversial likely due to variations in bioavailability after oral intake.

Purpose: In this study, we aim to investigate the potential inhibiting properties of liposomal DHA on atherosclerosis progression upon intravenous administration.

Methods: Four weeks old ApoE-/- and LDLr-/- mice were fed on athero-inducing high fat diet for 4 weeks and then randomly divided into two groups. The mice received either control liposomes (control group) or liposomal DHA (treatment group) via intravenous injection, twice a week for 8 weeks while still being fed on high fat diet. At the experiment endpoint, whole aortas were collected for Oil Red O staining to quantify plaque area or for biochemical analysis. Plasma was collected for total cholesterol measurement and lipidomic analysis. Aortic roots were used for histological analysis.

Results: Liposomal DHA suppressed lipopolysaccharide-induced inflammatory responses and oxidative stress in bone-marrow derived macrophages. As shown by IVIS imaging, upon intravenous injection, DHA-containing liposomes which accumulated preferentially in the atherosclerotic plaques. Compared to control, liposomal DHA treatment reduced the atherosclerotic plaque area in both atherosclerosis animal models, with the total plaque area decreased by 35.8% in ApoE-/- mice, ($p<0.0005$) and by 22.4% in LDLr-/- mice ($p<0.05$). Plaque composition analysis revealed that liposomal DHA treatment increased collagen content and reduced the number of macrophages and neutral lipid within the plaques, resulting in a lower plaque vulnerability index (1.095 for liposomal DHA treated group vs. 1.692 for control group, $p<0.05$). Among those plaque macrophages, as demonstrated by immunohistology,

M2 (anti-inflammatory) macrophages accounted for 4.44% in liposomal DHA treated mice and 2.24% in control liposomes treated mice ($p<0.05$). In agreement with the histology results, higher mRNA expression levels of anti-inflammatory markers (IL-10, CD206 and CD163) and collagen type 1 were determined in aortic tissue after liposomal DHA treatment. Moreover, liposomal DHA did not change total cholesterol level in the blood but significantly lowered plasma levels of several species of triglycerides.

Conclusions: Our findings demonstrate that incorporation of DHA in injectable liposomes is an effective way to increase the inhibitory effects of DHA on halt the progression of atherosclerosis via lowering circulating triglycerides, reducing plaque inflammation, and enhancing plaque stability. Intravenous administration of liposomal DHA may become an efficacious strategy for the treatment of atherosclerosis.

BACTERIAL NANOMOTIONS ASSISTED BY SUPERVISED MACHINE LEARNING ACCURATELY CLASSIFY ANTIBIOTIC SUSCEPTIBILITY

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Antimicrobial resistance (AMR) is a significant public health threat, reducing treatment options for infected patients. AMR is promoted by a lack of access to rapid antibiotic susceptibility tests (ASTs), leading to the use of extensive broad-spectrum antibiotics. Accelerated ASTs can enable clinicians to identify suitable narrow-spectrum antibiotics for treatment in a timely and informed manner. We describe a rapid growth-independent phenotypic AST by measuring bacterial vibrations using a novel nanomotion technology platform. For this, we use micromechanical sensors that are actuated by bacterial vibrations or, in other words, nanoscale motions. We apply supervised machine learning to extract the necessary information, i.e., signal parameters (SPs), from nanomotion recordings to generate classification models. We evaluated the response of a few hundred E. coli and Klebsiella pneumoniae strains from different hospitals to clinically relevant antibiotics using > 2500 individual recordings. Our AST correctly classified samples as susceptible or resistant at rates up to 100%, depending on the antibiotic. For the first time, information about antibiotic susceptibility from bacterial vibrations was extracted from such a diverse data set to create a reliable classification algorithm. It shows the potential of the nanomotion technology platform for future applications in discriminating phenotypic states of the cell.

SYNTHETIC BIOMOLECULAR CONDENSATES

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Biomolecular condensates act as membraneless organelles, compartmentalize particular proteins and/or nucleic acids in subcellular settings through liquid-liquid phase separation, and thus have been found to be crucial for numerous cellular functions. Studies correlating abnormal condensate location, composition, or physical characteristics to different human illnesses, such as cancer and degenerative disorders, are emerging rapidly.[1] Recent intriguing results suggest that manipulating the physicochemical features or phase behaviors of biomolecular condensates could be a novel approach to treating undruggable diseases.[2] However, the particular mechanisms of the actions remain unknown. Condensate-modifying medicines pose significant hurdles due to their extreme infancy and complexity. For unique design concepts, a fundamental knowledge of the "structure-dynamics-activity" relationship of biomolecular condensates in both living cells and model systems is critical.[3]

We recently reported a counterintuitive dilution-induced gel-sol-gel-sol cascade transition via competitive pathways using supramolecular polymers and surfactants.4 This provides insight into biomolecular condensates that undergo reentrant phase transitions in a concentration-dependent manner to form non-stoichiometric macromolecular assemblies through liquid-liquid phase separation. Condensates have heterogeneous structures that alter pharmacodynamics, and their liquid-solid metastable transition underlies severe protein aggregation disorders. We aim to advance the development by 1) engineering supramolecular active droplets that undergo stimuli-induced liquid-gel-solid transitions, which is critical for understanding how condensates contribute to both cell physiology and diseases, and 2) elucidating the interplay between drugs and supramolecular active droplets as well as some biomolecular condensates for therapeutics. This interdisciplinary research will help us comprehend the phase transition of biomolecular condensates and guide the development of innovative drugs for condensate-modifying therapies.

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FROM DATA TO ACTION – FROM EVIDENCE TO GLOBAL AMR POLICY DECISIONS

RALF SUDBRAK

Antimicrobial resistance (AMR) is one of the top 10 global public health threats facing humanity and an ever-present and growing socio-economic burden. Recent work by the Global Research on Antimicrobial Resistance Project estimates that 1.27 million deaths in 2019 were the direct result of drug resistant bacterial infections – a significant and increasing burden that is projected to grow globally, but with a disproportionate impact on low and middle income (LMIC) countries.

Given the urgency of the situation, there is a need for coordinated efforts in AMR across the value chain from research and development (R&D) efforts to the incentives being implemented globally that aim to improve the functioning of markets responsible for the development and distribution of priority health technologies for addressing AMR.

Recognising the critical nature of AMR has led to increased discussion of the AMR challenge at the national and international level, including within fora such as the UN General Assembly, the World Health Assembly, the G7, the G20, and by multi-lateral organisations such as the WHO, OIE and the FAO, resulting in high political interest and commitment. There is collective recognition that investments in R&D are crucial for developing solutions in terms of effective interventions and products resulting in the prevention, containment or reduction of AMR, including the use of nanomedicine tools and approaches. However, in order to promote the development of health technologies and strategies addressing AMR, resources need to be employed even more effectively than at present.

In July 2017, the G20 called for a new international R&D Collaboration Hub to maximise the impact of existing and new basic and clinical antimicrobial research initiatives, which led to the establishment of the Global AMR R&D Hub (www.globalamrhub.org). The Hub is catalysing the international momentum to support global priority-setting and evidence-based decision-making on allocation of resources for AMR R&D through the identification of gaps, overlaps and cross-sectoral collaboration.

THE NANOSTRUCTURE AND ADVERSE EFFECTS OF COVID-19 MRNA-LNP VACCINES: NEW FINDINGS AND CONCEPTS

JANOS SZEBENI, Semmelweis University, Dept of Translational Medicine, Nanomedicine Section, Budapest, Hungary

Abstract. Nanoscale structure analysis of the mRNA-LNP-based Covid-19 vaccine, BNT162b2 (Comirnaty), was carried out applying atomic force microscopy (AFM), in situ force spectroscopy, dynamic light scattering (DLS), transmission and cryo-transmission imaging (TEM and Cryo-TEM) and measurement of LNP pH gradient. The data led us to suggest a new model of vaccine nanoparticle (NP) molecular buildup, wherein the highly convoluted, thread ball-like mRNA is bound to the ionizable lipids or lipid clusters, mainly via hydrogen bonds, rather than ionic ones. The phospholipid membrane coat is either mono- or bilayer, although membrane-free surface areas also exist. Based on force spectroscopy, Comirnaty NPs are soft, highly compliant structures prone for disintegration in water to yield, among other fragments, mRNA lipoplexes. Another proposition presented relates to the acute adverse immune reactivity of Comirnaty, manifested in hypersensitivity reactions (HSRs) or anaphylaxis (ANA). Evidence is provided that anti-polyethylene glycol (PEG) antibodies (anti-PEG Abs) are playing a causal role in these processes by binding to PEG on the surface of vaccine NPs. Subsequent complement (C) activation with liberation of anaphylatoxins (C3a and C5a) explain the symptom of HSRs and ANA. The experimental setup provides a large-animal model for mRNA-vaccine-induced ANA in humans.

Introduction. Despite worldwide attention to mRNA-LNP-based Covid-19 vaccines, there are unanswered questions regarding their nanoscale structure and short- and long-term side effects. As for their structure, we found no attempt in the literature to reconcile the discrepancy between several copies of 1,414 kDa, 4,284 nucleotide mRNA in Comirnaty, having an extended length of ~1,500 nm, with the space available in ~60-100 nm diameter LNPs, also accommodating the ionizable lipid ALC-0315 present at 6-times larger number than the nucleotide count in the mRNAs [1]. Regarding the acute adverse effects of the vaccine, HSR/ANA, these reactions were suggested to be due to complement (C) activation-related pseudo-allergy (CARPA) [1, 2], but the mechanism of C activation has not been clarified. The long-term adverse symptoms, most importantly the chronic inflammatory processes in different organs, are also not understood. The goals of the lecture to be presented is to show experimental data that led us to make hypotheses on the mechanisms of the above side effects.

Methods. Individual and multiple vaccine NPs were visualized by AFM, DLS, Cryo-TEM and TEM, negative (uranyl acetate) stained TEM, and measurement of intra-LNP pH/pH gradient using the distribution of radioactive methylamine (^{14}C-MA) between the NPs and solvent medium [3]. The nanomechanical properties of Comirnaty was analyzed by in situ force spectroscopy carried out in contact mode on vesicles selected from a previously scanned AFM image [3]. PEGylated liposomal doxorubicin (Doxil) served as reference liposome. Anti-PEG Abs in the plasma of non-vaccinated and mRNA-LNP vaccine (Comirnaty and Spikevax) recipients were measured by ELISA [4]. The pig studies used the "porcine CARPA" model (4, 5).

Results and Discussion. Fig. 1A and E show that the freshly diluted vaccine, representing the inoculum used for vaccination of humans, consists of monodisperse, spherical NPs of about 120-150 nm apparent diameter and 40-60 nm topographical height, corresponding to slightly flattened surface-adsorbed NPs. One day storage of the diluted vaccine at 4 °C, i.e., samples not recommended for human use, led to notable deterioration of homogeneity (Fig. 1B, F). Specifically, both the size and shape of NPs became heterogeneous with extra small oval, and extra-large spheric structures, suggesting breakup and fusion. Freezing of stored, diluted samples, which is also disallowed, led to further dispersion and fusion of NPs with a substantial variety of fragment shapes in the ~5 to ~300 nm range (Fig. 1C, G). Doxil showed no such morphological changes (not shown) [3].

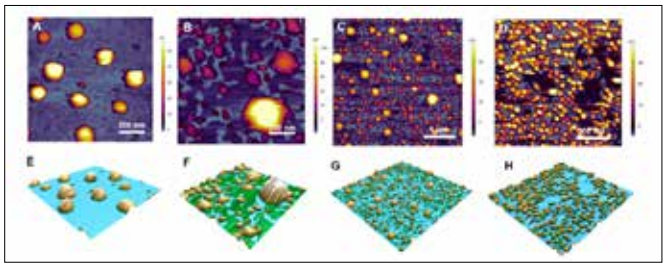

Fig. 1. AFM images (A–D) and corresponding 3D reconstructions (E–H) of Comirnaty and Doxil, immobilized on glass surface. (A) and (E) show a freshly diluted Comirnaty sample representing the jab inoculated into the deltoid muscle; (B) and (F) show a 1-day-old sample stored at 4 °C, (C) and (G) show a refrozen sample, representing unintended acceleration of fragmentation by refreezing the leftover vaccine; (D) and (H) show a freshly opened Doxil sample.

Fig 2 shows the force spectroscopy results, using the force-indentation curves (force curves) to quantify the NPs' mechanical resilience (stiffness).

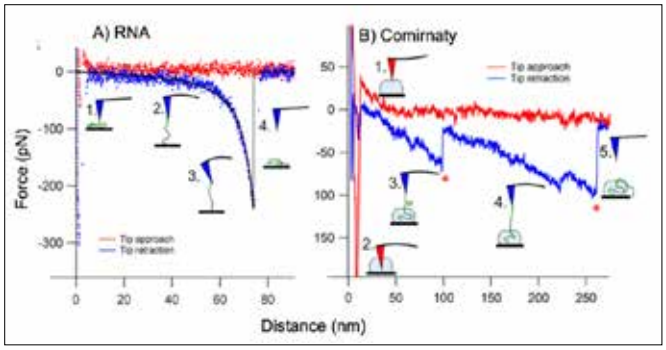

Fig. 2 Force–distance curves obtained by in situ force spectroscopy of an RNA molecule (A) and a Comirnaty NP (B) attached to glass support. The red and blue curves show indentation and RNA extraction, respectively. The numbered schematics along the curves illustrate the different stages of measurements.

As shown in Fig 2A, the force curve of an RNA molecule, attached to the specimen support glass surface, does not undergo major changes upon indentation by the tip of the cantilever, but it exponentially declines upon tip retraction. This suggests defiance against indentation, and exponentially increasing resistance against vertical stretching of the RNA. Similar nanomechanical testing of a Comirnaty NP shows a very different response, since the force curves revealed considerable indentation, and the rapidly declining retraction curves displayed sawtooth-like force transitions, indicating that molecular strands, most likely mRNA, can be pulled out from the NPs by stepwise rupture of mRNA–lipid bonds. Because these changes were induced at relatively low forces, it can be concluded that Comirnaty NPs are soft, compliant structures from which lipid coated mRNA (mRNA lipoplexes) can exit.

Consistent with the AFM findings (Fig 1A), cryo-TEM images of freshly diluted Comirnaty (Fig 3A) showed relatively homogeneous NP distribution in the 50-120 nm range. However, unlike the constant sharp rings around Doxil liposomes, corresponding to phospholipid bilayers (Fig 3B), the granular, solid cores of Comirnaty NPs are only

partly covered by phospholipid bilayers (red arrows in Fig 3A); most NPs are enclosed by weakly discernable coatings, assumably phospholipid monolayers, and there are uncovered surface regions as well. Also consistent with the AFM-inhomogeneity of stored vaccine, uranyl-stained TEM images of a stored sample showed a wide variety of odd-shaped LNPs and fragments not seen with cryo-TEM (Fig 3C). Higher magnification of some of these structures show circular and semicircular chains of 2–5 nm electron-dense spots that are aligned into strings, semicircles, or labyrinth-like networks in the LNP's interior most likely stabilized by intra-and inter-loop crosslinks (Fig 3D). Figure 3E is a snapshot of an mRNA leaving a NP in the form of a helix covered by the electrodense dots, which corresponds to mRNA-lipoplexes. The NPs pointed to by blue arrows in Fig 3C captures a fusion.

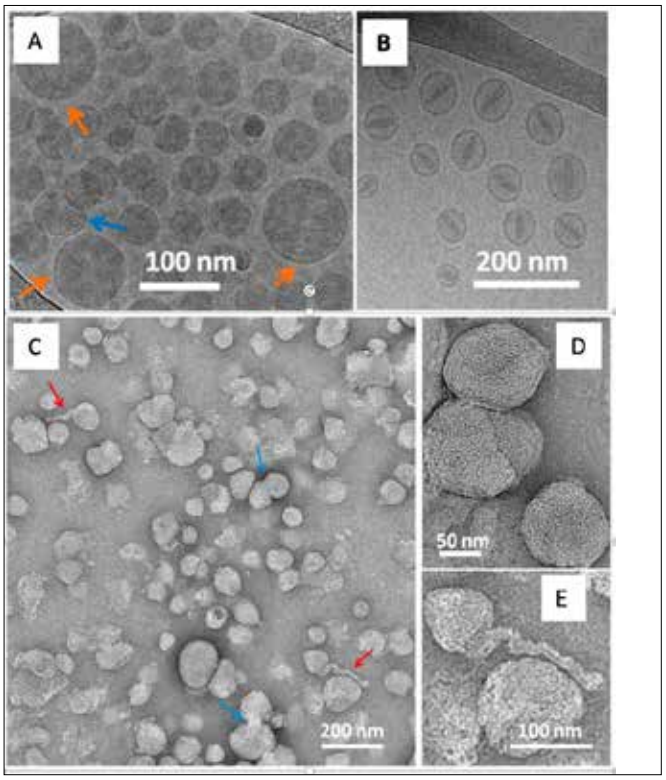

Fig. 3 Cryo-TEM images of freshly diluted Comirnaty (A) and Doxil (B). The orange arrows in panel A point to phospholipid bilayers. The blue arrow points at a bicompartmental NPs with an mRNA-containing bleb. (B) Cryo-TEM image of Doxil demonstrating equal coverages of liposomes by phospholipoid bilayers. (C-E), TEM images of a Comirnaty sample stored for 7 days in the refrigerator, diluted in water, and stained with acidic uranyl acetate. Blue arrows in (C) point at fusions of NPs. (D) Higher magnification image of some LNPs. (E) Zoom-in of one of the elongated helical-like structures extended from a remnant NP, most likely exit of a mRNA-lipoplex.

Regarding the assumed crosslinks stabilizing the 3D structure of mRNA lipid complex, the most abundant lipid component of Comirnaty, the ionizable ALC-0315, is positively charged only at low (~ 4) pH, where the mRNA and lipids are mixed during the manufacturing process. These lipids may have no net positive charge after thawing the vaccine stock and dilution in physiological saline. Thus, ionic bonds are unlikely to be the main force holding together the mRNA and lipids at the stage of its human use. This conclusion is also supported by the lack of pH gradient between Comirnaty NPs and its medium, as shown in an experiment measuring the accumulation of 14C-methylamine in Comirnaty [3]. On the other hand, a study on hydrogen bonds between mRNA and clusters of lipids similar to ALC-0315, amply discussed in [3], taken together with the fact that ALC-0315 displays free =O and −OH groups, and its size and -assumably- steric structure fit for bonding with the nitrogenous bases in the mRNA, suggest that the crosslinks stabilizing the NPs may be mainly hydrogen bonds. These data on the softness and time-limited stability of Comirnaty following dilution in water raises the possibility of disintegration of non-phagocytized NPs in tissue fluids or blood after inoculation into the deltoid muscle before all intact NPs would be taken up by antigen presenting cells. In case of disruption, the mRNA-containing NP remnants, or liberated mRNA polyplexes, may rapidly escape into the circulation and transfect host cells with the mRNA. Obviously, this is just a hypothesis which may deserve further studies.

Turning the attention to the HSRs and ANA problem, based on studies wherein 5X human doses of Comirnaty were injected in pigs, the hemodynamic changes, granulocytosis, lymphopenia and thrombocytopenia, seen in 6 of 14 pigs, with 1 ANA [2] pointed to CARPA as mechanism of physiological changes. When we immunized pigs by i.v. injection of PEGylated liposomes (Doxebo), massive production of anti-PEG IgM and IgG Abs were measured peaking around 1 week, indicating strong, type-2 (T cell independent) Ab response against PEG [4]. Injection of 1/3 of the human dose of Comirnaty into these anti-PEG "hyperimmune" pigs, all of 6 animals developed ANA, requiring resuscitation for survival [5]. In the control, non-immunized pig, even 5X human dose was without effect [5]. In animals developing ANA, significant rises of plasma C3a anaphylatoxin was measured in close parallelism with the hemodynamic changes [5], which supports the CARPA concept. These data also confirm a recent human study implicating anti-PEG Abs in mRNA-LNP vaccine-induced HSRs/ANA [6].

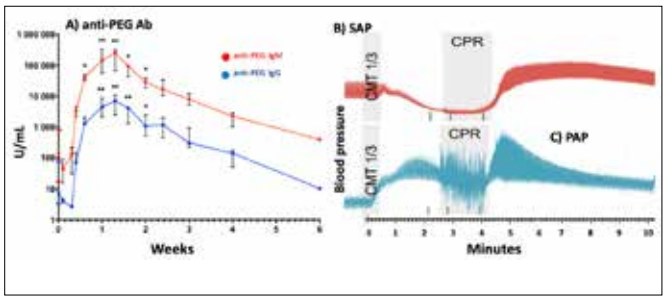

Fig. 4. Induction of anti-PEG Abs in pigs by Doxebo (A) and anaphylaxis caused by 1/3 human dose of Comirnaty (CMT), injected i.v. at the peak of Ab response. Panels B and C show real-life recordings of systemic and pulmonary arterial blood pressures (SAP, PAP), corresponding to anaphylaxis. CPR, cardiopulmonary resuscitation. Panel A is modified from Ref. [4], and B, from Ref [5].

Hypotheses: 1) The relative instability of Comirnaty may contribute to the broad dissemination of vaccine parts in the body, with a risk for delivery of the mRNA into the cytoplasm of cells in different organs. If the SP is expressed on the surface of host cells, C activation and/or an autoimmune response may underlie the local or disseminated sterile inflammation in a small fraction of people expressing long-term adverse effects. 2) CARPA caused by anti-PEG Abs may explain at least some of the HSRs/ANA caused by PEGylated Comirnaty and Spikevax. 3) The "hyperimmune porcine CARPA model" may allow better understanding of vaccine-induced ANA in humans and, hence, developing preventive measures against it.

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THERAPEUTIC APPLICATION OF MESSENGER RNA-LIPID NANOPARTICLES FOR IN VIVO GENE EDITING

YING TAM

Acuitas' is developing and optimizing a new generation of lipid nanoparticle (LNP) delivery systems to efficiently and safely deliver messenger RNA (mRNA)-based medicines. Through industry partnerships, academic collaborations and internal research, Acuitas is enabling mRNA in a broad range of therapeutic areas. The most advanced therapeutic application is as prophylactic vaccines against infectious disease but anti-cancer and tolerogenic vaccines, passive immunization, protein replacement for treatment of monogenic disease, therapeutic protein expression to treat chronic and acute disease and genomic engineering through expression of gene editing enzymes such as cas9, ZFNs or TALENs to address genetic diseases are highly active areas of research.

The challenge of safe and efficient delivery of nucleic acid-based medicines to appropriate target cells in the body can be addressed using non-viral LNP-mediated delivery. Currently, our focus is to express mRNA administered in LNP via different routes of administration in a range of target cells to enable a variety of therapeutic applications. LNP are able to accommodate larger nucleic acid payloads compared to alternative delivery approaches and provide additional potential safety benefits, particularly by avoiding risks associated with viral delivery systems.

Here, we will present data on key parameters impacting potency and safety of mRNA LNP. We will present our work with partners and collaborators illustrating the application of mRNA-LNP based drugs in wide range of therapeutic areas including results demonstrating the ability of mRNA-LNP vaccines to protect against infectious diseases, to express therapeutic proteins to treat acute liver disease and to engineer the genome through expression of gene editing enzymes to address genetic diseases.

NANO-FLOW CYTOMETRY AS A TOOL TO DETERMINE LNP QUALITY & ENCAPSULATION EFFICIENCY

ROB TEMPEST

Nano-flow cytometry using the NanoAnalyzer allows for simultaneous side scatter and fluorescence measurements to provide comprehensive data for nanoparticle samples from early development stages right up to quality control. The use of flexible labelling options allows for a range of presented or encapsulated cargo to be identified and measured at the single particle level. Multiparameter characterisation can be obtained in as a little as three minutes per sample and can determine values such as mean and median size, concentration, loaded vs. empty particle ratio, encapsulation efficiency and percentage positivity of fluorescently labelled targets. The rapid elucidation of these physical and phenotypic properties highlights the NanoAnalyzer as the ideal next-generation platform for comprehensive nanoparticle analysis.

FLASH NANOPRECIPITATION: A VERSATILE PLATFORM FOR MULTIFUNCTIONAL NANOCARRIERS

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Compared to conventional manufacturing methods, flash nanoprecipitation represents a promising bottom-up process for the production of nanocarriers. Based on a rapid mixing process using kinetically controlled nanoprecipitation, it involves three sequential steps: i) rapid mixing of a solvent with an antisolvent to achieve high supersaturation, ii) a nucleation phase and iii) nanoparticle growth/aggregation. The advantages of flash nanoprecipitation are its easily scalability, high efficiency and reproducibility. In addition, it is suitable for the manufacturing of a wide range of drug delivery systems [1–3]"ISSN":"15438392","PMID":"33213144","abstract":"Drug delivery systems (DDSs. These include, for example, micelles, polymer-based nanoparticles, polyelectrolyte complexes, drug nanocrystals and lipid-based nanoparticles [1,2,4,5]rapid and scalable method, the flash nanoprecipitation (FNP. Manufacturing is accomplished by means of controllable mixing devices such as microfluidic mixer systems, multi-inlet vortex mixers and (confined) impingement jet mixers (IJM). Thereby, IJM systems consist of pumps that transport two liquid streams into a mixer with two opposing liner jets. The two streams then collide at high speed in the mixing chamber and flash nanoprecipitation occurs [2,4]. Recently, IJM have attracted considerable attention as they were used for the production of Pfizer-BioNTech's mRNA-based vaccine [4,6]. However, a major challenge is the complex nature of the manufacturing process and its difficult application to complex nanocarrriers. Hence, the focus of the present study is to establish a comprehensive understanding of the manufacturing process. Therefore, a simple system consisting of oil in water (o/w) nanoemulsions stabilized by a non-ionic surfactant was selected.

For the preparation of the nanoemulsions, the IJM Nanoscaler (Knauer, Berlin, Germany) was used, which consisted of five im-

pingement jet mixers (i.e., IJM 1-5) with different inner diameters, three pumps (i.e., lipid pump, stabilizer pump and quenching pump) and two valves (Figure 1). The liquid lipid Labrafac™ lipophile WL 1349 (Gattefossé, Saint-Priest, France) was dissolved in ethanol absolute (Sigma-Aldrich, Vienna, Austria) and formed the organic phase in the lipid pump. The aqueous phase in the stabilizer pump consisted of a mixture of the non-ionic stabilizer Tween® 80 (Sigma-Aldrich) and MQ-water (Millipore S.A.S., Molsheim, France) and MQ-water as a quenching medium. Various process and formulation parameters (i.e., factors) including the total flow rate (i.e., 1 - 50 ml/min), the lipid concentration (i.e., 0.5 - 3 mg/ml in final formulation), the stabilizer concentration (i.e., relative to the applied lipid amount; 0.05 - 0.25 mg/mg lipid) and the amount of organic solvent (i.e., 0.1 - 0.25% corresponding to a ratio of organic to aqueous phase of 1:3 to 1:9) were investigated in a design of experiments (DoE). Using the program Modde 13.0 (Sartorius, Göttingen, Germany), a full factorial (2 levels) design with three center points and an interaction process model type (i.e., screening DoE) was applied. In total, 19 experiments were performed for each IJM (i.e., 1 - 5) in randomized order. Minimum and maximum limits for the mentioned factors were selected based on previous experiments. The results of droplet size investigations (i.e., d (0.9) and span) via dynamic light scattering (DLS, Litesizer 500, Anton Paar, Graz, Austria) with minimum, maximum and target values were set as responses. The responses were fitted as a function of the factors by means of partial least squares (PLS) and were analyzed to achieve optimized formulation and process parameters for each IJM. Thereby, outliers were excluded from the analysis and non-significant factors and interaction parameters were removed to simplify the models. The optimized nanoemulsions were re-prepared and further characterized by spatially resolved dynamic light scattering (SR-DLS) using the NanoFlowSizer (InProcess-LSP, 5349 AB Oss, The Netherlands) in comparison to DLS.

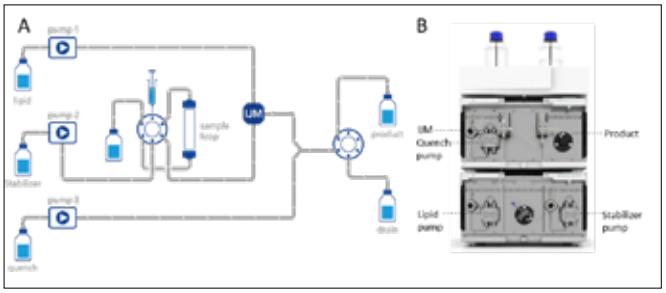

Figure 1: Schematic representation (A) and image (B) of the IJM Nanoscaler (Knauer, Berlin, Germany).

Good correlation coefficient values (i.e., R2 > 0.5) with good predictability (i.e., Q2 > 0.5) were observed for all DoE studies, indicating high agreement between the obtained data and the model and reliability of the prediction. The results revealed that the total flow rate had the greatest influence on the obtained droplet sizes and size distributions, regardless of the type of mixer used. Thereby, IJM 1 corresponded to the smallest inner diameter and IJM 5 to the largest inner diameter of the mixers. Figure 2 exemplarily shows the influence of the total flow rate and the stabilizer concentration on the size and size distribution (i.e., d (0.9) and span) for IJM 1 at a constant lipid amount of 1 mg/ml and an organic to aqueous phase ratio of 1:4. In general, flow rates of 50 ml/min exhibited the smallest sizes with the narrowest size distributions independent on the amount of lipid applied. The mean hydrodynamic diameters and the d (0.9) were between 130 and 170 nm and below 260 nm for all mixers, respectively. The span values of these samples ranged from 0.8 – 1.3. By contrast, nanoemulsions prepared with total flow rates of 1 ml/min were polydisperse and exhibited sizes of up to 6 µm. These results can be explained by the La Mer model and the droplet formation process during flash nanoprecipitation. At high flow rates, the mixing time is shorter than the nucleation and growth phases. Hence, small and uniform droplets are formed. However, when the flow rate is low, the mixing time is longer than the time of the precipitation process, causing the nucleus growth to dominate and the size of the droplets to increase [2,3]controllable, scalable, versatile, and cost-effective technique for the preparation of nanoparticles. In addition to the formulation of drugs, MINP has attracted tremendous interest in other fields. In this review, we highlight recent advances in the preparation of nanoparticles with complex nanostructures via MINP and their emerging applications beyond biomedicine. First, the mechanisms of nanoprecipitation and four mixing approaches for MINP are briefly discussed. Next, three strategies for the preparation of nanoparticles with complex nanostructures including sequential nanoprecipitation, controlling phase separation, and incorporating inorganic nanoparticles, are summarized. Then, emerging applications including the engineering of catalytic nanomaterials, environmentally friendly photovoltaic inks, colloidal surfactants for the preparation of Pickering emulsions, and green templates for the synthesis of nanomaterials, are reviewed. Furthermore, we discuss the structure-function relationships to gain more insight into design principles for the development of functional nanoparticles via MINP. Finally, the remaining issues and future applications are discussed. This review will stimulate the development of nanoparticles with complex nanostructures and their broader applications beyond biomedicine.","author":[{"dropping-particle":"","family":"Chen","given":"Tianyou","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Peng","given":"Yan","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Qiu","given":"Meishuang","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Yi","given":"Changfeng","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Xu","given":"Zushun","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Nanoscale","id":"ITEM-1","issue":"8","issued":{"date-parts":[["2023"]]},"page":"3594-3609","publisher":"Royal Society of Chemistry","title":"Recent advances in mixing-induced nanoprecipitation: from creating complex nanostructures to emerging applications beyond biomedicine","type":"article-journal","volume":"15"},"uris":["http://www.mendeley.com/documents/?uuid=f97967be-e9ae-4d76-88cc-8e98d82f3c91"]},{"id":"ITEM-2","itemData":{"DOI":"10.1016/j.apsb.2018.11.001","ISSN":"2211843","abstract":"Nanoparticles are considered to be a powerful approach for the delivery of poorly water-soluble drugs. One of the main challenges is developing an appropriate method for preparation of drug nanoparticles. As a simple, rapid and scalable method, the flash nanoprecipitation (FNP.

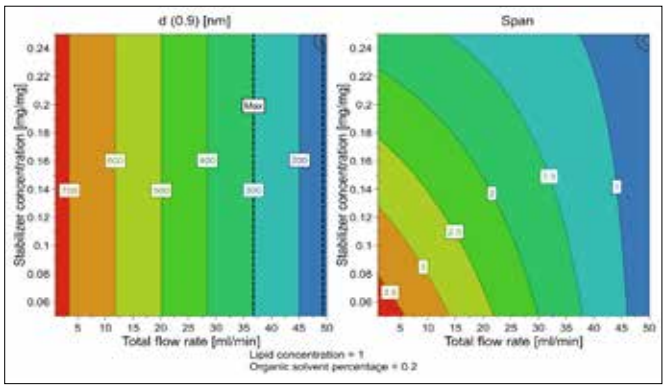

Figure 2: Representative response contour plots (i.e. d (0.9) and span) of IJM 1 at a lipid concentration of 1 mg/ml and a ratio of organic to aqueous phase of 1:4. Target and maximum values for d (0.9) and span were set to 150 and 300 nm and 0.1 and 5, respectively.

In addition, the applied stabilizer concentration significantly affected the droplet size, while the amount of lipid and the ratio of organic phase to aqueous phase only had a minor effect. These results were further confirmed in optimization studies and provide the basis for calculations of flow characteristics, dimensionless numbers and the preparation of nanoemulsions using other formulation components such as different liquid lipids or mixtures of solid and liquid lipids.

In conclusion, the present study revealed the feasibility of flash nanoprecipitation for the manufacturing of o/w nanoemulsions. Nevertheless, this process is highly complex, even using simple sys-

tems. In order to transfer the acquired understanding to more complex systems, further studies are required to investigate key parameters such as influence of mixer geometry, temperature, selection and ratio of solvent and anti-solvent.

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PHOSPHOLIPIDS AS NANOMATERIALS

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Based on the number of products and ongoing clinical trials with liposomes and lipid nanoparticles (LNPs), liposomes and LNPs can be considered as the most successful drug-carrier systems in nanotechnology so far. With the development of products such as Doxil®, AmBisome®, and Comirnaty®, the hurdles that had to be overcome enabling reproducible large-scale cGMP production of these liposome and LNP products were addressed. These products show clinically proven efficacy and low toxicity. Besides manufacturing factors, such as efficient incorporation of the drug in the lipid particles, control of the particle size and particle size distribution of the liposomes/LNPs, and handling of solvents during production, the large-scale availability of pharmaceutical (cGMP) grade phospholipids and “designer” lipids, such as ionizable or PEGylated ones, was certainly another unlocking key success factor enabling large-scale production of liposome and LNP products. For parenteral products based on lipid-carriers, natural phospholipids, derived from soybean and egg yolk, and synthetic phospholipids are being used. When required also cationic lipids including pH-sensitive amino-lipids may be applied. Alternative non-lipid carriers are not that advanced in pharmaceutical industry, because many of the hurdles described above have not been overcome yet. This seminar compares the benefits and state of the art of lipid-based drug carriers with alternative nano-carriers and draws the conclusion that academic and industrial research in nanotechnology carriers should focus on (phospho)lipid carriers.

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QUANTIFYING WEAK-BINDING KINETICS ON LIVING CELLS

SANDER VAN KASTEREN

Dr van Kasteren will focus on his recent work looking at carbohydrate-lectin interactions at the single molecule level on living cells. These interactions play an important role in a wide diversity of biological processes, but are hard to quantify due to the low affinities of the interactions and the overlapping specificities of different lectins. To study this in more detail, particularly on immune cells, he has developed a point-accumulation in nanoscale topography (PAINT)-based super-resolution microscopy method that can be used to capture the weak glycan-lectin interactions at the single molecule level in living cells (glyco-PAINT). This has allowed Dr van Kasteren to obtain on rates and off rates for lectins, and he is now using this to dissect the role of glycan interactions on downstream immune activation events. Overall, Glyco-PAINT represents a powerful approach to study weak glycan-lectin interactions on the surface of living cells that can be potentially extended to a variety of lectin-sugar interactions.

CROSSLINKED SELF-ASSEMBLED POLYPEPTIDE-BASED CONJUGATES OR POLYMERIC MICELLES?

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The use of rationally-designed synthetic polypeptide-based therapeutics has already demonstrated clinical benefit, with some examples in the market and many under clinical evaluation.[1] Polypeptide-based nanocarriers can be considered highly versatile, allowing architecture modification and introduction of bioresponsive elements that allow not only enhancing the solubility of the conjugated drug, but also increasing its stability, and enhancing its site-specific delivery.[2] Importantly, following a bottom-up strategy by means of the ’Ordinary-Extraordinary transition behavior’,[3] we obtained crosslinked self-assembling star-shaped polypeptide architectures yielding supramolecular nanostructures with interesting properties at the bio-nano interface.[2-4] This strategy and rational polymer-drug linker design, led to micellar structures with a lack of toxicity, enhanced *in vitro* internalization rate, greater terminal and accumulation half-life *in vivo*, and lymph node accumulation.[3] Therapeutic applications in cancer as well as neurodegeneration with these rationally designed therapeutics will be shown.[2-4] Finally, in order to scale up these materials and made them feasible for clinical development flow chemistry needs to be implemented as will secure not only batch to batch reproducibility, but also, cost-efficient processes.[5]

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APPRECIATING AND EXPLOITING THE MECHANICAL DESIGN OF PROTEINS

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Even though life is happening far out of equilibrium, very few cell signaling diagrams indicate which signaling pathways or protein functions are activated or destroyed by the stretching of proteins out of equilibrium. Mechanical forces drive essential life processes, from the first steps in fertilization all the way to shaping growing cellular assemblies into organisms. External and cell-generated forces also orchestrate tissue homeostasis in healthy organs, or if misbalanced, result in a range of degenerative diseases. Yet, our knowledge of proteins in biology, pharmaceutical sciences and medicine is still mostly based on knowledge of their equilibrium structure-function relationships. This notion is increasingly challenged as proteins can have amazing mechanical properties as well, as many extracellular and intracellular proteins serve as mechanochemical switches when stretched by cell generated forces. Reciprocal mechanical signaling between cells and their environment is thus key to the ability of cells to sense their 3D microenvironments and thus to the spatio-temporal coordination of tissue growth and regenerative processes. If miss-balanced, physical factors can even override chemical stimuli and this can tip cell niches towards pathological transformations. While enormous progress has been made in the last decades how biochemical factors regulate cell signaling pathways and the transcriptional responses of cells, combining the biochemical and mechanobiological viewpoints is essential to advance the field of regenerative medicine as will be illustrated here with recent discoveries.

LNP PRODUCTION FOR MRNA VACCINES, THERAPEUTICS AND FOR GENE-EDITING – PROOF OF CONCEPT FOR A VERSATILE PROCESS.

ANDREAS WAGNER
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Lipid nanoparticles (LNP) are the leading delivery systems for enabling the therapeutic potential of small interfering RNA (siRNA) as well as mRNA for systemic applications. Lipid nanoparticles, currently represent the most advanced platform for RNA delivery, which have now advanced to market products.
During the early days of the Covid-19 pandemic, industry partners reached out to Polymun to set up production processes for mRNA-LNPs together with the respective analytical test methods. Within weeks, a robust and scalable process has been developed, process conditions have been optimized and the process has been adapted to meet requirements for industrial scale. The LNP production process has to meet several requirements, such as simplicity, robustness, potential to scale up and easy handling. These processes are now applicable not just for vaccines, but also for mRNA therapeutics as well as for gene editing formulations.

PEPTIDE NANOSTRUCTURES CONTROLLING CELLULAR FUNCTIONS

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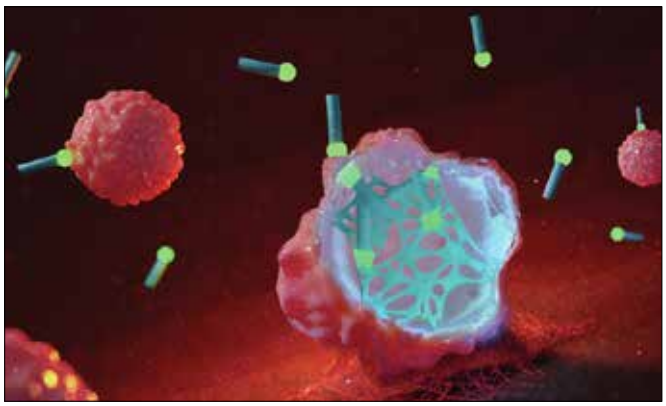
In my presentation, I will first discuss the controlled synthesis of peptide nanostructures through peptide cascade reactions in living cells[1-3]. I will then highlight the importance of computational approaches and screening for the identification and optimization of bioactive peptide nanostructures that stimulate neuron outgrowth or enrich virions at the cell membrane, which is attractive for applications in regenerative medicine and gene therapy.[4-5]
Our goal is to synthesize functional peptide biomaterials that exhibit many of the properties of living matter so that they can integrate and communicate with living systems to provide new avenues for medical challenges.



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ESTABLISHING A PATIENT-DERIVED GLIOBLASTOMA ORGANOIDS MODEL THAT MIMICS TUMOR HETEROGENEITY IN PATIENTS

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INTRODUCTION

Glioblastoma is the most frequent and malignant primary brain tumor. It carries a poor prognosis despite advances in surgery, radio- and chemotherapy. Major determinants in therapy failure include chemoresistance driven by the large heterogeneity at the intratumoral level, the tumor microenvironment, and the poor penetration of drugs across the blood-brain barrier (BBB). Therapeutic nanoparticles are a strong alternative to treat glioblastoma, since they can be used for targeted drug delivery across the BBB. To better evaluate the efficacy of different nanoparticles with potential anti-glioblastoma activity, it is necessary to have an experimental model that recapitulates the complexity of glioblastoma.
Here, we propose a micro-vascularized glioblastoma tumor model to screen for anti-cancer nanoparticles and drug-delivery systems. This tumor-on-a-chip model will comprise a patient tumor-derived organoid embedded in a network of microvessels and extracellular matrix, essential elements of the tumor microenvironment. The organoids will be fully patient-derived, with tissue taken from different tumor sub-regions to account for intra-tumor heterogeneity.

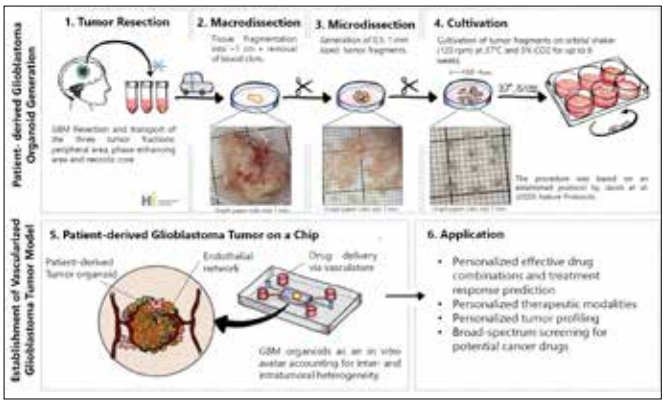

Figure 1: Workflow to gain patient-derived glioblastom organoids according to Jacob et al 2020.

RESULTS AND DISCUSSION

To establish the glioblastoma organoids model, we used patient navigated biopsies from three different sub-regions of glioblastoma (hypoxic/necrotic core, contrast-enhancing, and peritumoral infiltration zone). Figure 1 depicts the procedure which was based on an established protocol by Jacob et al.[1]. The patient material was provided by the Kantonsspital St. Gallen (KSSG) in Switzerland. The organoids were characterized with immunohistochemistry (H&E, GFAP, MAP2, CD45, CD3, CD68, and Ki-67) (Figure 2). The growth of the organoids was quantified from brigthfield microscopy pictures. A quality control was implemented based on the morphology and organoid-like appearance of the specimens.

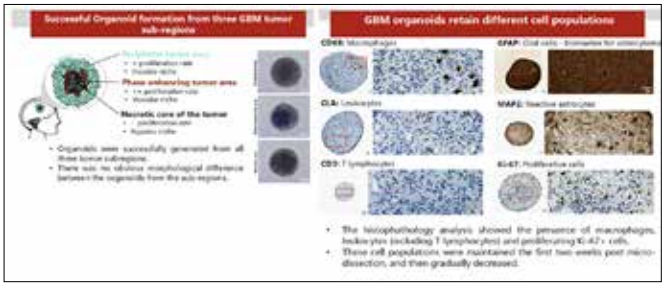

Figure 2: Patient-derived glioblastoma organoid formation and first characterization

The growth rate and quality of the organoids was patient-dependent. From two patients, we generated high-quality and fast-growing organoids. However, from the others we obtained low-quality organoids or nothing (from a recurrent case). There was no obvious morphological difference between the organoids from the three sub-regions. The histopathology analysis showed that the organoids preserved features from the original tumor tissue: abundant pleomorphic nuclei (like high-grade gliomas) and the presence of macrophages, leukocytes (including T lymphocytes) and proliferating Ki-67+ cells. These cell populations were maintained the first two weeks and then gradually decreased.
Organoids derived from more patients will be characterized. In parallel, we will generate an *in vitro* vascular network derived from glioblastoma endothelial cells.

CONCLUSION

This project sets the basis for a patient-relevant experimental system that can be used to screen for effective anti-cancer nanoparticles in a patient-specific manner, and to evaluate drug-delivery systems able to cross the BBB.

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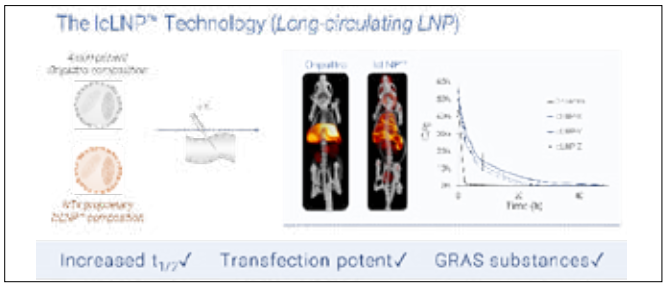
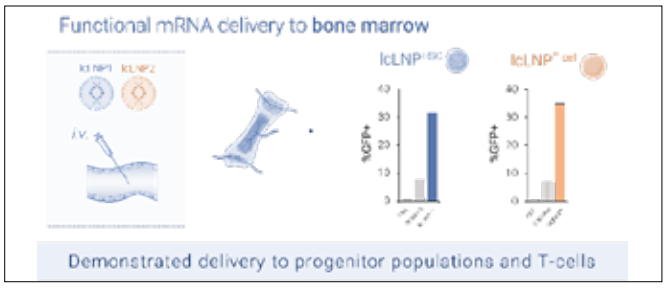
ADVANCING LIPID NANOPARTICLES FOR SAFE AND EFFICIENT NUCLEIC ACID DELIVERY TO EXTRAHEPATIC TISSUES.

DOMINIK WITZIGMANN, Chief Executive Officer and Co-founder NanoVation Therapeutics, Vancouver, BC, (CAN)





Lipid nanoparticles (LNPs) have emerged as the state-of-the-art non-viral delivery system for nucleic acid delivery. The successful clinical translation of LNP-based siRNA therapies, such as Onpattro used to treat polyneuropathies, and the LNP-mRNA vaccine platform for COVID-19, have paved the way for a medical revolution. LNP technology has enabled the protection of nucleic acids through encapsulation, delivery to specific tissues, as well as improved intracellular delivery. Intravenous administration of conventional LNP therapeutics (50/10/38.5/1.5mol% of ionizable lipid/helper lipid/cholesterol/PEG-lipid), however, is subject to liver accumulation and limited extrahepatic biodistribution due to rapid clearance. There is an urgent need for innovative LNP technologies to overcome this delivery barrier.
We have developed a novel long-circulating LNP (lcLNP™) system capable of delivering nucleic acids to a variety of extrahepatic tis-

sues, due to a substantially enhanced blood circulation half-life. By utilizing cutting-edge lipid chemistry and rational LNP design, we have developed LNP systems with a unique morphology. LNP-RNA systems prepared with high levels of "helper" lipids, such as distearoylphosphatidylcholine (DSPC), demonstrate a small interior "solid" core situated in an aqueous compartment that is bounded by a lipid bilayer. These liposome-like systems exhibit properties that are superior to the conventional LNP-RNA systems currently used in humans, with regard to RNA stability, systemic circulation, extrahepatic tissue delivery, as well as transfection potency.

To evaluate the effectiveness of our lcLNP™ system, we conducted library screens using *in vitro* and *in vivo* luminescence and fluorescence quantification assays. These assays allowed us to assess knockdown (siRNA), expression (mRNA), or editing (mRNA/sgRNA) in various cell types and isolated tissue samples. Additionally, we employed fluorescence-based lipid tracers, radiolabeling, and flow cytometry to evaluate the systemic blood circulation as well as tissue biodistribution. The results showed that upon intravenous administration, lcLNP™ RNA systems exhibit extended circulation lifetimes (t1/2 ~ 4-10h in mice) compared to LNP-mRNA systems containing 10 mol% helper lipids (t1/2 ~ 30min), resulting in improved accumulation in extrahepatic tissues such as bone marrow, spleen, skin, or solid tumors. The enhanced lcLNP™-mediated RNA delivery to extrahepatic tissues ultimately enables a significant improvement in gene silencing, expression, as well as editing. For instance, our lcLNP™ systems demonstrate significantly enhanced reporter gene expression in hematopoietic stem cells (HSCs) and T-cells following intravenous administration compared to conventional Onpattro-like LNP systems. Therefore, the rationally designed lcLNP™ system addresses a key barrier that impedes the extrahepatic delivery of genetic payloads. Our preclinical proof-of-concept studies demonstrate the potential to treat a variety of diseases with unmet medical needs, including genetic diseases and cancer.

FROM ORIGIN OF LIFE TO NEXT GENERATION THERAPEUTICS

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Specific structural features, located mainly on the periphery of ribosomes related to genetic diseases, as well as of antibiotics-resistant pathogens, are being used as locations for targeted next generation therapeutics.

In contrast, the site for peptide bond formation within the ribosome, the PTC, is located within a highly conserved internal pocket made exclusively of rRNA. This high conservation implies its existence irrespective of environmental conditions and indicates that it may represent a prebiotic RNA machine, which could be the kernel around which life originated. Lab constructs imitating this pocket possess capabilities for peptide bond formations, thus indicating that a molecular prebiotic bonding entity still exists and functions within ribosomes of all living cells.

CELLULAR NANOPARTICLES FOR ANTIBACTERIAL THERAPY AND VACCINATION

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The global incidence of infections caused by bacteria and viruses has been increasing, which imposes a major threat to public health given the high morbidity and mortality rates associated with these diseases. Nanoparticle technology has enabled a wide array of improvements in the treatment of infectious diseases, ranging from improved efficacy in drug delivery to enhanced immunogenicity of vaccines. Among the different bio-inspired nanotechnology strategies, utilization of cellular membrane material for nanoparticle preparation presents a unique top-down approach that offers the advantage of being able to completely replicate the surface antigens and functions of source cells.

Cell membrane-coated nanoparticles are made by wrapping natural cellular membranes onto synthetic nanoparticle cores. They leverage cell membranes to mimic some cell-like functions for biointerfacing, making it possible to enable novel biomedical applications. The targeting ability of these cell-mimicking nanoparticles is often mediated by specific proteins that are expressed on the source cells, and this bestows the nanoparticles with specific interactions with various disease substrates. On top of the natural biointerfacing capabilities of cell membrane-coated nanoparticles, their traits can be further enhanced by introducing exogenous moieties onto the membrane surface. One way to achieve this is to genetically engineer source cells to express desirable protein receptors, followed by membrane collection to prepare the nanoparticles.

Herein, I discuss the biological functionalization of polymeric nanoparticles with a layer of membrane coating derived from natural cells. Specifically, I will focus on the use of these cell-mimicking nanoparticles for the treatment of bacterial and viral infections.

CLINAM

European Foundation for Clinical Nanomedicine

14/2023

Basel, October 8 – 11

ABSTRACTS POSTERS

LIPOSOME- INDOCYANINE GREEN J- AGGREGATES AS BIODEGRADABLE AND HIGHLY STABLE PHOTO-THERMAL AGENTS FOR CANCER THERAPY

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Photothermal therapy has been proposed as a safe and effective approach to treat cancer. Its local application to cancerous tissues results in minimal systemic side effects. Furthermore, it has been combined with immunotherapy to boost its efficacy. Existing non-biodegradable photothermal agents, such as gold-based nanomaterials, exhibit slow liver clearance and biodegradability, raising long-term safety concerns. In the present work, we developed clinically relevant, biodegradable phospholipid-based photothermal agents that will be cleared from the body following systemic administration. Indocyanine green (ICG) is an FDA-approved near-infrared (NIR) fluorescent dye used in optical and optoacoustic imaging; however, its rapid photodegradation has limited its use as a photothermal agent. To overcome this issue, our group loaded clinically approved ICG into liposomes in the form of J-aggregate (parallel arrangements of the monomeric ICG molecules, IJA) that preserved the liposome characteristics while exhibiting superior photothermal stability in tumour tissues upon multiple irradiations with 808 nm laser. We have successfully optimized IJA formation into liposomes using different lipid compositions. Promisingly, the engineered IJA-liposomes exhibited a size below 150 nm with high stability over one month of storage at room temperature. They also showed minimal aggregation following freeze-drying. Furthermore, our IJA-liposomes exhibited high photothermal stability upon multiple irradiations with an 808 nm laser. They showed higher biocompatibility compared to free IJA and ICG in different cancer cell lines, with increased toxicity in combination with laser irradiation, resulting in high cell killing in a range of cancer monolayers and tumour spheroids. Finally, we determined the tumour-targeting capabilities and liver clearance of the optimal liposomal IJA formulation in tumour-bearing mice following intravenous administration, confirming their biodegradability and superior photothermal stability *in vivo* upon multiple irradiations. In conclusion, with both liposomes and ICG being clinically approved, our developed liposomal IJA constructs will offer a biodegradable and clinically relevant theranostic platform, enabling multimodal (fluorescence and optoacoustic) imaging and combinatory (chemo/immunotherapy and photothermal) cancer therapy.

ACKNOWLEDGMENT:

The Phospholipid Research Centre (WAJ-2021-097/1-1), Prostate Cancer UK (CDF-12-002 Fellowship), and the Engineering and Physical Sciences Research Council (EPSRC) (EP/M008657/1) for funding.

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PRAGMATIC CHALLENGES IN USING IN SILICO MODELING TO EVALUATE THE PHARMACOKINETICS OF IRON-CARBOHYDRATE PRODUCTS

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Intravenous iron-carbohydrate complexes are a heterogenous class of nanomedicines whose critical quality attributes (CQAs) have not been fully established. Despite being used in clinical practice for decades, the *in vivo* biodisposition profiles and mechanisms of biodegradation of the iron-carbohydrate complexes after uptake into the mononuclear phagocytic system are not established. The lack of mechanistic understanding of both plasma pharmacokinetics (PK) and tissue distribution prevents current application of physiologically-based PK (PBPK) modeling. There are three fundamental challenges that need to be addressed before predictive PBPK models can be developed and applied; 1) the lack of assays to quantitatively measure the serum concentration of intact iron nanoparticles and unbound iron species fundamentally limits the availability of data for computational modeling. 2) PBPK models need to include several parameters to describe iron-carbohydrate nanoparticle metabolism that are yet to be completely defined and those identified (e.g. ferritin) exhibit considerable interpatient variability. 3) modeling is further complicated by the lack of traditional receptor/enzyme interactions, iron is stored and released based on the individual's own iron homeostasis. The known parameters of bioavailability, distribution, metabolism, and excretion for iron-carbohydrate products will be reviewed and challenges that currently prevent the direct application of PBPK or other in silico modeling techniques will be discussed.

Bioavailability: Clinical iron indices currently used to evaluate plasma PK profiles of iron- carbohydrate products only measure the total serum iron (TI) or transferrin-bound iron (TBI) but cannot distinguish between nanoparticle-derived iron in the serum versus endogenous iron. For some products there is a pool of non-transferrin-bound iron or labile iron which depends on the patient's serum transferrin concentration and this can be affected by disease states. The pharmacologically relevant fraction of the iron-carbohydrate complex undergoes cell uptake and is effluxed from the iron storage cells (i.e. macrophages) to transferrin **(Figure 1).**

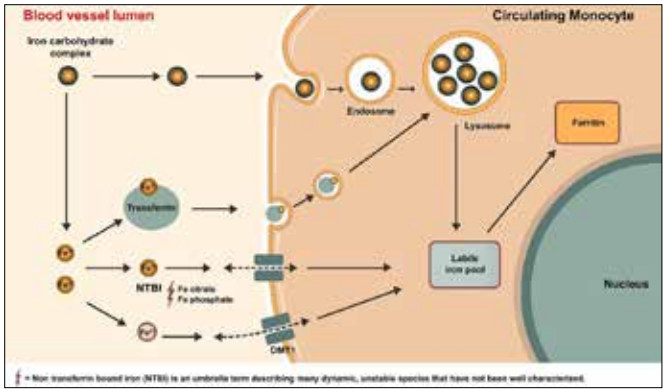

Figure 1. Hypothesized bioprocessing of iron-carbohydrate complexes

Distribution: Serum iron or iron carbohydrate nanoparticle concentration do not accurately represent tissue biodistribution. [Pau Clin PK] Methods to evaluate tissue biodistribution include radiolabeling the iron moiety or evaluating pre-clinical species. It has been suggested that radio-labeling a commercial iron-carbohydrate preparation may alter their 3-dimensional physical structure and change nano-bio interface and provide erroneous *in vivo* distribution data. This greatly limits the understanding the biodistribution in humans. Pre-clinical models can facilitate understanding of biodistribution *in vivo* using sensitive methods such as ICP-MS. However, translation of these data to humans, especially with regard to the impact of serum protein adsorption (ie protein corona) reamins unresolved.

Metabolism: Intravenous iron-carbohydrate complexes do not exhibit a classical drug-enzyme interaction that facilitates the breakdown of the drug into water-soluble species for excretion. Currently, very little is known about the biodegradation mechanism for iron-carbohydrate complexes.
However, the rate and extent of the biodegradation process are specific to each unique iron-carbohydrate product specific and are critical for the bioavailability of ferrous iron and furnishing the pharmacologic effect. As **Figure 1** depicts, the iron-carbohydrate nanoparticles are opsonized by peripheral blood macrophages which is influenced by each iron-carbohydrate nanoparticle's size, morphology and surface characteristics.
Excretion: Iron is typically highly conserved and recycled and therefore minimal excretion is predicted after intravenous dosing. Thus, excretion is not anticipated to be a key parameter in PBPK models.
Conclusion: The fundamental challenge to developing PBPK models is that there is not a fully validated assay to accurately measure nanoparticle-bound iron after intravenous administration of an iron-carbohydrate complex. Against this background, a better understanding of the influences of CQAs on the bioavailability, distribution, and efficacy of the iron-carbohydrate complexes is required. Improving scientific understanding of the interactions between the critical quality attributes of these products and multiple biological environments. Their complex PK in the context of the highly regulated and dynamic aspects of human iron metabolism requires more mechanistic research and validated *in vitro* techniques before they can be used to describe the *in vivo* disposition in more detail.

ANTI-TUMOR EFFICACY OF PEGYLATED LIPOSOMAL DOXORUBICIN TARGETED WITH CREKA PEPTIDE IN MURINE MELANOMA MODEL

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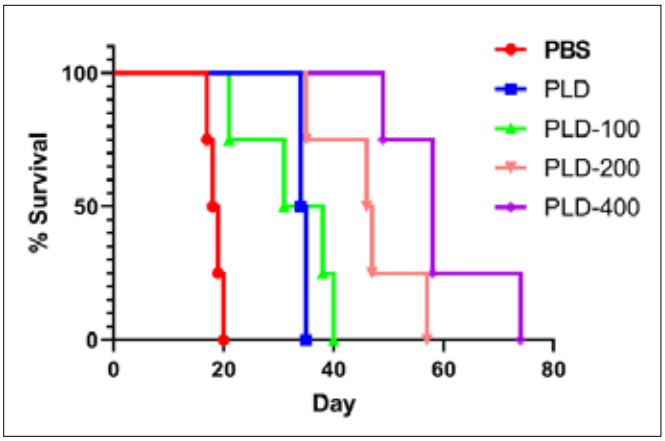
Objective: Malignant melanoma is a life-threatening skin cancer that originates from melanocytes, specialized pigmented cells found in the epidermis. In this study, we try to increase the specificity of PEGylated Liposomal Doxorubicin (PLD) formulation to melanoma tumor cells and reduce its side effects by CREKA peptide (Cys-Arg-Glu-Lys-Ala). CREKA is a tumor- homing peptide, which targets overexpressed fibrin and fibrin-fibronectin complexes found in tumor and vasculature stroma (1).
Methods: In order to have targeted PLDs, CREKA peptide was linked to DSPE-mPEG2000-maleimide phospholipid through thioether linkage. The targeted micelles with 50, 100, 200 and 400 peptide ligands, post- inserted into the preformed PLD. Following the physicochemical characterization (size and zeta potential using Zetasizer), the *in vitro* tests including cytotoxicity and cellular uptake were performed on the targeted formulations. Then, the therapeutic efficacy, biodistribution, and pharmacokinetics of the formulations were evaluated in B16F10 bearing C57BL/6 mice. The treated mice were evaluated for tumor growth and survival analysis. Graph Pad Prism 6 Software was used to analyze the data.
Results: Liposomal formulations showed appropriate physicochemical properties with size between 87 to 105 nm, and negative surface charge. The formulations were examined for cytotoxicity, and the targeted formulations showed a significant difference compared to PLD in terms of IC50. The presence of the peptide increased the toxicity of the formulations. In terms of cellular uptake, it was found that interactions of targeted formulations do not change significantly compared to PLD ($P \leq 0.05$). In animal studies (biodistribution and survival analysis) in melanoma model in C57BL/6 mice, no significant difference was observed in the distribution of targeted formulations in peripheral tissues. However, targeted formulations with 400 ligands (PLD-44) increased drug accumulation in the tumor tissue of mice. The longest survival period (Figure 1), the largest decrease in tumor size and drug accumulation at the tumor site ($P \leq 0.05$) were observed in formulations with 400 ligands (PLD-400).

Figure 1 Survival analysis of therapeutic groups were monitored by the multiple comparison log-rank (Mantel–Cox) test. Effects of treatments on survival time were monitored among B16F10 tumor model of C57BL/6 mice (n=4).



Conclusion: Overall, the results of this study showed that the use of CREKA peptide to decorate PLDs could result in the effective tumor regression and prolonged survival in C57BL/6 mice bearing B16F10 melanoma. This peptide enables nanoparticles to accumulate in target tissues at higher concentrations and can increase the efficiency of PLD formulation and reduce its side effects.
Keywords: PEGylated Liposomal Doxorubicin, CREKA, Tumor-homing Peptide, Targeted Drug Delivery, B16F10, Melanoma
Reference: 1- Zhang N, Ru B, Hu J, Xu L, Wan Q, Liu W, et al. Recent advances of CREKA peptide-based nanoplatforms in biomedical applications. Journal of Nanobiotechnology. 2023;21(1):77-.

NADENO – UNLEASHING THE POTENTIAL OF HARD-TO-DELIVER DRUGS

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NaDeNo is a Norwegian company founded on >10 years of nanomedicine research at SINTEF[1-3]. NaDeNo is developing a platform of proprietary polymeric nanoparticles to overcome drug delivery hurdles, with initial focus on repurposing of effective drugs in areas of high unmet medical need. One such drug is cabazitaxel, a highly potent taxane, which therapeutic potential has been limited due to its hydrophobicity, high systemic toxicity and the instability of the commercial formulation, Jevtana®[4]. Our lead candidate PACAB-002 is a proprietary combination of poly(alkyl cyanoacrylate) and cabazitaxel, for which highly encouraging preclinical data have been generated. PACAB-002 is intended for local administration in the peritoneal cavity for the treatment of peritoneal metastasis originating from ovarian cancer. This is a cancer indication with poor prognosis, for which there are currently no effective treatment options.

Through encapsulation in nanoparticles, we have shown even drug distribution and long drug retention time in the peritoneal cavity, tumor specific drug accumulation, low systemic toxicity and a significant reduction in tumor weight in mouse models (figure 1)[5]. PACAB-002 hence represents a safer and more efficient treatment option for peritoneal metastasis.

We are currently performing IND-enabling studies and process scale-up of PACAB-002, and preparing for clinical studies.

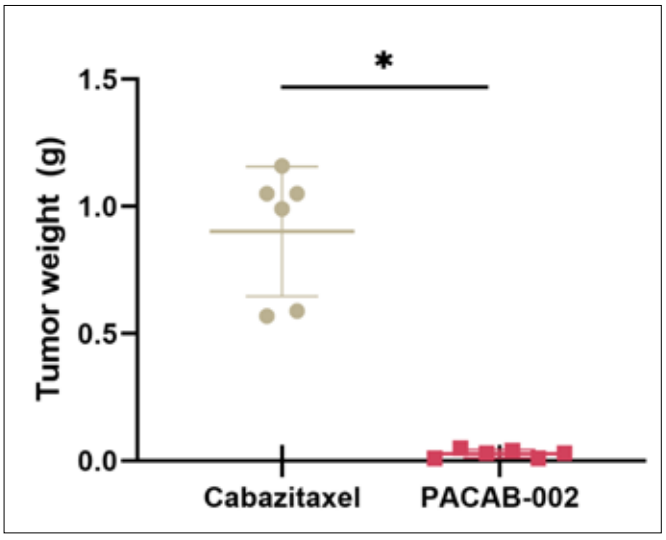

*Figure 1. Preclinical proof of concept for lead candidate PACAB-002 in mice bearing B76 xenografts originating from human ovarian cancer. Tumor weight at study end (day 38) after a single treatment by local injection in the peritoneum. Cabazitaxel is non-encapsulated cabazitaxel (Jevtana®-like formulation), whereas PACAB is cabazitaxel encapsulated in poly(alkyl cyanoacrylate) nanoparticles, n=6 animals per group, *P<.001*

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TARGETED NANO-FORMULATIONS FOR TREATMENT OF MRSA: A MULTICOMPONENT

ANDREAS ÅSLUND

platform for nano-formulated treatment of resistant microbial infections (LeadtoTreat)

DEVELOPING A PLATFORM FOR FUTURE TREATMENT OF MULTIDRUG-RESISTANT MICROBIAL INFECTIONS

The treatment of bacterial infections on a global scale is facing the enormous challenge of rapidly increasing predominance of antibiotic resistant strains. It is estimated that up to 50,000 lives in Europe and the US and 700,000 lives globally are lost each year due to drug-resistant microbes. Many bacteria that cause infectious diseases develop resistance to not only the primary antibiotic treatments available in the clinic but also to drugs of last resort which often require prolonged treatment periods and come with significant side effects. At the same time many promising lead compounds with high activity and wide therapeutic windows have failed to progress to clinical trials due to poor solubility, protein adsorption or other difficulties in formulation (e.g. low drugability). LeadtoTreat proposes a new solution to these challenges by introducing a platform for future infection treatment, enabling targeted delivery of novel lead compounds with low drugability, as well as synergistic combinations of antibiotics and potentiators in a nanoformulation.

The primary objective of LeadtoTreat is to develop a flexible, targeted nanoparticle system for delivery of synergistic antimicrobial treatments, demonstrated with multidrug resistant Staphylococcus aureus (MRSA) targeting nano-formulations of difficult-to-formulate-drug leads towards MRSA bacterial infections.

This platform technology will be demonstrated by converting a highly active, but water insoluble and protein binding, novel compound into targeted nano-formulations for treatment of MRSA infections with proven *in vivo* and *in vitro* safety. Furthermore, Lead-

toTreat aims to identify novel synergistic combinations of antibiotics and potentiators and convert these into highly active targeted nano-formulations for treatment of MRSA infections.
During Clinam 2023 we will present results from the first year of the project which includes data from the synergy screens of antibiotic/antibiotic and antibiotic/potentiator combinations and production of nanobodies towards selected strains of MRSA.

DYNAMICS OF BIODEGRADATION OF IRON CARBOHYDRATES IN MACROPHAGES, A CLUE TO UNDERSTAND THEIR THERAPEUTIC EFFECT

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Iron-carbohydrate complexes are the standard of care to treat severe iron deficiencies. Post-IV injection, these nanomedicines are cleared from blood circulation and can be found in the liver and spleen, mainly in macrophages. It is hypothesized that, in these cells, iron-carbohydrate complexes are internalized, biodegraded, and incorporated into the iron metabolism in the form of ferrous/ferric iron. The mechanism on how human macrophages perform this process is not fully understood. The why and how different iron-carbohydrate formulations trigger different physiological responses is also not known.
The main objective of this project is to unravel the mechanism of action of iron-carbohydrate complexes in human macrophages. The role of the carbohydrate ligands bound to the polynuclear iron cores is also studied, since it has been suggested that they are a key determinant in the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the nanoparticles.
For this purpose, we performed a dynamic characterization of the response of macrophages to three iron-carbohydrate complexes: iron sucrose (IS), ferric carboxymaltose (FCM), and iron isomaltoside-1000 (IIM). Primary human M2 macrophages (derived from blood monocytes from healthy donors) were our main model system. Upon treatment with a physiological concentration of IS, FCM or IIM, we measured at different time points: total iron uptake (ICP-OES), the labile iron pool (calcein and Turnbull's blue staining), intracellular ferric iron (Prussian blue-DAB staining), and ferritin production and release (ELISA). Besides, we performed TEM microscopy to observe intracellular structures containing iron and the structural changes induced by the treatments. Iron citrate and iron sulfate were used as controls.
The measured parameters allowed us to characterize the dynamics of biodegradation of each iron-carbohydrate complex. Although the type of responses to IS, FCM and IIM were similar, each nanoparticle formulation had a specific dynamic profile. For example, IS had a faster internalization rate compared to FCM and IIM. Besides, IS induced the production of ferritin (both intracellular and in supernatant) at earlier time points. Internalization of the nanoparticles was mediated by the endolysosomal system, as observed in the TEM images (Fig. 1). Using EDX spectroscopy, we determined that the observed nanostructures were composed of iron, confirming that the complete nanoparticles can be internalized by the cells.
Our results illustrate the impact that the physicochemical properties can have on the biological properties of these nanomedicines. Furthermore, our data show how human macrophages, a key player in the iron metabolism, effectively use these nanomedicines as a source of iron and convert it in bioavailable iron (e.g. ferric iron bound to ferritin) that can later be released to blood and be used in the bone marrow for hematopoiesis.

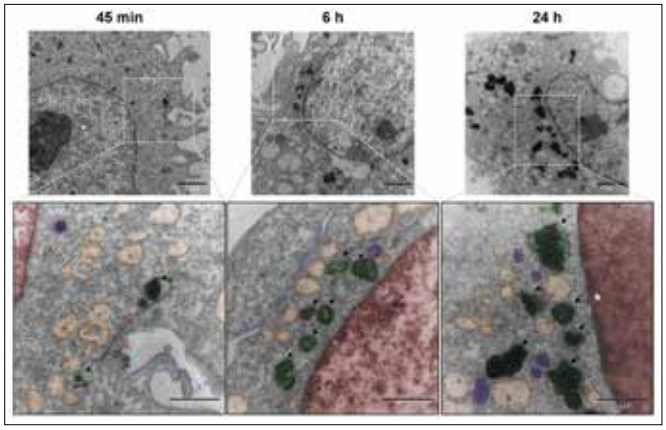

Figure 1. Iron sucrose (IS) nanoparticles internalized in macrophages via the endolysosomal system. Primary human M2 macrophages were treated with 1800 µM of IS for 45 min, 6 h and 24 h. The cells were then fixed and processed for TEM analysis. The images show lysosomes containing iron nanoparticles at the different treatment time points. Red: nucleus, blue outline: plasma membrane, violet: mitochondria, orange: endosomes, green/black arrows: iron nanoparticles in lysosomes, blue arrows: iron particles outside of cell, red arrow: endocytic process, pink arrow: ER.

NANOBIOTICS FOR MYCOBACTERIAL INFECTIONS: 'IT'S THE LITTLE THINGS THAT MATTER THE MOST'

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In 2017, the WHO published a list of antibiotic-resistant priority pathogens that are posing increasing risks to human health. This list included 12 classes of bacteria, plus Mycobacterium tuberculosis (Mtb) and Clostridioides difficile. Despite a relatively large preclinical pipeline (252 agents), the 50 antibiotics in clinical development were showing very little benefit over existing treatments. Since then, the lack of private investment and innovation efforts, allied to the events related to the COVID-19 pandemic, severely undermined any efforts to combat drug-resistant bacterial infections. In 2021 alone, 1.6 million people died of tuberculosis (TB), reversing the declining trend that had been observed between 2005 and 2019. Despite being an old foe (with reports of Mtb infection dating back thousands of years), it remains the most common and lethal airborne antimicrobial-resistant disease worldwide today. Irrefutably, there is a pressing need to think outside the box and develop new therapeutic alternatives.
By inhibiting cell wall mycolic acid synthesis, isoniazid (INH) has been the most important anti-TB drug since its development in the early 50s. However, as a free drug, it lacks the means for targeted delivery, being administered in high doses, rapidly egested, and highly toxic. We have developed an INH-based polymer by reacting its hydrazide group with the ketone group of an α-keto polyester. The antibiotic becomes active upon pH-triggered hydrolysis in the acidic environment of the endosomes to achieve the targeted release of a high drug payload (one molecule of antibiotic per monomeric unit of polymer) inside infected macrophages. This polymer was used to formulate nanoparticles (nanobiotics) containing a

second antibiotic (clofazimine; CFZ), with poor water solubility and poor caseum penetration, providing a mechanism of synchronous nanoscale delivery of hydrophilic and hydrophobic payloads, while preventing undesirable drug-drug interactions. We showed that the multi-drug nanobiotics efficiently targeted both intracellular and granuloma-resident mycobacteria *in vivo*, presenting statistically significant improved efficacy when compared to conventional therapy (Figure 1) [1].

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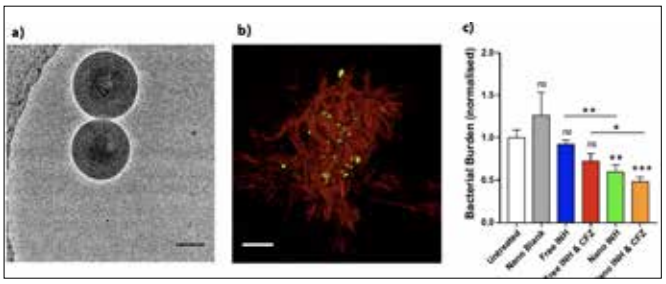

Figure 1 – a) Cryo-EM image of INH nanobiotics (scale bar, 100 nm). b) Confocal imaging showing the repartition and accumulation of coumarin 6-labelled Nano Blank (green) into a mycobacterial cord structure (scale bar, 5 µm). c) Effect of nanobiotics at 3 days post-infection on zebrafish infected with fluorescently labelled M. marinum assessed by the quantification of bacterial load (results plotted as mean ± SEM from 2 independent experiments; n = 21). INH: isoniazid. CFZ: clofazimine.

DEVELOPMENT AND CHARACTERIZATION OF SYNGENEIC TUMOR MODELS FOR HEPATOCELLULAR CARCINOMA IN IMMUNOCOMPETENT MICE

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INTRODUCTION

Hepatocellular Carcinoma (HCC) accounts for 90% of all primary liver tumors [1]. Cirrhosis, due to chronic organ damage, is characterized by a massive accumulation of scare tissue in the liver and is the most frequent risk factor for HCC [2] [3]. But incidences of HCC are also increasingly observed in patients with metabolic-associated steatohepatitis (MASH) without cirrhosis [4]. Common murine models for HCC are lengthy and tumor load tends to be heterogeneous as tumor induction takes around 20 weeks and less than 50% of mice bear tumors [5].

In this work, we introduce a rapid and easy-to-handle injection model for HCC in cirrhotic and non-cirrhotic livers, which recapitulates histological and molecular key features of HCC in patients [6].

RESULTS AND DISCUSSION

For the non-cirrhotic model, mice were intrasplenically injected with Dt81-Hepa 1-6 tumor cells (HCC cells), while for the cirrhotic model, mice were gavaged with profibrogenic CCl_4 for 6 weeks prior tumor cell inoculation. After 4 weeks, inoculated mice developed tumors exclusively in their livers (**Fig. 1a**). Interestingly, livers of the cirrhotic group had a significantly higher tumor load as indicated by higher liver weights (2.5-fold), higher Alpha-fetoprotein (AFP) sera levels and morphometric readouts of liver sections compared to non-cirrhotic mice (**Fig. 1b&c**). RNA-Seq analysis of HCC cells used in this work, revealed that HCC hub genes (AFP, MCM3, SPATS2, NT5DC2, MCM6) were significantly upregulated and tumor cells showed a distinct clustering compared to healthy hepatocytes (**Fig. 1d**).

3D multiphoton microscopy and 2D fluorescence microscopy revealed that extracellular markers (ECM), namely collagen 1-3, collagen-1, collagen-4, and fibronectin were significantly overexpressed in tumor tissues as compared to controls. Furthermore, the adjacent liver of the cirrhotic HCC model displayed fibrillar structures distributed throughout the non-cancerous tissue, suggesting an enhanced desmoplastic reaction of the tumor (**Fig. 2a**) 3D visualization of collagen structures via single harmonic generation (SHG) showed that collagen fibers are more abundant in the cirrhotic liver (**Fig. 2b**). Next, we analyzed the expression of markers for vasculature. All markers, namely CD31 (endothelial cells) and αSMA (pericyte, fibroblasts) were significantly overexpressed in tumor tissues (**Fig. 3**). No significant difference was observed between marker expression in the adjacent liver of HCC and fibrotic HCC.

Finally, we tested the novel HCC gold standard therapy atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) in our model. HCC mice were injected intravenously once weekly with the atezolizumab equivalent anti-hPD-L1 (8 mg/kg) and bevacizumab (5 mg/kg) (AtezoBev), respectively, starting at day 7 (**Fig. 3a**). AtezoBev showed an improved ($p<0.05$) antitumor effect as indicated by lower liver weights and AFP levels in the sera compared to control mice at day 28 (**Fig. 3b**).

CONCLUSION

We present an easy-to-handle murine model for HCC with high relevance for translational research. The model reflects characteristics of human HCC and showed a positive antitumor response to AtezoBev.

Figure 1. Model development of hepatocellular carcinoma. *a. Schematic representation of animal model development. The HCC model was generated by intrasplenic injection of Dt81-Hepa-1-6 cells. The fibrotic HCC model was generated by CCl4 administration for 6 weeks and subsequent injection of Dt81-Hepa-1-6 cells. Livers from healthy mice or mice only administrated with CCl4 were used as controls. b. Liver weight and measured AFP level in plasma mice revealed malignant lesion formation by substantial liver weight increase as well as an increase in AFP in the plasma of tumor-bearing mice. c. H&E staining of whole liver lobes reveal the difference between tumor lesions and the adjacent liver counterpart. d. RNA-Seq analysis of HCC cells used in this work. scale bar= 50 µm.*

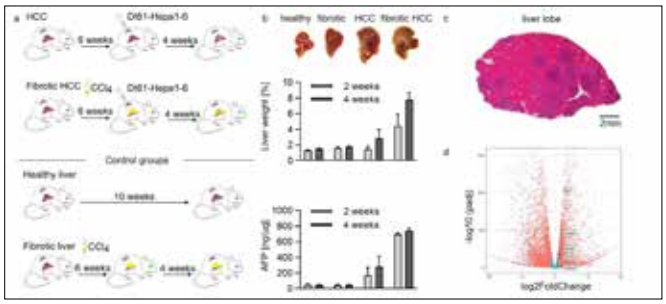


Figure 2. Deposition of extracellular matrix components in tumor and adjacent liver counterparts of HCC and fibrotic HCC model. *a. Representative fluorescence images of staining and analysis of different ECM components reveal that all components are heavily overexpressed in tumor tissue as compared to their respective liver counterpart. In the liver counterpart of fibrotic HCC fiber-like structures are visible which are absent in the liver counterpart of HCC. Together with the respective quantification it indicates an elevated matrix deposition in the former. b. Collagen fibers visualized in 3D via SHG, and quantified volumes confirm overproduction of dense fiber structures in tumors. scale bar = 50 µm, LC= liver counterpart, HCC= Hepatocellular carcinoma, F=Fibrotic, AF: Area fraction, VF: Volume fraction.*

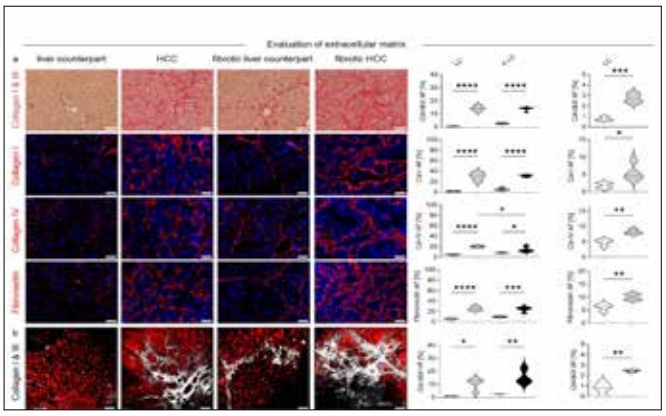


Figure 3. Expression of markers for vasculature and fibroblasts in tumor and adjacent liver counterparts of HCC and fibrotic HCC model. *Representative fluorescence images of staining and analysis of endothelilal cells (CD31) and pericytes (αSMA) revealed that all markers are upregulated in tumor tissues as compared to non-malignant counterpart. scale bar = 50 µm. LC= liver counterpart, HCC= Hepatocellular carcinoma, F=Fibrotic, AF: Area fraction, VF: Volume fraction.*

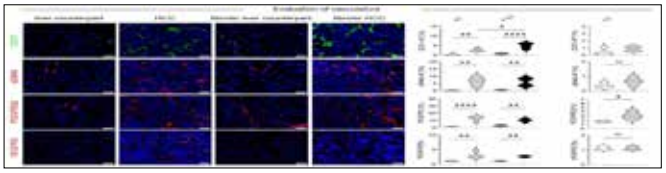
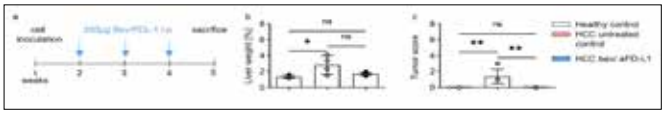


Figure 4: First line tumor-targeted therapy in HCC model. a. *Treatment schedule for checkpoint Bevazizumab/anti-PDL1 therapy on the non-fibrotic HCC model. The treatment resulted in an absence of tumor growth, which is reflected in non-increased liver weight (b) and a tumor score of 0 (c).*



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POLYETHYLENE GLYCOL (PEG) AS A BROAD APPLICABILITY MARKER FOR LC-MS/MS-BASED BIODISTRIBUTION ANALYSIS OF NANOMEDICINES

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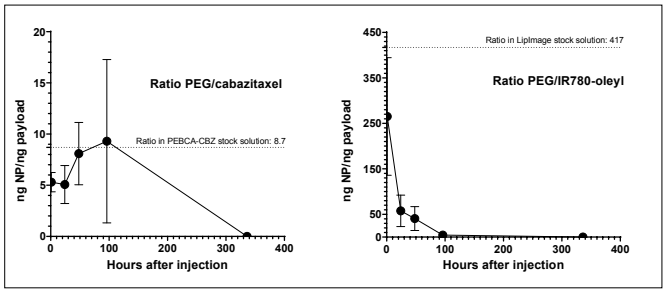
Polyethylene glycol (PEG) conjugation (PEGylation) is a well-established strategy to improve the pharmacokinetic and immunogenic properties of a wide variety of nanomedicines and therapeutic peptides and proteins. This broad use makes PEG an attractive 'all-round' candidate marker for the biodistribution of such PEGylated compounds. Here, we present the development of a novel strategy for PEG quantification in biological matrices. It is based on sample hydrolysis which both decomposes the sample matrix and degrades PEGylated analytes to specific molecular fragments more suitable for detection by LC-MS/MS. Method versatility was demonstrated by applying it to a wide variety of PEGylated compounds, including polymeric poly(ethylbutyl cyanoacrylate) (PEBCA) nanoparticles, lipidic nanoparticles (Doxil®, LipImage 815™ and lipid nanoparticles for nucleic acid delivery) and the antibody Cimzia®.

Method applicability was assessed by analyzing plasma and tissue samples from a comprehensive drug biodistribution study in rats, of both PEBCA containing the anticancer drug cabazitaxel, and LipImage 815™ nanoparticles containing the near-infrared dye IR780-oleyl. The results demonstrated the method's utility for biodistribution studies on PEG. Importantly, by using the method described herein in tandem with quantification of nanoparticle payloads, we showed that this approach can provide detailed understanding of various critical aspects of the *in vivo* behavior of PEGylated nanomedicines, such as drug release and particle stability. As seen from Figure 1, there is a very notable difference between how the polymeric and lipidic nanoparticles behave in liver tissue, when comparing the measured concentrations of nanocarrier base material (quantified as PEG) versus the encapsulated – and subsequently released – payload.

Together, the presented results demonstrate the novel method as a robust, versatile and generic approach for biodistribution analysis of PEGylated therapeutics.

Figure 1: Nanoparticle/payload ratios in liver tissue for PEBCA-CBZ (left graph) and LipImage (right graph). Dotted lines indicate ratios in original formulations prior to injection. Nanoparticles are quantified as PEG equivalents.



UNCOVERING THE DYNAMICS OF CELLULAR RESPONSES TRIGGERED BY IRON-CARBOHYDRATE COMPLEXES IN HUMAN MACROPHAGES USING QUANTITATIVE PROTEOMICS AND PHOSPHOPROTEOMICS

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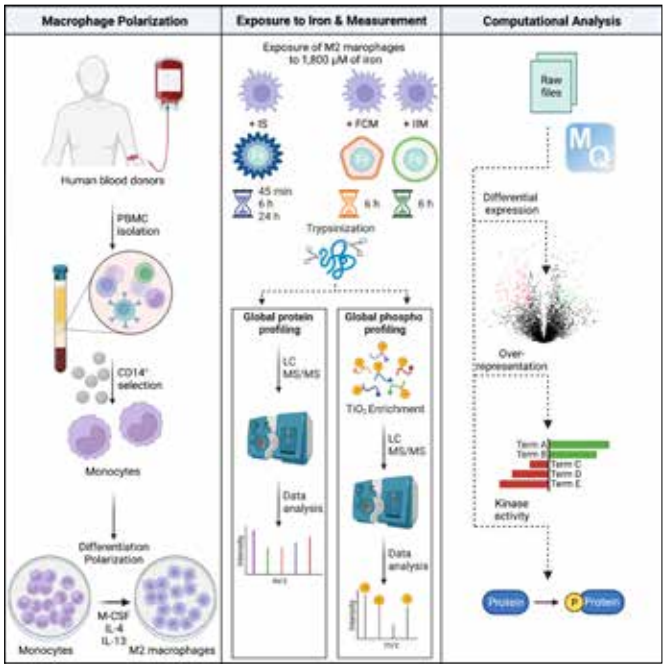
NEW

Iron deficiency is a common nutritional deficiency worldwide. In this context, the lack of iron is one of the main factors in the global burden of disease and, as a consequence, can lead to anemia. Left untreated, severe cases can cause serious health complications. To effectively replete iron in blood, intravenously (IV) administered iron-carbohydrate complexes are currently one of the best options, especially in cases where the use of oral iron salts is restricted due to tolerability, malabsorption, or the need for a rapid repletion. Upon administration of these nanomedicines, macrophages are presumed to play a critical role in their uptake, metabolization, and storage. However, the exact cellular and molecular mechanisms on how macrophages perform these processes are still unknown. After treatment of primary human M2 macrophages with clinical doses of iron sucrose (IS), the first approved iron-carbohydrate complex, we quantified differences in protein expression and phosphorylation, overrepresented cellular pathways, as well as enrichment of kinase activities using a bottom-up LC-MS/MS proteomics and phosphoproteomics approach (Figure 1). Treatment with iron sucrose altered the expression of various surface receptors and proteins related to the transferrin-related uptake mechanism, suggesting that a complex combination of entry mechanisms of complete particles and dissociated iron ions is responsible for the cellular uptake. A strong increase in intracellular ferritin levels showed the successful incorporation of iron coming from the nanoparticles into intracellular iron storage sites after cells were treated for only 6 h. Other changes indicated that iron derived from IS causes oxidative stress, which triggers protective mechanisms against ferroptosis and an autophagic response. Furthermore, we compared the response to IS with two other iron-carbohydrate complexes with different carbohydrate ligands, namely ferric carboxymaltose (FCM) and iron isomaltoside-1000 (IIM). Our data shows that iron-carbohydrate complexes with more robust pharmacokinetic properties (IIM > FCM > IS) are biodegraded at a slower pace by the macrophages. IS was associated with a loss of M2 polarization properties, that was not observed with FCM or IIM. Taken together, our analysis provides a comprehensive insight into the molecular cell responses to iron-carbohydrate complexes. It underlines the potential of global proteomics and phosphoproteomics approaches in the field of nanomedicines. The better understanding of molecular processes can support the development of new medicinal products with improved pharmacokinetic and pharmacodynamic parameters. In addition, the application of such technologies has the potential to be used to screen nanomedicines for desired and potentially toxic properties at the cellular level.

Figure 1. Experimental procedure. *The experimental procedure is based on the treatment of human M2 macrophages with iron-carbohydrate complexes and the subsequent profiling of differences in protein abundance and phosphorylation. Peripheral blood mononuclear cells (PBMCs) were isolated from human buffy coats, followed by a CD14 selection of monocytes. After differentiation, macrophage were polarized into the M2 state. M2 macrophages were treated with iron-carbohydrate complexes and characterized by global protein and phosphoprotein profiling. Generated data was investigated for differentially expressed entities, over-represented biological pathways and differences in kinase activities.*



STABILIZATION OF MRNA VACCINES BY LYOPHILIZATION

ROLAND BÖTTGER

Introduction: mRNA vaccines are suitable for tackling emerging pandemics due to their rapid development process, superior efficacy, and favorable safety profile. However, further maturation of mRNA technology is required to be competitive with other modalities on the regular drug market. A significant drawback of currently marketed mRNA products is their low stability requiring storage at negative temperatures, implying challenges in their transport and distribution, while consequently increasing their costs. The development of dry mRNA presentations has potential to increase stability and could enable storage at refrigerated or even ambient temperatures. We investigated novel LNPs with improved stability to enable dry storage at ambient conditions without loss of vaccination efficiency.

Methods: mRNA encoding the rabies virus glycoprotein (RAVG) was formulated in lipid nanoparticles (LNPs) with different lipid compositions and dialyzed against a range of lyoprotectants. The mRNA-LNPs were dried, stored under refrigerated, ambient, or elevated stress conditions, and reconstituted before analysis. The particle-size distribution, encapsulation efficiency and mRNA integrity were tested. Optimal formulations were selected and the virus neutralizing titers (VNT) as well as T-cell responses of splenocytes after two intramuscular injections into mice were tested in comparison with standard mRNA-LNPs stored as liquids at -80°C.

Results: The interdependence of LNP composition, lyoprotectant and drying process parameters was investigated. It was found that specific lipid excipients in LNPs are preferred to retain physicochemical parameters of mRNA-LNPs after lyophilization. In addition, optimal LNP compositions could resist more aggressive and faster drying potentially enabling an economic production process. The optimal formulation candidates could be stored at ambient conditions for at least 12 weeks with minimal change of the tested physicochemical parameters (Figure 1). In addition, these formulations showed no change in vaccination efficiency (Figure 2) and safety profile compared to identical mRNA-LNPs and state-of-the-art mRNA-LNPs stored as frozen liquids.

Conclusion: Lyophilization is a promising strategy to overcome stability issues and could help to increase opportunities for mRNA medicines on the post-pandemic pharmaceutical market.

Figure 1. Stability of lyophilized mRNA-LNPs stored at different temperatures measured as Z-Ave (A), PDI (B), Encapsulation Efficiency (C) and RNA Integrity (D).

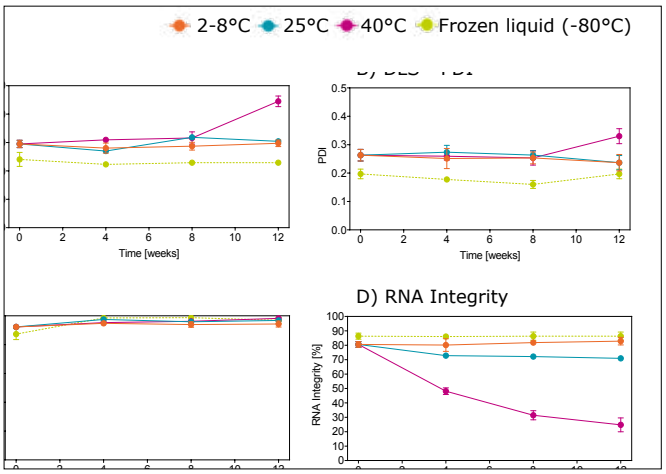
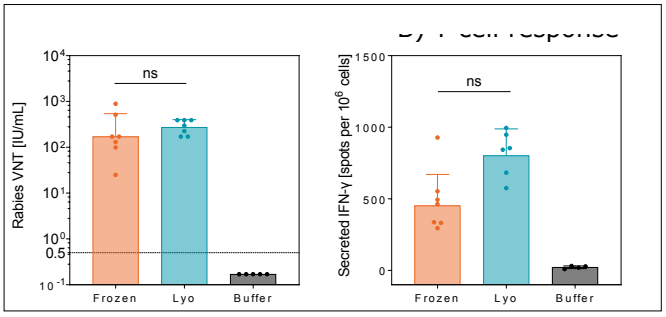


Figure 2. VNTs (A), and T-cell responses (B) in mice after two doses of 0.25 µg RAVG encoding mRNA-LNPs which have been stored as frozen liquid or lyophilized.



ASSESSMENT OF CELL PHENOTYPE AND GENE EXPRESSION CHANGES, FOLLOWING REPEAT EXPOSURE TO THE NRTIS FTC, 3TC AND LONG ACTING POLYMER LINEAR POLY(FTC) – RELEVANCE TO SUBCUTANEOUS ADMINISTRATION OF LONG-ACTING THERAPEUTICS

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HIV treatment requires chronic administration of antiretrovirals to suppress viral replication, with no current cure (Ruelas & Greene, 2013). Due to the lack of "forgiveness" in current antiretroviral regimens, in terms of viral breakthrough, long-acting antiretroviral medication can help to improve adherence to medication, resulting in better treatment outcomes (Chandiwana et al., 2021). Currently, the nucleoside reverse-transcriptase inhibitors (NRTIs), emtricitabine (FTC) and lamivudine (3TC), are being explored for use in long-acting delivery. A key question in this type of delivery is, with long-acting antiretrovirals, does repeated, and long-term, exposure to these drugs alter the functional capacity of human immune cells. Linear poly(FTC) is a long-acting polymeric prodrug of FTC, which is designed to be delivered subcutaneously as an implant that releases FTC slowly over time (Shakil et al., 2022). As such, it is important to assess its immunocompatibility with relevant immune cell types from the subcutaneous space. MUTZ-3 cells represent a useful model with which to study these interactions (Groell, Kalia, Jordan & Borchard, 2018).

The MUTZ-3 (human dendritic cell (DC) line) were cultured in either standard media or media containing either FTC (1.8 µg/mL), 3TC (2 µg/mL) or linear poly(FTC) (20 µg/mL) for 7 weeks, passaged twice-weekly, followed by an assessment of cellular phenotype, at week 7. Intracellular reactive oxygen species (ROS) were measured using CellROXTM Green Reagent, intracellular reduced glutathione was measured using ThiolTrackerTM Violet Dye and mitochondrial membrane potential (MMP) was assessed using JC-1 reagent (InvitrogenTM). Positive controls, specific to the cell type, were included in the evaluation, cells were treated with either LPS or Resiquimod (R848). Expression of a number of cell-surface markers in each cell line was assessed via multi-parametric flow cytometry. To related to possible subcutaneous administration of long-acting antiretrovirals, the MUTZ-3 cell line was assessed for changes in gene expression using the Nanostring™ metabolic pathways panel nCounter assay for differences between untreated cells and the cells treated with R848.

Intracellular ROS levels were significantly lower (18% and 21% lower respectively) in the FTC- and 3TC-cultured cells, than the untreated cells ($p<0.01$) (Figure 1a). However, linear poly(FTC)-cultured cells had significantly higher ROS levels, 19% higher than the untreated ($p<0.01$). FTC- and linear poly(FTC)-cultured MUTZ-3 cells displayed a significantly higher MMP, 29% and 33% higher than the untreated cells ($p<0.05$) (Figure 1c).

When compared to the untreated cells, the linear poly(FTC)-cultured cells showed a 262% significantly higher CD209 expression ($p<0.05$), the R848 treated linear poly(FTC)-cultured cells also showed a significantly higher expression of CD209 when comapred to the R848 treated cells, 56% higher ($p<0.05$) (Figure 1a). Linear poly(FTC)-cultured cells showed a 61% significantly lower expression of HLA-DR, when compared with the untreated cells ($p<0.05$) (Figure 1e). When compared to the R848 treated cells, the linear poly(FTC)-cultured R848 treated cells had a 76% significantly lower expression of HLA-DR ($p<0.05$) (Figure 1e). The linear poly(FTC)-cultured R848 treated cells had a 118% significantly higher CD274 expression ($p<0.01$) (Figure 1f). In the untreated-, 3TC- and linear poly(FTC)-cultured cells treated with R848 CD274 expression was also higher than the untreated counterparts, with 310% ($p<0.01$), 196% ($p<0.05$), and 516% ($p<0.01$) higher expression respectively (Figure 1f).

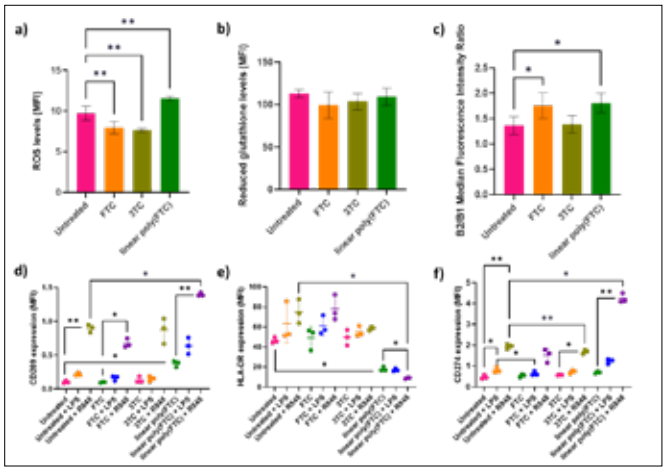

Figure 1: MUTZ-3 cells exposed to FTC and 3TC for 7 weeks and subsequent phenotypic assessment. a) Intracellular ROS, n=4, mean ± SD. b) Intracellular reduced glutathione, n=4, mean ± SD. c) MMP, n=4, mean ± SD. d) CD209 marker expression, n=3 ± SD. e) HLA-DR marker

*expression. f) CD274 marker expression, n=3 ± SD. P <0.0001 = ****, P< = ***, P <0.01 = ** and P <0.05 = *.*

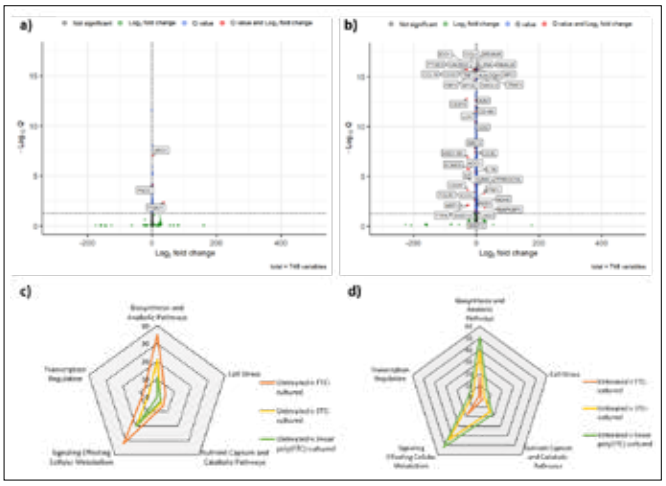

Figure 2: MUTZ-3 cells exposed to FTC and 3TC for 7 weeks and subsequent gene expression assessment. a) Significant gene expression changes when FTC-cultured R848 treated cells were compared to R848 treated cells (q<0.05). b) Significant gene expression changes when linear poly(FTC)-cultured R848 treated cells were compared to R848 treated cells (q<0.05). c) Number of genes within the metabolic pathways panel themes that had a higher expression. d) Number of genes within the metabolic pathways panel themes that had a lower expression.

Only 3 genes showed significantly altered gene expression when the FTC-cultured cells treated with R848 were compared to the R848 treated cells (Figure 2a). Many genes showed altered expression when linear poly(FTC)-cultured cells treated with R848 were compared to the R848 treated cells (Figure 2b). Of note significantly reduced gene expression of CCL4, TNF and CCL19 was seen in the linear poly(FTC)-cultured R848 treated cells compared to the R848 treated cells. Interestingly the CD274 gene expression contradicted what was seen in the marker expression results.

When compared to the untreated cells, the FTC-cultured cells had a greater profile of genes with higher expression and these tended to be within two main areas, Biosynthesis and anabolic pathways or Signalling effecting cellular metabolism (Figure 2c). 3TC then had a lower profile and followed by linear poly(FTC) and still in the same key areas (Figure 2c). Interestingly however when looking at genes that had lower expression, linear poly(FTC)-cultured cells had the largest profile of genes with lower expression and again in the same two themes, 3TC-cultured cells then had the next most changes followed by the FTC-cultured cells and again both within the same two themes (Figure 2d).

Responses to linear poly(FTC) in the MUTZ-3 cell line were marker specific, this is interesting as it indicates there is some activation, but also some inhibition of marker responses as a result of the cell sensing linear poly(FTC). Reduced expression of CCL4, TNF and CCL19 in the linear poly(FTC)-cultured R848 treated cells could suggest reduced activation of these cells. FTC exposure results in a greater profile of genes with higher expression, whereas linear poly(FTC) exposure results in a greater profile of genes with lower expression, this suggests there is a complex relationship between the repeat exposure of these three treatments and the effect on the cells, it is important to next understand the phenotype more clearly as the mRNA levels don't necessarily translate into increased protein levels, as was seen for CD274. Metabolic processes of these cultured cells should be explored further to better understand the consequences. Previously efavirenz and lopinavir have led to lower glucose uptake and caused bioenergetic modifications to cells, it would be useful to explore the methods used to determine if FTC, 3TC and linear poly(FTC) also alter these profiles (Heaton et al., 2022). These results have, potential, consequences for the development of future long-acting implants as they show possible effects of repeat exposure to antiretrovirals that may be used in such preparations and polymers of these antiretrovirals, and highlight the requirement for early assessment of biocompatibility, as highlighted elsewhere (Su et al., 2020).

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FROM BIOENGINEERING TO SURFACE MODIFICATION A CONCEPTUAL OVERVIEW OF LINKEROLOGY® METHODOLOGIES

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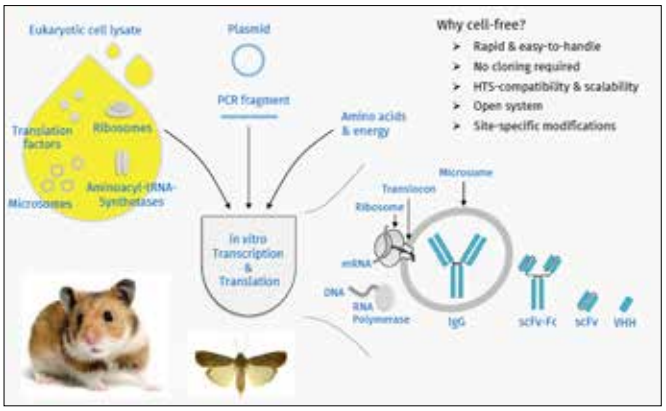
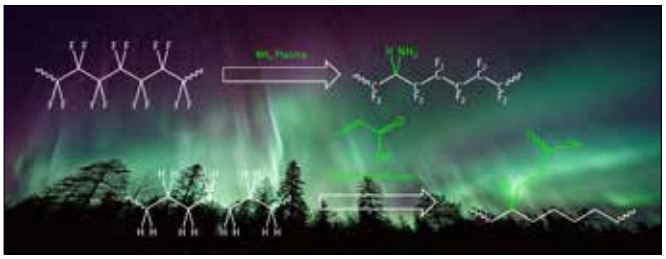


Conjugating highly potent small molecules to vastly target specific biomolecules (e.g. antibodies, single-chain, nanobodies) or other carriers has become a modern and sophisticated approach, particularly in the field of cancer therapy. As a result, the list of antibody-drug conjugates (ADCs) in clinics continues to grow. The choice of a linker for selective and site-specific control of payload release remains the major goal as premature release of a highly toxic payload would have fatal side-effects. Here we focus on preparing two

different classes of carriers for subsequent conjugation with the appropriate technology: (a) engineering biomolecules by cell-free synthesis and (b) treating plastic surfaces with plasma.
Cell-free synthesis (CFS) has attracted attention as a simple and controllable method for direct manipulation of protein expression to facilitate the synthesis of so far challenging or even inaccessible biomolecules, such as cytotoxic proteins, including site-specifically labeled proteins and protein-drug conjugates, or other complex membrane proteins. By using a lysate based on eukaryotic insect cells (Spodoptera frugiperda 21, Sf21), endogenous endoplasmic reticulum-derived structures (microsomes) are retained, enabling native-like protein maturation. The modular addition of protein-coding plasmids to the CFS allows a straightforward and defined study of protein assembly.
Plasma technology allows to equip inert polymers such as polyethylene (PE), polystyrene (PS), polytetrafluoroethylene (PTFE), or co-polymers thereof with functional groups like amine or carboxylate enabling further conjugations and applications.



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SELF-ASSEMBLING SUPRAMOLECULAR DENDRIMER NANOSYSTEMS AS POTENT ANTIBACTERIAL CANDIDATES AGAINST DRUG-RESISTANT BACTERIA AND BIOFILM.

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The swift rise and escalating widespread of multidrug-resistant bacterial pathogens demand the development of novel antibacterial agents that are highly effective, non-toxic, and do not induce resistance, while differing substantially from conventional antibiotics.[1] In light of this urgent need, amphiphilic dendrimers (Figure 1) have been emerging as innovative candidates as they can effectively combat bacterial AMR by emulating antimicrobial peptides, providing strong antibacterial effects while minimizing the risk of resistance development. [2]

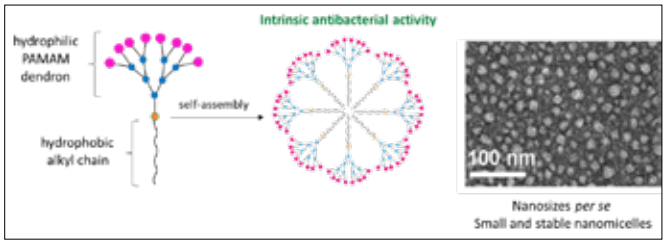

Figure 1. Supramolecular amphiphilic PAMAM dendrimers.

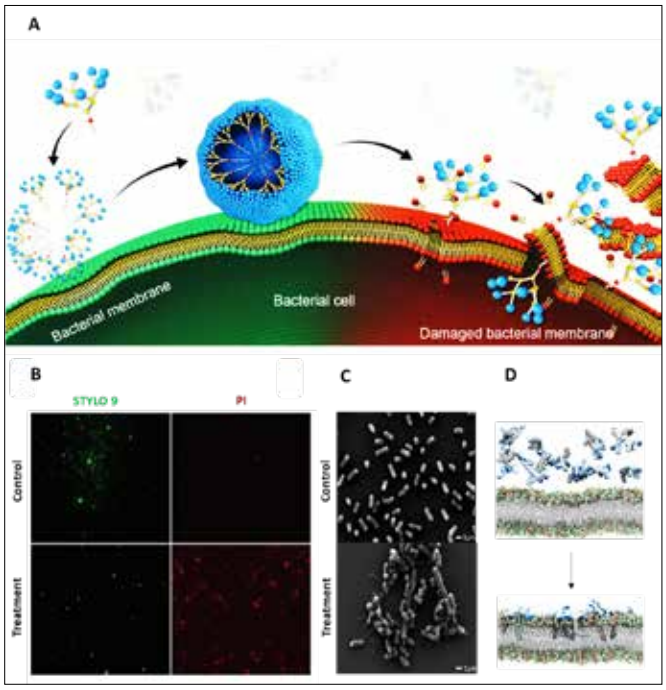

Figure 2.Membrane disruption effect of amphiphilic dendrimer with antibacterial activity; (A) Cartoon illustration of the antibacterial activity shown by amphiphilic dendrimer via membrane adsorption, self-assembling, interaction, insertion, disintegration and disruption; (B) Fluorescent microscopic imaging of live and dead cells upon treatment. Live and dead cells were stained using SYTO9 and propidium iodide respectively; (C) Scanning electron microscopic (SEM) images of bacterial membrane integrity and morphology upon treatment; (D) Simulating the interaction of the most active dendrimer with bacterial membrane using molecular dynamics (MD) simulations.

Through a structure/activity relationship analysis, different supramolecular dendrimers were designed, synthesized and evaluated for both Gram-positive and Gram-negative antibacterial activities as well as drug-resistant bacteria and biofilms eradication.[3] We provided compelling evidence showcasing the potential of a dendrimer carrying a long hydrophobic C_{18} alkyl chain and an amine-terminated poly(amidoamine) (PAMAM) dendron, effectively kill gram-negative and Gram-positive bacteria as well as multidrug-resistant bacterial strains and eradicate biofilm. This dendrimer demonstrated great antibacterial at a very low dose (MIC = 6.0 $\mu g.mL^{-1}$) without developing drug resistance, yet with a good safety and negligible cytotoxicity profile (SI over 30-fold). Notably, this dendrimer also demonstrated effective *in vivo* efficacy.[4] Mechanistic investigations revealed that this dendrimer targeted the membrane phospholipids phosphatidylglycerol of bacteria through membrane disruption (Figure 2A-D), leading to effective cell death. [4]
In conclusion, these small amphiphilic dendrimers could be used to specifically target bacterial membrane, offering a promising strategy to target drug-resistant bacterial pathogens in the view to addressing and leveraging the global antibiotic crisis. Because of their amphiphilic properties, these dendrimers can also be employed as

delivery systems[5] of and/or in combination with other antibacterial agents to achieve more targeted and effective treatment of drug-resistant bacterial infections.

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A MULTI-STAGE PULMONARY DRUG DELIVERY SYSTEM BASED ON SPOROPOLLENIN

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In this work, we have developed inhalable dry powder formulations from biomaterials such as chitosan or protamine, to improve the therapeutic effect of antibiotic treatment of lung disease by promoting local treatment of disease and reducing side effects. These polymeric nanocapsules (NCs) were prepared by solvent displacement method and were physico-chemically characterized and evaluated for their dissolution, permeability, stability, cytotoxicity, hemocompatibility, cell interaction and antimicrobial efficacy. Selected nanocarriers were also further incorporated into a microdelivery platform based on purified sporopollenin and the resulting microstructures were evaluated regarding their morphology, stability and aerodynamic characteristics.

Drug-loaded protamine and chitosan nanocapsules presented positive surface charge and a size range of 150 - 250 nm, with homogenous distribution and spherical chape. They were stable as suspension under storage, as well as in biological media and as a dry powder after lyophilization or spray drying (1). Nanocapsules showed a good safety profile and cellular uptake with no tolerogenic effect on macrophages, showed good compatibility with red blood cells and exhibited remarkable antibiotic efficacy in both RGM and SGM bacterial strains (2).

Chitosan and protamine nanocapsules were also successfully incorporated into a sporopollenin-based delivery platform to allow their pulmonary delivery (Figure 1.). This sporopollenin platform was obtained by the sequential purification of pollen grains, yielding highly porous, ultra-low-density microstructures with a microneedle-like morphology that is expected to promote interaction and enhance residence time upon mucosal administration (3).

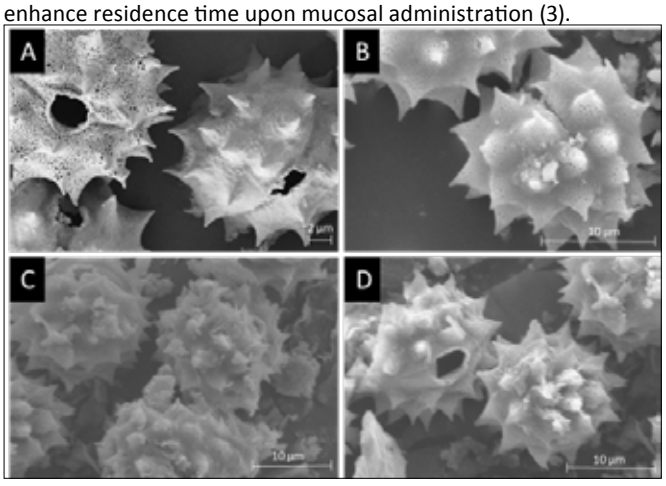

Figure 1. Scanning electron microscopy images of 5% rifabutin nanocapsules loaded into sporopollenin platforms (A, and B) and after their freeze-drying in the presence of excipients (C and D)

The resulting delivery system showed excellent physical and biological stability, high drug loading and the aerodynamic characterization indicated its potential for the pulmonary administration of the above developed rifabutin-loaded nanosystems.

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NONSPHERICAL MICROBUBBLES FOR ULTRASOUND-ASSISTED DRUG DELIVERY TO BRAIN

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INTRODUCTION

Microbubbles (MB) are 1-10 µm-sized gas-filled vesicles which are used for ultrasound (US) imaging and drug delivery applications. To enhance MB performance, several physicochemical features have been systematically optimized over the years, including size, surface chemistry and shell rigidity [1]. Shape is a feature that has thus far never been studied. All (pre)clinically used MB are spherical in shape. We here created rod-shaped MB and demonstrate that these non-spherical MB outperform spherical MB in multiple regards, including for *in vivo* blood-brain barrier (BBB) sonopermeation [2].

METHODS

Spherical MB were synthesized by anionic polymerization of n-butyl cyanoacrylate [3]. Rod-shaped MB were generated by stretching spherical MB unidirectionally above their glass transition temperature. We studied MB flow dynamics by assessing their propensity to marginate, i.e. move towards the vessel wall. We furthermore analysed MB acoustic properties, tumbling motion in flow, *in vitro* macrophage uptake, *in vivo* circulation kinetics, and *in vivo* active targeting to BBB endothelium (upon MB shell-functionalization with anti-transferrin receptor (CD71) antibodies). Finally, we evaluated the ability of targeted and untargeted spherical and non-spherical MB to permeate the BBB upon transcranial focused US, by visualizing and quantifying fluorescent model drug delivery to the brain.

RESULTS

Confocal and cryo-SEM microscopy confirmed the successful generation of non-spherical MB (Fig. 1A). Upon injecting MB in the presence of full blood into a straight microfluidic channel, we observed that rod-shaped MB flow closer to the vessel walls, and exhibit tumbling motion (Fig. 1B). We furthermore found that rod-shaped MB exhibit reduced macrophage uptake, contributing to longer *in*

vivo circulation times upon intravenous administration (Fig. 1C-D). Anti-CD71 antibody-targeted rod-shaped MB were found to bind more efficiently to blood vessels in the brain than their spherical counterparts (Fig. 1E). Exploiting all of the above features together, we finally demonstrated that non-spherical MB were significantly more effective than spherical MB in focused US-induced BBB opening and drug delivery to the brain (Fig. 1F).

CONCLUSIONS

We discovered that non-spherical MB can be stably generated. Rod-shaped MB outperform spherical MB, presenting with enhanced margination, reduced phagocytosis, improved circulation time, enhanced vascular binding, and increased BBB sonopermeation. Our efforts identify shape as a novel design parameter in the MB landscape, and they provide a framework for exploring the use of non-spherical MB for US-enhanced drug delivery and imaging applications.

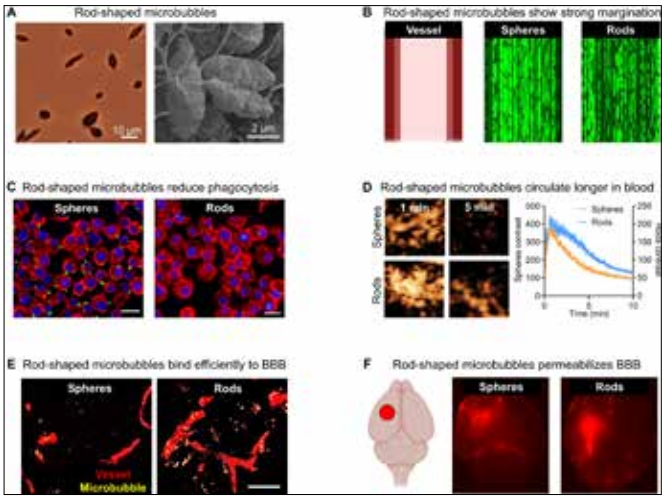

Figure 1. Generation and application of non-spherical microbubbles. *(A) Confocal and cryo-SEM confirm the formation of rod-shaped MB. (B) In vessel-like flow chambers, rod-shaped MB showed a higher propensity to marginate. (C) Upon incubating MB with macrophages, rod-shaped MB showed less phagocyte uptake. (D) Intravenous administration and US imaging demonstrated that rod-shaped MB have prolonged circulation times. (E) Ex vivo imaging of a brain section demonstrating that antibody-targeted rod-shaped MB strongly bind to the BBB. (F) Upon transcranial FUS, rod-shaped MB more efficiently permeate the BBB than spherical MB, thereby enhancing model drug delivery to the brain.*

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IL-12 DELIVERY THROUGH IMMUNOSTIMULATORY NANOPARTICLES ENHANCES INFLAMMATORY RESPONSE FOR GLIOBLASTOMA TREATMENT

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In the last decade, interleukin 12 (IL-12) has been studied as one of the most potent cytokines for anti-cancer immunotherapy because it stimulates the interferon-g production, decreases the angiogenesis and changes the cancer microenvironment from one that contains TH0 and M2-type phenotype macrophages to one richer in TH1 cells and inflammatory M1-type macrophages (1-2). However, the anticancer cytokine IL-12 cannot be used as a systemic cancer treatment due to its excessive toxicity, instability and short half-life (3). In glioblastoma, studies have showed that intracavitary levels of IL-12 are lower compared to anti-inflammatory cytokines (e.g. IL-8) or VEGF, which clearly make room for its delivery and to exploit its therapeutic effect (4). In this study, IL-12 was formulated and encapsulated into polymeric nanoparticles and intratumorally delivered to target TAMs and modulate the TAM landscape.

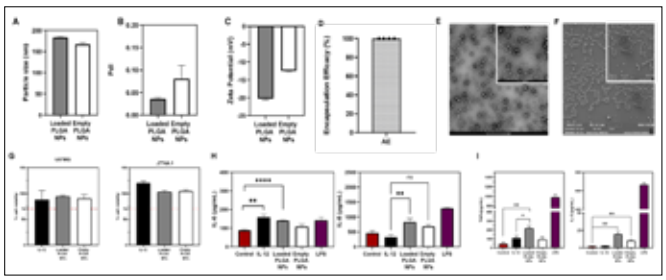
MATERIALS AND METHODS

Immunostimulatory nanoparticles were produced by double-emulsion technique and IL-12 was encapsulation. Nanoparticle size, PdI and Zeta Potential were obtained with DLS, while nanoparticle morphology was observed with SEM and TEM. In vitro assays were done with Glioblastoma cancer cells (U87) and murine macrophages (J774A.1). For the immunomodulation evaluation, ELISA, qPCR and FACS were used.

RESULTS AND DISCUSSION

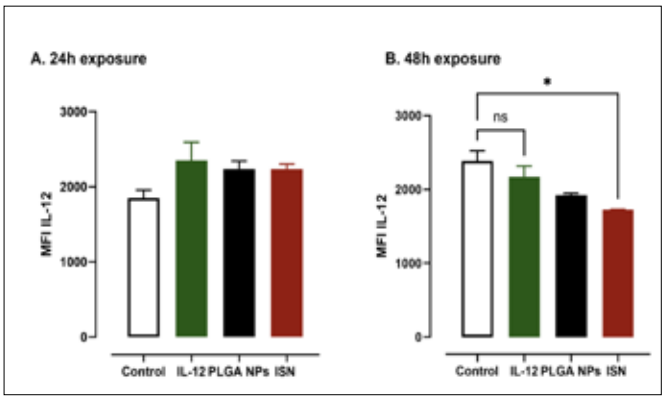
Immunostimulatory nanoparticle vaccine has an average particle size around 180 nm, a PdI of 0.05 and a negative zeta potential (-20 mV). The encapsulation of IL-12 was obtained successfully (around 98%). We studied the cell toxicity via LDH and *in vitro* data also show that the nanoparticle vaccine was not toxic for GBM cancer cells (U87MG cell line) or mouse macrophages (J774A.1 cell line). Importantly, presence of the nanoparticles simulated the production of pro-inflammatory cytokines (IL-6, IL-8) in GBM cancer cells and macrophages (TNF-α and IL-6), suggesting possible modulation of the TAM landscape. The same behavior was also observed by mRNA expression measured by RT-PCR for the cytokines IL-8 and IL-6, where PLGA nanoparticle vaccine increased the production of pro-inflammatory cytokines.

Figure 1. Preliminary results of PLGA nanoparticle vaccine containing IL-12. *Size distribution of nanoparticle vaccine were analyzed by (A) particle size, (B) PdI, (C) zeta potential. (D) Encapsulation efficacy of IL-12 within the nanoparticle vaccine. Morphology of the nanoparticles were analyzed by (E) transmission electron microscopy (TEM) and (F) scanning electron microscopy (SEM). Scale bar: 2 µm. Cell viability of U87MG (GBM cancer cell line) and J774A.1 (mouse macrophage cell line) after 24h of incubation with nanoparticle vaccine was evaluated by LDH (G). ELISA analysis of IL-6 and IL-8 production in culture supernatants of human U87MG with the indicated treatments. (I) ELISA analysis of TNF-α and IL-6 production in culture supernatants of J774A.1 macrophages with the indicated treatments. All data are presented as means ± SEM. Graphs represent pooled data from at least three independent experiments.*



Finally, we showed that ISN could modulate the intracellular levels of pro-inflammatory cytokines to allow an inflammation boost effectively. **Figure 2** showed a decrease in the intracellular levels of IL-12 upon ISN exposure to GBM cancer cells underlying downregulation of IL-12 secretion. These results open the door for deep fundamental research regarding the molecular mechanisms underlying GBM cytokine dynamics.

Figure 2. Intracellular delivery of IL-12 through PLGA nanoparticles after (A) 24h and (B) 48h of exposure. Flow cytometry analysis of U87 cell line for intracellular IL-12 expression. Representative mean fluorescence intensity (MFI) values for PE conjugated anti-human IL-12 antibody.



CONCLUSION

These data indicate that PLGA nanoparticle cointaining IL-12 is an effective drug delivery platform for glioblastoma treatment. To compare the interaction between GBM cancer cells and macrophages, *in vivo* work will be done further.

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DEVELOPING BIOMIMETIC NANOPARTICLES AS DRUG DELIVERY SYSTEM IN ACUTE MYELOID LEUKEMIA (AML)

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INTRODUCTION

In patients with acute myeloid leukemia (AML), despite treatment with intensive chemotherapy, relapse occurs in the majority of cases, often accompanied by a fatal outcome. It has become clear that a subset of cells, termed leukemic stem cells (LSCs), are notoriously therapy resistant leading to tumor relapse. Thus, novel strategies are needed that specifically target these resistant LSCs.

It is believed that one possible reason for the capacity of these cells to resist chemotherapy is their association with cells in the so-called "niche" in the bone marrow. Inside the niche in the bone marrow, LSCs communicate with mesenchymal stromal cells (MSCs) via secreted exosomes, direct cell-cell interactions and secreted factors. Normal hematopoietic stem cells critically depend on these niche interactions to control self-renewal, differentiation and stem cell quiescence. Within this context, we aim to develop biomimetic nanoparticles created using cell membranes extracted from MSCs and leukemia cells in order to deliver drugs to resistant cell populations.

EXPERIMENTAL METHODS

As a first step, procedures for cell membrane extraction using nitrogen cavitation have been optimized to establish a detergent free protocol for the generation of plasma membrane. Then, the extracted cell membranes are mixed with synthetic lipids which resemble the cell membrane composition and after freeze and thaw cycles, biomimetic nanoparticles are obtained by extrusion. These are characterized using dynamic light scattering and zeta potential measurements. Atomic force microscopy and proteomics are used to confirm the presence of proteins in the bilayer and study the mechanical properties of the bio-mimetic nanoparticles.

Next, the behavior of leukemia-derived bio-mimetic nanoparticles is investigated on both leukemia cells and mesenchymal stromal cells. Using flow cytometry, their association and uptake are quantified and compared to that of liposomes of the same composition without cell membrane components. Furthermore, the effect of membrane purity on the cell behavior of the resulting nanoparticles is also investigated.

RESULTS AND DISCUSSION

We show that the optimized method of membrane extraction using nitrogen cavitation and purification yields reproducible high purity membrane vesicles. Liposomes and bio-mimetic nanoparticles of similar size and charge can be reproducibly prepared **(Figure 1a)**. Flow cytometry results show that leukemia cells take up more bio-mimetic nanoparticles than control liposomes, suggesting that the insertion of membrane proteins promotes interaction with these cells (Fig.1b).

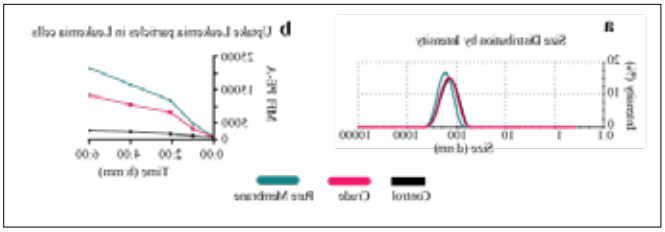

Figure 1. *a) DLS data of liposomes made without proteins, with a crude protein extract or a purified one. b) Kinetics of uptake of Leukemic bio-mimetic nanoparticles in leukemic cells.*

Atomic force microscopy confirms that the bio-mimetic nanoparticles are less susceptible to deformation, indicating that they are more rigid than the liposomes without membrane extract. The different mechanical properties are likely due to the incorporation of membrane lipids and proteins in the bilayer.

CONCLUSION

We have optimized a reproducible method for the preparation of high purity plasma membrane derived bio-mimetic nanoparticles. Our method does not use detergents and reduces the risks of chemical, mechanical or enzymatic degradation of the proteins in the membrane extract, which could interfere with the downstream analysis and evaluation of the generated nanoparticles. The method can be easily optimized and modified for other cells and tissues. The incorporation of cell membrane components changes the nanoparticle mechanical properties comparing to liposomes and the obtained biomimetic nanoparticles exhibit an increased association with leukemia cells.

INTERACTION OF ANTI-PEG ANTIBODIES WITH PEG

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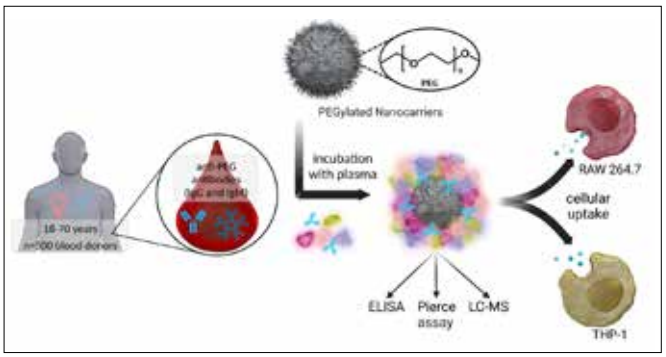
Nanocarriers (NCs) are a promising approach for efficient and targeted drug delivery. One major challenge, however, is to ensure sufficient circulation time of the NCs in the blood. Once the NCs enter the bloodstream, proteins accumulate on the surface and form a so-called protein corona, which determines the further course of the NCs.[1-2] Attachment of poly(ethylene glycol) (PEG) is the gold standard to reduce unspecific protein adsorption and prolong circulation time in the body. The polymer provides a stealth effect so that NCs are less recognized by the immune system and can circulate longer.[3-4]

However, in recent years, antibodies specifically targeting PEG have been identified.[5-6] An increasing prevalence of these anti-PEG antibodies has been described, and their presence has been correlated with reduced efficacy and, in some cases, severe allergic reactions to PEGylated therapeutics.[7-8] A high concentration of anti-PEG antibodies in the blood may affect the formation of the protein corona of NCs and thus their interaction with cells.

Nevertheless, the presence of anti-PEG antibodies in the protein corona and their effect on the cellular uptake of PEGylated NCs has not yet been investigated.

Therefore, we investigated pre-existing anti-PEG antibodies in healthy subjects, their accumulation in the protein corona of PEGylated nanocarriers, and their effects on cellular uptake behavior. Our results show a high concentration and prevalence of anti-PEG antibodies in the German population. Overall, 83% of 500 blood samples tested positive for anti-PEG IgG or IgM antibodies. PEGylated nanocarriers showed a higher concentration of anti-PEG antibodies in the protein corona compared to non-PEGylated ones. The increased anti-PEG antibody concentration in the protein corona lead to higher uptake of the nanocarriers in macrophages. Consequently, the anti-PEG antibodies in the protein corona could mitigate the stealth effect of PEG, leading to faster excretion from the bloodstream and undesirable side effects.

Figure 1: Overview of the experiment design: 1) anti-PEG antibody (IgG + IgM) plasma screening among 500 healthy individuals using ELISA. 2) Determination of anti-PEG IgG enrichment in the protein corona of PEGylated nanocarriers. 3) Monitoring the impact on cell interactions in RAW 264.7 (mouse) and THP-1 (human) macrophages depending on IgG presence in the protein corona.



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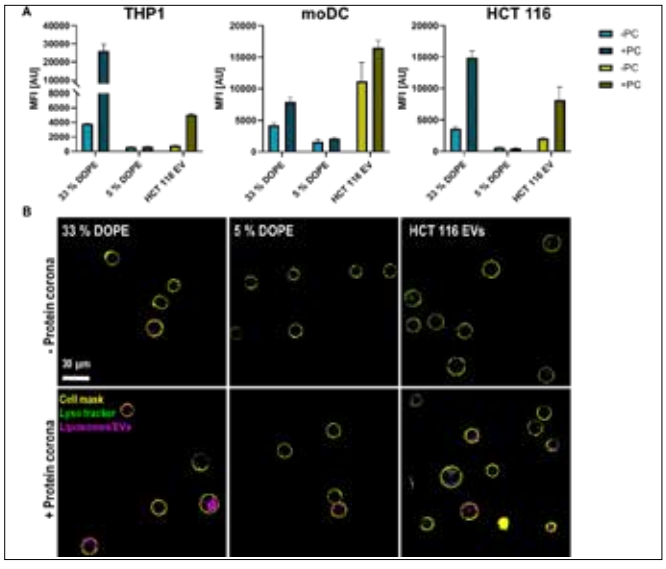
FORMING OF A PROTEIN CORONA ON EXTRACELLULAR VESICLES INCREASES UPTAKE INTO IMMUNE CELLS

LAURA DIETZ

Extracellular vesicles (EV) have attracted much attention as novel nanotherapeutic recently and first clinical trials are ongoing. Similar to synthetic nanotherapeutics, EVs acquire a protein corona upon contact with biological fluids that likely influences their biodistribution, cell targeting and in consequence therapeutic efficacy. Unlike for synthetic nanotherapeutics, little is known about the influence of the EVs' protein corona on all these processes. Therefore, we aimed to compare the influence of a protein corona on EVs directly to the protein corona on engineered liposomes.

First, we analyze the influence of the protein corona on EV uptake into human monocytes and compare it with the influence on the uptake of engineered liposomes. In Figure 1, we show that the adsorption of a protein corona increases the uptake of HCT 116-derived EVs in human monocytes and HCT 116 cells.

Figure 1: Uptake of liposomes and EVs with and without protein corona. (A) Flow cytometric analyses of particle uptake into THP1 cells, moDCs and HCT 116 cells after 16h. To account for the particle loss during protein corona preparation, MFI values were calibrated according to fluorescence measurements of the sample with and without protein corona. Means and standard deviations of median fluorescence intensities are shown (n=3). (B) Confocal laser scanning microscopy images of THP1 cells incubated with liposomes or EVs in presence (lower) and absence (upper) of a protein corona for 16 h. To account for the particle loss during protein corona preparation, cells were incubated with different dilutions of particle solutions calculated according to fluorescence measurements of the sample with and without protein corona.



Further, we use a proteomic approach in order to analyze the protein composition of the EVs themselves and the protein composition of a human blood plasma protein corona around EVs (Figure 2). The increased uptake of EVs in presence of a protein corona can be attributed to the presence of complement system proteins in the protein corona. Our results demonstrate the relevance of the protein corona for EV uptake, which will aid their use in therapeutic applications.

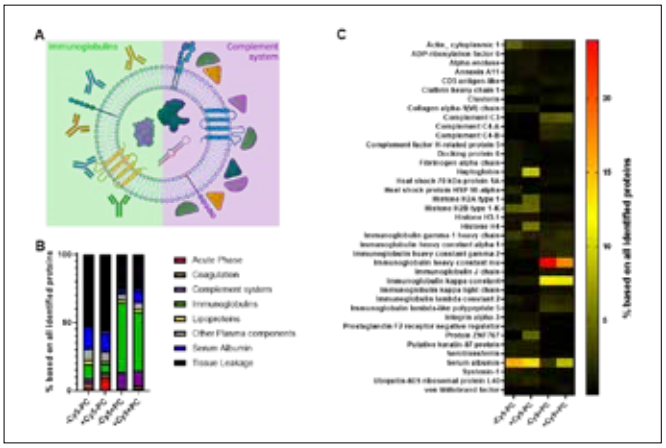


Figure 2: Proteomic analysis of EV and protein corona composition. A Graphic depiction of enrichment of immunoglobulins and complement system proteins in EV protein corona. B Assignment of proteins to different biofunctional classes. C LC-MS revealed the top 20 hard protein corona proteins of EVs with and without Cy5-labeling.

PEG LIPID ISOMERIZATION AS A SELECTIVE TOOL AGAINST ANTI-PEG ANTIBODY RECOGNITION IN LIPID NANOPARTICLES

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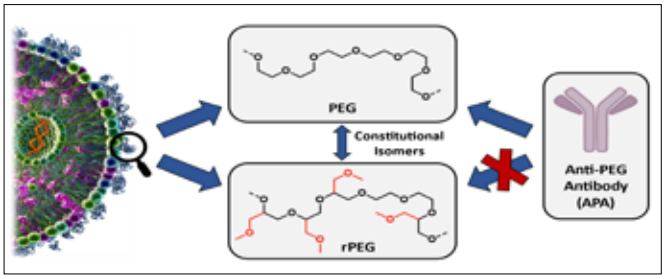

In the last decades, poly(ethylene glycol) (PEG) has been established as the most relevant pharmaceutical polymer in modern nanomedicine.[1] Its attachment, so-called PEGylation, to active pharmaceutical ingredients (API) or the surface of lipid nanoparticles (LNP) facilitates efficient water solubility, increases blood-circulation time (stealth effect) and inhibits aggregation of particles. As an example, PEG lipids are essential stabilizing excipients in LNP formulations to allow for efficient transport and transfection of nucleic acids. Despite these advantages, an increasing number of studies has led to concerns related to the presence of anti-PEG antibodies (APA) in a constantly growing part of the population. The presence of APAs results in the recognition and accelerated blood clearance of PEGylated therapeutics, diminishing the desired effect of PEGylation. This can even lead to anaphylactic shocks in severe cases.[2] As a result, various potential alternatives based on different polymer classes or proteins were investigated as substituents for PEG in the last couple of years.[3] We present isomerization of PEG as an efficient approach to inhibit APA interaction while preserving PEG´s main advantages and structure. Constitutional isomers of PEG (rPEG) were obtained via living anionic ring-opening (co)polymerization (AROP), offering well-defined structures with tailorable composition profile. Chain end functionalization of rPEGs with lipid anchor groups was conducted to obtain rPEG lipid structures. They were investigated via enzyme-linked immunosorbent assay regarding their interaction with APAs, leading to a detailed picture of the correlation between chain architecture and APA interaction. Further, formulation of various rPEG lipids in LNPs were investigated and compared to established PEG-based LNPs, showing similar particle sizes, transfection efficiencies and cell viabilities *in vitro*. In summary, the presented approach aims at the preservation of the highly efficient and seminal PEG-based nanomedicine for present and following generations.

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PEG ALTERNATIVES BASED ON BIOINSPIRED POLYMERS WITH SHIELDING PROPERTIES AS LIPID NANOPARTICLE (LNP) COMPONENTS.

AROA DURO, R. Miravet-Martí, S. Alonso, I. García, P. Martínez, G. Sogorb, L. Herrera, J. García, S. Esteban, V. J. Nebot

Polyethylene glycol (PEG) is a well-known polymer to enhance solubility and biocompatibility of APIs and nanomedicines in general acting as shielding to reduce immunogenicity and enhance blood circulation times [1]. Particularly, PEG-lipid conjugates are commonly applied in the formulation of the most successful nucleic acid delivery systems: lipid nanoparticles (LNPs) [2]. Indeed, PEG-shielded LNPs have positioned as key non-viral vectors for gene delivery with BioNTech/Pfizer and Moderna/ /NIAID mRNA COVID-19 vaccines leading the way [3,4]. However, the extensive use of PEG in marketed products has raised concerns related to immunogenicity and loss of efficiency of PEG-containing medicines due to the production of anti-PEG antibodies which clear PEGylated excipients from the bloodstream (Accelerated Blood Clearance, ABC) [5] . This is aggravated by the exposure of the population to PEG by the worldwide vaccination campaign against COVID, what has pushed the interest in PEG-free alternatives by pharma Industries.

To overcome this, at Curapath we work with a portfolio of proprietary bioinspired alternatives to PEG based on polypeptides and polypeptoids. Examples are the conjugate PSar-alpha tocopherol conjugate or diol modified PGA-lipid conjugates that have analogue solubility, macromolecular properties and interactions with water compared to PEG, with a wide and versatile terminus group functionality for (bio)conjugation [6]. Our proprietary PEG alternatives have been produced and scaled up in technical batches with demonstrated batch-to-batch reproducibility and QC compliance. We have investigated the advantages of their use in shielding strategies for LNP development. Stable formulations with adequate encapsulation efficiencies, suitable physic-chemical properties and good reproducibility are obtained when tuning the % of lipid-shielding. Our PEG-free alternatives when formulated in LNPs have shown superior transfection efficiency in immortalized and primary cell

cultures, lower immunogenic profile and comparable *in vivo* transfection to that of Benchmark PEG-containing formulations. These results make these PEG-free alternatives ideal shielding component for the delivery of biologics and nucleic acids. Envisaging what is ahead in the current nucleic acid delivery landscape, Curapath has engineered a library of different PEG replacement alternatives with the aim to not only overcome PEG drawbacks but to confer different surface features to help build the next generation LNPs.

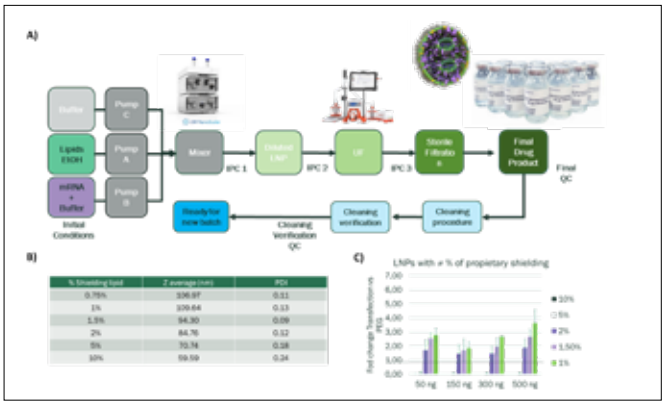

Figure 1. A) Overview of the formulation process. B) Examples of LNP formulations with size and PDI. C) In vitro transfection of LNPs with different % of proprietary shielding lipids.

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POLYMERIC NANOPARTICLES: HIGH THROUGHPUT SCREENING FOR FINDING THE RIGHT POLYMER FOR THE REQUIRED GENETIC MATERIAL

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Gene therapy has emerged as a versatile technique with the potential to treat a wide range of human diseases [1]. The vector used to carry genetic material across tissue-, organ-, and cell-associated barriers is a critically important component of this therapeutic modality. The transport and delivery of genetic material to a desired site of action remains a challenging prospect, with problems primarily relating to the physicochemical features of genetic material (i.e., hydrophilicity, high negative charge, and instability). The chosen vector must protect the genetic payload and enhance delivery to the desired subcellular site of action. Vectors must be chosen/designed to overcome various intracellular and extracellular barriers of differing nature and complexity, depending on the specific disease/disorder and the preferred administration route.

In contrast to viral vectors, polymeric non-viral vectors (NVVs) represent highly versatile and customizable chemical entities that effectively protect the genetic material and ensure efficient delivery [2]. Polymer based NVVs can deliver a wide range of nucleic acid cargo sizes and possess both flexible manufacturing processes and safe toxicity profiles. Under certain conditions, electrostatic interaction of polymeric NVVs and nucleic acids results in the formation of the so-called polyplexes that are well-defined solid-like colloidal polymeric nanoparticles (PNPs) [3].

Selecting the right vector families that best fits a nucleic acid cargo is complex, since each cargo has its own peculiarities. Thus, the Curapath product development team implemented a fit-for-all synthetic approach capable of producing more than 100 different polymers and ensured their quality by developing the necessary analytical methods, including purity, Mw, PDI, water content, counterion, and pKa, within 6 months. Once the polymers have been developed, an efficient, biodegradable polymeric nanoparticle is required. In order to reach these characteristics, several parameters must be taken into account. These parameters require of a scalable synthetic approach and extensive analytical development for the vector characterisation.

In order to achieve this challenging process, Curapath team has implemented some steps:

1. Optimisation every synthetic step including polymerisation of polypeptide backbone to be used as starting material, conjugation, and post- polymerisation modifications.
2. Optimise and standardise the PNP formulation set-up, composition, and protocol to achieve an acceptable target product profile in terms of physicochemical features and stability.
3. Evaluate the biological performance and provide feedback to the product development and formulation teams to create a structure-activity relationship study to identify the best NVV lead candidate.
4. Implement specific in-process controls for each synthetic stage both at drug substance and drug product stages to ensure quality throughout the process.
5. Develop analytical methods for final product release and stability descriptors.

The formulation team performed a high-throughput formulation procedure and essential analytical methods to assess the physicochemical parameters to ensure the suitability of the polyplexes for the indication. The parameters chosen were hydrodynamic diameter, PDI, API concentration, the needs of speeding up the process to have the highest range of information ad characterisation led to the use of Stunner. Stunner platform is a bult-in system that pulls together UV/Vis concentration and Dynamic Light Scattering (DLS) speeding up the process allowing to screen up to 96 samples in just 2 hours. Thanks to Stunner, Curapath monitored to achieve the best relation polymer/ API ratio (N/P ratio) and different excipients regarding to the previously defined physicochemical parameters. Apart of the aforementioned parameters, Curapath team implemented a cell culture process for *in vitro* functional and toxicity testing in order to screen the best candidates (Figure 1) [4].

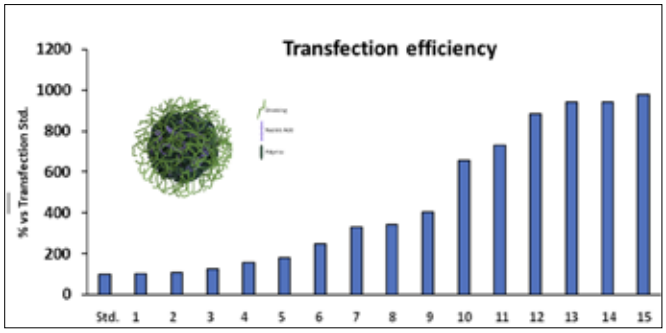

Figure 1. Transfection efficiency evolution of selected candidates related to transfection standard employing patient derived primary cell culture.

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MEASURING LIPID ORDER TO ASSESS CELL MEMBRANE PERMEABILITY, LIPID NANOPARTICLE STABILITY AND MEMBRANE DRUG INTERACTION

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INTRODUCTION

The state of synthetic and biological lipid systems can be characterized by the order of its lipid molecules. This phase state correlates with physical properties of the lipid system: For example, low lipid order goes along with low lipid packing density, low bending stiffness, high fluidity, high compressibility and vice versa.
Our aim is to exploit the dependency of application relevant properties such as cell membrane permeability, lipid nanoparticle (LNP) stability and membrane drug interaction on the lipid order.

MATERIALS AND METHODS

We use membrane embedded dyes such as Laurdan that are sensitive to the surrounding lipid order state and are incorporated either during the preparation of synthetic systems or in case of biological samples by dissolving them into the buffer using a solvent. The lipid order is quantified by measuring the fluorescent emission spectra and calculating the generalized polarization (GP) [1,2] our findings help to understand and predict cell membrane properties under physiological conditions as they explain the underlying physics of a broad order-disorder phase transition. Therefore, we analyzed the membranes of various cell lines, red blood cell ghosts and lipid vesicles by spectral decomposition in a custom-made setup in a temperature range from −40 °C to +90 °C. While the generalized polarization as a measure for membrane order of artificial lipid membranes like phosphatidylcholine show sharp transitions as known from calorimetry measurements, living cells in a physiological temperature range do only show linear changes. However, extending the temperature range shows the existence of broad transitions and their sensitivity to cholesterol content, pH and anaesthetic. Moreover, adaptation to culture conditions like decreased temperature and morphological changes like detachment of adherent cells or dendrite growth are accompanied by changes in membrane order as well. The observed changes of the generalized polarization are equivalent to temperature changes dT in the range of +12 K < dT < -6 K.”,”author”:[{“dropping-particle”:””,”family”:”Färber”,”given”:”Nicolas”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Westerhausen”,”given”:”Christoph”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”container-title”:”Biochimica et Biophysica Acta (BBA.

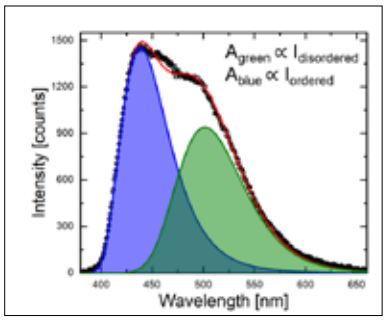

Figure 1 Left: Fluorescnce intensity of membrane-embedded Laurdan as funtion of wavelength. The blue part of the spectrum corresponds to lipids in the ordered state. The intensities in the green region arise from lipids in the disordered state. Right: The Generalized Polarization (GP) as measure for the membrane phase state.

Furthermore, cell membrane permeability is quantified by the uptake of a fluorescent dye into the cytosol while simultaneously measuring GP using fluorescence microscopy [3].
The change of lipid order in LNP suspensions is determined by determination of GP as function of time at different temperatures and serves as measure for LNP stability.
The interaction of cell membranes and drugs is evaluated by measuring GP after short and long-time drug exposure.

RESULTS AND DISCUSSION

First, we found that the permeability of cellular membranes is directly related to the plasma membrane order determined by GP measurement. This finding can facilitate the optimization of permeabilization and transfection protocols .

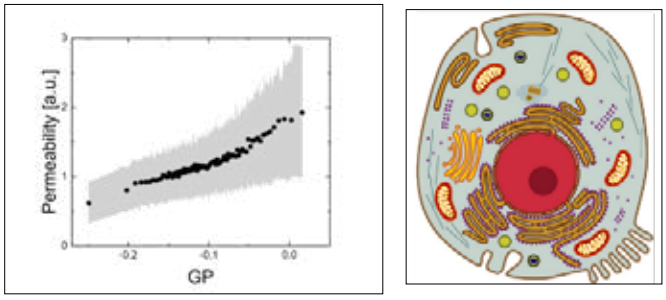

Figure 2 Cell membrane permeability of HeLa cells measured by the uptake of a fluorescent probe as function of the membrane phase state. The latter is determined by the fluorescent analysis of membrane-embedded Laurdan and quantified by the Generalized Polarization (GP).

Second, irreversible structural changes within LNPs can be determined measuring GP as function of time or temperature demonstrating that LNP stability can be optically assessed in situ under varying conditions even in the frozen state far below 0°C..

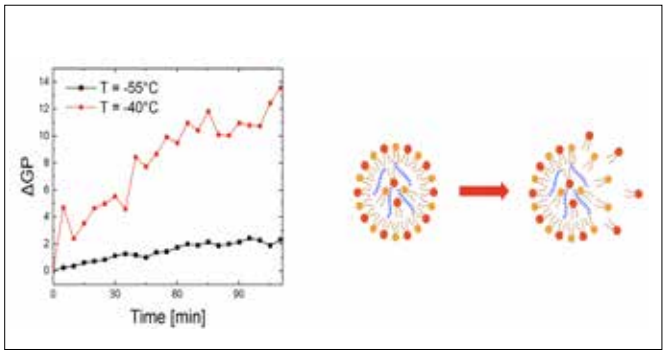

Figure 3 The lipid order of lipid nanoparticles (LNPs) quantified by the Generalized Polarization (GP) as function of time at two different temperatures. The change of GP indicates temperature dependent irreversible structural changes within the LNPs

Third, lipid phase transitions within cellular membranes are strongly influenced by short and long-time exposure of the drug tamoxifen. These results open up a new perspective on the mode of action and long-time adaptation effects of membrane targeted drugs

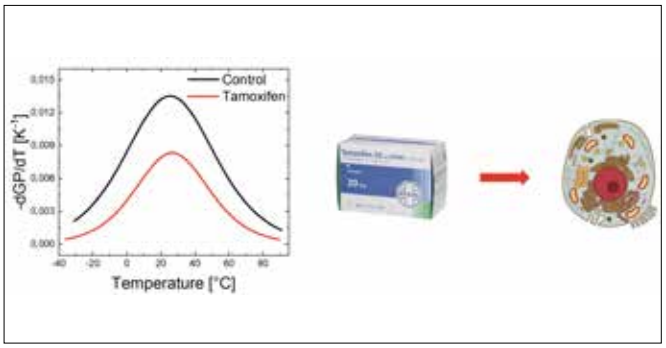

Figure 4 The derivative of the Generalized Polarization (GP) with respect to temperature indicates structural phase transitions within the membranes of HeLa cells. The sensitivity of these phase transitions to the exposure to the anti cancer drug tamoxifen shows that the working mechanism of the drug include a structural rearrangement of the membrane.

CONCLUSION

These observations might inspire researchers across different disciplines to include lipid order measurements in their studies. For this we provide detailed insight into the measurement procedure and introduce a custom-made device that facilitates this kind of studies.

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CATIONIC POLYMER NANOGELS FOR NUCLEIC ACID DELIVERY

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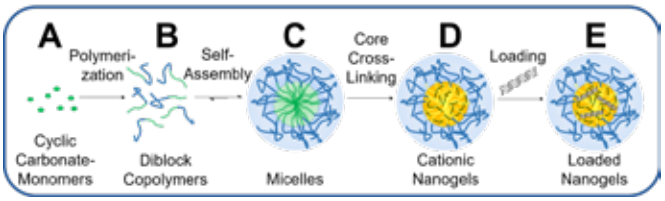
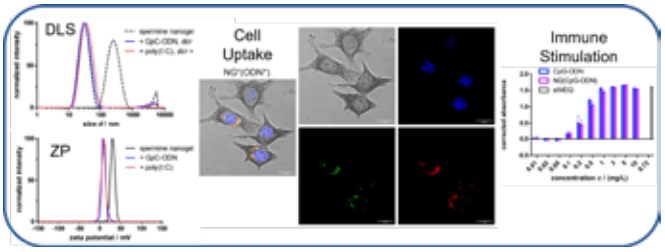


Degradable, cationic, core cross-linked, fully hydrophilic nanoparticles (nanogels) made of synthetic diblock copolymers consisting of a polyethylene glycol (PEG) and an aliphatic polycarbonate block can be loaded with various nucleic acids (RNA, DNA). Among these the class of immune stimulatory nucleic acids such as the Toll-like receptor (TLR) agonists CpG-ODN (TLR9) and Poly-(I:C) (TLR3) is of special interest. The transport system aims to alter biodistribution, lower toxicity, increase bioavailability and control activity of the nucleic acids at the target location with the final goal being an application as an adjuvant for cancer immunotherapy. The loaded nanogels can be synthesized using a versatile multi-step procedure (see top figure). They exhibit small defined sizes even upon loading with therapeutic cargo. The empty nanogels show positive surface charge which decreases substantially when nucleic acids are loaded. The nanogels are readily uptaken by cells, have no relevant toxicity and completely retain the immune stimulatory activity of their cargo (see bottom figure). We have developed a promising platform for nucleic acid delivery which is undergoing further chemical optimization to allow for specific targeting, cargo co-delivery, enhanced degradability and synergistic therapy.



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EVOLVING POLYETHYLENE GLYCOL (PEG): ISOMERIZATION OF PEG SUPPRESSES IMMUNE RECOGNITION

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Poly(ethylene glycol) (PEG) represents an essential building block for modern nanomedical applications, ranging from the treatment of chronic diseases to cancer therapy.[1] Nevertheless, reports regarding increasing prevalence of anti-PEG antibodies and related severe immune reactions raise growing concerns regarding the efficiency and safety of PEGylated therapeutics.[2–4] Given the importance of PEGylation, especially for modern nanomedicine, the occurring immunogenicity of PEG urges for alternatives to PEG in medical applications.
In recent years, different polymer classes besides polyethers have been investigated as potential alternatives for PEG, e.g., polyoxazolines or polysarcosine. As an alternative approach, in this work we aimed at preserving the underlying polyether class, while merely altering the polymer architecture *via* isomerization. Constitutional isomers of PEG (rPEGs) were synthesized based on the established synthesis technique for pharma-grade PEG, by the anionic ring-opening copolymerization of ethylene oxide (EO) with glycidyl methyl ether (GME). Well-defined, ideally random rPEGs with tuneable GME content and chain lengths as well as low dispersities (Đ= 1.06) were obtained. rPEGs show similar hydrophilicity to PEG, while retaining low toxicity and no immunogenicity. Anti-PEG ELISA with backbone- and endgroup-specific antibodies confirmed a suppression and for some copolymers complete elimination of antibody recognition with increasing GME content in the chains.

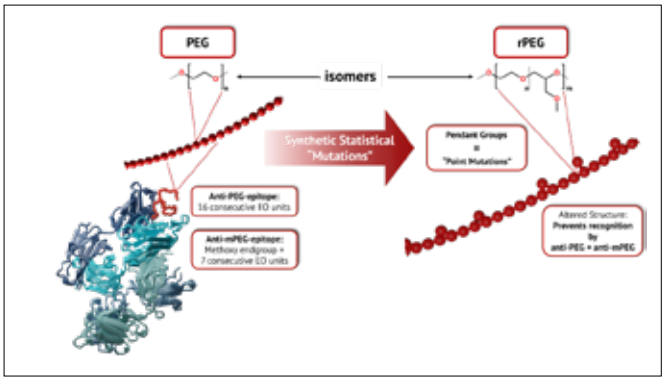

Figure 1: *Schematic of the working principle of rPEGs with randomly incorporated isomeric side chains along the PEG-backbone. These side chains act as synthetic "mutations" of the linear and highly regular polyether backbone, preventing recognition of the polymer as an epitope of anti-PEG antibodies.*[5,6]

The incorporated GME units introduce small pendant groups with high polarity along the otherwise linear polymer backbone which may be considered as "synthetic statistical point mutations" of the highly regular PEG structure. This alters the epitope of rPEGs beyond recognition by anti-PEG as well as anti-mPEG antibodies, while the beneficial properties of PEG such as high hydrophilicity and excellent biocompatibility are preserved.

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DENDRIMER NANOSYSTEMS HIJACK TUMOR-SECRETED EXTRACELLULAR VESICLE FOR SIRNA DELIVERY

CHRISTINA GALANAKOU

Nucleic acid therapeutics, if delivered effectively, hold great promise for precision medicine in cancer management [1,2]. However, nucleic acid delivery systems that can overcome tumor heterogeneity and evolutive nature while achieving deep tumor penetration are challenging to develop yet in high demand. We present here a delivery system based on self-assembling dendrimer nanomicelles for effective siRNA delivery **(Fig.1)** [3,4] via in situ tumor-secreted extracellular vehicles (EVs), an endogenous transport system that evolves with tumor microenvironment [5].

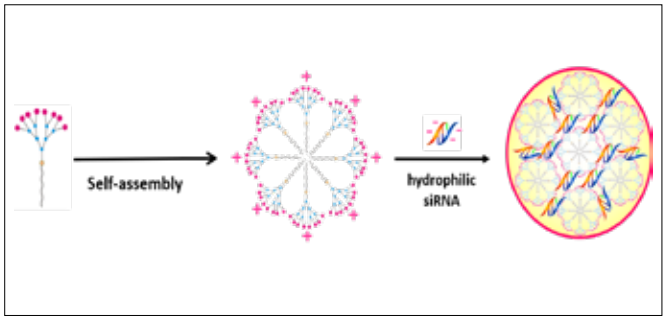

Fig.1. *Positively charged amphiphilic dendrimers self-assemble into nanomicelles and interact with negatively charged siRNA to create dendrimer/siRNA complex.*

These dendrimer nanosystems can prevent siRNA from degradation and promote cellular uptake. Notably, following cellular uptake, these dendrimer nanomicelles have their nucleic acid payload repackaged by the cells into EVs which are further transported and internalized by other cells to propagate delivery. By exploiting the intrinsic features of tumors alongside dendrimer supramolecular chemistry, we can develop smart and effective siRNA delivery system to overcome nucleic acid innate flaws, tumor heterogeneity and dynamic evolution thereby improving cancer therapy.

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VIRUS MIMICKING POLYSACCHARIDE NANOCOMPLEX WITH MACROPHAGE TARGETING CAPABILITY FOR POTENT GENE SILENCING

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INTRODUCTION:

Gene interference (RNAi) represents the next-generation treatment strategy for a myriad of indications. As a result of their advantages in biosafety, non-immunogenicity and economic feasibility, non-viral synthetic nanoparticles (NPs) have drawn attention as vectors to deliver RNAi molecules, including small interfering RNAs (siRNAs). In general, an ideal targeted siRNA delivery system should hold the following features: (i) efficient siRNA encapsulation efficacy to prevent its degradation under blood circulation; (ii) precise targeting properties to avoid off-target gene silencing; and (iii) efficient endosome escape capability to allow the siRNA reaching the cytosol. To fulfill these requirements, the development of targeted siRNA delivery nanosystem with maximally simplified synthetic scheme is desired. Herein, a virus-mimicking polysaccharide nanocomplex was developed which showed membrane destabilization behavior and macrophage targeting capability. Significant enhanced accumulation level of EEPG nanocomplex was observed in cardiac lesion site, indicating its exclusive targeting capability for ischemic heart diseases. Altogether, these findings suggest the designed EEPG nanocomplex is favorable for siRNA delivery, which might have translational potential as a versatile platform in inflammation-related diseases.

AIMS:

1. To design a versatile polysaccharide-based nanoplatform for gene delivery.
2. To optimize the release of siRNA during endocytosis by endosomolytic polymer.
3. To develop viral mimicry nanocomplex with precise targeting capability.

METHODS AND RESULTS:

β-glucan based nanoparticles were designed and synthesized for siRNA delivery (Figure 1a). Human hemolysis assay was conducted to assess the pH-responsive properties of NPs, suggested its hemolytic activity at pH 5.5 (Figure 1b). To gain a deeper understanding of the potential mechanism for the pH responsive membrane disrupting capability of EEPG and CEEPG NPs, we conducted molecular dynamics simulation to potentially interpret the interaction between EEPG and lipid membranes (Figure 1c), and the interaction between EEPG and chitosan within CEEPG NPs (Figure 1d) at the atomic level. Furthermore, fluorescently labeled CEEPG NPs (FITC-CEEPG NPs) were incubated with bone marrow-derived macrophages (BMDM). Compared with the control group, a substantial amount of relative cellular uptake (62.4%) was observed for FITC-CEEPG NPs, suggesting the targeting affinity of CEEPG NPs towards Dectin-1 receptor (Figure 2a, b).

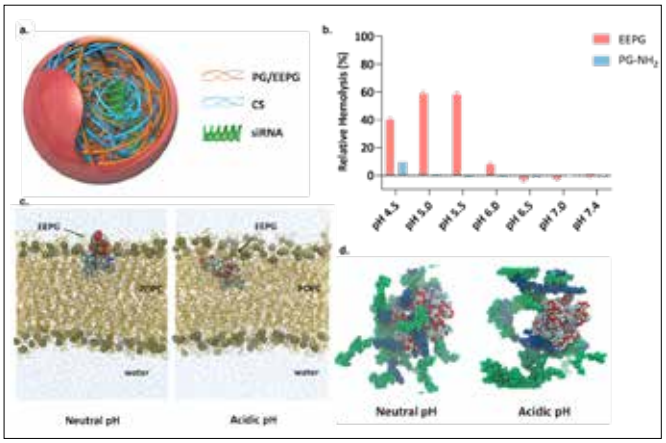

Figure 1. Endosomolytic activity of the synthesized polymer EEPG. *a. Schematic illustration of the formation of polysaccharide-based siRNA delivery system. b. Hemolytic behavior of EEPG and PG-NH$_2$. c, d. Molecular dynamic simulation of CEEPG NPs at acidic and neutral pH. Data are presented as mean ± SD of three independent measurements.*

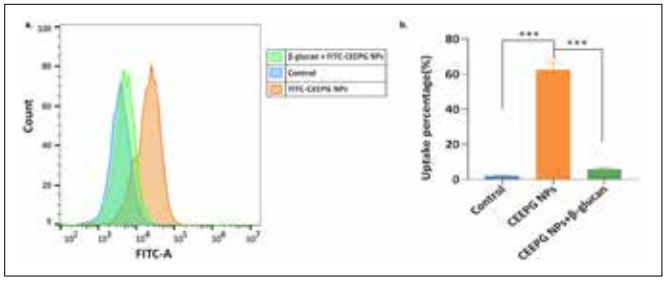

Figure 2. Targeting capability of the developed CEEPG NPs. *a. Flow cytometry analysis. b. Quantitative cellular uptake.*

For *in vivo* biodistribution and fluorescence assay, near infrared fluorescent dye Cyanine7 (Cy7) was used to conjugate with CEEPG NPs, a significantly higher fluorescent signal from heart was observed from Cy7-NPs group in mice with ischemic injury (Figure 3a). We further calculated the heart targeting index (HTI; average heart fluorescence emission/average liver fluorescence emission) for quantitative analysis of the enhanced cardiac accumulation of Cy7-CEEPG NPs, and a significantly higher fluorescent signal from heart was observed from the Cy7-CEEPG NPs under MI condition (37.3% enhancement, Figure 3b).

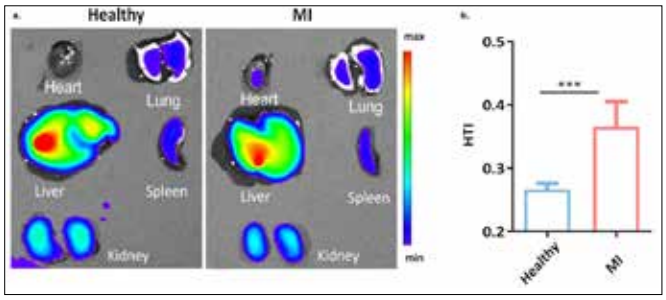

Figure 3. Biodistribution of Cy7-CEEPG NPs in MI murine model. *a. In vivo biodistribution analysis. b. Quantitative Heart targeting index (HTI).*

CONCLUSIONS

In summary, we fabricated a pH-responsive polysaccharide-based nanoplatform for siRNA delivery. Owing to the immune recognition of barley β-glucan by Dectin-1 receptor, this designed CEEPG NPs showed superior affinity to Dectin-1$^+$ macrophages. In a myocardial infarction murine model, the CEEPG NPs showed targeting efficacy and gene silencing effects in cardiac inflammatory lesions. Overall, the present study holds great potential for resolving inflammation progression in cardiovascular diseases.

MODULATION OF THE TUMOR MICROENVIRONMENT VIA PH-REGULATING LIPOSOMES

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High tumor metabolism and a non-uniform removal of the generated metabolites cause an extracellular hyperacidity in the tumor microenvironment (TME) compared to healthy tissue. This low pH outside of the tumor cells is linked to a poor prognosis in cancer patients of malignant melanoma, therefore it is desirably to restore

the extracellular pH within degenerative tissues. Additionally, immune cells like macrophages, which are called tumor-associated macrophages (TAM) are polarized towards an immunosuppressive M2-like macrophage type. Unlike M1-macrophages, M2-TAM promote tumor growth through secretion of soluble factors and the suppression of anti-tumor effector cells present in the TME. Accordingly, a low M1/M2 ratio in cancer patients also leads to a poor prognosis.

To implement the influence of pH in cancer tissue, we utilize specially adapted pH-regulating liposomes. By encapsulation of urease within our carriers, excretory urea is catalyzed to ammonia which is capable to raise the pH in the TME leading to increased anti-tumor effects or higher drug uptake of alkaline active drugs in cancer cells (e.g., doxorubicin).

We could show that our pH-regulating liposomes show functional activity being able to convert urea in *in vitro* systems and increasing the pH in the treated cultures. In addition, incubation of macrophages in culture media with different extracellular pH values confirmed that acidic conditions promote polarization towards an M2 type.

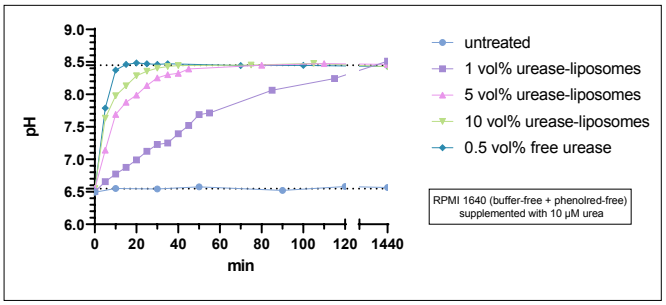

Fig. 1.: ***Change of pH with different urease-liposome concentrations in culture media****: pH/time-diagram of varying nanocarrier concentrations in cell culture media. Media were supplemented with 10 mM urea prior experiment. Medium (RPMI1640 + 10% FBS + 1% GlutaMAX + 0.2% Primocin) was used without HEPES buffer, sodium carbonate buffer and phenol red. Medium was acidified with 0.1 N hydrochloric acid to pH 6.5 prior experiment.*

With these experiments and future studies, we are investigating the influence of pH modulation via nanocarriers on the composition of immune cells and tumor cells in the TME and their anti-tumor functions as well as changes in efficacy of distinct drugs in more detail.

Our preliminary results show that the adjustment of extracellular pH in the TME via smart carrier systems is feasible and can lead to an increase of anti-tumor responses and might improve drug uptake and efficiency.

PERFORMANCE OF A NOVEL HIGH-THROUGHPUT NANOPARTICLE FORMULATION SET-UP

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INTRODUCTION

The relationships between nanoparticle design and physicochemical properties on one side and their biological activities on the other are highly complex. Although numerous exciting studies addressing nanoparticle biological behavior as a function of their properties exist[1], it remains poorly understood for most nanomedicines. As a result, the development of novel nanoformulations delivering therapeutics to specific tissues and cells is a challenging endeavour and today often based on extensive and costly nanoparticle library screening[2,3]. For example, in the case of lipid nanoparticles (LNP) delivering nucleic acids, laborious ionizable lipid and RNA-LNP screening studies form the basis for the recent first clinical approvals of this technology[2,4,5].

To allow for more cost-effective and efficient nanomedicine development pipelines, high-throughput (HT) nanoparticle synthesis and characterization have recently gained attention[6,7]. The most widely used approaches to synthesize RNA-LNP are based on mixing organic (containing lipids) and aqueous (containing RNA) phases. Mixing of these phases results in a change in polarity and solubility, which induces nanoparticle self-assembly through nanoprecipitation. Interestingly, various other lipidic and polymeric nanoparticle platforms can also be synthesized using this approach with the nanoparticle ingredients dissolved in the organic and aqueous phases.

In the so-called organic solvent injection method, mixing is achieved through bulk injection of the organic solvent into the aqueous phase. It has recently been demonstrated that this approach can be miniaturized and automated using robotic liquid handlers[6,7]. Although highly useful, the injection methodology does not provide a high mixing precision and faces reproducibility challenges, *e.g.* in terms of particle size and polydispersity[6]. Nanoparticle synthesis using flow mixing on the other hand, using for example microfluidic or T-junction mixers, allows for precise and rapid mixing at millisecond and nanoliter scales[8,9]. It is highly reproducible and scalable[8] and has become a widely used nanoparticle production technology[10]. Moreover, Particle Works (United Kingdom) has recently launched the first commercial HT nanoparticle formulation device based on flow mixing.

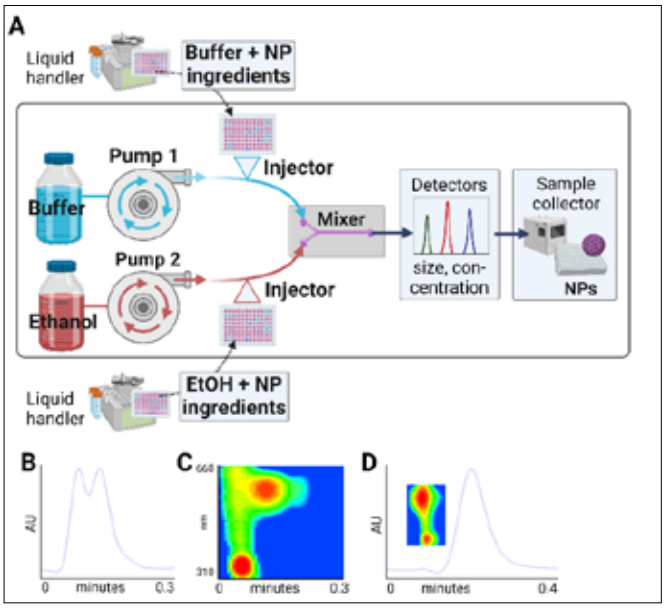
Here, we present a new HT flow-mixing based nanoparticle synthesis set-up consisting of commercially available chromatography instruments and microfluidic chips. We compare its performance with proven syringe pump-operated mixers and demonstrate that it provides highly reproducible and high-quality nanoparticle formulations.

METHODS AND RESULTS

High throughput nanoparticle synthesis set-up*:* We have assembled and custom-programmed commercial high-end liquid chromatography and online analytical modules into a HT nanoparticle formulation set-up, which we call the HT-mixer (**Figure 1A**). The set-up allows for miniaturization (<100 µL per nanoparticle batch), and dramatically increases the speed of manufacture (~3 minutes per batch). The repurposing of liquid chromatography units allows us to control flows and injection volumes (down to µL) very precisely in an automated and easily programmable sequence. The feeding pumps are run continuously, with injectors switching organic and aqueous phases with nanoparticle ingredients into the stream as liquid plugs. As a result, flow conditions in the mixer (pressure, flow geometry) are constant, which ensures maximum reproducibility and minimal waste of reagents. This continuous flow also provides extensive cleaning of the entire flow path between batches, with no carry-over. The mixer unit can easily be exchanged.

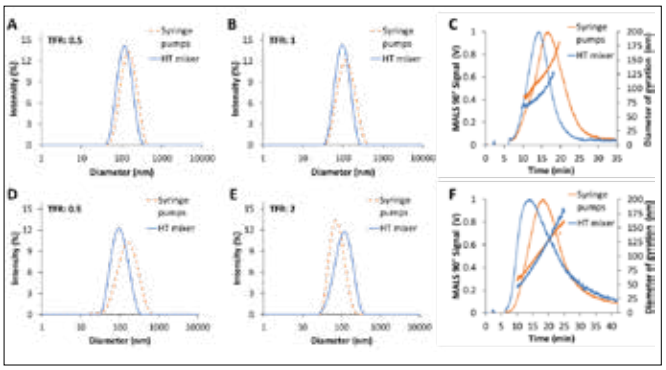
Figure 1*: High-throughput nanoparticle formulation set-up.* ***A****: Buffer and organic phase (exemplified with commonly used ethanol) are constantly pumped through the system. The injectors inject plugs of organic and aqueous solutions with nanoparticle ingredients, which meet in the mixer. Nanoparticles exiting the mixer can be lead through detectors and are finally collected in 96-well plates in an automated sample collector. The 96-well plates containing organic and aqueous aliquots with (for example varying types and concentrations of) nanoparticle ingredients can be prepared using our automated liquid handlers.* ***B-D****: To characterize plug-flow with an inline UV-detector and assure simultaneous arrival of the organic and aqueous liquid plugs with nanoparticle ingredients in the mixer, we injected the dyes coumarin and nile red in the organic and aqueous channel, respectively.* ***B****: Absorption at 210 nm showed 2 peaks, indicating asynchronous arrival of the dyes in the UV-detector, and hence mixer.* ***C****: UV absorption spectra confirmed that the two peaks originated from nile red (max abs. 580 nm) and coumarin (max abs. 350 nm).* ***D****:*

Tuning injection timepoints resulted in synchronous injections in the two channels. Figure 1A was created with Biorender.com.



***Benchmarking performance of high throughput nanoparticle synthesis set-up**:* To assure the nanoparticles produced using our HT-mixer, we prepared oil-in-water emulsions and mRNA-LNPs with the same microfluidic chip (Darwin microfluidics, product #: LFT-012.00-4264) in the HT mixer and in a mixer set-up with syringe pumps. We measured hydrodynamic diameter (z-avg) and polydispersity index with dynamic light scattering (DLS, Zetasizer Pro, Malvern). Furthermore, a selection of nanoparticle batches was characterized using multi-detector field flow fractionation (MD-FFF).
Oil-in-water emulsions were prepared in water using DSPC:Cholesterol:PEG2000-DSPE at molar ratios of 57:33:10 at a final lipid concentration in the emulsion of 2 mM. 2 mg of Miglyol 812 N per 1 µmole of lipid was added and made up the dispersed phase of the emulsion. We prepared various batches of only 50 to 100 µL at a total flow rate (TFR) of 0.5 and 1 mL/min on both mixer set-ups. The emulsions prepared with the HT-mixer were consistently smaller in particle size than the ones prepared with the syringe pumps. Nevertheless, emulsions from both mixer set-ups were reproducible in size, see **Table 1** and **Figure 2 A-C.**

***Figure 2**: Examples of DLS and MD-FFF results. **A-C**: DLS (**A** and **B**) and MD-FFF (TFR 0.5, **C**) demonstrated that emulsions prepared with the HT-mixer were nearly identical to, but slightly smaller than emulsions prepared with the syringe pump set-up. The DLS showed a single population of emulsions, which was confirmed by the high-resolution MD-FFF analysis. **D-F**: Also for mRNA-LNPs, the formulations were similar for the two mixer set-ups and consisted of a single population as observed with DLS (**D** and **E**) and MD-FFF (TFR: 1, **F**).*



mRNA-LNPs were prepared in pH 4 acetate buffer using D-Lin-MC3-DMA:DSPC:cholesterol:PEG2000-DMG at molar ratios of 50:10:38.5:1.5 at a final lipid concentration of 2.5 mM. N/P used was 6, resulting in a final mRNA concentratin of 89 µg/mL. We prepared various batches of only 50 to 100 µL at a TFR of 0.5, 1, and 2 mL/min on both mixer set-ups. mRNA-LNPs from both mixer set-ups consisted of single populations and were similar in size. Furthermore, the size and polydispersity of mRNA-LNPs produced with the HT-mixer were reproducible, see **Table 1** and **Figure 2D-F**

***Table 1**: Summary of various nanoparticle batches produced on the HT-mixer and the syringe pump set-up*

NP type	Mixer set-up	organic : aqueous flow rate ratio	Total flow rate (ml/min)	Final lipid concentration (mM)	DLS Z-AVG (nm)	DLS PdI (a.u.)	MD-FFF Diameter of gyration (nm)
Emulsion	Syringe	1:3	1	2	107	0.17	105
Emulsion	Syringe	1:3	1	2	111	0.15	112
Emulsion	HT mixer	1:3	1	2	76	0.08	64
Emulsion	HT mixer	1:3	1	2	88	0.14	80
Emulsion	Syringe	1:3	0.5	2	128	0.17	n.a.
Emulsion	Syringe	1:3	0.5	2	130	0.18	150
Emulsion	HT mixer	1:3	0.5	2	108	0.16	n.a.
Emulsion	HT mixer	1:3	0.5	2	109	0.14	90
mRNA-LNP	HT mixer	1:3	0.5	2.5	84	0.21	80
mRNA-LNP	HT mixer	1:3	0.5	2.5	91	0.18	85
mRNA-LNP	HT mixer	1:3	1	2.5	87	0.24	78
mRNA-LNP	HT mixer	1:3	1	2.5	97	0.23	67
mRNA-LNP	HT mixer	1:3	2	2.5	103	0.22	85
mRNA-LNP	HT mixer	1:3	2	2.5	108	0.21	82

CONCLUSION AND OUTLOOK

We present a proof of principle for our HT nanoparticle formulation and characterization approach that is capable of automated production of very small batches (<100 µL) of various nanoparticle types at 3 minutes per batch by state-of-the-art microfluidics mixing. This does not only save a significant amount of time in nanomedicine development, at a batch size of <100 uL, it also reduces material costs substantially. Combined with, 1) our liquid robotic handlers to prepare (differential) organic and aqueous solutions of nanoparticle ingredients, and 2) online characterization (only UV-detector shown, online sizing in progress), this allows us to formulate and characterize nanoparticles in a high-throughput fashion. Although full integration in our nanomedicine development pipeline is ongoing, we envision this will become a valuable tool for us and our collaborators.

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NOSE-TO-BRAIN DELIVERY OF ACYL-GHRELIN PEPTIDE GOLD NANOCONJUGATES FOR TREATMENT OF NEURODEGENERATIVE DISEASES

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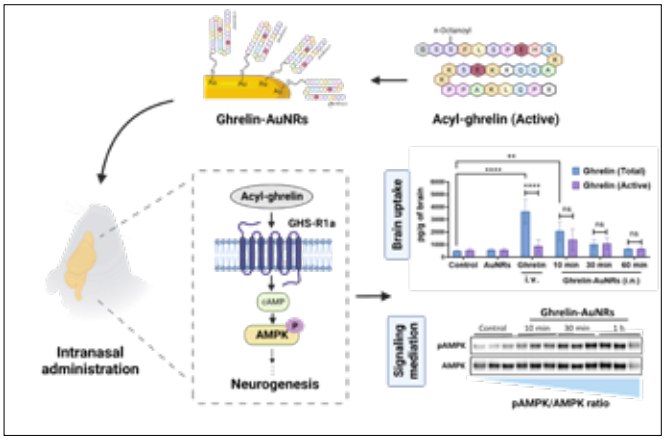

Scheme 1. Graphical abstract

INTRODUCTION

Neurological disorders are an important cause of mortality worldwide. Acyl-ghrelin, a 28-amino acid peptide, which has a n-octanoic acid at the third serine residue, demonstrates beneficial effects for the treatment of neurodegenerative diseases. Acyl-ghrelin undergoes deactivation by endogenous esterase enzymes. Although ghrelin can penetrate the BBB, the short plasma half-life of ~10 minutes and non-specific distribution make systemic administration of acyl-ghrelin inadequate for neurological disorder treatment. Our previous work demonstrated gold nanorods (AuNRs), as drug carriers, can reach the brain in a rapid and effective manner with minimal systemic exposure following intranasal administration. It is hypothesized that intranasal AuNRs could be a suitable platform for acyl-ghrelin brain delivery.

METHODS

Acyl-ghrelin was conjugated to AuNRs *via* PEG linkers using EDC/sulfo-NHS chemistry. The hydrodynamic size, zeta potential and morphology of Ghrelin-AuNRs were assessed by nanoparticle tracking analysis (NTA), electrophoretic mobility measurement and transmission electron microscope (TEM), respectively. Ghrelin conjugation was confirmed by proton nuclear magnetic resonance (^{1}H-NMR). To test brain uptake, Ghrelin-AuNRs was intranasally administered to mice before culling at 10, 30 and 60 min post administration. Total and active ghrelin in brain tissues were quantified using enzyme-linked immunosorbent assay (ELISA). Intravenous acyl-ghrelin group was included as a positive control. *In vivo* biological activity was assessed by measuring phosphorylated AMP-activated protein kinase (AMPK) to AMPK ratio by Western blotting.

RESULTS

Ghrelin-AuNRs was successfully synthesised and characterised. Ghrelin conjugation to AuNRs increased the hydrodynamic size to ~130 nm. All particles maintained the rod morphology. Our results confirmed that Ghrelin-AuNRs was successful in delivering ghrelin to the brain at all time points tested with highest achieved at 10 min post administration. Contrary to intravenous free ghrelin peptide where the ghrelin activity dropped to 25% of the total, AuNRs maintained comparable levels between total and active ghrelin. pAMPK/AMPK ratio was higher in Ghrelin-AuNRs treatment compared to the control in a time-dependent manner.

CONCLUSIONS

This is the first study to report the feasibility of ghrelin nanoparticle conjugates for intranasal administration for brain targeting opening new prospective for treatment of neurodegenerative diseases.

ACKNOWLEDGEMENT

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MODULATION OF IMMUNE RESPONSE THROUGH DENDRIMER FUNCTIONALIZATION

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Many nanoparticles in the blood activate complement system, an integral part of innate immune system that render nanoparticles susceptible to phagocytosis by immune cells like polymorphonuclear leukocytes and tissue macrophages [1]. Nanoparticle-mediated complement activation is multiparametric and is modulated by physicochemical properties including size, shape, and surface characteristics as well as non-specific protein binding [1–3]. Recently, we showed poly(amido amine) dendrimers evade complement activation due to Angstrom-scale spacing arrangement (the ASSA phenomenon) of their surface functional motifs [4]. Considering this, we hypothesize immune cells might also respond differently to nanoparticles that display surface ligands/functional groups in ASSA arrangement. Here, we extend our studies by functionalizing polymeric nanoparticle surfaces with a library of fully characterized dendrimers and assess surface properties with a wide range of state-of-the-art biophysical modalities. The results show how precision surface patterning with dendrimers can control and modulate immune responses through assessment of serum protein deposition by shot-gun proteomics and macrophage challenge. *Supported by the European Union's Horizon 2020 programme funded under H2020-EU.1.3. – Excellent Science – Marie Skłodowska-Curie Actions, grant agreement ID. 956544 (DIRNANO: Directing the Immune Response through Designed Nanomaterials).*

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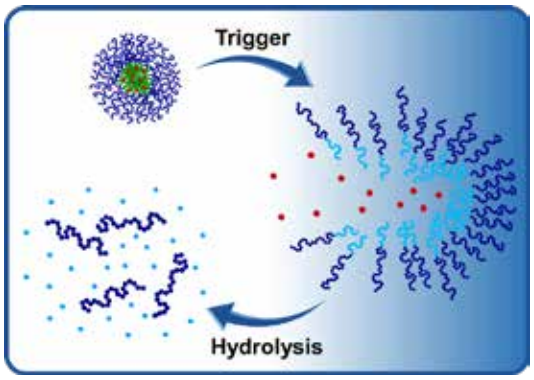
TRIGGERABLE POLYCARBONATES AS POTENTIAL IMMUNODRUG NANOCARRIERS

ADRIAN HAUCK

Over the past decade immunotherapy has gained much attention as promising fourth pillar in oncology. In addition to chemotherapy, radiotherapy and surgery, immunotherapy has the potential to complement these prevailing cancer treatments as a highly efficient alternative.[1]

Despite intensive immune stimulation, many promising immunodrug applications are restricted, due to adverse properties such as poor aqueous solubility, systemic inflammation and toxicity. Nanocarrier guided drug delivery has the potential to overcome these obstacles by solubilizing hydrophobic small molecule drugs and selectively delivering them into the tumor micro environment (TME) and its draining lymph nodes, therefore reducing off-target immune responses.[2] To further promote this effect, smart nanocarriers are developed which feature a chemical trigger that responds to environmental changes, releasing their cargo primarily at the target site.



To design biodegradable smart nanocarriers, we make use of biocompatible materials namely poly(ethylene glycol)-*b*-poly(carbonates) and customize the chemical structures for accessing novel unique properties. These block copolymers themselves do already have appealing properties. Due to their amphiphilic character, the polymers assemble into nanometer-sized polymeric micelles that can be used for hydrophobic drug encapsulation. In addition, these compounds exhibit low toxicity, and the aliphatic carbonate block provides hydrolytic degradability.[2] In order to extend the nano carrier with a stimulus responsive functionality, a polymer system was developed bearing an acid degradable ketal linker in the carbonate side group. Nanocarriers formulated from these polymers enable a selective release of active cargos in acidic environments, as it is found in tumor tissues and intracellular lysosomes. The synthesis of a novel carbonate monomer was demonstrated, as well as the polymerization of amphiphilic block copolymers and the formulation of polymeric micelles, derived from this novel monomer. The acidic release mechanism of the carrier was investigated on the polymers themselves, as well as on the formulated polymeric micelles confirming high stability of the carrier under physiologic conditions and gradual degradation at mild acidic pH levels. Furthermore, phagocytosis of polymer end group dye labeled micelles by macrophages was confirmed and the delivery of TLR7/8 agonist CL075 into these cells affords a pronounced immune cell maturation.

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INVESTIGATING THE IMMUNOLOGICAL RESPONSES OF HEPATIC AND IMMUNE CELLS LINKED TO THE BIORETENTION OF IRON OXIDE NANOPARTICLES

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Depending upon their intended application, nanoparticles exhibit a wide variety of physical and chemical characteristics. They can be synthesised to produce variations in size, shape, surface charge and coated with various polymers, rendering them highly adaptable for a wide range of medical applications [1, 2]. Superparamagnetic iron oxides (SPIONs), known for their distinctive physical and chemical properties, are currently under investigation for their potential role in hyperthermia treatments targeting solid mass tumours and have made it through to clinical trials [3-5]. However, as with any nanoparticle formulation, the immunocompatibility of SPIONs has raised concerns, given the diverse characteristics of nanoparticles, such as size and surface charge, which are closely tied to chemical features like surface coating [6-8]. Assessing their compatibility with the immune system poses a challenge due to this variability, exacerbated by the absence of appropriate immune-competent tissue models, impeding their progress in research. The current study investigated the impact of commercially available SPIONs (carboxylic (CAR) or amine (AMI) terminated, as well as PEG coated) on human immune (THP-1) and liver cells (Hep G2), specifically focusing on pro-inflammatory cytokines and underpinning bioenergetic profiles, utilising defined cytocompatible concentrations. Additionally, our previous work, using physiologically-based pharmacokinetic modelling (PBPK), has shown the possible hepatic bioretention of SPIONs of up to 7 days following a single administration. As such, we used both 24- and 168-hour exposure times, to assess impacts of longer exposure times. Cytocompatibility and cellular responses, were assessed using a number of methodologies including, but not limited to, Luminex® Multiplex Assays and Agilent Seahorse XF Real-Time ATP rate assay to determine monoculture responses as a move towards development of appropriate immune-competent tissue models.

Table 1. Panel of ligands used in experiments.

Ligand	Target
Phytohemagglutinin-M (PHA)	cell division and metabolic activity
LPS-EK Ultrapure	TLR4
Oligodeoxynucleotide (ODN) 2006	TLR9
2'3'-cyclic guanosine monophosphate adenosine monophosphate (cGAMP)	STING pathway
Poly(dG:dC)/LyoVec	cytosolic DNA sensor (CDS)
Herpes Simplex virus (HSV)-60/LyoVec	cytosolic DNA sensor (CDS)
Poly (I:C) HMW	TLR3

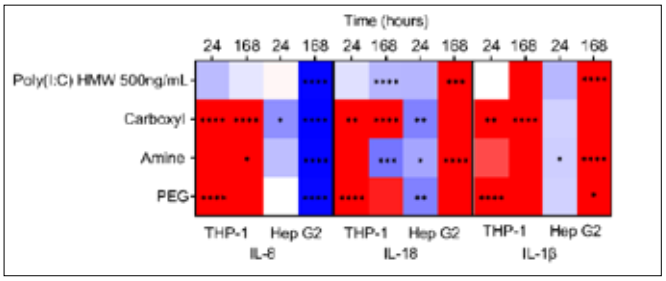
Responses of the human immune and liver parenchymal cells to experimental SPIONs and immunostimulatory ligands: THP-1 and Hep G2 cells were pre-exposed to cytocompatible concentrations of SPIONs and known ligands for 24- and 168-hours. Following incubation, responses were measured via a Luminex assay (Figure 1) and CellROX green.

At 24-hours in the THP-1 cells, treatment with the SPIONs lead to significantly greater IL-8 secretion (CAR = 18.26-fold, AMI = 2.20-fold, PEG = 40.14-fold), IL-18 secretion (CAR = 23.00-fold, AMI = 3.04-fold, PEG = 37.14-fold) and IL-1β secretion (CAR = 3.24-fold, AMI = 1.71-fold, PEG = 5.89-fold) when compared to the untreated. When linking this to the 168-hour treatment, for IL-8 and IL-1β, the changes were amplified further (IL-8 CAR = 229.11-fold, AMI = 22.59-fold, PEG = 46.78-fold; IL-1β CAR = 2907.74-fold, AMI = 11.08-fold, PEG = 1873.13-fold) when compared to the untreated. Treatment with SPIONs led to significant differences in IL-8 (AMI

p=<0.0001) and IL-1β secretion when comparing the 24- and 168-hour profiles (CAR p=<0.0001, AMI p=0.0018, PEG p=<0.0001) in THP-1 cells.

At 24-hours in the Hep G2 cells, treatment with the SPIONs lead to significantly lower IL-8 secretion (CAR = 1.79-fold), IL-18 secretion (CAR = 1.98-fold, AMI = 1.55-fold, PEG = 1.98-fold) and IL-1β secretion (AMI = 1.24-fold) when compared to the untreated. Further relating this at the 168-hour treatment, there was significantly lower secretion for IL-8 (CAR = 364.36-fold, AMI = 107.18-fold, PEG = 48.99-fold). For both IL-18 and IL-1β, significantly greater secretion was recorded in response to AMI (IL-18 = 5.49-fold, IL-1β = 10.98-fold) when compared to the untreated. PEG treatment at 168-hours also lead to significantly greater IL-1β secretion (6.38-fold greater) when compared to the untreated. Treatment with AMI led to significant differences in IL-1β secretion when comparing the 24- and 168-hour profiles (p=0.0009) in the Hep G2 cells.

*Figure 1. Secretion profiles of three cytokines, IL-8, IL-18 and IL-1β from THP-1 and Hep G2 cells exposed to SPIONs and Poly (I:C) HMW for 24- and 168-hours; n=3, * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.*



ROS production over 24 hours in THP-1 cells in response to treatment with immunostimulatory ligands and SPIONs: ROS production (Figure 2) measured via kinetic 24-hour measurements differed in response to immunostimulatory ligands and SPIONs. THP-1 cells treated with ligands and SPIONs showed a significant increase in AUC than untreated control cells. For example, cells treated with Poly(dG-dC) significantly increased the AUC by 1.94-fold, which was further increased by the addition of CAR (+ Poly(dG-dC)) to 2.37-fold when compared with the untreated (p =<0.0001) when compared to the untreated.

*Figure 2. Reactive oxygen species (ROS) in THP-1 cells in response to immunostimulatory ligands and SPIONs over 24 hours. Data displayed as average of n=4 ± SD. Statistical significance when compared to untreated displayed as * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.*

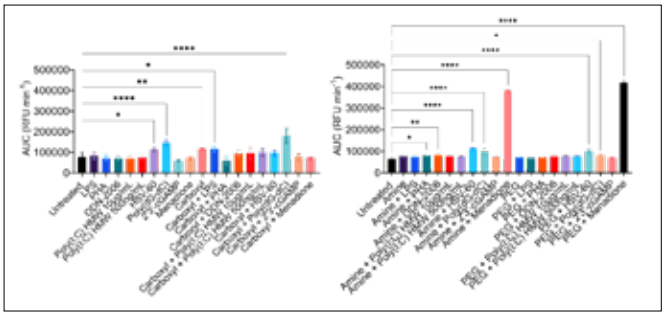
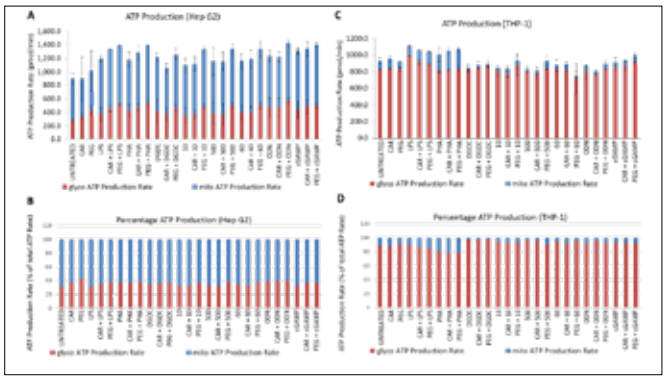


*Figure 3. ATP production rate assessment on THP-1 and Hep G2 cells following 24-hour incubation with immunostimulatory ligands, CAR and PEG. (**A**) ATP production in Hep G2 cells (**B**) Percentage of ATP production in Hep G2 cells when compared to untreated, 100% is total production (**C**) ATP production in THP-1 cells (**D**) Percentage of ATP production in THP- cells when compared to untreated, 100% is total production. Graphs displayed as an average (n=4) ± SD. Blue bars displaying ATP produced via oxidative phosphorylation and red bars displaying ATP produced via glycolysis.*



These results support reports that the materials can cause oxidative stress, with associated inflammation; dependent on the material, cell type and what was measured, there are different responses to the known ligands and the IONPs. It is also clear that longer exposure times, linked to physiologically relevant exposures, can lead to sustained inflammatory profiles in immune-competent as well as professional immune cells; requiring their greater consideration in compatibility profiling. Further clarification over extended exposures will help us to clearly define these impacts and possible consequences for immune responses, using physiologically relevant models and realistic exposure times.

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DUAL DRUG LOADED TARGETED DELIVERY OF MULTIFUNCTIONAL LIPOSOME AGAINST HUMAN GLIOMA ORTHOTOPIC MODEL

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Several limiting factors including the blood-brain barrier, the highly invasive nature of gliomas, and drug resistance challenges with delivering therapeutic agents specifically to the tumour cells, which results in a poor prognosis, make it difficult to treat malignant gliomas. To deliver targeted payloads with favourable pharmacokinetics and take benefit of cellular targeting for increased specificity, and enhanced effectiveness, nano-based therapeutic compounds are now in extensive use. In the present investigation, dual drug-loaded multi-targeting liposomes were formulated aiming for an efficacious targeted delivery to the tumour, in an intracerebral glioma model. Multifunctional liposomes were engineered with a combination of standard anticancer drug, Temozolomide (TMZ) and a chemosensitizer O6-Benzylguanine (O6-BG) along with ligand

transferrin to bypass the blood-brain barrier as well as with a glioma tumour cell (U-87 MG Luc$^+$) surface targeting moiety, anti-integrin antibody. The engineered nano-liposome showed high drug entrapment efficiency with a particle size of 187 ± 10 nm and have uniform size distribution **(Figure 1a).** The optimized formulation was tested *in vitro* as well as *in vivo* against the intracerebral glioma model to evaluate the pharmacokinetics of the nanocomposite in comparison with pure drug combinations.

Our findings indicated that the cellular uptake of functionalized liposomes was prominent **(Figure 1b)** which further enhanced the glioma cellular death through apoptosis. Further, the pharmacokinetic study showed an efficient intracerebral uptake of nanocomposite with a controlled release of the drug up to 48 h after intravenous infusion and subsequent time-dependent depletion in contrast to that of pure TMZ which was seen clearing from the brain within 4 h, demonstrating the possibility of improved pharmacodynamics.

(a)

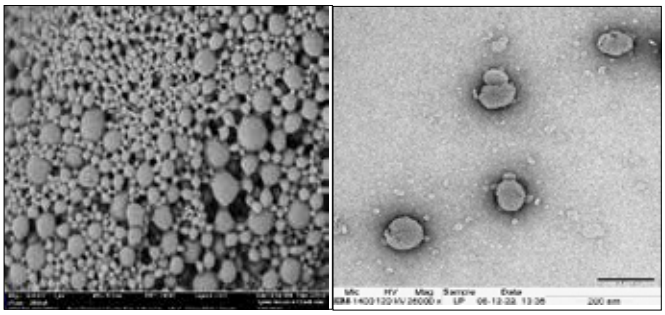

Figure 1: (a) *Physicochemical characterization of ligand conjugated liposomes; representative image of liposome vesicles by Field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The nanocomposite has uniform size distribution and is spherical;* **(b)** *cellular uptake of liposome; the representative confocal laser scanning microscopy images of U87 cells incubated with dye alone, Coumarin 6-loaded liposome and Coumarin 6-loaded LP-Tf-ITG (10 µg/mL dye eq.) for 4h, scale bar is 25µm.*

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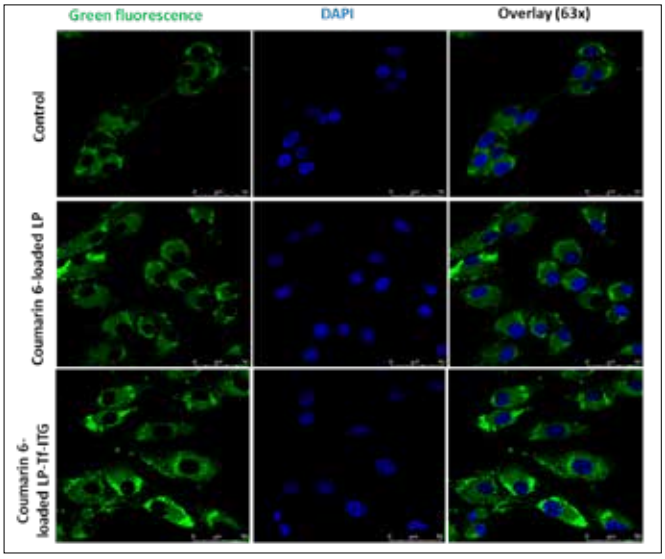
(b)



SURFACE-FUNCTIONALIZED HUMAN SERUM ALBUMIN FOR MODULATING TUMOR MICROENVIRONMENT

LIFAN HU, Darijan Schüler, Seah Ling Kuan, Tanja Weil

The altered metabolic biology, is a hallmark of cancer cells that support their activities and malignant properties [1]. Warburg effect indicates that cancer cells tend to undergo glycolysis rather than oxidative phosphorylation (OXPHOS), even in aerobic environment, resulting in tumor immunosuppressive environment. Consequently, cancer metabolism has emerged as a vital area in cancer research to develop new treatment that are more effective.

Small molecule inhibitors have been developed for inhibiting key rate-limiting enzyme or functional protein in cancer metabolism network. For example, 2-DG which can competitively inhibit glucose transport or FX11 which can inhibit the activity of lactate dehydrogenase (LDH, conversion of pyruvate into lactate) have been proposed for cancer therapy [2]. However, there are shortcomings such as limited stability and off-target toxic side effects from non-specific targeting need to be addressed.

Human serum albumin (HSA) as a natural protein attracted much attention, due to its excellent biocompatibility and biodegradability, high availability, and abundant groups for chemical modification[3]. Herein, we present a HSA-based therapeutic system containing covalently attached inhibitors that exhibits selective uptake in acidic tumor microenvironment of tumor tissue, to modulate cancer metabolism.

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MULTICOMPONENT SUPRAMOLECULAR PLATFORM FOR THE DESIGN OF GLYCOCONJUGATE ANTITUMOR VACCINES

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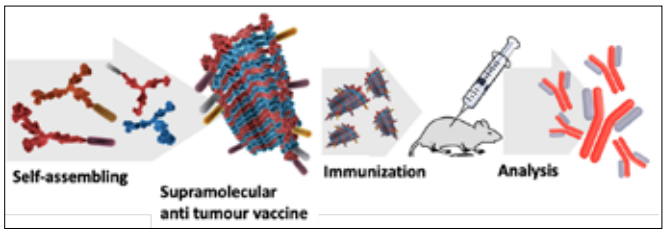

Peptide secondary structures can be harnessed to design monomers capable of self-assembling into nano-scaled supramolecular structures in aqueous media.[1,2] Decorating the surface with immunogenic molecular patterns results in pathogen-mimicking entities and potential vaccine candidates.[3] In the context of antitumor vaccines, the challenge is to overcome self-tolerance mechanisms to enforce an immune response against endogenous, tumor-associated glycopeptide motifs.[4] For this purpose, co-stimulation of B cells with T helper cells is mandatory, which we aim to achieve by co-presentation of different epitopes and immunostimulant agents on

the surface of multicomponent supramolecular polymers. The use of thermoset supramolecular hydrogels as a vaccine depot also allows for sustained immune stimulation and could be an alternative to the adjuvants required in conventional vaccination strategies.
B-cell epitopes derived from either breast tumour-associated MUC or melanoma-associated CSPG4 surface proteins and co-presented multivalently with "universal" immunostimulatory T-helper cell epitopes. Mucin 1 (MUC1) is known to undergo O-glycosylation changes during tumourgenesis, making it an excellent tumour-associated target for immunotherapy. Fully synthetic 22 amino acid MUC1-derived glycopeptides bearing sialylated STN tumour associated carbohydrate antigens are therefore being used.[5-7] Chondroitin sulfate proteoglycan 4 (CSPG4) is a surface proteoglycan that has been observed to be highly expressed on tumour cells, whereas expression on healthy tissue is limited, making it a highly potential target for the difficult-to-threat melanoma.[9-10] Future investigations will also be based on fully synthetic derivatives of this marker. T-cell stimulation is achieved by incorporating a small fragment of highly immunogenic tetanus toxin (p30). In addition, an imidazoquinoline, a potent TLR7/8 agonist,[11] has been synthesized as an immunomodulator. Mannose moieties can be attached to the surface of the nanorods to further recruit and stimulate macrophages as accessory cells. These epitopes were conjugated to supramolecular monomers and mixed in aqueous solution to yield polymeric vaccines. High antibody titers of the IgG type were observed in all mice. Furthermore, FACS analysis confirmed the high binding affinity of the generated antibodies to T47D tumor cells. These results support the potential of this modular supramolecular platform approach for the development of antitumor vaccines.

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STUDIES OF CABAZITAXEL-LOADED POLY(2-ALKYL CYANOACRYLATE) NANOPARTICLE

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Despite many recent advances in nano-engineered cancer therapeutics, strategies to overcome toxicity and drug resistance are still demanding. Biodegradable poly(2-alkyl cyanoacrylate) (PACA) nanoparticles (NPs) have obtained large attention during the last years. We previously reported that cabazitaxel (CBZ) loaded poly (2-ethylbutyl cyanoacrylate) NPs (PEBCA-CBZ NPs) showed promising results in a patient derived xenograft (PDX) model of triple negative breast cancer, and this was associated with a decrease of M2 macrophages (Fusser *et.al.* 2019). Now, we report comparative preclinical testing of these NPs and a new variant made of PEHCA (poly (2-ethylhexyl cyanoacrylate), which are either unconjugated or conjugated to folate and loaded with CBZ. *In vitro* studies were performed in different breast cancer cells, where the cellular uptake and cytotoxicity were assessed (figure 1). All NP variants showed similar pattern of toxicity in a cell dependent manner even though their internalization differed. Their biodistribution in mice appeared to be similar. *In vivo* efficacy studies performed in the HBCx39 triple negative PDX model showed enhanced effects of drug-loaded PEBCA variants compared to free drug (figure 2). The folate-conjugated PEBCA variant did not show enhanced effects compared to the unconjugated counterpart, which might be due to a buried orientation of the folate moieties on the NP surface. In conclusion, drug-loaded PEBCA NPs are promising for their use in triple negative breast cancer therapy.
This Poster is based on a manuscript in preparation with the following co-authors: Remya Valsalakumari, Abhilash D. Pandya, Lina Prasmickaite, Audun Kvalvaag, Anne Grethe Myrann, Andreas K.O. Åslund, Marianne Kjos, Cristina Fontecha-Cuenca, Hajira B. Haroon, Rita Ribeiro, Jutta Horejs-Hoeck, S. Moein Moghimi, Ýrr Mørch, Tore Skotland, Kirsten Sandvig, Gunhild Mari Mælandsmo and Tore-Geir Iversen

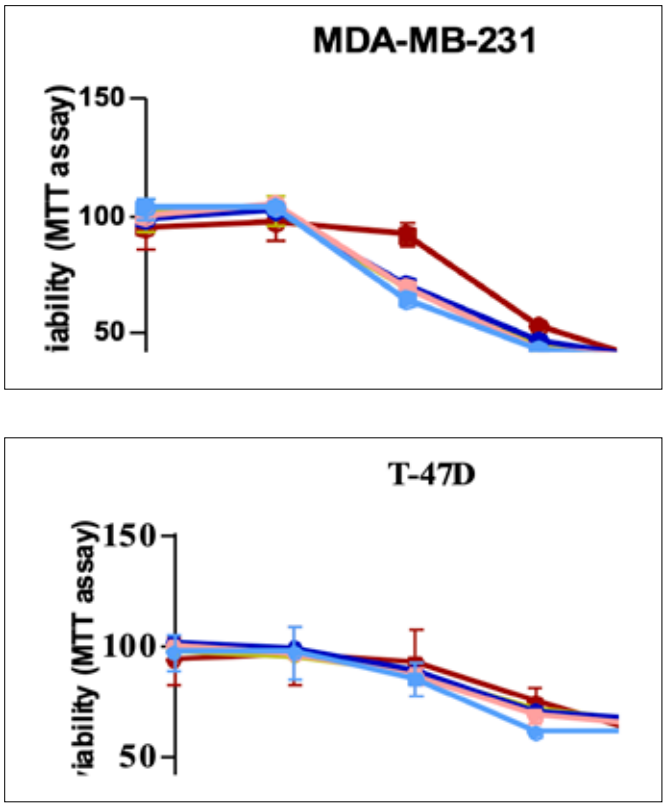

Figure 1 Toxicity induced by CBZ-loaded PACA-NP variants in breast cancer cell lines. Cell viability measured by MTT assay after exposing

the cells to increasing concentration of CBZ-containing NP variants for 72 h at 37 °C. Data shown are representation of one out of three independent experiments. Values were expressed as mean ± SD.

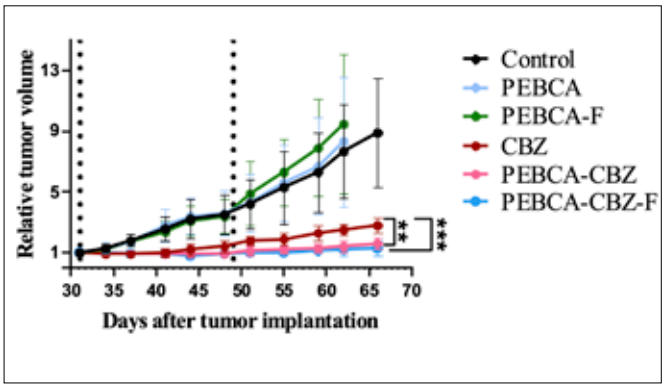

*Figure 2. In vivo efficacy of PACA NP variants in orthotopic HBCx39 PDX model of breast cancer in athymic nude mice. Treatment with various CBZ loaded NPs/free CBZ (i.v. administration) was started when palpable tumors were developed and reached a diameter of around 5-6 mm. Treatment doses equivalent to 20 mg/kg b.wt of CBZ was given as first dose and half of this dose was administered later as a second dose, approximately on week 7 after implantation (indicated by dotted lines). Data represented as mean of relative tumor volume ± SD. Statistical analysis was based on p-values generated by one-way ANOVA followed by Tukey's multiple comparison test (**p=0.006, ***p=0.0006).*

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WELL CHARACTERIZED LIPID NANOPARTICLE LIBRARY ACCELERATES DEVELOPMENT OF NEXT GENERATION GENOMIC MEDICINES

NIKITA JAIN

Co-authors: Sedigheh Nazaripour, Zhengyu Chen, Suraj Abraham, Leanna Yee, Sams Sadat, Gayatri Mehar Namala, Helena Son, Kobe Tickner, Gertrude Henneh, Tony Wu, Sijo Chemmannur, Malathi Anantha, Ruchi Sharma, Srinivas Abbina, Seetalakshmi Thambatti, Vinay Mayya, Emily Soon, Jay Paquette, Anitha Thomas
Precision NanoSystems ULC

INTRODUCTION:

Ionizable amino lipids are a major constituent of the lipid nanoparticles for delivering nucleic acid therapeutics (e.g., DLin-MC3-DMA in ONPATTRO®, ALC-0315 in Comirnaty®, SM-102 in Spikevax®). The scarcity of lipids that are suitable for cell therapy, vaccination, and gene therapies continue to be a problem in advancing many potential diagnostic/therapeutic/vaccine candidates to the clinic. Herein, we describe the development of novel ionizable lipids to be used as functional excipients for designing vehicles for nucleic acid therapeutics/vaccines *in vivo* or ex vivo use in cell therapy applications.

METHODS:

We studied the transfection efficiency (TE) of LNP-based mRNA formulations of novel ionizable lipid candidates in primary human T cells and established a workflow for engineering of primary immune T cells towards non-viral CAR T therapy. Lipids were also tested in rodents for vaccine applications using self-amplifying RNA (saRNA) encoding various antigens. We have then evaluated safety and efficacy of various ionizable lipid candidates and their biodistribution using various LNP compositions. Further, using ionizable lipids from the library, we have shown gene editing of various targets in rodents.

RESULTS:

Proprietary ionizable lipids and novel LNP compositions showed very high TE over MC3- LNPs in primary human T cells. Following IV administration of human erythropoietin (EPO) mRNA encoded LNPs, human EPO was generated resulting in an increase in hematocrit. Proprietary lipids comprising LNP vaccine candidates using self-amplifying RNA encoded for SARS-CoV-2 spike protein showed IgG levels specific to spike protein. The IgG levels were comparable to to that of SM-102 and ALC-0315 lipids used in the current mRNA vaccines. Preliminary results show case gene knock out of transthyretin (TTR) and PCSK9 protein (Figure 1).

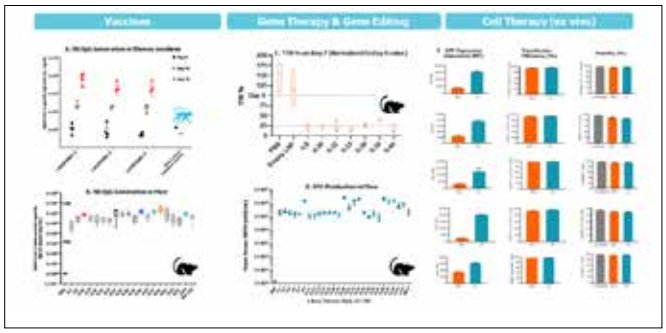

Figure 1. Proprietary ionizable lipids enable key applications in genomic medicine.

Finally, we were able to show that physico-chemical profiles of ionizable lipids are going to be different for cell therapy, protein replacement, gene editing and vaccine applications.

CONCLUSION:

PNI proprietary lipid and or LNP compositional library can be used to mitigate the challenges in genomics medicine development by plug and play nature of the technology. We believe that these studies will pave the path to the advancement in nucleic acid-based therapeutics and vaccines, or for cell & gene therapy agents for early diagnosis and detection of cancer, and for targeted genomic medicines.

STRATEGIES FOR PRODUCING CLINICAL AND COMMERCIAL RNA-LNP DRUG PRODUCTS

IAN JOHNSTON, D. Singh; A. Braun; B. MacDougall; B. Ma; A. Lazic; L. Yee; A. St. Quintin; C. Robin; F. Yuen; P. Harvie; S. Abraham; S. Clarke
Affiliations: Precision NanoSystems ULC, Vancouver, Canada

INTRODUCTION

The RNA-lipid nanoparticle (LNP) vaccines for the SARS-CoV-2 pandemic highlight the impact of genomic medicines deployed at scale. RNA-LNP applications also include gene editing, oncology, and rare diseases. Despite this growing momentum, developing RNA-based vaccines and therapeutics still faces significant manufacturing challenges. The mixing process to encapsulate RNA within LNPs is among the most difficult unit operation to scale up to commercial throughput rates and batch sizes, while maintaining critical quality attributes (CQAs) such as size and biological potency. Therefore, we have recently developed a new single-use mixer, enabling pre-dilution RNA-LNP flow rates of up to 800 mL/min. Additionally, we have developed two systems with software that enables 21 CFR Part 11 compliance* (*pending third party audit) and are ATEX/IECEx** (**pending final certification) rated, enabling batch sizes from 0.5 to 3200 L. In this work, we characterize the performance of the new mixer and instrumentation with liposomes and with representative nucleic acid-LNPs. Furthermore, we designed a new SARS-CoV-2

RNA-LNP vaccine candidate, and utilized the complete NanoAssemblr® platform to rapidly scale-up from the research bench to a commercially relevant process.

METHODS

POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine): cholesterol liposomes were formulated at flow rates between 2 and 20 mL/min using the NxGen™ mixer; 50 and 200 mL/min using the NxGen 500 mixer; and 200 and 1000 mL/min using the novel NxGen 48 L/h mixer. The size and polydispersity of the liposomes were measured with dynamic light scattering (DLS). Next, plasmid DNA (pDNA) encoding enhanced green fluorescent protein (eGFP) or self-amplifying mRNA (saRNA) encoding the SARS-CoV-2 spike protein, and a custom lipid composition were used as models for an RNA-LNP vaccine. The flow rate for pDNA-LNP formulation was kept constant at 800 mL/min while the pre-dilution formulation volume was stepwise increased from 5 to 50 L using the NxGen 48 L/h mixer. The saRNA-LNP formulation flow rate was stepwise increased from 12 mL/min using the NanoAssemblr® Ignite+™ through to 800 mL/min using the new NanoAssemblr® commercial formulation system and modular commercial formulation skid. N/P ratio, flow rate ratio, and post-formulation diluent were kept constant. The pDNA- and saRNA-LNPs were concentrated with tangential flow filtration (TFF) and diafiltered into cryo-preservation buffer. The formulation and downstream processing steps were accomplished within a 6-hour shift. Size and polydispersity index (PDI) were measured with DLS. For the saRNA-LNP formulations, size and morphology were determined by cryo-electron microscopy. Nucleic acid encapsulation efficiency was determined with either the RiboGreen assay or the PicoGreen assay for saRNA- and DNA-LNPs, respectively. The saRNA integrity, post-encapsulation, was assayed through capillary gel electrophoresis. In vitro expression of the SARS-CoV-2 spike protein was assessed in BHK 570 cells while *in vivo* expression was assessed in a BALB/c mouse model. Immunization was done by intramuscular injection, with a booster on day 28. Tail bleeds were 1 day before each immunization and mice were culled on day 42. IgG titers were determined by ELISA.

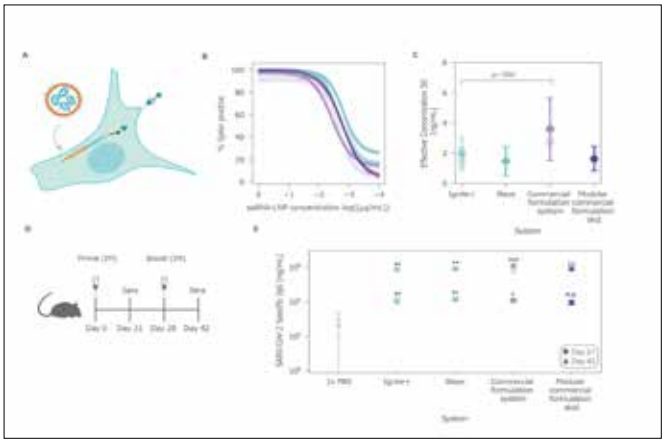

Figure 1. Expression of SARS-CoV-2 antigen and immune response for saRNA-LNPs prepared using NxGen technology.

RESULTS

To probe the quality of mixing within the NxGen series of mixers, we used POPC:cholesterol liposomes, which have a size that is sensitive to subtle changes in mixing. We found that 40 nm limit-size liposomes could be formed at flow rates above 6 mL/min on the NxGen mixer, above 80 mL/min on the NxGen 500, and above 400 mL/min on the NxGen 48 L/h. To demonstrate the NxGen 48 L/h mixer with a representative formulation, we then used pDNA-LNPs as a surrogate for an mRNA-LNP vaccine and prepared three batches of increasing volume. The pDNA-LNPs were of high quality from all three batches with particle sizes ranging from 81 to 85 nm, PDI < 0.16, and DNA encapsulation efficiency >98%. Next, we designed a SARS-CoV-2 saRNA-LNP vaccine candidate and stepwise increased the flow rates and batch volumes to mimic the drug development process from discovery through to commercial production. The saRNA-LNPs were of similar quality with sizes ranging from 61 – 83 nm, PDI < 0.18 and RNA encapsulation efficiency > 94%. One way analysis of variance testing showed no significant differences in size, PDI, or encapsulation efficiency when comparing instrument or mixer. *In vitro* potency assays showed similar dose response curves between samples and consistent EC_{50} values between 1.3 to 3.6 ng/mL. Finally, *in vivo* immunization studies showed a robust SARS-CoV-2 specific IgG response in all instrument-mixer combinations with titers varying between $1.94x10^5$ to $2.83x10^6$ ng/mL.

A) Schematic of *in vitro* testing by transfection of BHK 570 cells. The cells were stained with an anti-spike conjugated AlexaFluor488 antibody for fluorescence imaging. B) Percentage of cells expressing SARS-CoV-2 spike protein in BHK 570 cells as a function of saRNA dose for each system and mixer condition with 95% confidence intervals in shaded areas. C) EC50 values plotted as functions of system. Error bars are 95% confidence intervals. D) Schematic of *in vivo* immunization study design with initial and booster dose noted along with sera collection. E) SARS-CoV-2 specific IgG response in serum from BALB/c mice at day 21 and 42 post-injection for each condition. Error bars are 1 standard deviation. 1X PBS versus instrument comparison p-value for a given time point using post-hoc Tukey test after one-way ANOVA (p≤.05: *, p≤.01: **, p≤.001: ***, p≤.0001: ****).

CONCLUSIONS

This work demonstrates that production of nucleic acid-LNPs with consistent CQAs can be achieved on a wide range of scales using the NanoAssemblr series of instruments and NxGen series of mixers. Additionally, the NxGen 48 L/h and NanoAssemblr commercial formulation system and modular commercial formulation skid can prepare RNA-LNPs at throughputs needed to meet commercial manufacturing product goals. Broadly, the introduction of these two new instruments will simplify the production of RNA-LNP drug products for a wide range of applications.

Acknowledgements: *No funding acknowledgements*

ACHIEVING DENDRITIC CELL SUBSET-SPECIFIC TARGETING IN VIVO BY SITE-DIRECTED CONJUGATION OF TARGETING ANTIBODIES TO NANOCARRIERS

CARINA JUNG

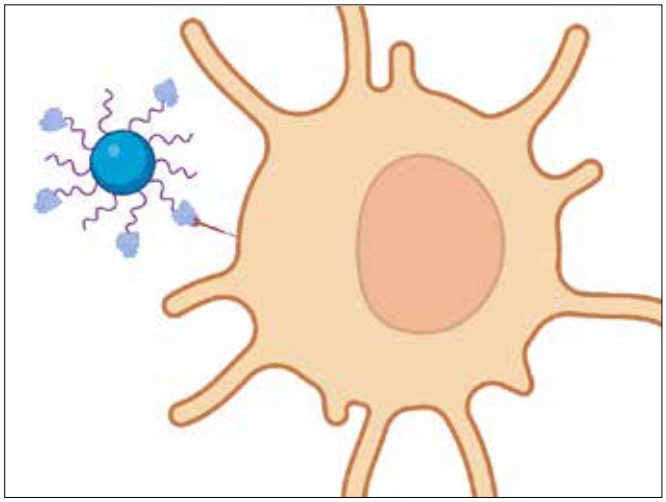

Figure 1: Nanocarrier conjugate binding to receptor on dendritic cell surface.[1]

aIn recent years, nanomedicine, especially in the field of immunotherapy, has emerged as promising treatment approach in the ongoing fight against cancer. An interesting target cell type within the immune system are dendritic cells, due to their ability to cross-present antigens and, thus, induce a T cell response. To trigger this process, it would be favorable to actively deliver an antigen to a dendritic cell, which can be done by introducing a targeting moiety to nanocarriers. However, the design of a suitable carrier system for this purpose, especially one able to efficiently target dendritic cells in the complex *in vivo* environment, is quite challenging.
Here, we functionalize magnetite based nanocarriers with antibodies and their derivatives, and investigate the resulting conjugates in terms of their behavior *in vitro* and *in vivo.*
The functionalization was achieved by enzymatic modification of the antibody, followed by a linker attachment to the nanocarrier surface and a subsequent linking by copper-free azide-DBCO click chemistry (**Figure 2**).

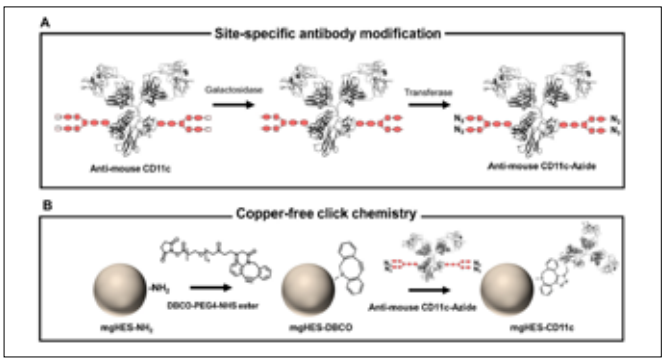

Figure 2: *Synthesis of nanocarrier-antibody conjugates. A) Azide modification of antibodies by enzymatic pathway; B) attachment to nanocarriers via copper-free click chemistry.*[2]

The optimized system shows a highly efficient targeting of dendritic cells *in vitro*, as well as *in vivo* in a biodistribution assay performed in a mouse model (**Figure 3**).

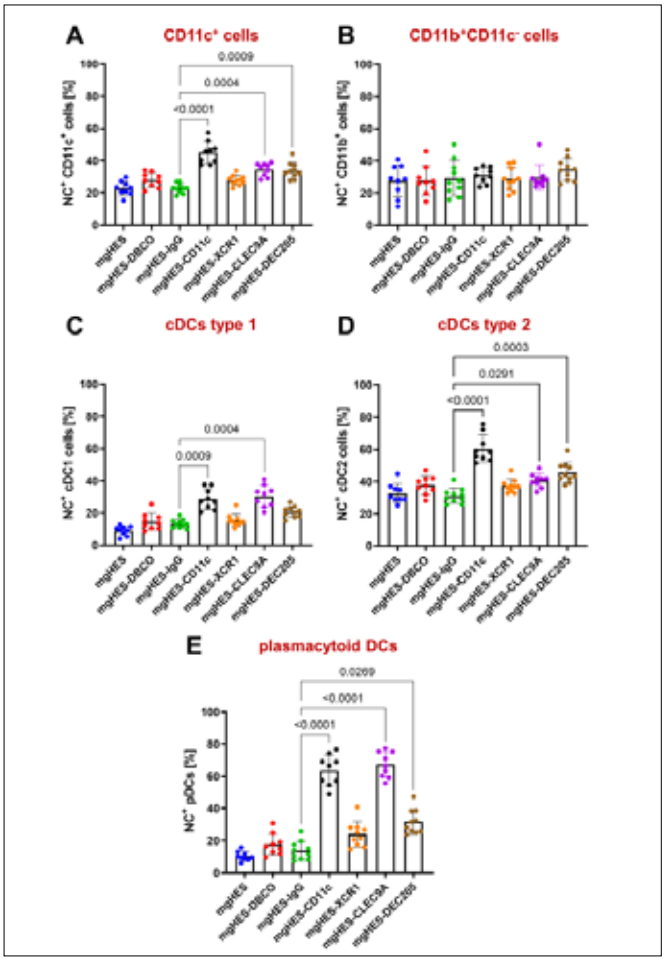

Figure 3: In vivo biodistribution results. Increased uptake of CD11c, Clec9a and DEC205 modified nanocarriers in comparison to their isotype controls was observed in all dendritic cell subtypes.[2]

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EFFECT OF POLYASPARTAMIDE-BASED POLYELECTROLYTES ON CELLULAR UPTAKE

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Polyelectrolytes have been getting increasing attention for their potential applications for drug and RNA delivery. The versatile chemical structure of polyelectrolytes provides multiple opportunities for the nanoformulation of drug molecules as well as nucleic acids to increase blood circulation and cellular uptake and also to achieve endosomal escape. Furthermore, polyelectrolytes exhibit various reactive groups that provide the opportunity for further modification whit targeting agents to explore new targeting strategies. Within this context, this work aimed to study the effect of the chemical structure of polyaspartamide-based polyelectrolytes on the cellular uptake by HeLa cells [1,2].
Polyaspartic acid and polyaspartamide (PASP) based polyelectrolytes (PEs) are biocompatible and biodegradable polymers derived from polysuccinimide (PSI) by nucleophile addition. Due to the high reactivity of the PSI, polyaspartamide-based polyelectrolytes (PASP-PEs) can be synthesized with versatile chemical structures and modified with different biologically active molecules such as drugs or targeting agents [3]antimycobacterials, biocompatible cell encapsulants and tissue adhesives is highlighted. Polyelectrolytes (PELs. Furthermore, PASP derivatives proved to be biocompatible and biodegradable, and these can be likely attributed to their peptide-like structure [4,5]"ISBN":"1944-8252 (Electronic. Due to these features, the number of biomedical applications of PASP-PEs has increased significantly in the last decade [3]antimycobacterials, biocompatible cell encapsulants and tissue adhesives is highlighted. Polyelectrolytes (PELs.
In this present research, we aimed to coat silica nanoparticles (SiNP) with different PASP-PEs and investigate the effect of a polyelectrolyte coating on cellular uptake. Polyelectrolytes have been shown to interact in different ways with proteins and different cells since their protein binding ability depends on the ionic strength and pH of the environment, thus may provide novel properties to nanoparticles when used as a coating[6]. To study this phenomenon, polyanions and polycations were synthesized with different chemical structures and targeting agents from polysuccinimide by nucleophile addition. In addition, commercially available branched polyethylene imine (PEI) was used as a comparison. Silica nanoparticles were used as a nanoparticle core and were coated with the PASP-PEs by adsorption. The size, charge, and stability in cell culture conditions of the PE-coated silica were determined. Finally, HeLa cells were used for *in vitro* experiments in order to determine the effect of the PE-coating on the interaction with cells and uptake efficiency. To follow the cellular uptake, flow cytometry analysis, and confocal microscopy measurements were carried out, while preliminary studies were made with different inhibitors to characterize the uptake mechanism.

The results showed that the polyanion coating had no significant effect on the average size of the Si NPs but coating with polycations induced a slight increase in the average diameter. In both cases, however, the PE coating strongly changed the zeta potential of the Si NPs, further confirming the deposition of the PEs. Thus, the PE-coated silicas which were stable in the cell culture medium were used for further experiments with HeLa cells.

Cell uptake studies showed that the differently charged polyelectrolyte coatings influenced the cellular uptake of the Si NPs differently. While PASP-based polyanion coating had no significant influence on cellular uptake with respect to the bare silica, coating with polycations highly increased the cellular uptake of the Si NPs, as commonly observed for positively charged materials (Figure 1). Application of a second polyanion layer on the polycation layer reverted the effect and led to a slightly decreased uptake in respect of the polycation-coated silica according to the flow cytometry analysis. Interestingly, cell viability results revealed that the PASP-based polycations in solution have very high cytotoxicity, however, when the same polycations were adsorbed on the surface of the Si NPs no significant impact on cell viability was observed. Since adsorbed PASP-based polycations have no relevant effect on viability but strongly increase the uptake of NPs, polycation coating might open unique internalization routes, which is the future perspective of the project.

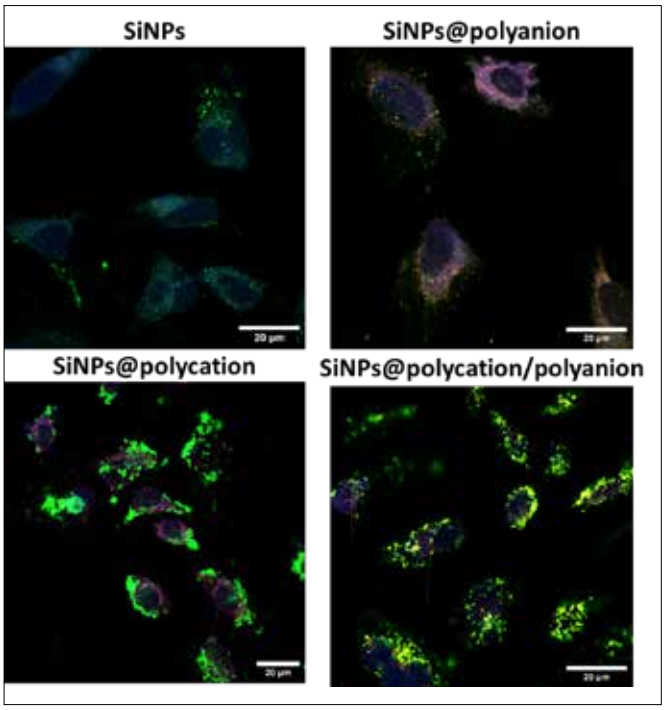

Figure 1.: Confocal microscopy images of HeLa cells treated with differently coated SiNPs

At a broader level, this study showed that different polyelectrolytes could be adsorbed on silica nanoparticles and strongly altered the zeta potential of the original nanoparticles. Experiments on cells revealed that the uptake of the SiNPs can be significantly influenced by using different PASP-based polyelectrolytes in single or multiple layers depending on the final charge of the particles, and that adsorption of a positively charged PE lead to increased uptake, but without the toxicity usually observed with positively charged nanoparticles. These findings may open up new ways to use PE-coatings to increase uptake by cells.

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EMULSION TEMPLATED PROTEIN NANOCAPSULE FORMATION BY INTERFACIAL DENATURATION FOR THE EFFICIENT ENCAPSULATION AND DELIVERY OF ADJUVANTS FOR CANCER IMMUNOTHERAPY

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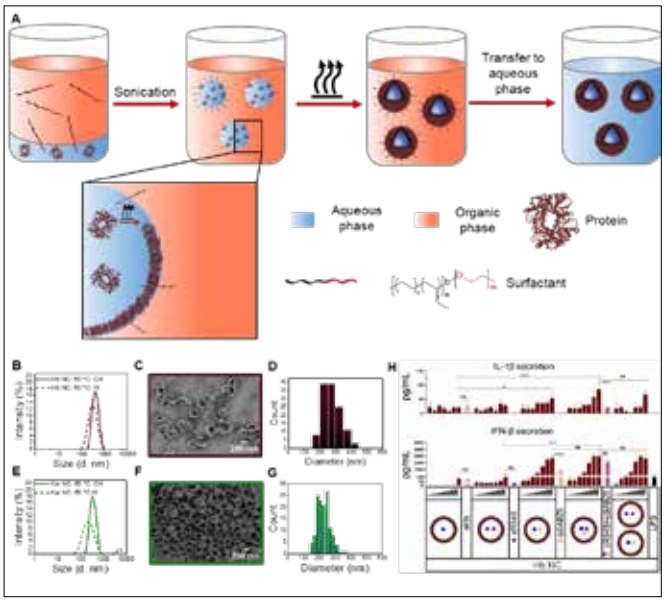
While various types of intracellular delivery nanocarriers have been developed and reported, only a few have successfully been translated to clinical use due to the complex chemistry and rigorous procedures involved in their development and production.[1] Utilizing miniemulsion technique, protein nanocapsules can be synthesized from different types of proteins, for Eg: human serum albumin, ovalbumin, haemoglobin etc. Previously our group have developed various synthetic strategies for protein nanocapsules using cross-linking reactions such as azide alkyne copper free click reactions.[2-4] However in these cases, the additional reagents required for cross-linking complicates the synthetic process. Recently we designed a new class of versatile protein nanocapsules (PNC) using miniemulsion technique, exploiting inherent properties of proteins; denaturation and disulfide bonds, without any additional chemical reactions. Abundantly available natural proteins; haemoglobin, ovalbumin and keratin formed PNC by interfacial confinement and interfacial denaturation in an inverse miniemulsion. While haemoglobin and ovalbumin denatures below 90 ⍰, keratin only denatures at very high temperatures. At the water-oil interface of the inverse miniemulsion, proteins get confined owing to their hydrophobic and hydrophilic amino acids, which results in the partial denaturation of the proteins at the interface and forms PNC shell. Increase in temperature formed stable nanocapsules with haemoglobin (Hb NC), whereas keratin nanocapsules (Ker NC) formation was not influenced by temperature. A model antigen (Ovalbumin) was incorporated in Hb NC and Ker NC easily by mixing the protein and antigen together, without any modification or conjugation. All the NC showed very high encapsulation efficiency, excellent uptake by dendritic cells and had minimal cell toxicity. With the combination of encapsulated super additive adjuvants and the antigen, a novel nanovaccine for cancer immunotherapy was developed. Because there is no complex reactions or reagents involved in the synthesis, we believe the clinical translation of these nanocarriers will be easier compared to others.

Cancer is a complex and multifactorial disease that affects millions of people worldwide. Immunotherapy has emerged as a promising approach to cancer treatment, as it harnesses the patient's own immune system to fight cancer cells. Cancer vaccines are the one approach to cancer immunotherapy, using tumor cell-associated antigens, to awaken the body's immune system against cancer. Several studies have already shown that Toll-like receptor 7 (TLR7/8) agonist R848 or STING agonist diABZI-loaded nano vaccines can elicit immune responses to achieve the desired effect of cancer treatment[5,6]. In this study, combined STING and TLR 7/8 agonists were encapsulated in the nanocapsules to enhance the immune response to cancer vaccine. To assess the ability of adjuvant-loaded PNC to stimulate bone marrow derived dendritic cells (BMDCs), cytokine secretion and expression of CD80 and CD86 co-stimulatory molecules were measured after 24 h of incubation with various PNC formulations. The results showed that the combination of R848 and diABZI can synergistically enhance the cytokines' expression compare to R848 alone. Consistent with the results obtained from cytokine expression analysis, dual adjuvants loaded NCs can synergistically upregulate co-stimulatory molecules' expression in BMDC. In short, dual adjuvant encapsulated PNC showed their capacity to enhance the immune response.

Figure 1: A) Synthesis of protein-nanocapsules (PNC) by miniemulsification method. Proteins dissolved in the dispersed phase (water) and

*surfactant P((E/B)-b-EO) (low HLB surfactant) dissolved in the continues phase (cyclohexane) was emulsified using ultrasonication. The emulsion was heated to obtain PNC in the organic phase. In this step, protein get confined and denatured at the oil water interface. After purification to remove excess of the surfactant, PNC in cyclohexane was transferred to water using SDS as a surfactant (high HLB surfactant), cyclohexane was evaporated at room temperature to obtain PNC exclusively in water and then SDS was removed by centrifugal filtration. Dynamic light scattering (DLS) chromatograms of B) haemoglobin nanocapsules (Hb NC), E) keratin nanocapsules (Ker NC) obtained at 60°. Their corresponding C, F) SEM images and D, G) size distribution obtained from SEM measurements, brown-Hb, green-Ker, CH (cyclohexane), W (water). H) Evaluation of the efficacy of adjuvants loaded Hb NC in inducing cytokine secretion by bone marrow-derived dendritic cells (BMDCs). To achieve this, BMDCs were exposed to varying concentrations (0.1, 0.3, 1, 3, 10, 30, 100 µg/mL) of empty or adjuvant-loaded Hb NC, soluble haemoglobin (sHb), soluble R848 (sR848, 600 ng/mL), soluble diABZI (sdiABZI, 1.1 µg/mL), or LPS (100 ng/mL) for 24 hours. The concentration of soluble adjuvants used for treatment was equivalent to amount present in 100 µg/mL of Hb NC. The supernatants of BMDCs were collected to detect IL-1β, and IFN-β expression (mean ± SD; n=3). All statistical analyses were performed using one-way ANOVA with Turkey's post hoc test. (ns, not statically significant *P ≤ 0.05; **P ≤ 0.01; ****P ≤ 0.0001).*



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REPURPOSING CU(DDC)$_2$-LIPOSOMES AS ANTIBACTERIAL AGENT FOR STAPHYLOCOCCI INFECTIONS

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INTRODUCTION

Staphylococcus aureus and *Staphylococcus epidermidis* are pathogens associated with life-threatening infections, such as bacteraemia and surgical site infections. Standard treatment with antibiotics frequently fails due to the rise of antibiotic resistance and the formation of biofilms. Biofilms are clusters of bacteria embedded in a matrix that acts like a shield against antibiotics and the immune system. Therefore, new antibacterial treatments are urgently needed [1].

We previously showed that a combination of diethyldithiocarbamate (DDC^-) and copper ions (Cu^{2+}) is a promising new antibacterial strategy against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and *S. epidermidis*. The effects against bacteria and biofilms were linked to the formation of the $Cu(DDC)_2$ complex (2:1 molar ratio of DDC^- and Cu^{2+}) and an excess Cu^{2+} [2]. However, $Cu(DDC)_2$ is water insoluble, limiting its practicality in the clinical setting and requiring the development of a pharmaceutical formulation [3].

To improve the solubility of $Cu(DDC)_2$, nanoparticles including liposomal formulations of $Cu(DDC)_2$ have been developed and successfully used in pre-clinical experiments as therapeutically active agents against cancer cells [4]. Inspired by this, we aim to develop a drug delivery vehicle for $Cu(DDC)_2$ + Cu^{2+} by repurposing $Cu(DDC)_2$-liposomes as antibacterial agents and investigating the *in vitro* and *in vivo* activity against staphylococci in combination with Cu^{2+}.

MATERIALS AND METHODS

Chemicals and bacterial strains

DDC^-, Cu^{2+} and cholesterol were purchased from Sigma-Aldrich (Steinheim, Germany) and the lipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycerol)-2000] (DSPE-mPEG2000) were donated by Lipoid GmbH (Ludwigshafen, Germany). The bacterial strains *S. epidermidis* ATCC 35984 and MRSA Mu 50 were purchased from the American Type Culture Collection (Manassas, VA, USA).

Liposomes preparation

Cu^{2+}-liposomes composed of DSPC, cholesterol, and DSPE-$mPEG_{2000}$ at a molar ratio of 50:45:5 were prepared by the thin-film hydration method and purified by size exclusion chromatography using a Sephadex™ G-50 fine (GE Healthcare Life Science) column. Cu^{2+}-liposomes were collected in sucrose-HEPES buffer. $Cu(DDC)_2$-liposomes were prepared by remote loading of DDC^- into Cu^{2+}-liposomes and purified by centrifugation using Vivaspin® Turbo 4 filtration units (100 kDa MWCO, Sartorius AG, Göttingen, Germany)[4].

Prior to use, Cu^{2+}-liposomes and $Cu(DDC)_2$-liposomes were sterile filtered with a 0.2 µm cellulose acetate filter (VWR International) and the hydrodynamic diameter and polydispersity index of the liposomes were determined by dynamic light scattering using a ZetaPals (Brookhaven Instruments Corporation, Holtsville, NY, USA). The $Cu(DDC)_2$-liposomes + Cu^{2+}-liposomes were mixed in a 1:6.2 molar ratio and used at a final concentration of 128 µM.

In vitro antibacterial activity of liposomes

S. epidermidis and MRSA biofilms were grown in a black-walled 96-well microtiter plate for 24 h at 37 °C on a rotating platform. Rinsed biofilms were then incubated with $Cu(DDC)_2$-liposomes, Cu^{2+}-liposomes, free Cu^{2+}, $Cu(DDC)_2$-liposomes + free Cu^{2+} or $Cu(DDC)_2$-liposomes + Cu^{2+}-liposomes or free $Cu(DDC)_2$ + Cu^{2+} for 24 h. Biofilm

viability was assessed following incubation with 10% alamarBlue cell viability reagent (Thermo Fisher Scientific) by fluorescence measurements on a TECAN Spark plate reader (Männedorf, Switzerland) at $\lambda_{excitation}$ = 530 nm/ $\lambda_{emission}$ = 590 nm. The percentage of biofilm viability was calculated based on the difference in fluorescence intensity between treated and untreated biofilms [5].

***In vivo* antibacterial activity of liposomes**

Galleria mellonella larvae (Angel-Zentrum, Freiburg, Germany) were injected in the last left proleg with micro-fine (30 gauge) needle insulin syringes (BD, Franklin Lakes, NJ, USA) containing a mixture of *S. epidermidis* with Cu(DDC)$_2$-liposomes + free Cu^{2+} or Cu(DDC)$_2$-liposomes + Cu^{2+}-liposomes. Larvae were incubated in the dark at 37 °C and survival was visually assessed over 4 days (Figure 1). Controls included non-injected larvae (control), uninfected or infected larvae treated with 0.9% NaCl (vehicle control), and uninfected, treated larvae (toxicity control). Results were analysed using Kaplan-Meier survival curves with significant differences between groups determines by log-rank test, significance was Bonferonni-Holm-corrected for multiple comparisons.

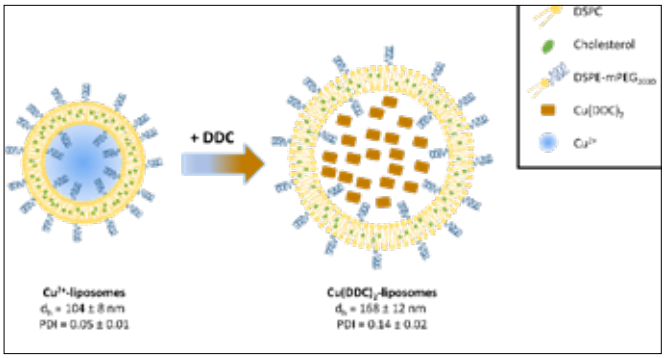

Figure 1*: Infection and injection of Galleria mellonella (left); Visual assessment of survival after 4 days: living larvae (middle) and dead larvae (right).*

RESULTS

Liposome characteristics

While the hydrodynamic diameter was below 200 nm for both liposomes, Cu^{2+}-liposomes were smaller than Cu(DDC)$_2$-liposomes. In addition, the PDI of Cu^{2+}-liposomes and Cu(DDC)$_2$-liposomes was below 0.2, indicating a homogenous population of liposomes (Figure 2).

Figure 2*: Size (as hydrodynamic diameter d_h) and polydispersity index (PDI) of Cu^{2+}-liposomes and Cu(DDC)$_2$-liposomes.*

Antibiofilm activity of liposomal Cu(DDC)$_2$ + Cu^{2+}

No reduction in biofilm viability was observed when MRSA and *S. epidermidis* biofilms were treated with Cu(DDC)$_2$-liposomes, Cu^{2+}-liposomes, free Cu^{2+}, and Cu(DDC)$_2$-liposomes + Cu^{2+}-liposomes. Only treatment with Cu(DDC)$_2$-liposomes + free Cu^{2+} and Cu(DDC)$_2$ + Cu^{2+} significantly reduced the biofilm viability of MRSA and *S. epidermidis*.

Efficacy of liposomal Cu(DDC)$_2$ + Cu^{2+} in *Galleria mellonella.*

Treatment of the uninfected larvae with Cu(DDC)$_2$-liposomes + free Cu^{2+} or Cu(DDC)$_2$-liposomes + Cu^{2+}-liposomes indicated no toxicity of the liposomes. *S. epidermidis* infected larvae that were injected with the vehicle control resulted in a survival rate below 30%. However, a significant increase in survival was observed in *S. epidermidis* infected larvae treated with Cu(DDC)$_2$-liposomes + free Cu^{2+} or Cu(DDC)$_2$-liposomes + Cu^{2+}-liposomes ($p < 0.01$), indicating treatment efficacy.

CONCLUSION

The repurposed Cu(DDC)$_2$-liposomes combined with free Cu^{2+} showed *in vitro* antibacterial activity and combined with free or liposomal Cu^{2+} demonstrated non-toxicity and efficacy *in vivo*. These nanoparticles present a novel treatment strategy against staphylococci infections and a mammalian animal study is warranted to further investigate efficacy and safety.

ACKNOWLEDGMENTS

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DESIGNING NANOMEDICINE LIBRARIES VIA CUSTOM-MADE 3D-PRINTED MICROFLUIDICS FOR APPLICATIONS IN HEMATOLOGICAL MALIGNANCIES

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INTRODUCTION

Nanomedicines have shown significant value in solid malignancies, offering promising solutions for targeted therapies that result in

enhanced treatment efficacy and reduced toxicity. However, their potential applications in (rare) hematological malignancies remain comparatively less investigated. Regarding the existing treatments, most hematological malignancies rely on palliative approaches, while in certain cases only allogeneic stem cell transplantation offers a curative solution. Hence, exploring the nanomedicine potential against (rare) hematological malignancies will offer new possibilities for improved patient outcomes. Nanoparticles are traditionally composed via top-down approaches, which, although well-established, are time-consuming for preclinical research. In contrast, bottom-up strategies (e.g., rapid development of nanoparticle libraries via microfluidics) can accelerate formulation production and evaluation in preclinical research. In this study, we prepared a nanoparticle library consisting of lipid-based nanoparticles (liposomes (LP), lipid nanoparticles (LNP), and nanoemulsions (NE)) by using custom-made microfluidic chips and via a Design-of-Experiment (DoE) approach (**Fig. 1A**). Our aim is to **(i)** to investigate the significant input variables and their impact on the output (size and PDI) to predict and control nanoparticle (NP) characteristics more efficiently, and **(ii)** evaluate the engagement of the various nanomedicines against hematological malignancies.

METHODS

Microfluidic chips with an internal architecture enabling passive micromixing of solutions were fabricated using 3D printing. The internal architecture and the compatibility with different organic solvents used in nanoparticle manufacturing were evaluated via computed tomography (CT) scans conducted after each 40 ml of total injection. The CT scans were repeated up to a total volume of 720 ml, which corresponds to approximately 180 formulations. Then, a nanomedicine library was composed of three types of nanoparticles, including LP, LNP, and NE. The properties and relationship between input parameters and size/PDI of lipid-based nanoformulations were analyzed using a DoE based on Box-Behnken Design (BBD), as the most efficient DoE design. Finally, the cytocompatibility was assessed using an apoptosis assay, while cell uptake of the formulations was studied in hematological malignancies representative cell lines, including K562, THP.1, MM.1S, and 32D through FACS analysis.

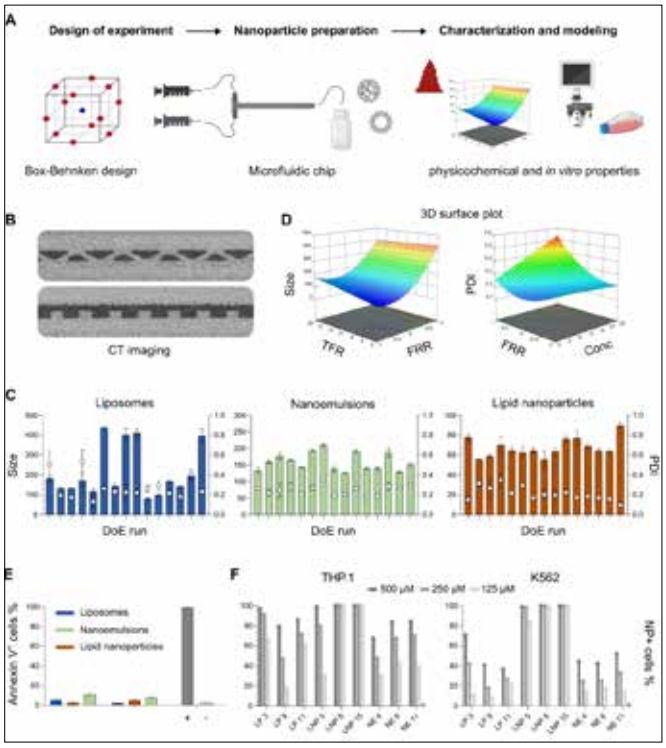
RESULTS

CT imaging revealed a robust meander-like internal architecture of the polypropylene-made chips, which exhibited excellent tolerance upon exposure to PBS, ethanol, methanol, chloroform, and tetrahydrofuran (**Fig. 1B**). Dynamic light scattering measurements on 15 initially designed formulations per category revealed size and dispersity variations, which were dependent on the selected manufacturing parameters (**Fig. 1C**). Subsequent modeling of such parameters, including concentration, total flow rate, and flow rate ratios elucidated the contribution of such parameters to the characteristics of the final product (**Fig. 1D**). The accuracy of the modeling procedure was cross-checked by developing additional formulations, using parameters tested for the first time. Furthermore, cytocompatibility assays showed no significant toxicity of the empty nanoformulations on the tested cell lines (**Fig. 1E**). The cell uptake of the nanoformulations was influenced by the size of the formulations. Nanoparticles within the optimal size range demonstrated significantly higher uptake compared to the larger nanoparticles, primarily due to differences in uptake pathways. Additionally, LNPs demonstrate considerable uptake compared to other formulations, attributed to the inclusion of cationic lipids that enhance cellular uptake (**Fig. 1F**).

CONCLUSIONS

Our study focuses on the rapid and versatile production of a nanoparticle library using a bottom-up strategy, ensuring reproducibility. Mathematical modeling played a crucial role in predicting the nanoformulation properties based on the initial settings. Notably, the formulations exhibited considerable uptake without showing any toxicity. The variability in uptake highlights the importance of selecting the appropriate formulations for specific cell lines, emphasizing its potential engagement with such cells in an *in vivo* scenario.

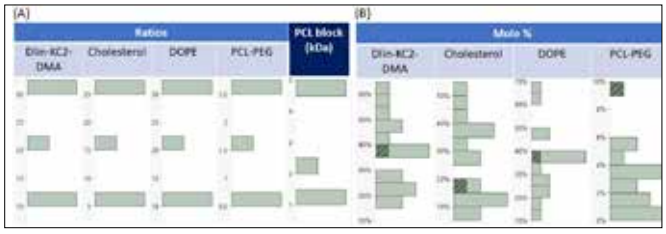
Figure 1. A. *Nanomedicine libraries study design.* **B.** *Coronal and sagittal view of the 3D-printed microfluidic chip.* **C.** *Size and dispersity variations of the nanoformulations.* **D.** *3D surface plot of Box-Behnken modeling.* **E.** *Apoptosis assay.* **F.** *Cell uptake study.*



DEVELOPMENT AND OPTIMIZATION OF NEXT-GENERATION LIPID NANOPARTICLES FOR IN-SITU CAR-T PRODUCTION

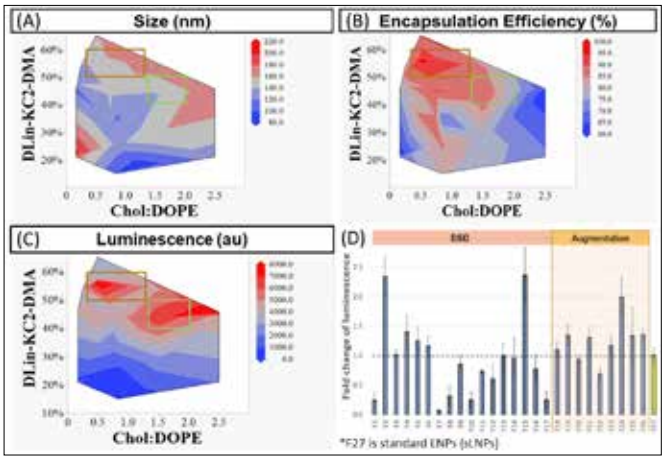
BUMJUN KIM
and Robert Prud'homme

Current chimeric antigen receptor (CAR) T cell therapy employees the ex-vivo engineering of T cells, which require expensive and complicated CAR-T cell and viral vector manufacturing facilities. In-situ engineering of T cells via synthetic lipid nanoparticles (LNPs) may obviate the needs for the complex manufacturing processes and facilities. However, targeting non-hepatic cells and tissues remain the major challenge for LNP-based therapeutics. One of the major mechanisms of liver targeting of LNPs is a result of the shedding of poly(ethylene glycol) (PEG)-lipids and adsorption of apolipoprotein E (ApoE) on LNPs, leading to low-density lipoprotein receptor (LDLR)-mediated uptake by hepatocytes.[1] PEG-lipid shedding occurs at a rate of 26%/h.[2] Despite the recent promise of antibody (Ab)-directed targeting of LNPs toward T cells[3], anchoring the large Abs onto the LNP surface by the weak anchoring energy of lipid tails will result in partitioning the Ab-conjugate lipids off of the circulating LNPs even more rapidly than PEG-lipids. To reduce the liver targeting and improve the T cell engineering *in vivo*, a next-generation LNP (ngLNP) needs to be developed.

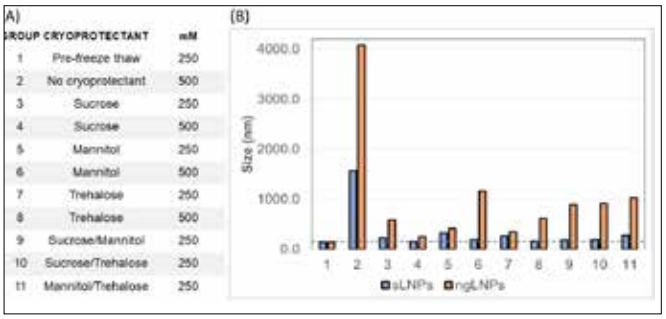


Here, we replace the lipid-PEG with poly(ε-caprolactone)-block-poly(ethylene glycol) (PLC-b-PEG) and optimize the formulation for T cell transfection. By anchoring PCL-b-PEG that has a larger hydrophobic block compared to lipid tails in PEG-lipids, "shedding off" event will be minimized even after the attachment of hy-

drophilic Abs. Using design of experiments (DOE), we selected 26 unique LNP formulations out of large LNP design space (35 =243 possible formulations) (**Fig 1A-1B**). We identified the design space where the LNPs are smaller than 160 nm, achieve high encapsulation of pDNA (>85%), and exhibit two times higher transfection in Jurkat cells compared to standard LNPs (stLNPs) (**Fig 2A-2D**). The optimized ngLNP formulation did not compromise the *in vitro* T cell transfection. Sucrose improved the freeze-thaw stability of ngLNPs (**Fig 3A-3B**).



Future study will involve the conjugation of anti-CD3 Abs onto ngLNPs; the biodistribution and the efficiency of *in vivo* CAR-T production will be evaluated in comparison to stLNPs conjugated with Abs.Acknowledgement: I would like to thank Dr Robert Prud'homme for the guidance on this project I would also like to thank Genentech and Bill Melinda Gates Foundation for supporting this project



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PREPARATION OF SMALL MULTILAMELLAR VESICLES USING DUAL CENTRIFUGATION

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Dual Centrifugation (DC) is a new in-vial homogenization technique for the preparation of liposomes with high entrapping efficiencies (EE) of above 50%. The DC process is based on the additional rotation of vials filled with a viscous lipid-buffer mixture during conventional centrifugation, which results in strong sample movements and thus efficient homogenization [1]this additional rotation constantly forces the sample material towards the center of the centrifuge. This unique combination of two contra rotating movements results in shear forces and thus, in efficient homogenization. We demonstrated that it is possible to prepare liposomes by DAC, by homogenizing a rather concentrated blend of hydrogenated phosphatidylcholine and cholesterol (55:45 mol%. Here we show that increasing lipid concentrations during DC-homogenization (10 – 80%) lead to a continuous increase in the lamellarity of the resulting liposomes. If the lipid concentration is higher than needed for the formation of densely packed small unilamellar vesicles (SUVs), the excess lipids form additional inner membranes inside the resulting vesicles. At optimal lipid concentration, which depends on the lipid composition used for DC-homogenisation (about 50 – 70%), predominantly small multilamellar vesicles (SMVs) are formed. Those vesicles are not only characterized by the number of membranes and a very small aqueous core but also by a rather small size of approximately 150 nm and very low PDI values [2,3]. The low PDI values of the SMVs can be explained by the complete filling with membranes that cooperate and make the SMVs much stiffer than unilamellar vesicles.

To determine the lamellarity, a rapid inaccessible surface (IAS) assay has been developed based on the quenching of a membrane-bound fluorescence marker. Cryo-TEM images prove the existence of SMVs and confirm the IAS assay results [3]. The cooperative membrane effects of SMVs were investigated by differential scanning calorimetry (DSC).

SMVs are easily accessible by DC and appear to be a promising carrier for the delivery of lipophilic drugs.

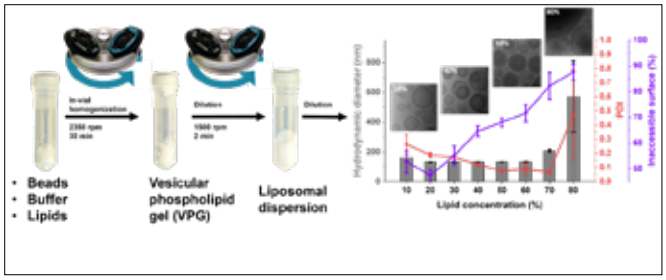

Figure 1: Preparation process of liposomes by DC and resulting liposome characteristics after dilution of the vesicular phospholipid gel. The hydrodynamic diameter, size distribution (PDI), and the inaccessible surface and thus liposome lamellarity are strongly dependent on the lipid concentration used during DC-homogenization. Published in Koehler et al., 2023, Pharmaceutics [3]

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DEVELOPMENT OF PH-RESPONSIVE LIPID-BASED NANOTRANSPORTERS AIMED AT EFFECTIVE SIRNA DELIVERY

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INTRODUCTION

The use of lipid-based RNA delivery nanosystems has seen a considerable breakthrough in recent years with FDA approval of the earliest so formulated siRNA drug ONPATTRO for the treatment of transthyretin-mediated amyloidosis [1] followed by authorizing COVID-19 mRNA vaccines developed by Pfizer/BioNTech and Moderna [2]. One of the key factors for their successful implementation in clinical practice was good efficiency of RNA release rate at the site of action undoubtedly caused by the presence of pH-responsive ionizable lipids facilitating endosomal escape of a payload [2, 3]. Importantly, each lipid-based nanocarrier must be modified with a coating polymer to increase its biocompatibility and avoid interaction with immune system elements, for which polyethylene glycol (PEG), the only FDA-approved surface moiety so far, is usually employed [4]. However, it has been reported that ordinary PEG coating hinders drug release by limiting endosomal escape. Therefore, it is essential to incorporate a pH-responsive anchor into the nanocarrier structure to make the PEG shed with the decreasing pH in an endosome [5]. Lipid-based nanotransporters used in this contribution contain oxime linkage that meets the pH-sensitivity, biocompatibility and hydrolytic stability requirements [6]. Nanostructures so formulated can solve one of the main actual pitfalls in nanomedicine, namely figuring out effective RNA-drug release at a desired spot once in a cell [7].

MATERIALS AND METHODS

As a first step, cationic empty lipid nanoparticles were prepared by combining appropriate aliquots of four different lipids, e.g. DODAG, DOPE, cholesterol and CPA in desired molar ratio. Thereafter, siRNA was entrapped based on the opposite charge between the nucleic acid and cationic lipid. PEGylation of so formed lipoplexes was accomplished by click chemistry-based coupling of aldehyde group of mPEG2000 and aminoxy group of CPA lipid forming pH-sensitive oxime linkage. To explore their structure at neutral pH environment and structural changes and behaviour at mildly acidic conditions simulating the local endosomal environment, lipid-based nanotransporters were imaged by cryo-TEM. Functionality studies were performed using target hepatocellular carcinoma cells expressing hepatitis B virus (HBV) functional viral particles (HepG2.2.15 cell line) where gene silencing efficiency was quantified by RT-PCR within 6 days post transfection with comparison of the knockdown effect between two anti-HBV siRNAs (1407 and chemically modified 1407-ALN) and corresponding controls (1407C and chemically modified 1407C-ALN) entrapped in prepared lipid nanotransporters. These were also screened for their impact on HepG2.2.15 cytotoxicity. Last but not least, the subsequent confocal laser scanning microscopy was employed to monitor double-labelled lipid nanotransporters (Cy5 labelling the lipids, AlexaFluor 488 labelling the siRNA) in HepG2 cells upon the cellular uptake.

RESULTS

Hereby we present the study focused on basic characteristics of formulated pH-responsive lipid-based nanotransporters to efficiently deliver and release siRNA API *in vitro.* They are designed to be intact at neutral pH and hydrolysable under slightly acidic conditions, which trigger PEG shedding (Figure 1A). This was confirmed by cryo-TEM revealing their stable and bilamellar structure at physiological pH, whereas with the local pH decrease lipid-based nanotransporters turned into multilamellar fused structures presumably due to exposure of lipid surfaces upon PEG shedding (Figure 1B).

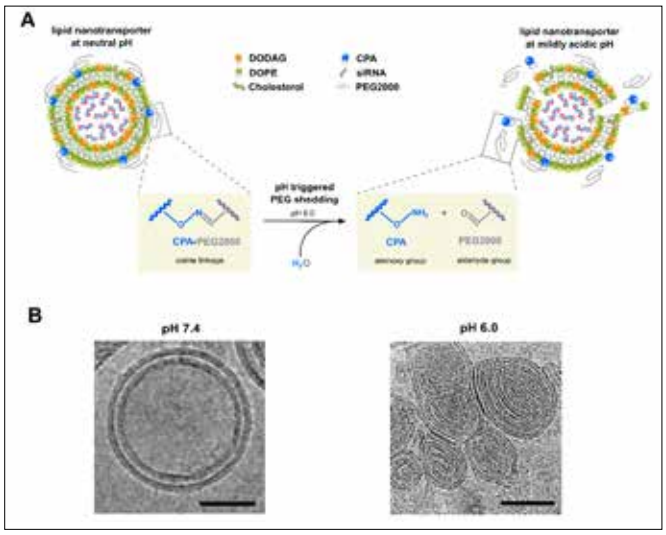

Figure 1. Behaviour of lipid nanotransporters with entrapped siRNA upon pH decrease. *(A) Schematic depiction including pH-triggered click chemistry-based PEG shedding reaction. (B) Representative cryo-TEM images of lipid nanotrasporters under two different pH conditions. Scale bar: 50 nm.*

Cellular uptake was monitored in HepG2 cell line at different time points. Images taken by high-resolution confocal laser scanning microscopy demonstrated that double-labelled siRNA-carrying lipid nanotransporters were accumulating in the cells 8 hours post transfection, while 24 hours of incubation resulted in increased AF488-siRNA fluorescence signal with capturing its diffusion to the surrounding environment staining the entire cells, which indicates siRNA release (Figure 2A). The investigations of targeting siRNA gene silencing effect in HepG2.2.15 cells showed a significant drop of the target viral gene expression down to 40%/45% one day after the transfection with 1407/1407-ALN siRNA entrapped in lipid nanotransporters, with maintaining their target gene down-regulating effect for the next four days, compared to the effect of control siRNAs (Figure 2B). The very high cell viability levels proved no general impact of any siRNA on HepG2.2.15 cytotoxicity upon nanotransporter-mediated transfection.

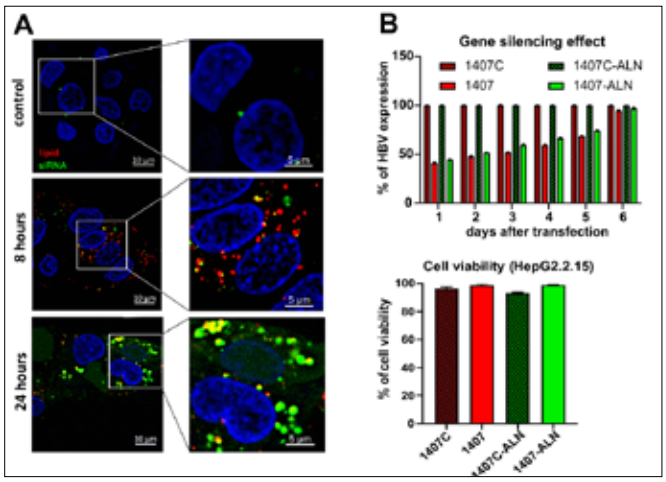

Figure 2. Lipid nanotransporter-mediated siRNA delivery to target cells. *(A) Cellular uptake by HepG2 cells imaged by confocal laser scanning microscopy at two different time points visualizing Cy5-labelled lipid (red), AlexaFluor 488-labelled siRNA, their colocalization (yellow) and Hoechst-stained nuclei (blue). Scale bar of the original images: 10 µm. Scale bar of zoomed cut-outs: 5 µm. (B) Anti-HBV siRNAs knockdown effect in HepG2.1.1.15 cells (top panel) and cell viability (bottom panel).*

CONCLUSION

In conclusion, this work presents the insight into the development of new generation lipid-based pH-responsive nanotransporters

fully enabling functional intracellular delivery of therapeutic siRNA to mediate mRNA knockdown *in vitro* with a strong potential for *in vivo* use.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support from The Czech Health Research Council (project no. NU20-03-00477). We also acknowledge the Cryo-Electron Microscopy and Tomography Core Facility supported by the Czech Ministry of Education, Youth and Sports (MEYS CR, LM2018127) and support by JCMM (Brno Ph.D. Talent).

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FUNCTIONALISED LIPOSOMES FOR AUTOMATED FLUORINE-18 SURFACE RADIOLABELLING AND IN VIVO PET IMAGING

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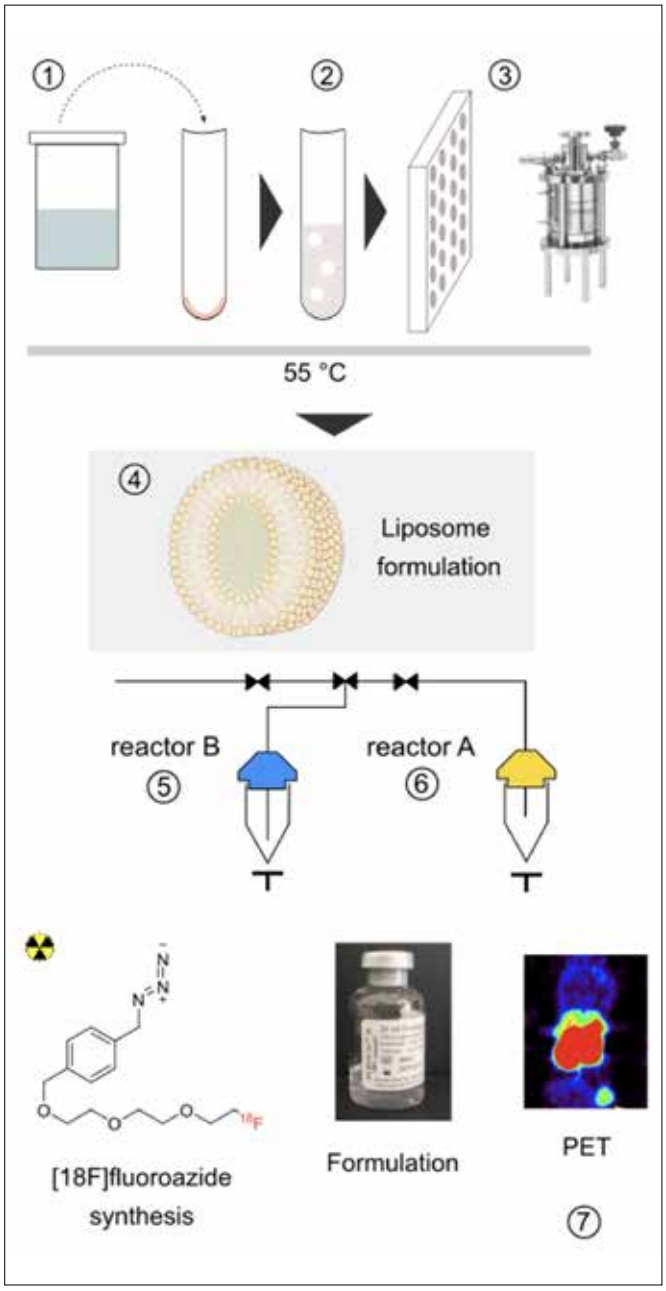
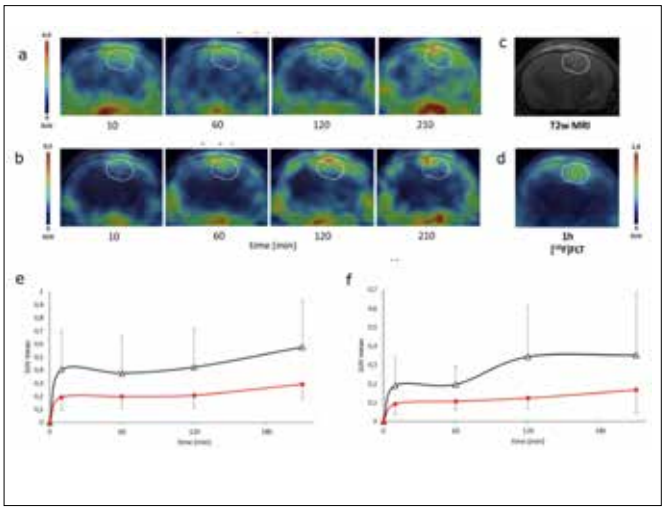
RATIONALE AND AIM

Liposomes were functionalised with a peptide derived from the receptor-binding domain of the apolipoprotein E (mApoE), useful to promote the blood-brain barrier (BBB) crossing, and with an MMP-sensitive lipopeptide (MSLP) for an MMP-triggered drug release. An automated liposome surface radiolabelling was performed both via copper-catalysed azide-alkyne cycloadditions (CuAAC) and copper-free cycloaddition, using a fluorine-18 labelled azide on alkyne-DOPE constructs embedded in liposomes and. A glioma model obtained with Gli36ΔEGFR cells was used for the *in vivo* biodistribution studies.



Scheme of automated radiolabelling of liposomes. (1) Film formation and (2) hydration followed by (3) downsizing using a thermobarrel extruder; (4) liposome physicochemical characterization. Fluorine-18 production in a reactor (5), liposome surface radiolabelling (6), and in vivo biodistribution (7).

Liposomes have great potential as imaging agents for positron-emission tomography (PET) due to their drug delivery and controlled-release capabilities. With such imaging technique, labelled liposomes can non-invasively be tracked, providing functional information on pharmacokinetics and biodistribution parameters. Here, liposomes were functionalised with a peptide derived from the receptor-binding domain of the mApoE, useful to promote the BBB crossing, and with an MSLP for an MMP-triggered drug release; an automated liposome surface radiolabelling was performed both via copper(I)-catalysed alkyne-azide cycloaddition (CuAAC) and copper-free alkyne-azide cycloaddition approaches, both using a fluorine-18 labelled azide ([18F]B). Radiosynthesis was entirely automated on a radiosynthesis system, from cyclotron-produced [18F]B to the final [18F]-Lip products, here called 18F]C-Lip, obtained by CuAAC, [18F]D-Lip and [18F]E-Lip obtained by copper-free alkyne-azide cycloaddition radiolabelling. [18F]E-Lip is the dual-functionalised formulation. High radiochemical purity and suitable yields (5 to 10% according to the cycloaddition approach) were obtained with [18F]-Lip endowed with a hydrodynamic size smaller than 200 nm



PET images of [18F]D-Lip (a) and [18F]E-Lip (b) from 10 to 210 min. Representative T2w MRI image (c) and PET image at 1h for [18F]FLT (d). The white line indicates the tumour area depicted on MRI and transferred to PET images. [18F]FLT PET imaging was used as a radiotracer clinical standard on orthotopic glioma model obtained with Gli36ΔEGFR cells. Tumour uptake quantification of [18F]D-Lip (e) and [18F]E-Lip (f). Data are expressed as SUV mean.

low-medium dispersity, and negative ζ-potential. As expected, the liposome's charge increased as a function of m-DOPE concentration, although the liposome's particle size, polydispersity, and Span values did not change. Nanoparticle's intracranial and systemic biodistribution has been evaluated *in vivo* in an orthotopic mouse model of glioma with PET up to three hours post-injection (n=4). Tumour uptake of [18F]-LIP was slightly higher than functionalised [18F]-Lip but with lower tumour-to-normal brain parenchyma ratio levels. This data suggest that functionalisation prevents the uptake in non-target brain tissue.

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OVERCOMING IMMUNE SUPPRESSION: REPOLARIZING TUMOR-ASSOCIATED MACROPHAGES WITH PH-MODULATING CAPSULES FOR ENHANCED CANCER THERAPY

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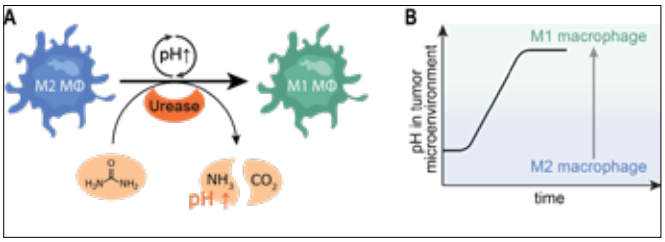
Tumor-associated macrophages emerge as major suppressors of the innate immune response. Specifically, the prevalence of anti-inflammatory M2 macrophages presents a significant hurdle, dampening the immune system's ability to target cancer cells effectively. A potential solution lies in the repolarization of these M2 macrophages into pro-inflammatory M1 macrophages, a process that can be induced by increasing the pH value of the tumor's microenvironment. [1]

The application of antibody-urease conjugates has already demonstrated a pH-modulating effect by converting endogenous urea into ammonia *in situ*.[2] However, a considerable limitation of these antibody-enzyme conjugates is the rapid enzymatic degradation, which affects delivery efficiency and inhibits a permanent pH-modulation. Here we discuss the preparation of urease-loaded capsules and their application for the pH-modulation of the tumor microenvironment.

Fig. 1: Repolarization of anti-inflammatory M2 macrophages to pro-inflammatory M1 macrophages by increasing the tumor's pH. A Catalytic reaction of urea into ammonia and carbon dioxide using urease. B Graphical illustration of the pH value in time and repolarization of macrophages.



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NEBULIZATION OF SIRNA: ESTIMATING THE IMPACT OF THE TRANSITION FROM POLYPLEX TO MICELLEPLEX

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Respiratory diseases such as COPD, asthma bronchiale, infections or lung cancer are one of the leading causes of death worldwide. Short interference RNA (siRNA)-based therapy is a promising approach for treating all these diseases. However, even with numerous siRNA-based therapies being investigated in clinical trials, only a few were actually approved, all of which can only be administered through injection1. Up until now, no direct inhalation-based delivery system for RNA achieved approval. This is mainly due to the fact that lipid nanoparticle (LNP) based systems such as Onpattro® were optimized for injection-based delivery and without optimization are severely altered by nebulization2. In this study, we present our approach to applying different commercially available vibrating mesh nebulizers (VMN) on polymer-based siRNA delivery agents. Our aim was to evaluate the impact of the Pari eFlow® rapid and Aerogen® Pro nebulizers on physicochemical and in-vitro performance parameters of RNA nanoformulations.

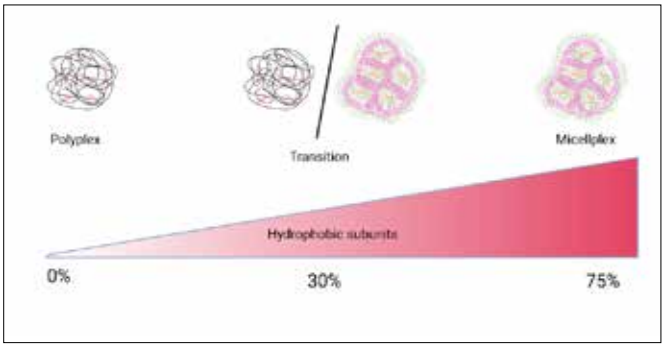

Figure 1. Schematic transition of polyplexes to micelleplexes

By varying the number of hydrophobic subunits within our polymers, we can convert the resulting nanoparticle systems from polyplexes to micelleplexes (Figure 1.). By doing so, we get insights into different behaviors of polyplexes and micelleplexes during nebulization.

We, therefore, applied polyethyleneimine (PEI) as an example of a well-characterized polyplex. For the transition to micelleplexes, poly(β-amino ester) (PBAE) copolymers with hydrophobic and poly-cationic subunits were applied. By increasing the ratio of hydrophobic to hydrophilic subunits within these polymers, we can simulate the transition from polyplex to micelleplex. This transition was proven experimentally and allowed conclusions about wheth-

er electrostatic or hydrophobic interactions are more susceptible to nebulization-associated stress and which key parameters are impacted the most.

Furthermore, we showcase the in-vitro performance of the formulations that seem to be most stable after nebulization.

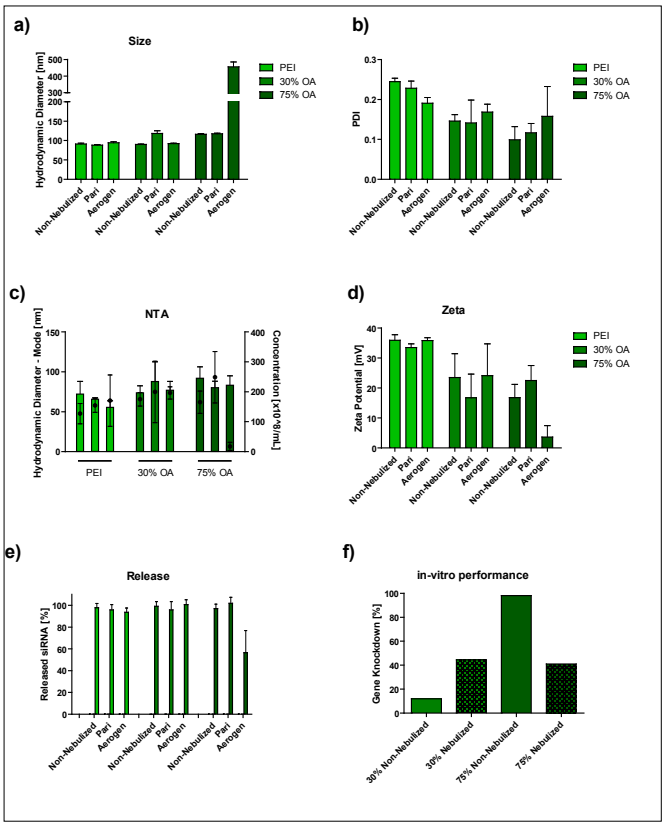

Figure 2. Physicochemical characteristics of poly- and micelleplexes a) Hydrodynamic Diameter and b) Polydispersity index of PEI polyplexes, 30% Oleylamine and 75% Oleylamine containing micelleplexes. c) Nanoparticle tracking analysis of samples, diluted 1 to 100 in formulation buffer, of PEI polyplexes, 30% Oleylamine and 75% Oleylamine containing micelleplexes. d) Zeta potential of samples, diluted 1 to 10 in formulation buffer, of PEI polyplexes, 30% Oleylamine and 75% Oleylamine containing micelleplexes. e) Release of siRNA from nebulized PEI polyplexes (left), 30% Oleylamine (middle) and 75% Oleylamine (right) containing micelleplexes after incubation with Heparin and Triton-X (Results represent 3 individual experiences each consisting of 3 replicates). f) gene knockdown of eGFP in H1299 cells incubated for 48h with nebulized and non-nebulized micelleplexes

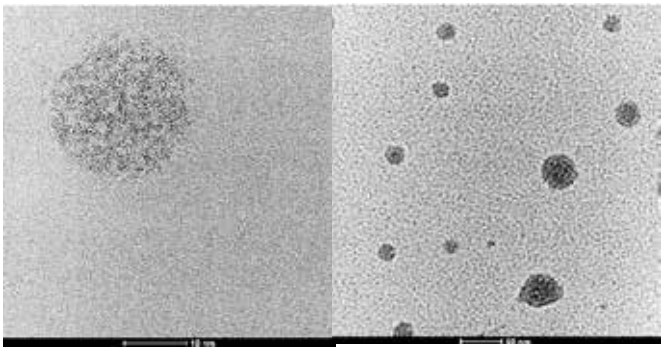

Figure 3. Transmission electron microscopy of 75% OA Nanoparticles (Scalebar 50 nm (left) and 10 nm (right))

Our results demonstrate that nanoparticles with varying hydrophobic proportions react differently to shear and temperature forces in our tested nebulizers. 30% oleylamine (OA) monomer nanoparticles were more severely impacted by the Pari eFlow® rapid. The Aerogen® Pro seemed to have no impact on the physicochemical characteristics. Micelleplexes with 75% OA subunits were more susceptible to the Aerogen® Pro-associated stress and showed almost no impact through the Pari eFlow® Rapid nebulizer (Figure 2. a-e).

Surprisingly, despite observing particles with comparable physicochemical characteristics such hydrodynamic diameter, Zeta potential, particle concentration and siRNA encapsulation, their in-vitro performance can still differ. This contradicts the common “same properties, same performance” hypothesis. We, therefore, further investigated our lead candidate (75% oleylamine containing micellplex) via TEM as well as in-vitro and ex-vivo. With this study we introduce a promising lead candidate for nebulized RNA therapy.

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PROTEIN CORONA BIO-INSPIRED MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES FOR LIVER REGENERATION

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INTRODUCTION

The regenerative and immunomodulatory properties of extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) have been demonstrated in both clinical and pre-clinical studies of liver pathology. We have previously described the liver accumulation of intravenously administered EVs, but knowledge of their cellular distribution within the liver and factors affecting this are limited.

METHODS

Herein, we derive EVs using two culturing methods allowing for the collection of EVs generated under different protein environments. We hypothesise that, under these culture conditions, EVs will form distinct protein corona (PC) leading to modulation of their pharmacokinetic profile. MSC EVs were obtained from cells maintained in the presence or absence of serum (EV_1 and EV_2, respectively).

RESULTS

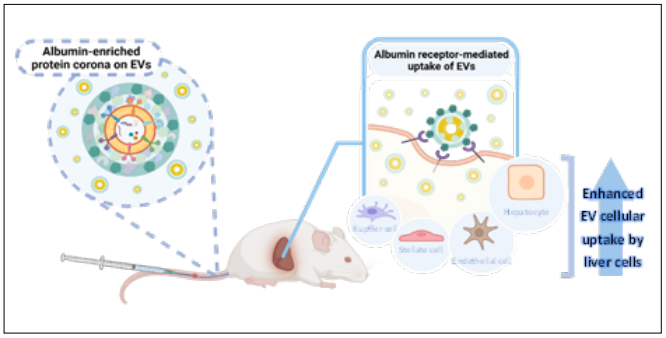
In each case, EVs exhibited comparable physicochemical properties and marker expression but different protein compositions. When EVs were incubated with fetal bovine serum (FBS), to model the secondary corona formed upon systemic delivery, further PC binding patterns could be resolved by liquid chromatography-mass spectrometry. In healthy mouse models, EV_1 and EV_2 accumulated in the liver and kidney, respectively. Using flow cytometry, it was determined that both EVs were comparably taken up by Kupffer cells. EV_1, however, exhibited higher uptake in hepatocytes, liver sinusoidal endothelial cells, and stellate cells. Quantitative proteomics, gene ontology enrichment analysis, and principal component analysis identified that the composition of the PC in EVs was responsible for the differential organ/cellular distribution.

CONCLUSIONS

These findings identify the potential of modifying cell culture conditions as a simple means of retargeting therapeutically relevant EVs to organs or cells. Such an approach offers a solution to a critical challenge facing intravenously administered.

Acknowledgement

Revadee Liam-Or is an awardee of King's PGR international scholarship.



Scheme 1. Graphical abstract

UNRAVELLING GENE THERAPY'S POTENTIAL IN ALZHEIMER'S DISEASE VIA THE BRAIN-BLOOD BARRIER

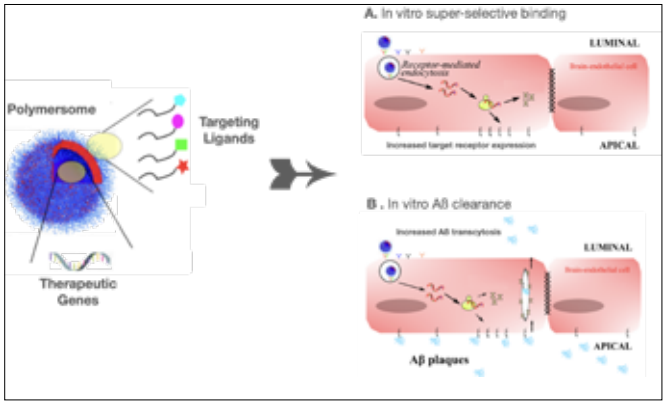
CATIA LOPES

Alzheimer's disease (AD) is a genetic and sporadic neurodegenerative disorder that causes a progressive cognitive impairment, apathy, and dependence on personal daily activities. There is no cure available for AD, and there are only two classes of approved drugs that are effective in treat some of the cognitive symptoms and potentially delay the clinical decline. It is the most common cause of dementia (60-80% of cases) with a prevalence near 50 million people. If no effective treatment is established soon, the incidence and prevalence of AD are expected to double in Europe by 2050, in great part due to the growing global ageing of the population. Despite the considerable progress achieved in the research of best performing disease-modifying agents, the main clinical challenge remains the accomplishment of an efficient and safe therapeutic option that can arrest the disease progression and prevent cognitive failure. Thus, a better understanding of AD pathogenesis and identifying key disease targets that prevent, halt or cure the disease has become an urgent priority within the research and medical communities worldwide.

To contribute to ameliorate the AD pathophysiology, we are currently developing an innovative gene therapy approach to target and modulate the protective barrier of the brain. To mediate this gene therapy approach we are currently establishing super-selective polymersomes capable of targeting the blood-brain barrier (BBB) and allow the modulation of the Aβ clearance mechanism across the BBB (Figure 1). The proposed Aβ lowering intervention relies on the re-establishment of proper expression levels of a novel key intervenient in the process of Aβ clearance across the brain endothelium by combining nanomedicine and gene therapy and, therefore, this approach is expected to modulate the progression of the AD positively.

Here we will share our latest *in vitro* results regarding the: i) development of a biocompatible and super-selective brain endothelium-targeted nanosystem to carry the genes of interest in a safe way; ii) modulation of target genes expression levels in BECs; iii) improvement of Aβ transcytosis across the brain endothelium.

Figure 1. Nanomedicine and gene therapy synergy for targeted Alzheimer's Disease modulation. The approach advanced by our group focuses on the development of a biocompatible and super-selective polymersome incorporating ligands targeting the brain endothelium. This nanovector will be explored to carry therapeutic genes to the brain endothelial cells that, when upregulated, may be capable to improve the overall Aβ transcytosis from the brain parenchyma to the bloodstream for clearance.



IN VITRO SYNERGISTIC EFFECT OF DUAL-LOADED BUDESONIDE AND SERPINE1 SIRNA LIPID-POLYMER HYBRID NANOPARTICLES FOR THE TREATMENT OF INFLAMMATION AND FIBROSIS IN MACROPHAGES INVOLVED IN TISSUE INJURY CONDITIONS

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INTRODUCTION:

Tissue injury is a complex condition caused by the overuse or injury of a tissue and characterized by inflammation and fibrosis, which hamper the tissue regeneration process [1]. In specific tissue injury conditions such as tendinitis, macrophages are immune cells that play a key role in the different healing stages, since they are responsible for the resolution of the inflammation at early stages of the tendon regeneration process and of promoting extracellular matrix remodelling at later stages [2]. Specifically, macrophages present an M1 phenotype during early staged of the healing process and they must shift the phenotype to M2 for inflammation resolution [3,4]. Nevertheless, macrophage dysfunction can lead to impaired inflammation resolution and the shift of phenotype to M2 might lead to the formation of scar tissue, which impedes the recovery of the mechanical properties of the tissue [5]. In the past, traditional thera-

pies based on non-steroidal anti-inflammatory drugs and corticosteroids have focused on reducing inflammation, but they do not prevent the formation of fibrotic tissue and neither promote proper remodelling of the ECM. In this context, a nanoplatform of dual-loaded hybrid lipid-polymer hybrid nanoparticles was optimized in a previous work to efficiently load and simultaneously deliver a hydrophobic small molecule drug and an siRNA to macrophages (**Figure 1**). Following on that, in the present work, budesonide and an siRNA against a relevant pro-fibrotic gene are loaded in this nanoplatform and the nanosystem is evaluated in terms of its efficacy to reduce inflammation and fibrosis induced by macrophages, showing that the nanosystem can reduce the inflammatory state of macrophages and prevent the expression of pro-fibrotic genes, increasing the expression of matrix metalloproteinases involved in tissue remodelling.

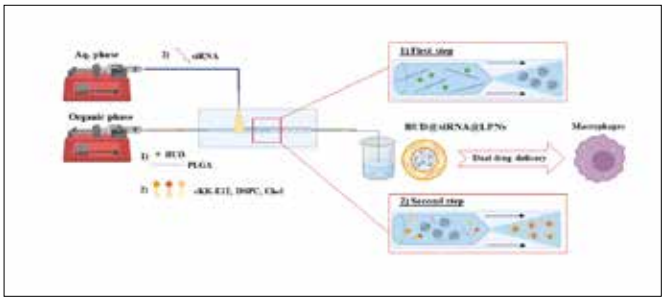

Figure 1. *Co-flow microfluidics approach optimized for the preparation of lipid-polymer hybrid nanoparticles co-loaded with budesonide and a model siRNA for dual delivery to macrophages.*

METHODOLOGY:

The efficacy of the already optimized nanosystem is tested *in vitro* in both human and murine macrophage cell lines. Firstly, qPCR and Western blot are performed to evaluate the changes in the gene and protein expression of macrophages pre-treated with LPS and/or TGF-β1 and incubated with the dual-loaded LPNs. In addition, ELISA and macrophage polarization studies confirmed the ability of the nanosystem to shift macrophages to the M2 phenotype, while matrix metalloproteinases studies support the prevention of fibrosis in spite of the induction of fibrosis with TGF-β1. In addition, the interactions of the nanosystem with other immune cells, such as T cells, were also evaluated by assessing the activation of toll-like receptors (TLRs).

RESULTS:

Budesonide and pro-fibrotic gene siRNA were efficiently loaded in already optimized LPNs. The expression of pro-inflammatory genes like nf-κβ1 and tnf-α is decreased, while the expression of pro-fibrotic genes and matrix metalloproteinases is also modulated in the direction of promoting the degradation and remodelling of ECM (**Figure 2**). These changes in gene expression were confirmed by Western blots studies, which evaluated the protein expression of the most important inflammatory and pro-fibrotic proteins in this study, *i.e.*, nf-κβ1 and TGF-β1. Furthermore, ELISA assays of IL-4 and IL-1 were conducted to confirm the anti-inflammatory potential of these nanosystem, and macrophage polarization studies supported the shift of phenotype towards M2 by assessing the expression of the main receptor expressed in M1, *i.e.*, CD86 and in M2 macrophages, *i.e.*, CD206. Finally, the immune-safety of the nanosystem was assessed by evaluating the interaction of the nanosystem with TLRs in T cells co-cultured with macrophages after incubating with the dual-loaded LPNs, providing insights into the interaction of LPNs with other cells of the immune environment present in the tissue injured.

CONCLUSION:

Budesonide and pro-fibrotic gene siRNA dual-loaded LPNs were tested *in vitro* in both human and murine macrophage cell lines. The synergistic anti-inflammatory and anti-fibrotic effect of the nanosystem was confirmed by the observed modulation of the gene and protein expression of relevant inflammatory genes. In addition, the production of anti-inflammatory cytokines was increased while the expression of pro-inflammatory cytokines was decreased. Moreover, macrophages were shifted to the M2 phenotype, which favours inflammatory resolution. Simultaneously, the gene and protein expression of pro-fibrotic genes and matrix metalloproteinases was modulated towards a matrix remodelling direction, which was further confirmed by the increase in the activity of a matrix metalloproteinase. Furthermore, the production of collagen I and III was modulated after treatment with dual-loaded LPNs, favouring the tissue healing in the later stages of the repair process. Finally, the interaction of LPNs with other immune cells of the immune environment of injured tissues was evaluated, and the low interactions between LPNs and TLRs confirmed the immunosafety of the nanosystem, encouraging his application for local administration in injured tissues.

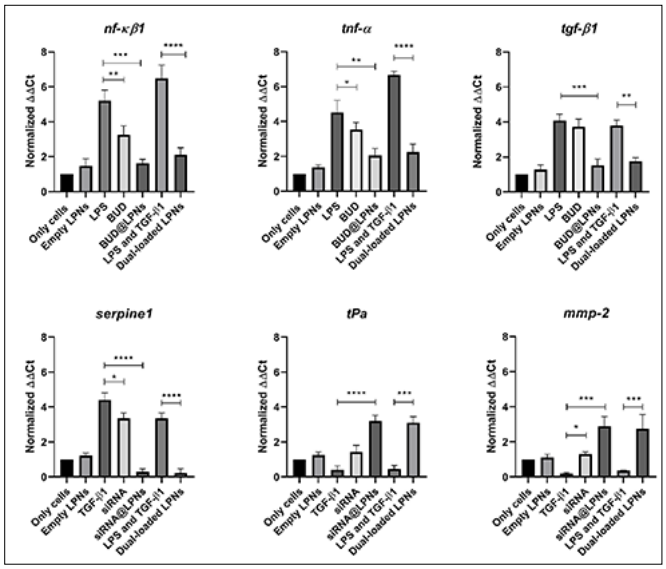

Figure 2. *Gene expression modulation of pro-inflammatory and fibrosis-related genes after induction of inflammation or fibrosis and incubation with the single-loaded and dual-loaded LPNs in RAW 264.7 cells. A one-way ANOVA was conducted and the level of significance was established at ***p < 0.001 for comparison between LPS vs. BUD@LPNs and BUD, LPS + TGF-β1 vs. dual-loaded LPNs and TGF-β1 vs. siRNA@LPNs and siRNA.*

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CHEMORADIATION THERAPY USING LIPID NANOCARRIERS

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Introduction: Chemotherapy is widely used for cancer treatment, but it can cause undesired side effects in patients. Radiotherapy is a widely used modality for cancer therapy that utilizes ionizing radiation to target and destroy tumor cells. However, there is a need for innovative strategies to overcome limitations such as tumor resistance and systemic toxicity. Drug-loaded nanoparticles that respond to radiotherapy have emerged as a promising tool for enhancing the efficacy of cancer treatment[1–3]. These nanoparticles can be designed to release therapeutic agents selectively within the tumor, exploiting the radiation-induced changes in nanoparticles for precise drug delivery. This integration of radiotherapy and nanoparticle-based drug release holds significant potential to improve the therapeutic outcomes in cancer patients while minimizing adverse effects, representing a promising advancement in cancer treatment strategies. In this study we explored the design of a drug-loaded nanocarrier system that releases its cargo by application of ionization radiation. The nanocarrier is based in liposomal systems containing the drugs and sensitizer agents that can be activated with ionizing radiation.

Methods: Liposomes were synthesized by a thin film hydration method followed by extrusion. Several lipids were used in the preparation of liposomes: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-stearoyl-2-linoleoyl-sn-glycero -3-phosphocholine (SLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). Formulations included 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000 (DSPE-PEG2000) and cholesterol (CHOL) to increase stability at a molar ratio of 64.5:33:2.5 (Lipid:CHOL:DSPE-PEG2000). Protoporphyrin IX (PPIX), Verteporfin (VP), Cercosporin (CERC), Hypericin (HYP) and Rose Bengal (RB) were studied as potential radiosensitizers[3–5]. Hydrophobic sensitizers were added before thin film formation while hydrophilic ones were added at the lipid hydration step. Liposomes were hydrated with a fluorescent dye (Carboxyfluorescein, CF) and irradiated with UV light and photons (137Cs irradiator). CF release was monitored after irradiation as well as the formation of conjugated dienes due to lipid peroxidation. In vitro studies were performed on HeLa cells in order to assess cellular uptake and radiosensitization effects when incubated with liposomes. Cell internalization was monitored using confocal fluorescent microscopy and cell survival rates were obtained performing clonogenic studies assays after irradiation with photons.

Results: First, we studied liposomes prepared with DLPC and different radiosensitizers. The CF release and lipid peroxidation obtained during 80 minutes of irradiation with UV light and after irradiation with \gamma photons with doses ranging 0-10 Gy are shown in figures 1 and 2 respectively.

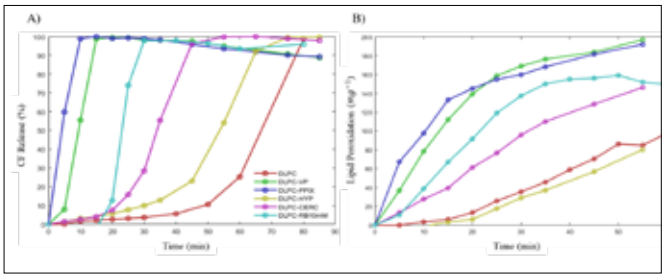

Figure 1. CF release (A) and lipid peroxidation (B) of liposomes including different radiosensitizers irradiated with UV light during 80 minutes.

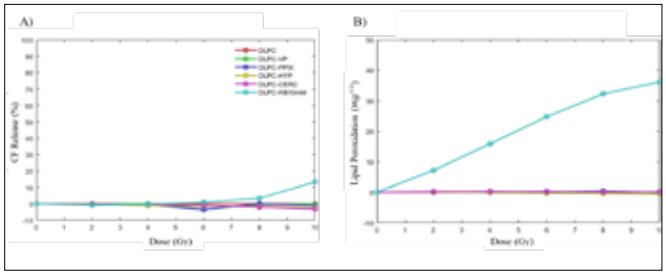

Figure 2. *Instant CF release* **(A)** *and lipid peroxidation* **(B)** *of liposomes including different radiosensitizers irradiated with photons (0, 2, 4, 8, 10 Gy).*

VP and PPIX induced the fastest CF release and lipid peroxidation under UV irradiation due to their photosensitizing capabilities and their location within the lipid bilayer. RB also triggered a rapid release of CF even though it is located in the lumen of the liposome. On the other hand, only RB induced lipid peroxidation and CF release when irradiated with photons, although only mild instant CF release was obtained for all radiation doses. Passive CF release after irradiation of DLPC-RB liposomes was monitored for 24 hours as shown in figure 3 exhibiting earlier induced release for higher radiation doses. We further investigated other RB-loaded liposome formulations including DLPC liposomes with different sizes (200, 50 and 30 nm) (see figures 3 and 4), DLPC liposomes with different RB concentrations (see figure 3), and liposomes prepared with other lipids (SLPC, DOPC) (see figure 5).

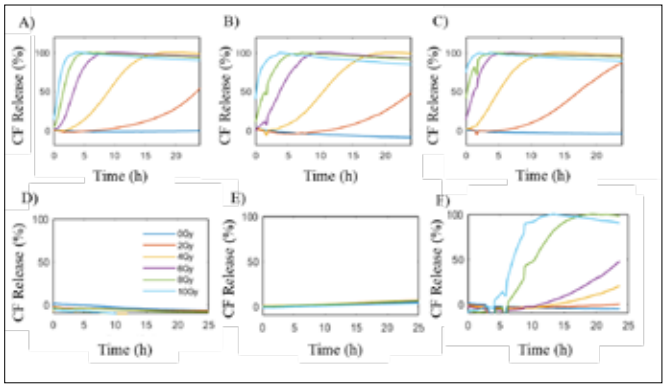

Figure 3. *Passive CF release from liposomes monitored during 24 hours after irradiation (0, 2, 4, 6, 8, 10 Gy): DLPC-RB(10mM)-200nm* **(A)**, *DLPC-RB(10mM)-50nm* **(B)**, *DLPC-RB(10mM)-30nm* **(C)**, *DLPC-200nm* **(D)**, *DLPC-RB(1mM)-200nm* **(E),** *and DLPC-RB(5mM)-200 nm* **(F)**.

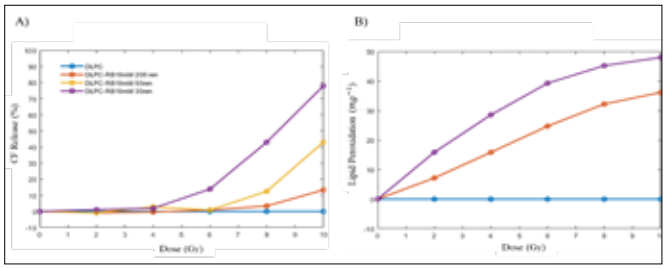

Figure 4. *Instant CF release* **(A)** *and lipid peroxidation* **(B)** *of DLPC-RB liposomes with different sizes irradiated with photons (0, 2, 4, 6, 8, 10 Gy).*

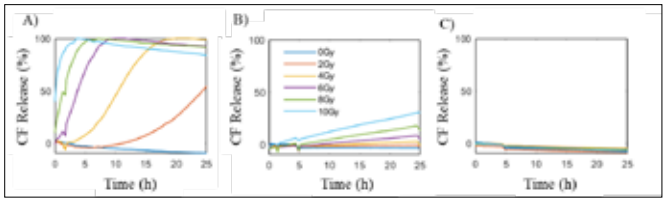

Figure 5. *Passive CF release from liposomes monitored during*

24 hours after irradiation (0, 2, 4, 6, 8, 10 Gy): DLPC-RB(10 mM)-200 nm **(A)**, *SLPC-RB(10 mM)-200 nm* **(B)** *and DOPC-RB(10 mM)-200 nm* **(C)**.

Smaller liposomes accelerated radiation induced release while SLPC and DOPC showed only a minor response. Figure 3 shows that the fastest CF release was obtained for liposomes with a RB concentration of 10 mM. Cell internalization of DSPC-RB liposomes after 24 h incubation is shown in figure 6. A small radiosensitizing effect of DSPC-RB liposomes is shown after 8 Gy of irradiation (figure 6).

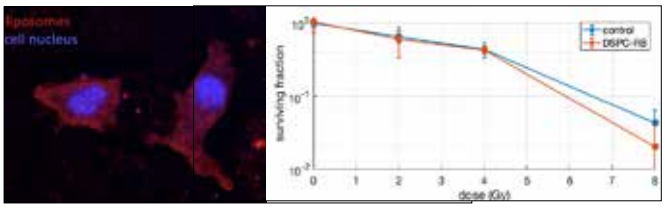

Figure 6. (A) *Cell internalization of DLPC-RB liposomes (544 nm/590 nm, red) in HeLa cells (24 h incubation). Cell nuclei were stained with DAPI (blue). (B) Survival fraction of HeLa cells after irradiation (0, 2, 4, 8 Gy).*

Conclusions: We identified RB as a good radiosensitizer to induce lipid peroxidation in liposome membranes under radiotherapy leading to the synchronous release of their cargo. Moreover, minor differences on CF release was found for liposomes loaded with different RB concentrations when irradiated with UV light while large differences were obtained when irradiated with photons. We also verified that induced release is faster for smaller liposomes probably due to the higher curvature of the surface which leads to increased exposure of unsaturated lipids to reactive species. Other important aspect learnt from this study is that lipids containing polyunsaturated fatty acids like DLPC or SLPC are more sensitive to radiation damage. Finally, we showed the internalization of RB liposomes on HeLa cells while only a minor radiosensitising effect was obtained. Further work is needed to increase the radiosensitizing effect of the liposomes and to explore their chemoradiation capabilities on *in vitro* studies.

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OPTIMIZATION OF PROCESS PARAMETERS FOR INNOVATIVE LIPOSOME PRODUCTION FROM NANOEMULSIONS

LARISSA LUBITZ

Glioblastoma multiforme (GBM) is the most common and also the most aggressive form of high-grade gliomas, i.e. malignant primary brain tumors (Paolillo et al., 2018). Standard therapies include surgery, radiotherapy and chemotherapy, leaving patients with acute side effects and a reported survival from diagnosis of less than 15 months (Rajaratnam et al., 2020). In order to treat tumor cells by receptor-specific targeting ligands, one approach is transporting active pharmaceutical ingredients (APIs) to the site of action in small biodegradable capsules, so-called liposomes as drug delivery systems (Akbarzadeh et al., 2013). Liposomes consist of a phospholipid bilayer and contain biocompatible and low-toxic lipids, making them an ideal carrier for hydrophilic and lipophilic molecules (Guimarães et al., 2021). In addition to various physiological benefits, liposomal drug delivery systems also enable some procedural advantages. For example, tissue specificity can be enabled by adding functional groups such as amine, carboxylic acid or maleimide to PEG, to which ligands such as small molecules, peptides or antibodies can be conjugated to achieve active drug targeting (Saw et al., 2015). For the targeted release of the encapsulated drug, properties such as long-term stability and encapsulation efficiency are of importance since liposomes are exposed to significant chemical stresses in a patient's body. To offer patients the safest available treatment, the optimal physicochemical liposome composition is essential (Kelly et al., 2011).

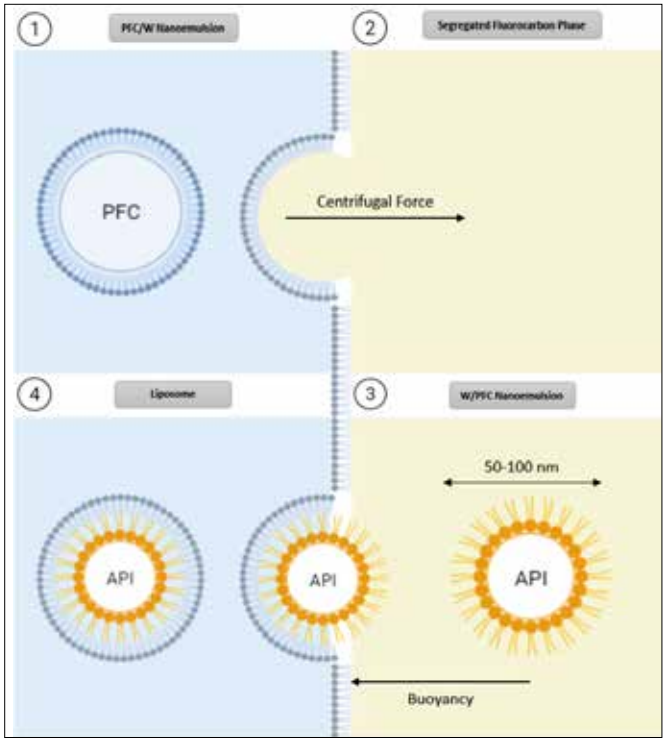

Figure 1: Schematic workflow of the centrifugation process for the production of asymmetric liposomes.

The aim of this work is the manufacturing optimization and the investigation of the stability of a perfluorocarbon-in-water emulsion as an intermediate for further use to produce asymmetric liposomes via a centrifugation process (Ullmann et al., 2021). This centrifugal process could be done either in batch mode using small tubes or in a continuous centrifuge where both emulsions are overlaid in a countercurrent.

As shown in Figure 1, asymmetric liposomes are prepared by centrifugation of two inverse nanoemulsions. For this purpose, in a batch process, a water-in-perfluorocarbon nanoemulsion (W/PFC) is placed in a tube (see Figure 1, item 3) and overlaid with a PFC-in-water nanoemulsion (PFC/W, see Figure 1, item 1).

The following aspects come into consideration in the process:

I. Centrifugal forces cause sedimentation of the PFC droplets due to their density. The density of the used PFC is twice that of water. The phospholipids, which stabilize the PFC droplets as a monolayer, are released at the phase boundary and form a monolayer, with their head groups oriented towards the upper water phase.
II. In parallel, the buoyancy of water droplets from the lower W/PFC nanoemulsion occurs, which are also stabilized with a monolayer of phospholipids. The buoyancy of these droplets is also caused by the density differences already mentioned. During the phase transfer of the water droplets, a second phospholipid layer is formed by the released phospholipids of the PFC droplets located there.

Using the thin-film method for phospholipid suspension preparation and pre-emulsification with Ultra- Turrax®, a PFC/W nanoemulsion was prepared. A membrane extruder or high-pressure homogenizer such as the LV1 microfluidizer was used as the manufacturing method.

Therefore, the following aspects were considered:

A For membrane extrusion: comparison of pre-emulsification methods, number of passages per membrane, number of membranes, as well as the impact of cooling and the influence of sonication.
B For high-pressure homogenization: selection of homogenization pressure, number of cycles and the emulsion composition regarding phospholipid concentration, selection of phospholipid and molar ratio to cholesterol as well as the percentage of PFC and type of PFC.

Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter (Z-Ave), the polydispersity index (PdI) and the derived count rate (DCR).

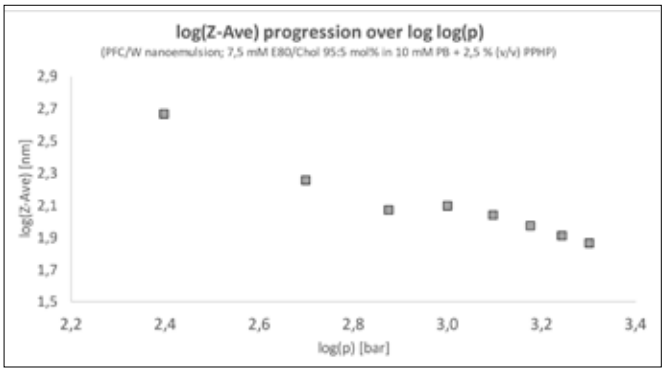

Figure 2: Logarithmic representation of the final Z-Average (Z-Ave) of a PFC/W nanoemulsion after six cycles as a function of the process pressure used.

To evaluate the influence of the process pressure on the perfluorocarbon-in-water nanoemulsion, the pressure was varied from 250 bar to 2000 bar in which the emulsion was homogenized for six cycles. Here, a double logarithmic plot following Qian and McClements showed that there is a process shift between 750 bar and 1000 bar (Figure 2) (Qian and McClements, 2011). This is presumably due to a transition from laminar to turbulent flow, which, however, cannot yet be derived from estimated Reynolds numbers in a simplified linear tube model (Table 1).

Table 1: Calculated pressure-dependent Reynold numbers for the Y-Channel of the LV1.

Pressure [bar]	Estimated Reynolds number in simplified linear tube model
250	$1{,}210^4 \pm 0{,}4$ %
500	$2{,}210^4 \pm 6{,}1$ %
750	$3{,}010^4 \pm 5{,}8$ %
1000	$2{,}910^4 \pm 0{,}3$ %
1250	$3{,}310^4 \pm 4{,}1$ %
1500	$4{,}010^4 \pm 6{,}2$ %
1750	$4{,}110^4 \pm 9{,}2$ %
2000	$5{,}110^4 \pm 3{,}2$ %

This process transition becomes evident not only for the droplet sizes (Z-Average) of a PFC/W nanoemulsion processed for six cycles at the LV1, but also when evaluating the volume flow and resulting velocity in the process chamber as well as the product temperature (Figure 3 a and b). A slit width of the Y-channel of 75 µm was used to calculate the product speed.

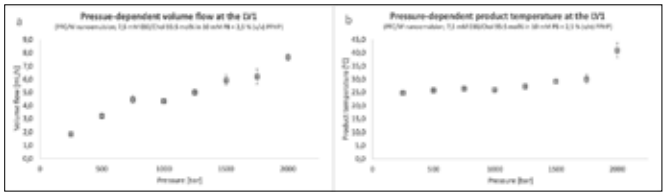

Figure 3: a) Pressure-dependent volume flow and b) pressure-dependent product temperature at the LV1 for a PFC/W nanoemulsion processed with a volume of 6 mL.

As we will show in our poster, high-pressure homogenization of pharmaceutical emulsions not only comprises the interaction of fluid dynamics with colloidal chemistry and physics, but also includes fluid-structure interactions. The elastomechanics of high-pressure devices leads to extreme conditions in a very short time with lasting colloidal structures being formed under such conditions. Based on our results, the field of mathematical modeling for the conceptual design of optimal emulsions based on physical process parameters in interaction with experimental process optimization opens up.

CALCIUM PHOSPHATE NANOPARTICLES AS POTENTIAL CARRIERS FOR VACCINES

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Keywords: vaccine, calcium phosphate nanoparticles, ovalbumin, flame spray pyrolysis

Subunit vaccines have emerged as a promising approach to prevent infectious diseases, offering a safer alternative to conventional live attenuated or killed vaccines. Moreover, they are cost-effective and easily producible on a large scale. However, these vaccine formulations still face challenges such as limited immunogenicity and stability. To address these issues, nanoparticles (NPs) can be utilized as a delivery platform for vaccines. NPs possess the ability to protect the antigen cargo, enhance immunogenicity, and precisely deliver the cargo to the desired location (Pati et al., 2018).

Among the numerous NP delivery systems developed, calcium phosphate (CaP) NPs stand out as highly promising candidates. They exhibit biocompatibility and biodegradability, making them well-tolerated by the body. CaP NPs have also demonstrated potential as vaccine adjuvants, capable of eliciting both cell-mediated and humoral immune responses (Lin et al., 2017). As a result, CaP NPs effectively address the issue of poor immunogenicity associated with recombinant protein/antigen vaccines. Furthermore, CaP NPs have demonstrated a high loading capacity for diverse proteins and peptides (Tsikourkitoudi et al., 2020), making them a promising nanocarrier for protein-based vaccines.

Here we use flame spray pyrolysis (FSP), a scalable nanofabrication technique that allows easy tuning of NPs properties such as composition, sizes, crystallinity, etc. These parameters are critical factors as they determine the mode of cellular uptake by Antigen presenting cells (APCs) as well as the internalization efficiency (Pati et al. 2018). We have synthesized amorphous CaP NPs with Ca to P ratio of 1.5 and varying silica content to screen the nanocarrier based on optimum hydrodynamic sizes, antigen loading capacity, and cell cytotoxicity. We obtain a very high specific surface area of greater 150m2/g for all these NPs. Although only CaP NPs with 25 wt% or above silica content yield a NPs suspension of 100-200 nm mean hydrodynamic diameter (shown in figure 1).

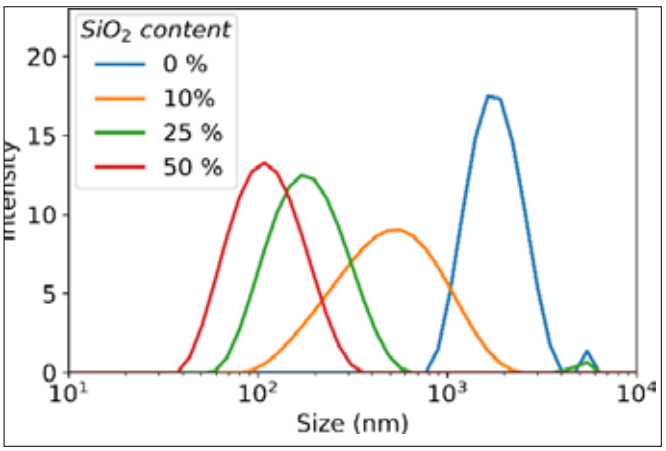

Figure 1: Hydrodynamic sizes of CaP nanoparticles with varying silica content measured using dynamic light scattering.

Upon loading ovalbumin (OVA), a model protein antigen, we achieve loading capacity values of up to 60 µg/mg NPs for 25 wt% silica CaP particles. These values are even higher in case of bare CaP NPs, up to 400 µg/mg NPs. These particles also display pH dependent solubility with more than 50% dissolution in 2 hrs at pH 4. Remarkably, none of the NPs exhibit cytotoxic effects on human lung epithelial cells, with cell viability of more than 90%.

After screening, we used 10% & 25% SiO2 containing CaP NPs to study the OVA uptake and the immunomodulatory effects (cytokine production, dendritic cell activation) on bone marrow derived dendritic cells (BMDCs). As per our preliminary studies, NP:OVA complexes were successfully internalized by BMDCs. Furthermore, there was an upregulation of CD80 and CD86 expression on BMDCs in the case of NP:OVA conjugates, as compared to water and ovalbumin alone (depicted in Figure 2) thus indicating DC activation. Lipopolysaccharide mixed with OVA was used as positive control for activation.

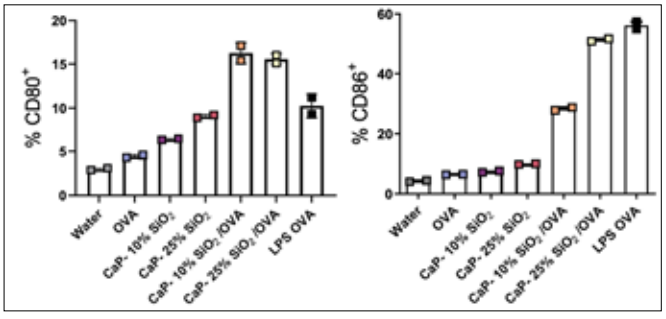

Figure 2: Percentage of BMDC expressing CD80 and CD86 when incubated with 10% & 25% SiO2 containing CaP NPs (with and without ovalbumin) for 18 hrs. LPS OVA was used as a positive control.

In conclusion, FSP can be used for scalable production of calcium phosphate nanoparticles with good control over its properties. These particles were able to load antigen on the surface and help in the internalization by dendritic cells. The NP-antigen conjugate also resulted in the activation of dendritic cells.

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NANOZYMES-ARMED MICROBES FOR ALLEVIATING INTESTINAL INFLAMMATION AND MICROBIOTA DYSBIOSIS

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Inflammatory bowel disease can be caused by the dysfunction of the intestinal mucosal barrier and dysregulation of gut microbiota. Traditional treatments use drugs to manage inflammation with possible probiotic therapy as an adjuvant. However, current standard practices often suffer from metabolic instability, limited targeting and result in unsatisfactory therapeutic outcomes. Here we report on nanozyme-modified Bifidobacterium longum probiotics for reshaping a healthy immune system in inflammatory bowel disease. Probiotics can promote the targeting and retention of the biocompatible artificial enzymes to persistently scavenge elevated reactive oxygen species and alleviate inflammatory factors. The reduced inflammation caused by nanozymes improves bacterial viability to rapidly reshape the intestinal barrier functions and restore the gut microbiota. The therapeutic effects are demonstrated in murine and canine models and show superior outcomes than traditional clinical drugs.

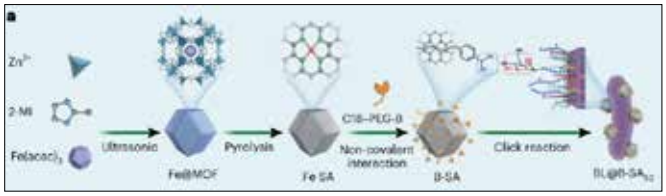

Figure 1. BL@B-SA is composed of nanozymes (Fe SA), BL probiotics and linkers (C18–PEG-B).

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MULTI-DETECTOR FIELD-FLOW FRACTIONATION FOR THE ASSESSMENT OF CRITICAL QUALITY ATTRIBUTES OF NANO-SIZED DRUG DELIVERY SYSTEMS

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The COVID-19 pandemic has sparked the application of lipid nanoparticles as delivery platform for mRNA-based vaccines (mRNA-LNPs) with BioNTech/Pfizer and Moderna being at the forefront of their commercial breakthrough. Besides the recent success story of mRNA-LNPs there are several other platforms that are either already well-established or currently under investigation as potent drug delivery systems including e.g., vesicles (e.g., liposomes, exosomes) or viruses (e.g., AVs, AAVs).[1] One thing that all these delivery platforms have in common is their size in the nanometer regime, which enhances efficacy and selectivity to ultimately deliver their cargo load to specific target sites in the human body.
Several analytical tools are available to determine critical quality attributes (CQA) of these delivery systems such as e.g., particle size distribution, concentration, aggregation behavior, surface Zeta potential, corona formation, payload, or encapsulation efficiency to eventually ensure their safe and efficient use. Field-Flow Fractionation (FFF) is among the most promising techniques to determine these CQA.[2]
FFF comprises a family of flow-based techniques, where an external force field enables the fractionation of nano-sized sample constituents in suspension. In Asymmetrical Flow FFF (AF4) for example, fractionation by hydrodynamic size is induced by a second flow field. Adding an electrical field to AF4 also enables additional fractionation by electrophoretic mobility (EAF4) while in Centrifugal FFF (CF3) sample constituents are separated according to differences in their buoyant masses. Like in liquid chromatography, FFF can be coupled downstream with multiple detection systems to derive information about CQA such as size distribution (via multi-angle light scattering (MALS) or dynamic light scattering (DLS)), concentration (e.g., via UV/Vis and/or refractive index (RI) detection or nanoparticle tracking analysis (NTA)), shape (via combining MALS and DLS) or payload (e.g., via combining UV/Vis and RI) of the analyzed sample constituents.[3]
We here present the application of multi-detector AF4, EAF4 and CF3 to determine several CQA of nano-sized drug delivery platforms. The first example describes the application of EAF4-MALS-NTA to derive size distribution, concentration, and surface Zeta potential of liposomal Doxorubicin (Doxil®) incubated in cell culture medium (DMEM + 10% FCS) to study the potential formation of a protein corona under physiological conditions (**Figure 1**).[4]

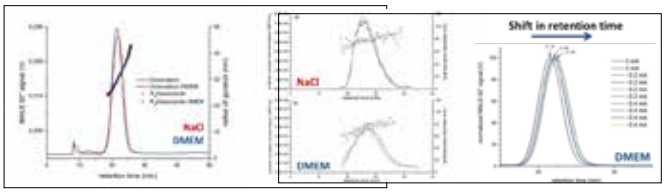

Figure 1*: Left: Particle size distribution as radius of gyration (R_g) of Doxil® in NaCl (red trace) and DMEM (blue trace) obtained from EAF4-MALS. Middle: Particle number distribution (hydrodynamic diameter, dots) and concentration (lines) of Doxil® determined by EAF4-NTA. Right: Shift in retention time of Doxil® to determine the surface Zeta potential of Doxil® in DMEM analyzed by EAF4-MALS.*

In the second example, CF3-MALS was used to determine the payload of exosomes filled with BSA as cargo. Here, differences in the measured retention times (tr) of empty and filled exosomes can be translated into a size-resolved mass distribution (m' α tr) turning CF3-MALS into an ultrasensitive balance that can quantify the BSA cargo mass down to the low attogram range (Figure 2).

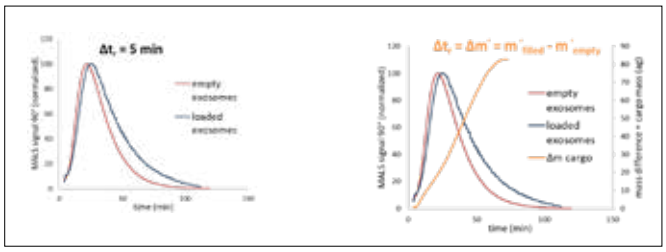

Figure 2*: Left: CF3-MALS fractograms of empty (red trace) and BSA-filled exosomes (blue trace) displaying a 5 min shift in retention time (t_r) between the two samples. Right: Quantification of the size resolved BSA cargo mass (orange trace; m' approx. 10-80 attogram).*

In the last example, Frit-Inlet AF4-UV-MALS-RI-DLS was used to determine the LNP size distribution and to quantify their size-resolved mRNA content (Figure 3).[5] Payload determination was performed by compositional analysis using the UV/Vis detector (λ = 260 nm) to quantify the mRNA and the RI detector to quantify the UV-inactive lipids. With a priori knowledge of the refractive index increments (dn/dc) and extinction coefficients (ϵ) of mRNA and lipids (at the recorded UV wavelength and in the used medium) and considering potential scattering contributions to the UV-absorption signal, mRNA payload could be determined to 8.08 % ± 0.42 % by solving the following two equations with respect to the mRNA concentration (cmRNA):

$$\rightarrow \boldsymbol{RI\ signal} \propto c_{mRNA} \cdot \left(\frac{dn}{dc}\right)_{mRNA} + c_{Lipids} \cdot \left(\frac{dn}{dc}\right)_{Lipids}$$

$$\rightarrow \boldsymbol{UV\ signal} \propto c_{mRNA} \cdot \epsilon_{mRNA} + c_{Lipids} \cdot \epsilon_{Lipids}$$

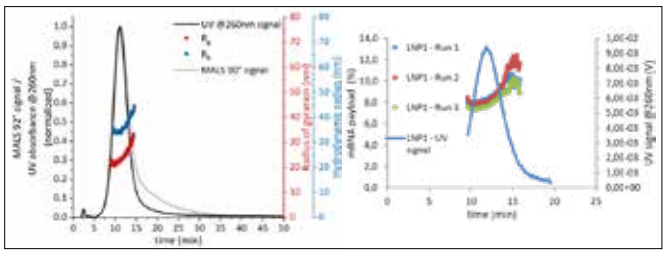

Figure 3*: Left: mRNA-LNP size distribution calculated from MALS (red dots: radius of gyration R_g) and DLS analysis (blue dots: hydrodynamic radius) after fractionation by Frit-Inlet AF4. Right: Size-resolved mRNA payload determined from three replicates.*

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MICRONEEDLE-ENHANCED DELIVERY OF NANO-CRYSTALLINE IMIQUIMOD FOR TRANSCUTANEOUS IMMUNISATION– MANUFACTURING, CHARACTERISATION AND PERMEATION

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The development of effective therapeutic drugs requires consideration beyond just the chemical structure or pharmacological profile; ultimately it is about reaching the target destination within the body. The human skin, which is characterised by a high density of immune cells, presents an attractive target site for drug delivery via dermal formulations such as transcutaneous immunisation. Due to their high potential for clinical use, microneedle arrays (MNAs) are an increasingly popular subject of research when developing formulations for transdermal drug delivery. MNAs for dermal drug application were first described in the late 1990s. Ever since, the number of publications on the topic has been growing every year.

Here we show our developments in the manufacturing process, especially focusing on the appropriate incorporation of a suspended drug in nano-scale and characterization of the fast-dissolving MNAs. To achieve this, we embedded nanocrystalline imiquimod, a toll like receptor 7-agonist, in a polyvinyl-alcohol matrix.

In an approach to show the advantages of MNAs over other dermally applied formulations, we manufactured three different formulations, all containing nanocrystalline imiquimod. In detail, we investigated two semisolid formulations versus our MNA. To show the impact of MNA on drug delivery, *ex vivo* permeation experiment was conducted using a Franz diffusion cell (Fig. 1 (a)). Through the use of MNAs, we were able to achieve the same amount of drug permeation after 24 hours although only using 7% of the dose of nanocrystalline imiquimod compared to the dose used in the semisolid formulations (figure 1 (c), (d)).

By gaining a deeper understanding of the drug permeation process and the influence of different formulations on drug permeation, promising avenues for the development and exploration of biopredictive *in vivo* studies are opened. Such studies hold the potential to further enhance the efficiency and efficacy of drug delivery systems.

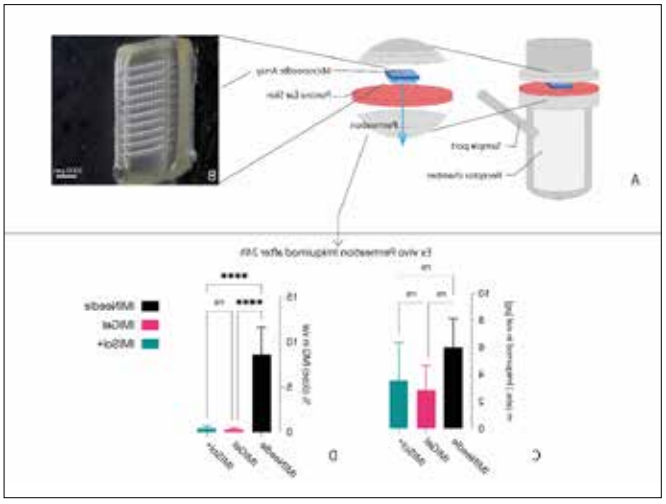

Figure 1: *Ex vivo permeation study of imiquimod (IMQ) microneedle arrays versus two semisolid formulations (IMIGel, IMISol+). (a) Franz diffusion cell; (b) IMQ-loaded microneedle array before insertion (z-stack) (Zeiss Discovery V 12 stereomicroscope); (c), (d) Summary of ex vivo permeation data of IMQ across porcine cadaver ear skin (n = 5) over 24 hours: Comparing MN (dose = 70 µg IMQ) to two semisolid formulations of IMQ, showing means ± SD; one-way ANOVA followed by Tukey's post-hoc analysis (significant difference (p < 0.001))*

UNLEASHING MR1'S POTENTIAL: PEPTIDE-FUNCTIONALISED POLYMERSOMES FOR TARGETED TUBERCULOSIS THERAPY

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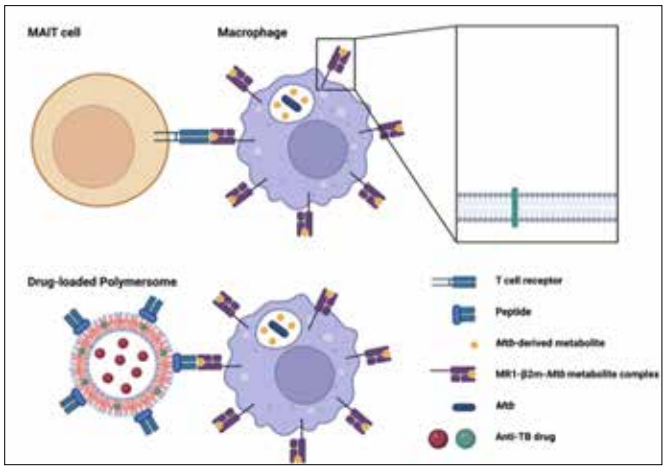

Figure 1 – MAIT cell mimetic polymeric nanotherapeutics able to simultaneously deliver drug combination therapy and specifically target infected host cells through their unique metabolic profiles.

The global increase in infection outbreaks poses significant challenges to public health. Factors such as the overuse and misuse of antibiotics and the scarcity of novel drugs have contributed to the emergence and spread of drug-resistant bacteria. Among these, Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis, remains a serious threat, causing over 1.6 million deaths annually. Mucosal-associated invariant T (MAIT) cells, a distinct subset population of T cells, are able to recognise bacterial-derived metabolites, including those produced by Mtb, through their presentation by the MHC class I-like related (MR1) protein. MR1, a conserved protein primarily located in the endoplasmic reticulum, translocates to the cell surface upon metabolite binding and association with β2 microglobulin (β2m), thus serving as a sensor for intracellular infections. We have shown that polymeric nanoparticles containing isoniazid and clofazimine present lack of toxicity, dose-dependent response, and improved therapeutic efficacy when compared to free drugs in an *in vivo* model of mycobacterial infection [1]. However, the targeted delivery to infected cells remains a challenge. Using phage display technology, we are now screening for peptides capable of binding specific MR1-metabolite complexes

to subsequently direct antibiotic-loaded polymersomes functionalised with these peptides to infected cells (Figure 1). We successfully expressed and purified MR1 Ectodomain-β2m complexes both with and without acetyl-6-formylpterin (Ac-6-FP), an intermediate in bacterial folate biosynthesis pathways, which has been shown to enhance MR1 presentation on the plasma membrane of cells *in vitro* (Figure 2). These purified complexes are now being used as targets in our phage display experiments. Additionally, we are using THP-1 cells exposed to bacterial supernatants or Ac-6-FP for on-cell phage display panning. This study paves the way for the development of innovative approaches for targeted drug delivery through MR1-metabolite presentation, a system that is now known to be relevant not only in the context of bacterial infections but also in cancer and autoimmune disorders.

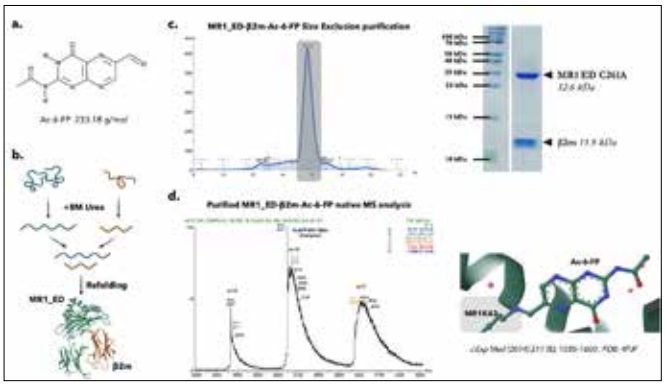

Figure 2 – a. Acetyl-6-formylpterin (Ac-6-FP). b. MR1_ED and β2m inclusion bodies were denatured using 8M urea and the proteins were refolded with a buffer containing 2mM EDTA and 0.4M of arginine. c. (Left) The refolded sample was purified by DEAE anion exchange (not shown) and subsequently by size exclusion chromatography (Superdex 200 10/300 GL). (Right) SDS-PAGE confirmed that the fractions collected from SEC contained MR1_ED and β2m. d. The purified proteins were analysed by native mass spectrometry to confirm the metabolite binding to the MR1_ED-β2mheterodimer.

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PEG HYDROGEL TOOLBOX – REALIZING VARIOUS RELEASE TIMEFRAMES OF VACCINE NANOPARTICLES FROM HYDROGELS INTENDED FOR IMPROVED QUALITY OF HIV IMMUNIZATION

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Purpose: Today about 37.9 Mio people are living with the human immunodeficiency virus (HIV) [1]. An HIV-vaccine for prophylaxis would be the most effective approach to fight the global pandemic [2]. The viral envelope protein (Env) of HIV plays a key role in broadly neutralizing antibody elicitation and therefore protection against infection [3]. We and others have shown that the immobilization of Env on the surface of nanoparticles (NPs) is more effective compared to vaccination with the soluble protein [4,5]. Another aspect is that a prolonged delivery of Env antigens e.g. from mini-osmotic pumps mimicking the kinetics of natural infections, elicit enhanced humoral responses compared to traditional bolus immunization [6]. Our goal is to combine both principles namely the particulate and prolonged delivery of Env antigen. Hydrogels have already proven to be a suitable platform for the release of proteins with different kinetics while maintaining the integrity of the proteins. [7-9]. Poly (ethylene glycol) (PEG) as material for the fabrication of hydrogels in particular has the immense advantage that it is easy to functionalize, and thus different release kinetics can be realized by varying the macromolecular chain length and the branching factor. Therefore, antigen carrying silica nanoparticles (SiNPs) were incorporated into PEG hydrogels for release over a prolonged period.

Methods: PEG hydrogels were fabricated by cross linking furyl and maleimide functionalized multi-arm PEG macromonomers (8armPEG40k/20k/10k, 4armPEG40k, and 4armPEG40k-Lysine-hexanoic acid [4armPEG40k-Lys-AHX]) via the Diels-Alder reaction. Env trimer (Env_{tri}), Env monomer (Env_{mono}), as well as human serum albumin (HSA) as model antigens were used for immobilization on amino-functionalized SiNPs (100 nm) [6]. Hydrogels composed of different hydrogel precursors were loaded with antigen@SiNP and characterized in terms of mesh size, mechanical property (Young's modulus, measure of hydrogel stiffness) rheology, regarding gelation time (t_{gel}: time coincides with the gel point [cross over of storage (G') and loss modulus (G'')] and indicates the transition from a liquid-like to a solid-like behavior), and *in vitro* release. The impact of potential antigen PEGylation during hydrogel formation was investigated by the affinity to a broadly neutralizing antibody (Ab) by microscale thermophoresis (MST), as well as analyzed by HPLC, respectively.

Results: To allow for the formation of hydrogels, we successfully functionalized PEG macromonomers with furyl and maleimide groups (conversion: 82% and 61%, respectively). Hydrogels were loaded with antigen decorated SiNPs (HSA@SiNP [135 nm], Env_{mono}@SiNP [157 nm], and Env_{tri}@SiNP [186 nm]) by mixing with the polymer precursor solution. Investigating the impact of SiNP loading on hydrogel integrity, rheological experiments revealed that gelation time (t_{gel}) and hydrogel strength were not negatively affected (Figure 1).

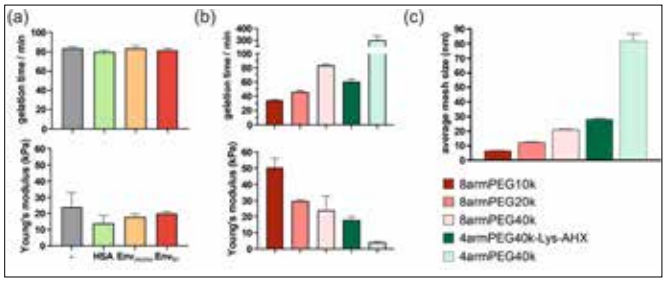

Figure 1: Hydrogel characterization. If indicated, hydrogels were loaded with 10 µg antigen@SiNP and analyzed rheologically and mechanically. a) Hydrogel loading with antigen@SiNP had no impact on tgel as well as on hydrogel stiffness. b) With increasing polymer chain length at constant polymer concentration (10% w/V) tgel decreased. The young's modulus decreased with increasing molecular weight and decreasing branching factor. c). With increasing molecular weight and decreasing branching factor, the average hydrogel mesh size increased.

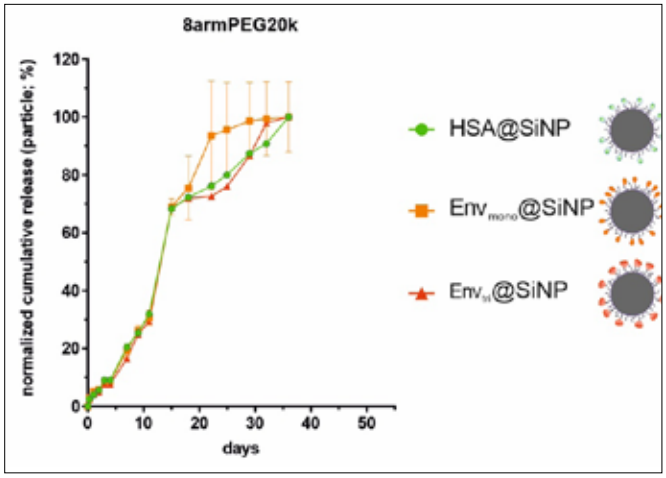

Figure 2 shows a representative release profile of antigen decorated NPs from an 8armPEG20k hydrogel. NPs were released in a controlled

manner over a time frame of about 35 days. No differences were observed between the three types of antigen decorated NPs.
Figure 2: *in vitro release of antigen labeled SiNP from 8armPEG20k hydrogel (10% w/V).*

Incorporation of decorated SiNPs could potentially lead to PEGylation of the antigen. Therefore, to test the integrity of the antigens before loading the SiNPs into the hydrogels and after their release, binding studies to antibodies were performed. To this end, the broadly neutralizing antibody VRC01 was chosen. The antibody affinity to Env_{mono}@SiNP and Env_{tri}@SiNP before loading and after release were quite similar (Env_{mono}@SiNP: before hydrogel loading: K_d = 5 nM, after release: K_d = 4 nM; Env_{tri}@SiNP: before hydrogel loading: K_d = 15 nM, after release K_d = 3 nM) and not negatively affected.

Conclusion: We believe that PEG hydrogels may serve as toolbox for the controlled release of vaccine NPs improving the immune response. An important step will be to demonstrate biological activity of released antigen carrying SiNPs *in vivo*.

Acknowledgements: This work was supported by a grant (grant no. 16GW0363K) from the German Federal Ministry of Education and Research (BMBF, Project HIVacToGC).

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NANOTECHNOLOGIES FOR TARGETING THE TUMOR MICROENVIRONMENT IN THE COLORECTAL CANCER

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Background: Colorectal cancer (CRC) is a complex disease characterized by a diverse tumor microenvironment comprising immune and non-immune components, with macrophages being the most abundant tumor-infiltrating cells that exhibit distinct phenotypic states, usually referred to as tumor-promoting M2 macrophages, or antitumoral M1 macrophages (PMID 34209703). Macrophage polarization represents the activation state of a macrophage at a specific moment. However, owing to the remarkable plasticity of macrophages, their polarization status is dynamic and can be modified by integrating multiple signals from the neighboring milieu (PMID 31530089). The dynamic interplay between tumor cells and macrophages plays a critical role in shaping the immunomodulatory properties of the tumor microenvironment, affecting tumor progression and therapeutic responses (PMID 36253762). Moreover, the impact of both conventional and innovative therapies on the tumor microenvironment, where immune cells, including macrophages, play a crucial role, is often overlooked. The macrophage plasticity phenomenon allows for exploring novel therapeutic strategies aimed at reprogramming them from the M2 to M1 phenotype.

Aim: This study aims to explore potential nanotechnology- and drug-based strategies for targeting macrophages in the tumor microenvironment of colorectal cancer. The novelty of this work lies in its comprehensive approach to the heterogeneity and complexity of the tumor microenvironment.

Methodology: To address the need for innovative therapeutic strategies, nanotechnologies and photodynamic therapy were explored for their potential to target macrophages and cancer cells. A novel theranostic nanocomplex, composed of quantum dots and a photosensitizer, previously shown to accumulate in human skin mesenchymal cells (PMID 34499462), was tested for its efficacy in targeting macrophages and CRC cell lines. The induced transcriptomic changes, reflecting macrophage polarization state, were later compared to the gene expression profile induced by several small molecule inhibitors, designed for targeting the stemness pathways.

Results: We demonstrated that the theranostic nanocomplex accumulates uniformly in all CRC cell lines, regardless of their molecular subtype, as well as in macrophages. This suggests the potential utility of nanotechnologies for targeting tumor-associated macrophages in CRC. After prolonged incubation with nanocomplex, no major transcriptome changes were induced in the CRC cells on the molecular level. Macrophages, on the other hand, responded by downregulating their M2-related gene expression and upregulating the M1-related gene expression, suggesting the potential repolarization from the protumoral M2 type to the antitumoral M1 type. Stemness inhibitors demonstrated ambiguous effects on macrophages, inducing changes in gene expression associated with both M1 and M2 phenotypes, and allowing for further combining several treatment strategies for obtaining the desired response.

Conclusions: Novel therapeutic approaches, such as theranostic nanoparticles or stemness inhibitors, can act as immunomodulatory agents for repolarizing M2 macrophages towards an antitumor phenotype (Figure 1). These findings justify further investigation to elucidate the underlying mechanisms and explore potential combination therapies for improved clinical outcomes in CRC treatment.

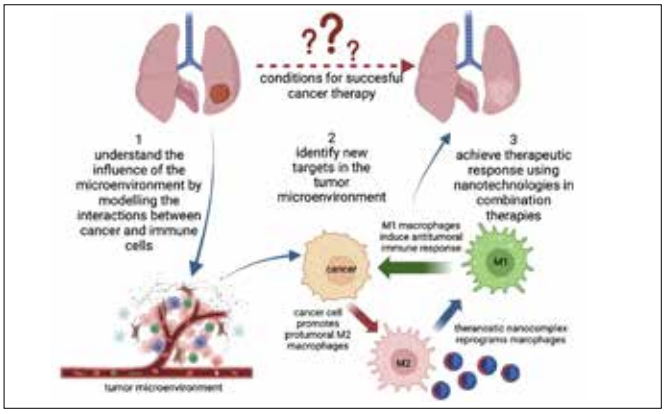

Figure 1. The conditions for successful cancer therapy might lie in the tumor microenvironment, composed of different host cell types. One of the most abundant populations – tumor-associated macrophages – are usually protumoral M2-like. However, our initial findings suggest that theranostic nanocomplex can repolarize M2 macrophages into their antitumoral M1 state and consequently exert an active immune response.

Acknowledgements: This work was financially supported by the Research Council of Lithuania, Grant No. S-MIP-22-31.

LOCAL DELIVERY OF LIPID LIQUID CRYSTALLINE FORMULATION OF DOXORUBICIN TO CANCER CELLS

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Introduction

Despite considerable advancements in developing efficient drug delivery strategies, cancer still remained one of the leading causes of death worldwide. The serious challenges are the low concentration of the cytotoxic agent at the tumor microenvironment and the systemic toxicity of the chemotherapeutics. With this in mind, we developed an injectable lipid liquid crystalline (LLC) based formulation of doxorubicin with

sustained release pattern which provide sufficient dose of doxorubicin at the target tissue.

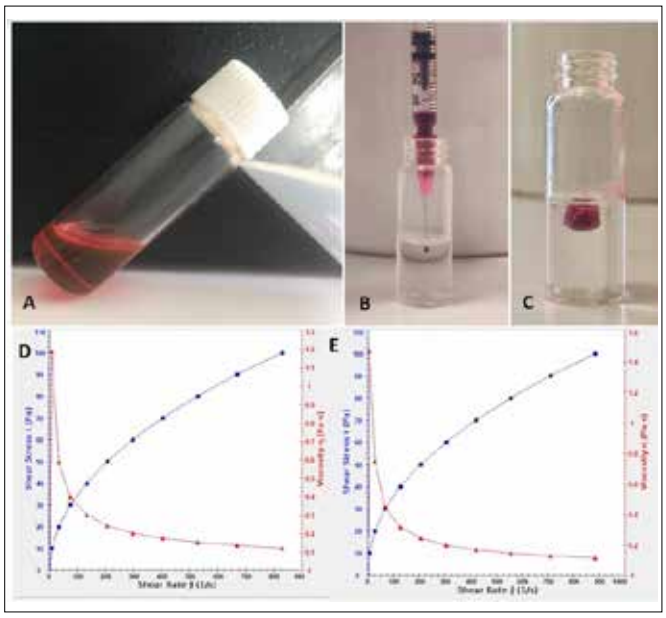
Methods

18 different formulations of LLC loaded with doxorubicin were synthesized via different ratios of phosphatidyl choline (PC): sorbitan monooleate (SMO), N-Methyl-2-pyrrolidone (NMP) and tween 80. Afterwards, physicochemical characteristics of the formulations were studied. Then, *in vivo* tumor inhibitory effect of the selected formulations in C26 tumor bearing mouse model was investigated.

Results

The results revealed that F_T (DOX loaded PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%)) and F (DOX loaded PC: SMO/NMP (50:50/50 w/w%)) showed pseudoplastic behavior as well as being syringeable (Figure 1)

Figure 1. F_T Formulation (DOX loaded PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%) was in sol state at room temperature (A). The syringeability of the formulation (B). The formation of gel state while injecting the formulation into phosphate buffered saline (pH:7.4) solution (C). Rheograms showing the pseudoplastic behaviors of F_T (DOX loaded PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%) and F (DOX loaded PC: SMO/NMP (50:50/50 w/w%)(E&F).



Additionally, doxorubicin was released in a sustained manner for up to 60 days in both formulations (Figure 2).

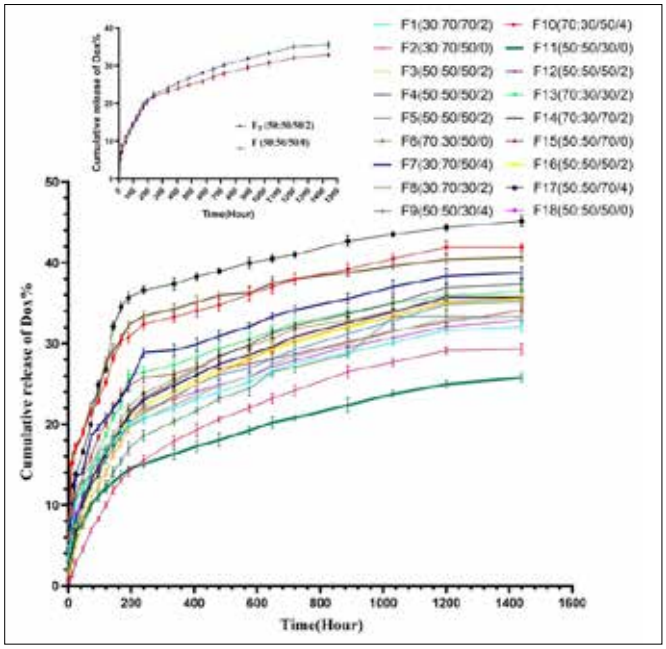


Figure 2. The release study of various formulations with different ratios of PC: SMO/NMP/Tween 80. Additionally, a separate chart showing the release pattern of the selected formulations is shown at the top.

After intratumoral administration of the formulations, the results indicated a significant decrease in tumor size in both formulations compared to intravascular administration of doxorubicin. Prolonged release of the formulations were proved by animal imaging studies. Besides, histopathological studies demonstrated that the developed formulations showed no systemic toxicity.

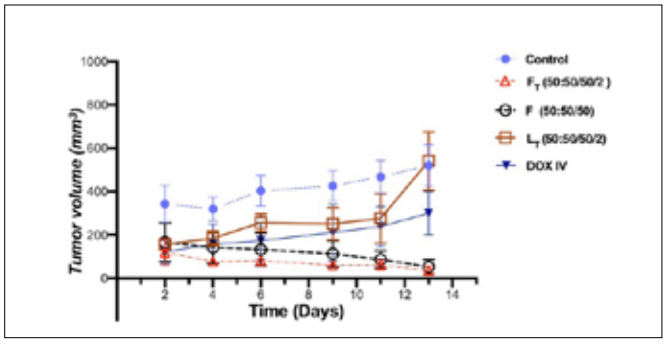


Figure 3. The tumor volume of Balb/c tumor bearing mice model after administration of PBS (control group), intravascular DOX (DOX IV), intratumoral FT (DOX loaded PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%), F (DOX loaded PC: SMO/NMP (50:50/50 w/w%) and LT which is lipid liquid crystalline formulation made of PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%).

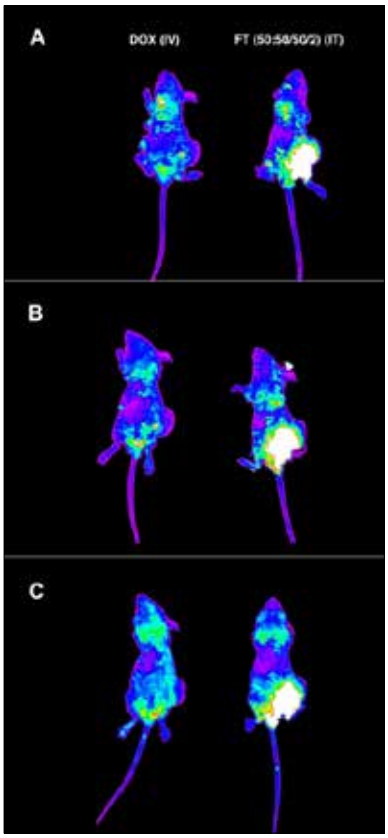


Figure 4. Animal imaging study of Balb/C tumor bearing mice after intravascular and intratumoral administration of DOX and F_T (DOX loaded PC: SMO/NMP/Tween 80 (50:50/50/2 w/w%).The study was done 4h (A) 24 h(B) and 72 h after the administration of the treatment.

Conclusion: We believe that doxorubicin loaded lipid liquid crystalline formulations could efficiently eradicate cancers cells in C26 tumor bearing mouse models without systemic cytotoxicity.

Keywords: Lipid liquid crystalline, In situ forming, Injectable, Doxorubicin

BRAIN-TARGETED LIPOSOMES LOADED WITH ANTI-ALPHA-SYNUCLEIN MONOCLONAL ANTIBODY FOR TREATING PARKINSON'S DISEASE IN ITS EARLY STAGES

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Parkinson's disease (PD) is one of the most common neurodegenerative condition, with limited treatment options. The disease is characterized by the loss of dopaminergic neurons and abnormal accumulation and propagation of the neuronal protein alpha-synuclein (AS). An anti-AS antibody (SynO4) has previously shown a high affinity to AS aggregates,[1] suggesting that it can be used as a therapeutic agent to slow PD progression. However, SynO4's penetration into the brain, similarly to other antibodies, is very limited by the highly selective blood-brain barrier (BBB) (only around 0.01% of the injected dose penetrates the BBB), thereby curbing its therapeutic efficiency.[2] In addition, antibodies are limited with their ability to enter cell membranes and specifically neurons. This is a major obstacle for an effective reduction of intracellular AS aggregates and oligomers. During PD, the transferrin receptor is overexpressed on the BBB and in CNS neurons.[3] To overcome the brain-penetration challenge, we encapsulated the therapeutic Syn-O4 antibody within 100-nm lipid nanoparticles decorated with transferrin on their surface (Figure 1).

Figure 1. Brain targeted liposomes deliver anti-alpha-synuclein monoclonal antibody to reduce aggregation of alpha synuclein in early stage Parkinson disease mouse model. *Through receptor-mediated transcytosis, the liposomes cross the BBB and are taken up by disease neuron cells; the antibody payload then targets the AS aggregation to inhibit neuron cell death.*
Transferrin nanoparticles loaded with SynO4 demonstrated enhanced penetration across a supported BBB model and higher neuron cellular uptake and target engagement to intracellular neuronal alpha-synuclein aggregates compared to free SynO4.

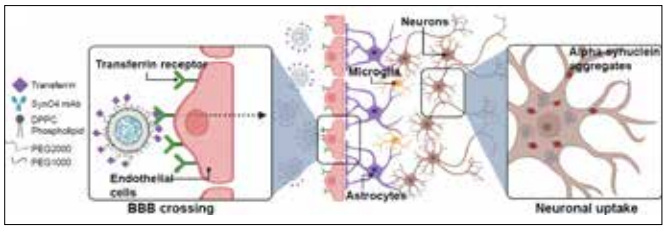
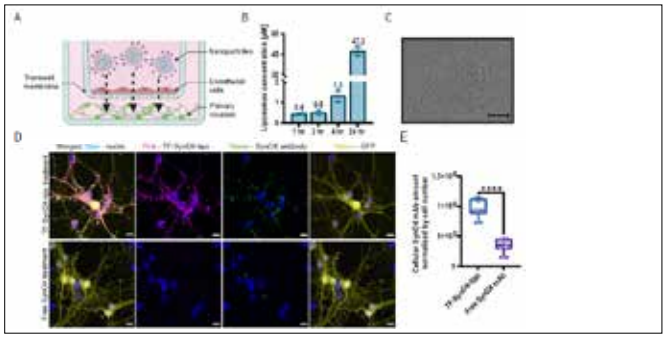


Figure 2. Penetration of TF-lipo across an *in vitro* model of BBB and cellular uptake of TF-SynO4-lipo in PD *in vitro* neuron models. *Representation of the transcytosis of TF liposomes across an in vitro model of BBB composed of BMECs and primary neurons co-culture in a noncontact manner* **(A)**. *Cy5-TF liposomes were administrated to a monolayer of BMEC cells (apical side) of a transwell. The particle concentration on the other side of the transwell increases over time and reaches 47.3±3.2 ug/ml after 24 hours* **(B)**. *A cryo-TEM image of the medium out of the insert shows that the liposomes are intact after crossing* **(C)**, *(scale bar is 100 nm). Confocal images of uptake of TF-SynO4-lipo or free SynO4 mAb in infected PD neurons after overnight incubation. The liposomes were labelled with Cy5 (pink), the antibody was labelled with Cy3 (green), and the PD primary neuron cells were marked with GFP (yellow)* **(D)**, *(scale bar is 10 um). Analysis of cellular SynO4 mAbs amount normalized to cell number by IMARIS imaging software* **(E)**.



The efficacy of the TF-SynO4-lipo was tested in primary cortical neurons infected with a viral vector overexpressing A53T alpha-synuclein. The cells were treated overnight with either TF-SynO4-lipo or the free form of the mAb. TF-SynO4-lipo treatment reduced AS aggregation level significantly compared to PD-induced cells or free mAb treatment.

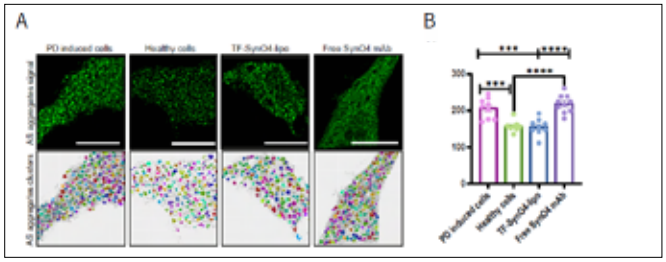


Figure 3. Therapeutic effect of TF-SynO4-lipo in PD in vitro neuronal model. *dSTORM images of PD-infected neurons treated overnight with TF-SynO4-lipo or free SynO4 antibody; the neurons were marked with GFP (green)* **(G)** *(scale bar 9 um). Analysis of AS clusters using HDCSCAN algorithm* **(H)**.
Furthermore, in vivo studies show that systematic administration of transferrin-targeted liposomes efficiently crossed the BBB and were delivered to the neuronal cells in an AAV-based PD-like mouse model. The nanoparticles improved the therapeutic efficacy, including reducing AS aggregation and neuroinflammation.

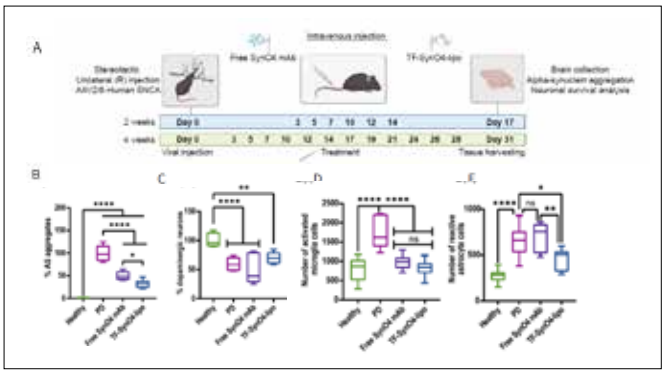


Figure 4. TF-SynO4 liposomes' capacity for reducing alpha-synuclein aggregation and slowing the degeneration of dopaminergic neurons in AAV-based PD mice model. *An illustration of the therapeutic efficacy experiment* **(A)**. *The percentage amount of aggregated alpha-synuclein in different treatments after four weeks* **(B)**. *The percentage of dopaminergic neurons survival after four weeks* **(C)**. *The number of activated microglia cells four weeks* **(D).** *The number of reactive astrocyte cells after four weeks* **(E)**. *Results of B, C, D and E (four to five independent repetitions performed in 2-3 technical replicates) are presented as mean±SD. One-way ANOVA and was used for statistical analysis; *p≤0.02 **p≤0.007 ***p≤ 0.0005 ****p< 0.0001.*

Taken together, the use of transferrin SynO4 liposomes and their ability to encapsulate therapeutic antibodies represents a promising therapeutic approach and a novel platform for effective drug delivery into the brain. Thereby, they can be considered an improvement of the treatment of PD and other neurodegenerative and CNS disorders.

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MODULATING THE TUMOR MICROENVIRONMENT WITH NANOMEDICINE AND METRONOMIC THERAPY TO ENHANCE TREATMENT EFFICACY OF IMMUNOTHERAPY

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INTRODUCTION

Nanoparticle formulations are considered to be advantageous compared to conventional chemotherapy owing to their preferential accumulation in tumor tissues as a result of the enhanced permeability and retention (EPR) effect and their prolonged circulation in the blood [1, 2]. Modest survival benefits of clinically approved nanoparticles are in large part attributed to the fact that the abnormal tumor microenvironment (TME) poses physiological barriers that can significantly decrease the delivery of nanoparticle formulations throughout tumors [1].

A metronomic approach, using a lower and more frequent dose schedule, has emerged as an alternative to the standard maximum tolerated dose (MTD) chemotherapy schedule [3, 4]. This approach has been shown to yield better survival than MTD in several preclinical tumor models as has the potential to normalize the TME, improve blood vessel function and perfusion, and increase the cytotoxic activity of immunotherapy. Interestingly, in recent studies, CRLX101 nanoparticles have shown similar to the normalization effects of metronomic chemotherapy. Even though CRLX101 failed in clinical trials, similarities between nanomedicine and metronomic therapy suggest that these two approaches can be compared using the same unified theoretical framework of inducing normalization of TME to enhance therapy (Table 1). Here, we hypothesize that nanoparticle formulations can trigger the same cascade of activities as metronomic therapy [5]. To test our hypothesis, we tailored our mathematical framework to model nanoparticles and analyzed the experimental data on the effect of the nanoparticles on the growth of mouse models of breast cancers. To validate model predictions, we performed *in vivo* experiments in breast tumor model by employing the clinically approved nanoparticle formulation Doxil. During Doxil treatment we monitored changes in tumor stiffness and perfusion using ultrasound shear wave elastography (SWE) and dynamic contrast enhanced ultrasound (DCEUS), respectively. We found that low doses of Doxil can effectively reduce tumor stiffness and improve perfusion thus, inducing normalization of the TME and enhance the efficacy of immunotherapy.

Table 1. Similarities and differences from the use of nanomedicines and metronomic chemotherapy to treat solid tumors.

Similarities	Differences
i) maintain effective drug levels in the blood for long duration	i) nanomedicine can exhibit improved accumulation to tumor tissue (EPR effect) and target to cancer cells
ii) induce vascular normalization	ii) nanomedicine are likely to have less adverse effects on normal tissues, except the liver
iii) improve tumor perfusion and oxygenation	iii) nanomedicine can exhibit limited penetration into tumor tissue owing to their large size
iv) improve drug delivery and treatment efficacy	iv) nanomedicines require less frequent dosing
v) potential to make the TME more immune-supportive	v) a few nanoparticles have been approved for clinical use, but metronomic therapy is not yet approved

RESULTS AND DISCUSSION

The mathematical framework for tumor growth and response to therapy and its components are presented in Figure 1A, B. To test our hypothesis that nanoparticles have normalization effects similar to metronomic therapy, we compared model predictions of tumor growth to experimental data for CRLX101 particles from two independent studies [6, 7]. The model parameters were specified based on the control/untreated groups and model predictions were compared with experimental data for the treated groups (Figure 1C, D). Our model predictions were in very good agreement with the experimental data of tumor growth for both doses when vascular normalization (i.e., reduction in vessel leakiness) was considered (Figure 1C,D), whereas it significantly over-predicted final tumor volume when vascular normalization were not taken into account. Therefore, the results of both studies can be well explained when nanoparticle treatment works via similar processes as metronomic therapy.

Guided by model predictions, we next set out to test their validity in 4T1 breast tumors where we investigated the normalization effects of the Doxil. The treatment protocol is summarized in Figure 2A. To monitor the effects of Doxil on normalizing the mechanical TME, SWE and DCEUS were performed in the beginning of the treatment and before the initiation of the second cycle. Doxil treatment with two lower and more frequent doses was able to reduce tumor stiffness and interstitial fluid pressure, improve perfusion (time to peak

of contrast agents) and decrease tumor growth whereas the higher, less frequent dose reduced stiffness and improved perfusion in a less efficient way (Figure 2B-E). For the second cycle of Doxil treatment, an aPDL1 immune checkpoint inhibitor was added to the treatment protocol to investigate whether pretreatment with a metronomic/normalizing dose of nanomedicine can improve the efficacy of nano-immunotherapy. Immunotherapy alone had no effect on tumor mechanical properties, on perfusion (Figure 2B-E) and on tumor growth. On the other hand, the optimal efficacy of combinatorial treatment of Doxil with aPDL1 was observed when nanomedicine was used in a metronomic and normalizing dose (Figure 2B-E).

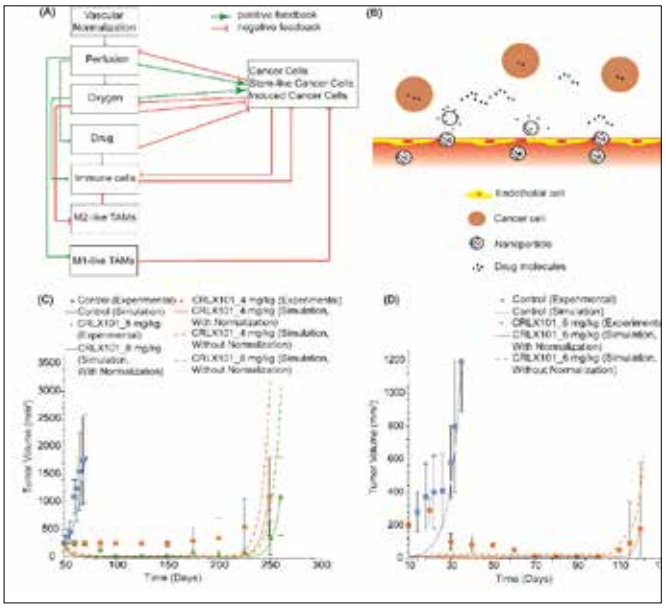

Figure 1. (A) Schematic of mathematical model components and their interactions. (B) Schematic for nanoparticle delivery to tumors without binding. (C) Model predictions for the experimental data from [6] and (D) model predictions for the experimental data from [7].

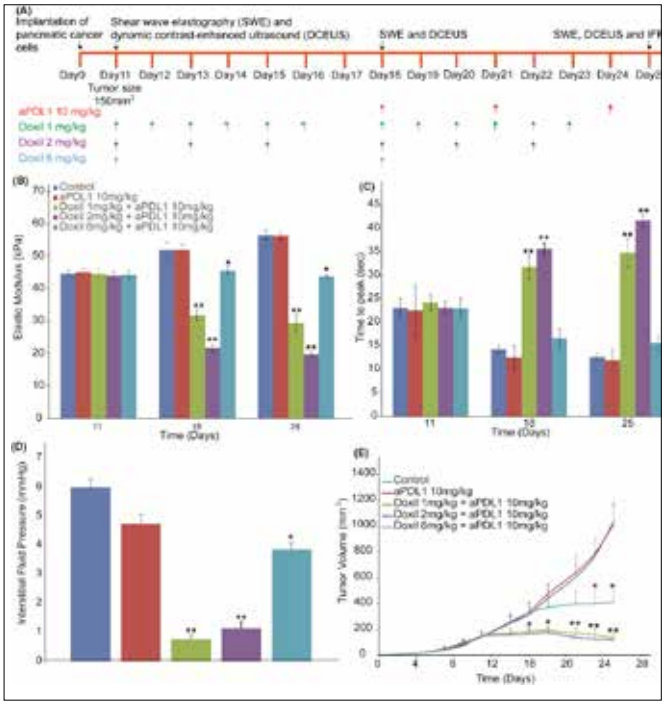

Figure 2. Low and more frequent doses of the nanomedicine Doxil normalize the mechanical TME. (A) Treatment protocol. (B) Quantification of the average elastic modulus of the tumors and (C) Time to peak of contrast agents for the different treatment groups on Days 11, 18 and 25. (D) Interstitial fluid pressure levels at the end of the treatment protocol. (E) Tumor volume data. Data presented as mean ± SEM (n = 6 mice per group).

CONCLUSION

Our analysis of preclinical experimental data suggests that nanomedicine and metronomic therapy can be viewed using the same unified framework as strategies that normalize the TME by delivering lower, but more sustained levels of drug to the tumor than achieved with MTD. Additionally, we showed experimentally that low and more frequent doses of Doxil -compared to high and less frequent doses- decrease tumor stiffness, improves perfusion and exhibits enhanced anti-tumor effects either alone or in combination with immunotherapy.

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HIERARCHIC POLYMERIC MICROPLATES FOR DELIVERY OF SMALL MOLECULES AND NANOPARTICLES

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Introduction: In the last decades, polymeric microparticles have found application in different biopharmaceutical fields, especially as a controlled drug delivery system [1]. The top-down fabrication methodology is one of the currently employed techniques to fabricate particles, allowing for tuning the size, shape, particle density and surface area. Applying this procedure, we developed in our lab the Microplates (μPL), polymeric microparticles owing a peculiar shape, defined by a square base of 20 × 20 μm and 10 μm height, homogeneous in size, shape and surface area and developed to deliver a wide variety of payloads [2-4]. Recent optimizations of particle fabrication methods led us to develop a novel porous version of μPL, with different physiochemical and biopharmaceutical properties.

Methods: For this novel design, the top-down methodology was implemented under lower pressure and also developing novel compositions, based on Poly(D,L-lactide-co-glycolide) (PLGA) as main polymer and a copolymer able to modify particle configurations and properties. Both direct and post-production loading strategies were valued to develop a hierarchic system; Curcumin (CURC), a compound with anti-inflammatory and anti-cancer properties [5], was directly loaded into the particle structure, while fluorescent small molecules (such as Rhodamine B), polystyrene beads nanoparticles (50 and 200 nm) and fluorescent liposomes were loaded after particle preparation.

The morphology of the porous-μPL was investigated by the Scanning Electron Microscopy (SEM), Confocal Microscopy and Fluorescence Microscopy, and the two latter were also applied to study drug and nanoparticle distributions within the particle structure (Fig.1). The size distribution and particle concentration were determined using a Multisizer system. Drug encapsulation efficacy (EE) was determined by HPLC and UV-Vis analyses.

Results and Discussion: Porous-μPL showed a well-defined squared shape, characterized by a homogeneous alveolar structure with size range from 0.4 to 1.5 μm. This sponge-like structure leads to a large specific surface area which results in a high loading capacity based on different mechanisms, such as physical adsorption, H-bond formation, hydrophobic or ionic interactions and even formation of covalent linkage. The loading of CURC allows to better

observe the porous structure of the system; the distribution of signals from RhB, nanoparticles and fluorescent liposomes, confirmed the ability of these particles to act as a hierarchic system able to housing different pharmaceutical entities. Moreover, the yield of the fabrication process was 57.58 ± 4.96, while EE resulted 12.53 ± 1.08 % and 11.38 ± 1.08 % for CURC and RhB, respectively, and ranged between 40-70% for beads of different sizes.

This new fabrication approach allowed to maximize the favorable properties of these systems, especially in terms of morphology and drug loading. Moreover, their strategic features widen the spectrum of application to several therapeutic strategies, from the simultaneous delivery of drugs owning different physiochemical properties, to haul nanoparticles or liposomes for sustained drug releases. However, further investigations are required to understand the role of co-polymer and its correlation with the number, structure, and pore diameter, and how these features might affect the properties of the systems (e.g., drug EE and release rate).

In conclusion, novel µPL characterized by a peculiar porous structure have been obtained. The preliminary collected results define them as a promising hierarchic platform for multidrug delivery and suitable for various therapeutic applications.

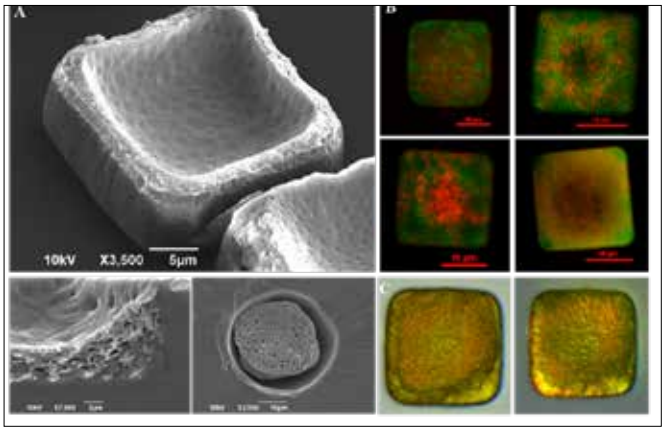

Figure 1: Geometrical characterization of porous µPL: A) SEM image; B) Confocal microscopy (40X) of µPL loaded by CURC and RhB, Beads 50 or 200nm and liposomes; C) Fluorescence microscopy image (40X).

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THERMORESPONSIVE CHIRAL-NEMATIC LIQUID CRYSTALS AS MULTIFUNCTIONAL NANO-STRUCTURED MATERIAL FOR SKIN DRUG DELIVERY

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Among the different chiral soft templates, liquid-crystalline soft matter is highly attractive for guiding the self-assembly of nanoscale functional building blocks into high-order chiral nanomaterials due to their inherent long-range ordered molecular assemblies [1]. In fact, chiral liquid-crystalline nanoarchitectures are widely described as functional optical materials, as well as charge-, energy- and ion-transporting soft templates. Nowadays, many researchers have devoted themselves to the design and synthesis of advanced chiral functional nanomaterials using lyotropic liquid crystals (LCs) but only few investigations are dedicated to a biomedical application of thermotropic (thermoresponsive) LCs.

While novel and more powerful drugs continue to be designed and synthesized, a growing focus is being given to the techniques by which these active ingredients are administered. To address this issue, research in recent decades has attached importance to controlled drug-delivery systems [2]. For that matter, advanced drug-delivery systems for skin applications should allow for variable or on-demand drug release, ideally in response to some specific, (patho)physiological stimulus. In this regard, LCs are promising candidates, as they represent multifunctional nanostructured materials that are responsive towards stimuli by temperature, light, magnetic or electric fields. Out of the two LC types, the lyotropic systems as typically amphiphilic mesogens have been extensively studied as delivery vehicles for a wide range of drugs [3]. In contrast, the investigations on thermoresponsive LCs for such purposes are much more limited, notably those marked by phase transition within the human body temperature. Hence, the current work addresses an innovative strategy to implement the anisotropic properties of thermotropic LCs for extending their application in drug delivery. In this regard, we fabricated novel compositions with a thermosensitive core based on natural products – cholesteryl esters and mono-/bicyclic terpenoids (Fig. 1). The distinctive feature of aforementioned systems is their temperature-induced on/off switchable permeability as result of their transition to the LC state at normal human skin temperature.

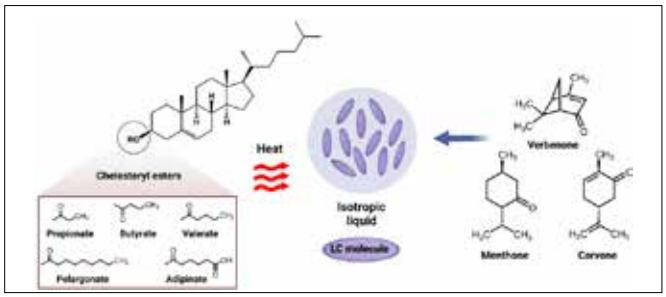

Fig. 1. *Schematic representation of liquid crystal (LC) system preparation based on cholesteryl esters and terpenoids.*

LC mixtures were prepared by mixing of cholesteryl esters according to a multiplicity of mass ratios leading to the formation of four basic formulations – systems **S1–S4** (Fig. 2). In order to achieve the phase transition crystalline (Cr) to chiral nematic (N*) in the range of normal human skin temperature, the basic systems were optimized by doping with mono-/bicyclic terpenoids of 5% or 10%

concentration (w/w). In total, 24 novel LC systems containing menthone, carvone and verbenone were designed as penetration/permeation enhancers for drug delivery.

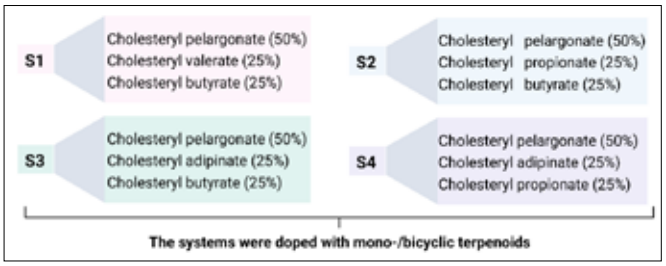

Fig. 2. *Compositions of liquid crystal systems.*

According to the data of differential scanning calorimetry, phase transition (Cr–N*) corresponding to normal human skin temperature was achieved predominantly when incorporating 10% of terpenoids into the LC systems. This mesomorphic behavior is exemplified by liquid crystalline properties of system **S1** comprising terpenoid compounds as depicted in Fig. 3, where a shaded area reflects the mesophase range of LC mixtures. The convergence of melting and isotrope curves and, accordingly, narrowing of temperature range for N*–isotropic (N*–Iso) phase transitions is observed for system S1 when adding terpenoids in the following manner: verbenone > carvone > menthone. As depicted in Figure 4, the addition of verbenone (10%, w/w) to the system **S1** leads to phase transitions Cr–N* and N*–Iso overlapping at the temperature point 36.9 °C, indicating the disappearance of mesophase with terpenoid content over 10%. Thus, we may conclude that the range of LC phase existence is extremely sensitive to both terpenoid chemical structure and its concentration.

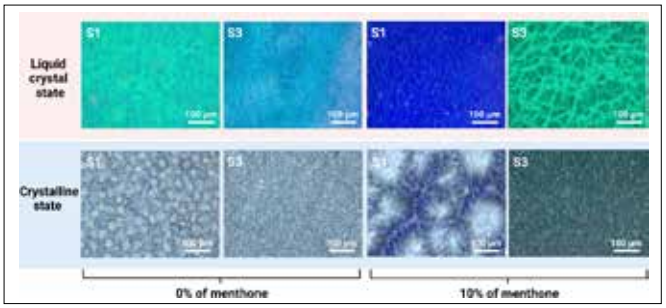

Fig. 3. *Temperatures of phase transitions "crystal – mesophase" (–– squares) and "mesophase – isotropic liquid" (–– circles) for system* **S1** *containing 5–10% of menthone (A), carvone (B) and verbenone (C); – liquid crystal state.*

To further explore the mesogenic behavior of LC systems, their optical texture was estimated when imaged with polarized optical microscopy (POM, Fig. 4). For all of LC systems, two phase transitions of solid state-mesophase and mesophase-isotropic phase were clearly observed on heating and cooling. When pure systems **S1** and **S3** were heated to 43–45 °C, the focal-conic texture of cholesteric phase began to appear indicating the birefringence properties of materials. It should be noted, however, that adding of terpenoids (menthone, as an example) serving as chiral dopants affects the mesophase temperature region of the N*-LC. Namely, it becomes narrower as the dopant concentration increases, eventually resulting in destruction of the mesophase when the concentration reaches a critical value. When incorporating menthone (10%, w/w) in the LC systems **S1** and **S3**, a different colorful planar texture of the cholesteric phase was observed as illustrated by POM images.

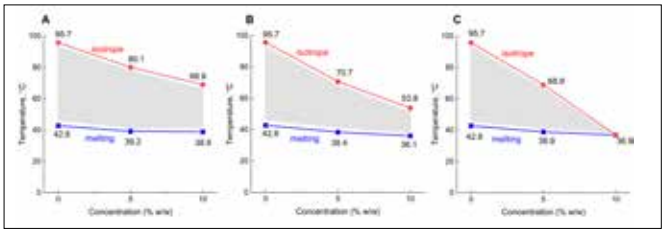

Fig. 4. *Optical polarizing microscope textures. Crystalline and chiral nematic liquid crystal structure of system* **1** *(***S1***) and system* **3** *(***S3***) in their pure form and after incorporation of 10% of menthone.*

Furthermore, we describe the dependence of helical pitch on LC formulation for various ternary cholesteric systems doped with terpenoids, suggesting that these chiral dopants are nominally untwisting. Modification of cholesteric helical pitch leads to a visual color change of the LC systems upon their melting on the skin surface. In this context, we may propose that terpenoids are incorporated into quasi-nematic layers formed by orientationally ordered molecules of cholesteryl esters contributing to the untwisting effect on the pitch (Fig. 5).

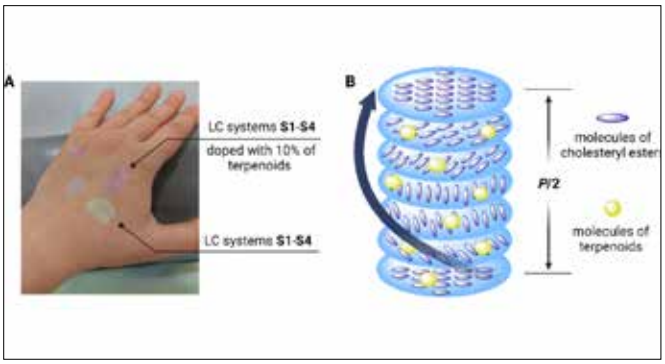

Fig. 5. *Phase transition of pure liquid crystal (LC) systems* **S1–S4** *and those containing 10% of terpenoids on the skin surface (A). Schematic representation of helical pitch in the cholesteric liquid crystals doped with terpenoids (B).*

To prove the basic concept regarding the thermoresponsive LCs as drug-delivery systems for skin applications, we explored their potency by studying *in vitro* and *ex vivo* penetration across artificial Strat-M® membrane and full human skin, respectively. By incorporating model drugs with diverse molecular structures and physicochemical properties into such LC matrix, we explored their potential exploitation for both transdermal and intradermal drug delivery. To the best of our knowledge, this is the first report concerning the LC-templated chiral nanomaterials such as thermoresponsive LCs based on cholesteryl esters and terpenoids as tunable soft-matter systems for skin drug delivery. Prospectively, we intend to exploit these triggerable LC compositions, releasing drugs at elevated human skin temperature as a drug reservoir system.

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EXPLOITATION OF THE 2D GRAPHENE OXIDE BIOMOLECULE CORONA IN SECRETOME-BASED CANCER BIOMARKER DISCOVERY

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Nanotechnology has emerged as a promising tool for cancer biomarker discovery. Nanoparticles (NPs) undergo rapid modification once they come into contact with the biological milieu to form a "biomolecule corona" due to their interfacial reactivity[1]. Analysis of the biomolecule corona by mass spectrometry-based proteomics has shown an enhanced discovery of low-abundant proteins and has attracted significant interest as a promising technology in cancer biomarker discovery[2,3].

Graphene oxide is a two-dimensional (2D) nanomaterial with a distinctively large surface area and high surface reactivity[4]. In this study, the biomolecule corona formed around graphene oxide nanosheets is exploited to provide an in-depth analysis of the secretome and identify unique proteomic signatures of different cancer cell lines. The cancer cell secretome consists of proteins released by cancer cells and provides a highly specific analyte for biomarker discovery. However, the utility of the secretome is limited by the low abundance of secreted proteins within the milieu of the culture medium, which mainly contains highly abundant foetal bovine serum (FBS) proteins. Therefore, this study aims to utilize the graphene oxide biomolecule corona to enrich low-abundance secretome proteins within the milleu of FBS-containing conditioned media.

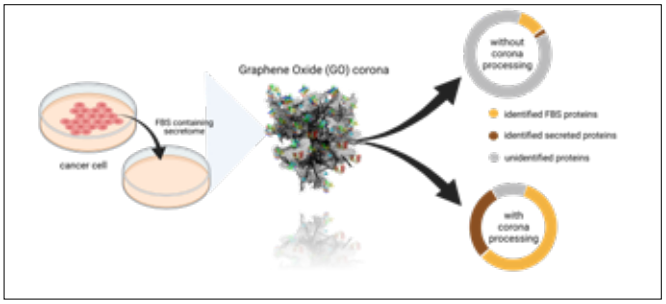

Figure 1. Graphical illustration of graphene oxide (GO) enrichment of secretome proteins in FBS-containing media

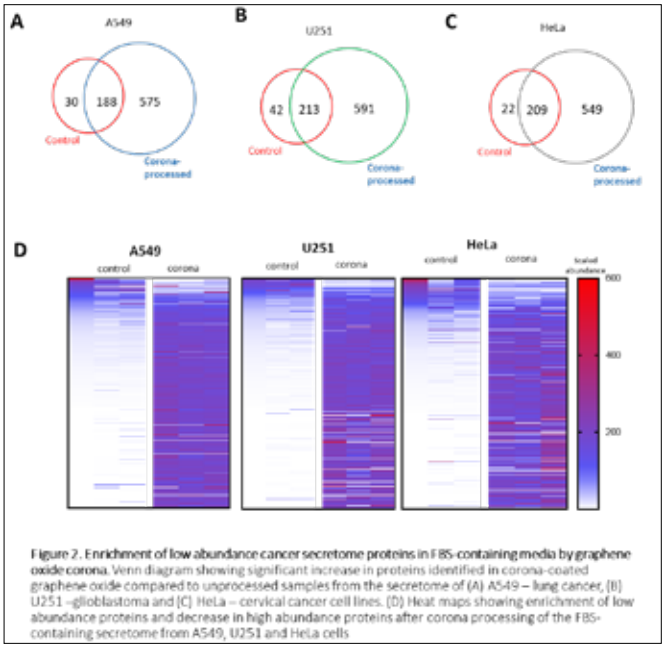

Figure 2. Enrichment of low abundance cancer secretome proteins in FBS-containing media by graphene oxide corona. Venn diagram showing significant increase in proteins identified in corona-coated graphene oxide compared to unprocessed samples from the secretome of (A) A549 – lung cancer, (B) U251 –glioblastoma and (C) HeLa – cervical cancer cell lines. (D) Heat maps showing enrichment of low abundance proteins and decrease in high abundance proteins after corona processing of the FBS-containing secretome from A549, U251 and HeLa cells

The secretome of lung cancer (A549), glioblastoma (U251) and cervical cancer (HeLa) cell lines was obtained by collecting the conditioned media of the cultured cancer cells. The collected secretome was incubated with graphene oxide nanosheets to form the biomolecule corona. Using the 2-step NanoOmics purification protocol[5], the graphene oxide biomolecule corona was isolated via a combination of size exclusion chromatography and membrane ultrafiltration.

Proteomic mass spectrometry analysis of the isolated biomolecule corona showed a significant increase in the number of identified proteins in the corona-processed secretome of all the cancer cell lines when compared to the unprocessed secretome samples (Fig 2B-D). Heat map of the relative abundance of ech protein identified in corona samples and control samples showed significant enrichment of low-abundance secreted protein due to the corona-processing (Fig 2E). Pathway analysis of secreted proteins identified pathways unique to each cancer cell type. Ultimately, the graphene oxide corona-processing protocol enhanced the discovery of uniquely secreted proteins from different cancer cell lines.

In the future, we plan to utilize the graphene oxide biomolecule corona platform to correlate the cancer secretome proteomic fingerprints with proteomic analysis of plasma samples obtained from cancer patients, with the ultimate goal to identify highly-specific blood biomarkers for cancer early detection.

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RE-ENGINEERING THE TUMOR MICROENVIRONMENT WITH POLYMERIC MICELLES TO IMPROVE THE EFFICACY OF NANO-IMMUNOTHERAPY IN BREAST CANCER

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INTRODUCTION

Nano-drugs combined with immunotherapy (nano-immunotherapy) has high potential to improve cancer patient outcomes, as already demonstrated in triple negative breast cancer with the combination of nanoparticle albumin-bound paclitaxel and the immune checkpoint blocker (ICB) antibody, atezolizumab [1]. This regimen, however, has a modest effect on survival, with median survival lasting less than two years. Compromised therapeutic outcomes are likely to be caused in part by abnormalities in the tumor microenvironment (TME) that inhibit the delivery of nanoparticles and antibodies and induce hypoxic and immunosuppressive conditions that fuel tumor progression, metastasis and drug resistance [2]. TME abnormalities

in triple negative breast cancers include the dense tumor interstitial space, abundant in cancer-associated fibroblasts (CAFs), collagen and hyaluronan, which causes stiffening of the tumor and accumulation of mechanical forces [3]. The development of such mechanical forces within the TME leads to tumor vessel compression and thus, the formation of a dysfunctional vasculature that limits tissue oxygenation and drug delivery [4].

To improve efficacy, research focuses on drugs that reprogram CAFs to improve therapeutic delivery and immunostimulation. These drugs, however, have a narrow therapeutic window and cause adverse effects. Developing novel strategies that increase CAF-reprogramming while limiting adverse effects is urgent. Here [5], taking advantage of the CAF-reprogramming capabilities of tranilast, we developed tranilast-loaded micelles. Strikingly, a 100-fold reduced dose of tranilast-micelles induces superior reprogramming compared to free drug owing to enhanced intratumoral accumulation and cancer-associated fibroblast uptake. Combination of tranilast-micelles and Doxil with immunotherapy increases T-cell infiltration, resulting in cures and immunological memory in mice bearing immunotherapy-resistant breast cancer.

RESULTS AND DISCUSSION

Tranilast loaded micelles were prepared by mixing PEG-*b*-poly (benzyl-L-glutamate) copolymer (PEG-PBLG) and tranilast in tedrahydrofuran (THF) (Fig. 1A). The THF in the mixture was then evaporated by using a rotatory evaporator, and the polymer and tranilast were resuspended by using MilliQ water to form tranilast-loaded micelles (Tranilast/m). The micelles were sonicated and filtered by using a 0.22 µm filter. The free tranilast that was not loaded in the micelles was removed by dialysis against water. The micelles exhibited minimal batch-to-batch variation with an average size ~ 95 nm and polydispersity index values close to 0.12, as assessed by dynamic light scattering(Fig. 1B). The transmission electron microscopy observation of the micelles was done after staining with 1% n acetate (Fig. 1C)

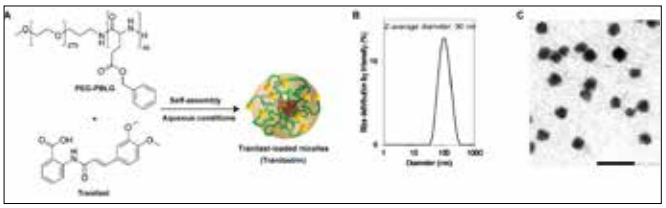

Figure 1. (A) Schematic of Tranilast/m preparation. (B) Size distribution of Tranilast/m determined by dynamic light scattering. (C) Transmission electron microscopy observation.

Next, we set out to test our hypothesis that the Tranilast/m can potentiate normalization of the tumor stroma when administered in amounts significantly lower than those of the free agent. All orthotopic models for murine mammary tumors were generated by implantation of 5×10^4 4T1 or E0771 cancer cells into the third mammary fat pad of 8-week-old BALBC and C57BL6 female mice, respectively. Mice were treated either with free tranilast or the Tranilast/m. Free tranilast was administered at the optimal dose of 200 mg/kg orally every day, or by intravenous injection (i.v.) at the dose of 2 mg/kg daily. Tranilast/m were administered i.v. following two protocols: a daily administration of 2 mg/kg or a 4 mg/kg dose every other day. From the two Tranilast/m protocols, we found that only the daily 2 mg/kg dose managed to reduce fluid pressure and tumor elastic modulus (Fig. 2A,B). Elevated interstitial fluid pressure and stiffness in desmoplastic tumors, such as the tumor models considered in our study, depend on large part on hyaluronan and collagen levels. We assessed the levels of these two key structural components using fluorescence immunostaining (Fig. 2C-F). Significantly, we showed that Tranilast/m treatment suppressed collagen levels more effectively compared to free tranilast (Fig. 2D,F). Given these changes in tumor composition and stiffness, we reasoned that the abnormalities in vascular perfusion would be restored. Immunostaining of the endothelial cells confirmed improved vascular perfusion (Fig. 2G,H).

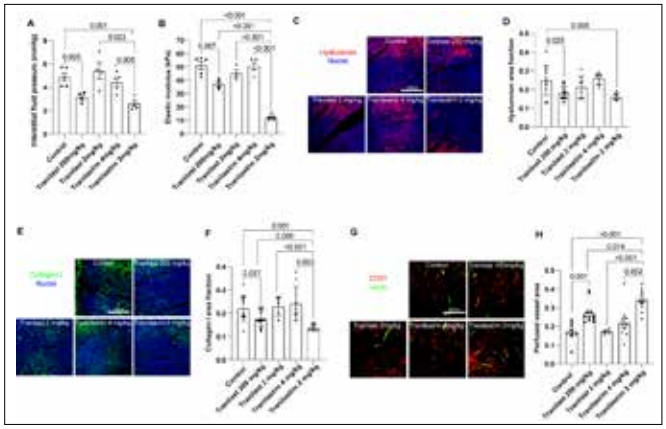

Figure 2. (A) Quantification of interstitial fluid pressure using the wick-in-needle technique in E0771 tumors (B) Ex vivo elastic modulus quantification of E0771 tumor specimens. (C) Representative fluorescence images of tumor tissue sections stained with anti-collagen I (green) antibody and DAPI (blue). Scale bar 100 µm. (D) Quantification of the area fraction positive for collagen I staining treated as indicated. (E) Representative fluorescence images of tumor tissue sections stained with anti-hyaluronan (red) antibody and DAPI (blue). Scale bar 100 µm. (F) Quantification of the area fraction positive for hyaluronan staining treated as indicated. (G) Immunofluorescence staining of E0771 tumor tissues using the endothelial cell marker anti-CD31 (red) and biotinylated lectin (green) as a measure of vascular perfusion indicated by the colocalization of CD31 and lectin protein (yellow). Scale bar 200 µm. (H) Perfused vessel fraction normalized to total CD31 positive staining.

Finally, we explored the antitumor efficacy of immune checkpoint blockers (ICBs) combined with Doxil nanomedicine alongside with the tranilast micelles in orthotopic E0771 tumors. Primary tumors were removed on day 21 and animals were monitored for their survival rate. Treatment with Tranilast/m, ICB and Doxil monotherapies as well as ICB-Doxil combination failed to provide any survival advantage or tumor regression compared to control group (Fig. 7a). Treatment with all three drugs yield to a 100% survival. On day 90, all surviving mice from the Tranilast/m-Doxil and Tranilast/m-Doxil-ICB combination groups, along with a group of eight naïve mice were challenged with E0771 cells. As expected, all naïve mice succumbed while all mice of the triple combination treatment group successfully rejected E0771 cells. Tumor-free mice were challenged again on day 130 with a subcutaneous injection of $2.5\ x10^5$ cells from the unrelated MCA205 fibrosarcoma tumor line. Notably, none of the mice in either group was able to reject this irrelevant tumor line, indicating that the long-term immune memory acquired is tumor specific (Fig. 7b-d).

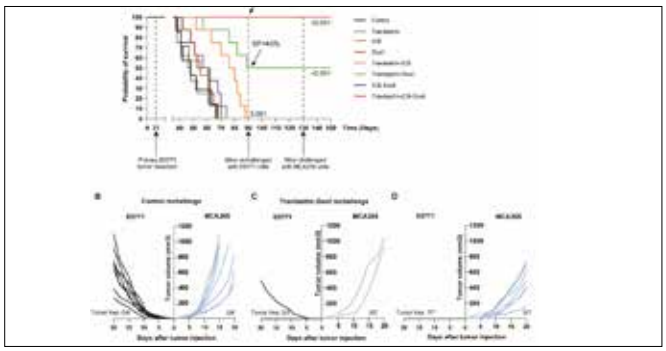

Figure 3. (A) Kaplan-Meier survival curves for the various treatment considered (n=7-8 mice). (B-D) Individual growth curves of naïve mice (B), cured mice of Tranilast/m-Doxil group (C) and Tranilast/m-ICB-Doxil group (D) challenged with E0771 (left) and MCA205 (right) tumor cells. The number of tumor free mice is also demonstrated in each study.

CONCLUSION

We developed a micellar nanoformulation of the mechanothera-

peutic tranilast. Employing two syngeneic triple negative breast tumor models, tranilast micelles were found to be more effective than free tranilast when administered in a dose 100 times lower than that of the free drug. The micellar formulation of tranilast was also found to boost the delivery and efficacy of Doxil, overcome immune checkpoint resistance, induce tumor immunogenicity and improve the efficacy of nano-immunotherapy leading to cure.

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A NEW APTAMER DELIVERY SYSTEM TRANSPORTING NUCLEIC ACIDS TO T-CELLS FOR GLIOBLASTOMA IMMUNOTHERAPY.

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BACKGROUND:

Glioblastoma (GBM) is a primary grade IV brain cancer, which is the most aggressive lethal brain tumour in humans.[1] GBM comprises 16% of all primary and central nervous system tumours.[2] GBM is still incurable due to its resistance to standard therapies and there is a lack of effective drug carriers able to target the tumour cells specifically and penetrate the tumour,[3] leading to the current survival length of 15 months.[4] Given the poor survival with currently approved treatments for GBM, new therapeutic strategies are urgently needed. The stable lipid nanoparticle (SNALP) delivery platform is becoming increasingly popular to encapsulate RNA therapies and deliver them to their chosen target.[5] So far, the use of nucleic acids to block or stimulate the ICPs of T-cells has also not been too successful, due to them being hard to transfect. A challenge that is faced is delivering the SNALPs to the correct locations and losing therapeutics to non-desirable cells. Conjugating a specific targeting agent to the outside of the SNALPs could be a way to tackle this, as the targeting agent guides the SNALPs to the desired T-cell, such as aptamers. Aptamers are single-stranded oligonucleotides that bind strongly and specifically to diverse targets.[6] Aptamers can be targeted drug carriers, increasing efficacy and minimising side effects.[7]

AIMS:

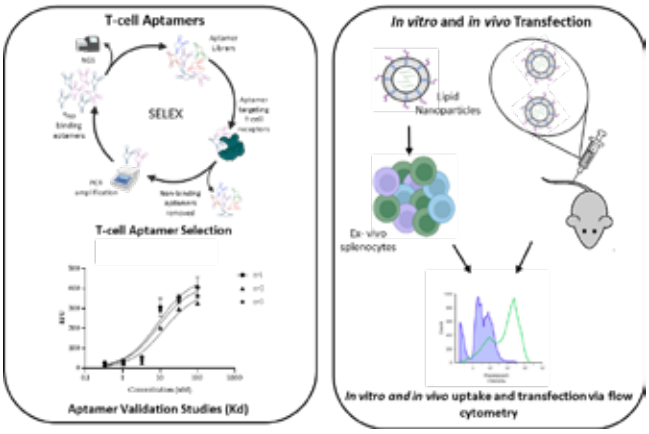
This research aims to create a therapeutic delivery system using novel T-cell targeting aptamers to improve the therapeutic effect of SNALPs-mOX40 being delivered to immune cells in GBM *in vivo* models. Delivering mOX40 to T-cells will overexpress this marker and trigger an immune response with the T-cells and their reciprocal APC.

RESULTS:

Two T-cell surface proteins that exert immune regulatory functions have been identified that have no published aptamers targeting them, so designing aptamers for targeting or therapeutic purposes is considered novel and innovative. Currently, 8 cycles of SELEX have been done on the two T-cell surface proteins recombinant protein, using a randomised DNA library of 30 nucleotides followed by Next Generation Sequencing. This has produced 6 top aptamer candidates, which have undergone validation studies of *in vitro* and ex vivo binding affinity assays across both human and mouse T-cells. These have produced Kds ranging from 0.33- 16.66 nM. SNALPs have been produced that were 140-150 nm in size with >90% encapsulation efficiency (%EE) and offered protection against RNAse treatment. In vitro and ex vivo preliminary transfection studies with SNALPs-mOX40 without an aptamer have been undertaken to get a baseline of transfection potency. The transfection has been observed using flow cytometry. The data shows OX40 has an increased expression of ~9% across both cell lines (splenocyte T-cells and human jurkat cells). We have also tested *in vivo* transfection of T-cells in the spleen after intravenous (I.V.) injection to 6 healthy C57BL/6 mice at mOX40 dose of 13 µg and confirmed comparable transfection to that obtained *in vitro*.

CONCLUSION:

SELEX has been performed successfully to produce five high-affinity novel aptamers selected against two T-cell targets, in the desirable K_d range of 0.33- 16.66 nM. SNALPs encapsulating mOX40 have been formulated and successfully transfected *in vitro* and *in vivo* T-cells, increasing the OX40 expression. Future testing will focus on conjugation of aptamers to SNALPs. We will compare mRNA transfection to that obtained using non-targeted SNALPs following intravenous administration. The long-term goal of this work is to develop a T-cell targeted delivery system delivering nucleic acids for better outcomes of cancer immunotherapy, more particularly brain cancer.



Scheme 1. Overview of T-cell targeted aptamer delivery system to GBM. *T-cell targeting aptamer selection via SELEX followed by aptamer validation studies including binding affinity assays. The formulation of SNALPs followed by transfection of OX40 in vitro and in vivo.*

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MODULAR AND ADAPTIVE SELF-ASSEMBLING DENDRIMERS FOR NANOMEDICINE

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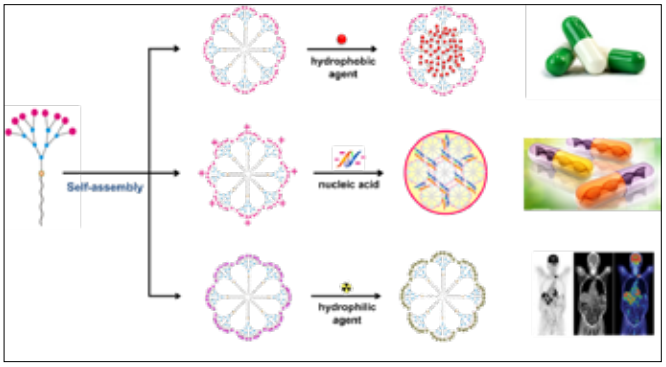

Figure 1: *Cartoon representations of the self-assembly of amphiphilic dendrimers into supramolecular dendrimer nanosystems for the delivery of hydrophobic anticancer drugs and hydrophilic imaging agents as well as negatively charged nucleic acid therapeutics.*

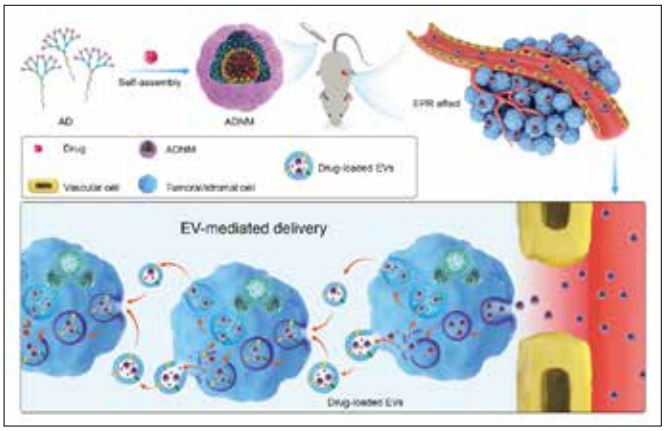

Figure 2: *Amphiphilic dendrimer nanomicelles (ADNMs) encapsulate the anticancer drug and induce tumor-assisted drug delivery via extracellular versicle (EV)-mediated intercellular transport for overcoming tumor heterogeneity and dynamic evolution.*

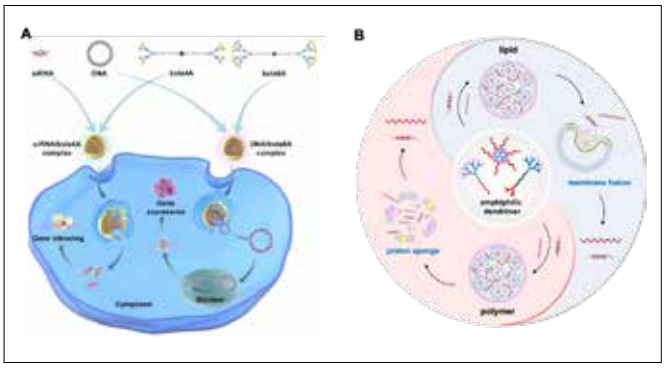

Figure 3: *(A) Selective delivery of nucleic acid cargos mediated by bola-amphiphilic dendrimers of different generations. (B) Amphiphilic dendrimers for nucleic acid delivery by making use of the delivery advantages of both lipid and polymer vectors*

The application of nanotechnology is widely expected to bring breakthrough in medicine for disease treatment and diagnosis. Dendrimers are ideal materials for elaborating nanomedicine by virtue of their well-defined structure, multivalent cooperativity and nanosize *per se*. We have recently established modular and adaptive self-assembling dendrimer nanosystems[1] for the delivery of imaging agents,[2] anticancer drugs[3] and nucleic acid therapeutics[4] for cancer detection and treatment (Figure 1). Remarkably, these dendrimer nanosystems are able to exploit the *in situ* tumor-secreted extracellular vesicles for intercellular delivery and deep penetration in tumor tissue, overcoming tumor heterogeneity and dynamic evolution (Figure 2).[3] Also, selective delivery of nucleic acid cargoes can be achieved using amphiphilic dendrimers of different generations yet benefiting the delivery advantages of both lipid and polymer vectors (Figure 3).[4] Our findings offer a fresh perspective for exploiting the advantageous features of supramolecular dendrimers to reach the ultimate goal of smart nanomedicine.

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MACROMOLECULAR IMMUNODRUG DELIVERY GUIDED BY SELF-IMMOLATIVE NANOBODY MODIFICATIONS

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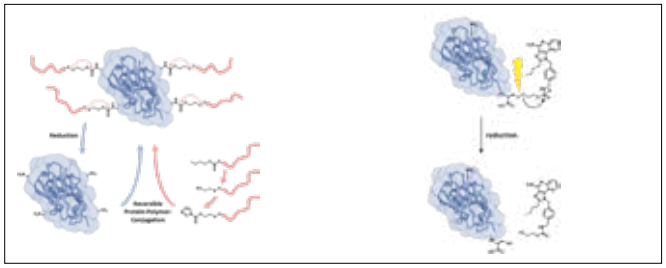
Macromolecular modifications of bioactive compounds can improve their site-specific delivery, enhance their residence in the body and reduce unwanted side reactions, which is highly relevant for immune modulating drugs.[1] On the other hand, such modifications can be accompanied by a reduction in their activity. To circumvent this, only a transient covalent conjugation between drug and macromolecule would be advantageous to combine the benefits of both for suitable delivery applications.[2]
In this regard, reductive-responsive self-immolative linkers can be employed. They allow a site-selective conjugation of small molecules to synthetic polymers or even nanobody proteins as macromolecular precision targeting carriers. Moreover, such drug-loaded nanobodies can be further decorated transiently with synthetic polymers to enhance their blood circulation. Upon reductive trigger a traceless release of the bioactive compounds occurs, and full bioactivity is restored.[3]



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ADVANCED NANO AND MICRO MEDICINES TO TACKLE NEUROLOGICAL DISORDER

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Introduction: Poly(D,L-lactide-co-glycolide) (PLGA) is the main component of a plethora of drug delivery systems (in the form of nanoparticles, microparticles, and implants), that have been proposed for a variety of biomedical applications (1), including brain diseases (2). Neurological disorders, which comprise developmental and degenerative diseases, ischemic stroke, and brain tumors, represent a major and increasing global health challenge that requires dedicated resources gathering (3). With the aim of finding new therapeutic approaches for those diseases, fast and efficient long-term treatments are needed to speed up the possibility of new cures. A platform based on square polymeric microparticles called PLGA-microPlates (μPL), has been developed to deliver a wide range of payloads. They are characterized by homogeneous size, shape and surface area (4, 5). μPL loaded with the antipsychotic drug risperidone (RSP) demonstrated sustained drug release of the over several weeks for the treatment of schizophrenia (6). Schizophrenia is a disorder characterized by cognitive impairment and psychotic symptoms that fluctuate over time and can only be mitigated with the treatment of antipsychotics. Interestingly, a novel top-down fabrication process has been proposed to obtain square-defined PLGA μPL, as a versatile platform for the sustained drug delivery of new chemical entities for the treatment of neurodevelopmental disorders (NDDs) (in preparation). PLGA is the principal polymer of another technology developed for intra-vascular administration. PLGA-based Discoidal Polymeric Nanoconstructs (DPN) carrying the clinical formulation of the tissue plasminogen activator (tPA) were proposed as new thrombolytic agent (tPA-DPN) (7) (Figure 1).

Methods: All the platforms have been characterized in terms of mechanical, physico-chemical, and pharmacological properties. The quality assessment consisted of: a) morphology evaluation, performed by scanning electron microscopy and fluorescence or confocal microscopy; b) loading, encapsulation efficiency, and the drug release profiles up to 3 months, under physiologically relevant conditions, by analytical and molecular assays. The morphology of the porous-μPL was investigated by the Scanning Electron Microscopy, Confocal Microscopy and Fluorescence Microscopy, while the size distribution and particle concentration were determined using a Multisizer system. HPLC and UV-Vis analyses were used to quantify drug encapsulation efficiency.

Results: For the μPL configurations, a well-defined shape and high fabrication yielding (50-70%) were documented. Drug release profiles were sustained for all the loaded drugs. Tall μPL realize the slowest release documenting up to 3 months compared to short μPL. The therapeutic efficacy of one single intraperitoneal injection of RSP loaded μPL is compared to the daily administration of free RSP in a preclinical mouse model of cognitive and psychiatric liability. In temporal order object recognition tasks, mice treated with RSP-μPL outperform those receiving free RSP. About the other technology for vascular applications, tPA-DPN preserve over 70% of the tPA original activity after 3h of exposure to serum proteins. Under dynamic conditions, tPA-DPNs dissolve clots more efficiently than free tPA. At about 1/10 of the clinical dose, tPA-DPNs still effectively dissolve 70% of the clots. *In vivo*, tPA-DPNs outperform the lytic activity of free-tPA in terms of both percentage of successful recanalization events and clot area reduction. These systems have been also characterized in terms of toxicity and impact on cell metabolism and expression of pro-inflammatory markers.

Discussion and future prospective: RSP-loaded μPL are a promising platform for improving symptoms associated to schizophrenia, indeed the sustained release of antipsychotics from a single injection of μPL can rescue cognitive impairment up to several weeks. Moreover, the long-term efficacy with one single administration could be of clinical relevance in terms of patient's compliance and adherence to the treatment regimen. tPA-DPN are promising nanotools for enhancing potency and safety of thrombolytic therapies, especially for those brain conditions already impaired (e.g. recurrent stroke or comorbidities). This is due to the conjugation of tPA with preserved lytic activity, the deformability and blood circulating time of DPN together with the faster blood clot dissolution. Further investigations are ongoing for the validation of the efficacy of this technology in a preclinical ischemic animal model (in preparation). We here propose a versatile platform obtained with fabrication process relying on soft lithographic techniques and leading to microsystems with a peculiar size, shape, surface, and tunable mechanical properties, critical aspects for the sustained delivery of new chemical entities, which could be effectively used in treating neurological conditions where small therapeutic doses can be provided continuously over several weeks upon a single administration, increased compliance with the reduction of the administration's frequency, and lower side effects.

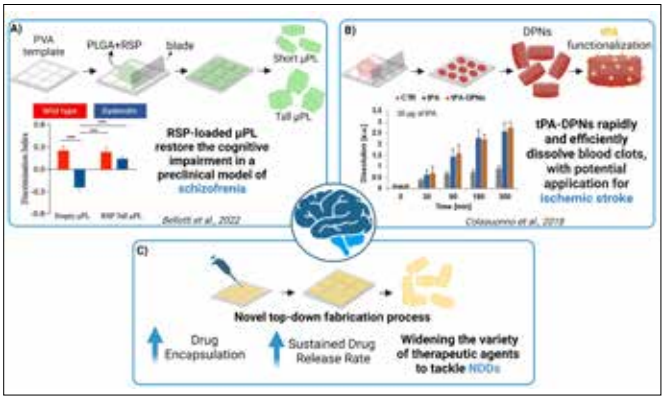

Figure 1: Schematic representation of PLGA-based drug delivery systems developed to tackle neurological disorders including schizophrenia (A), ischemic stroke (B) and NDDs (C).

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EMULSION TEMPLATED PROTEIN NANOCAPSULE FORMATION BY INTERFACIAL DENATURATION FOR THE EFFICIENT ENCAPSULATION AND DELIVERY OF ADJUVANTS FOR CANCER IMMUNOTHERAPY

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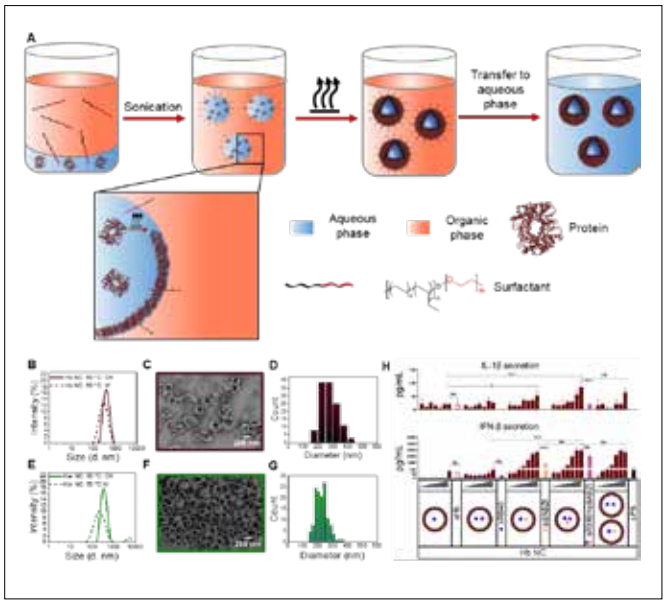
Presenters, * corresponding authors

While various types of intracellular delivery nanocarriers have been developed and reported, only a few have successfully been translated to clinical use due to the complex chemistry and rigorous procedures involved in their development and production.[1] Utilizing miniemulsion technique, protein nanocapsules can be synthesized from different types of proteins, for Eg: human serum albumin, ovalbumin, haemoglobin etc. Previously our group have developed various synthetic strategies for protein nanocapsules using cross-linking reactions such as azide alkyne copper free click reactions.[2-4] However in these cases, the additional reagents required for cross-linking complicates the synthetic process. Recently we designed a new class of versatile protein nanocapsules (PNC) using miniemulsion technique, exploiting inherent properties of proteins; denaturation and disulfide bonds, without any additional chemical reactions. Abundantly available natural proteins; haemoglobin, ovalbumin and keratin formed PNC by interfacial confinement and interfacial denaturation in an inverse miniemulsion. While haemoglobin and ovalbumin denatures below 90°, keratin only denatures at very high temperatures. At the water-oil interface of the inverse miniemulsion, proteins get confined owing to their hydrophobic and hydrophilic amino acids, which results in the partial denaturation of the proteins at the interface and forms PNC shell. Increase in temperature formed stable nanocapsules with haemoglobin (Hb NC), whereas keratin nanocapsules (Ker NC) formation was not influenced by temperature. A model antigen (Ovalbumin) was incorporated in Hb NC and Ker NC easily by mixing the protein and antigen together, without any modification or conjugation. All the NC showed very high encapsulation efficiency, excellent uptake by dendritic cells and had minimal cell toxicity. With the combination of encapsulated super additive adjuvants and the antigen, a novel nanovaccine for cancer immunotherapy was developed. Because there is no complex reactions or reagents involved in the synthesis, we believe the clinical translation of these nanocarriers will be easier compared to others.

Cancer is a complex and multifactorial disease that affects millions of people worldwide. Immunotherapy has emerged as a promising approach to cancer treatment, as it harnesses the patient's own immune system to fight cancer cells. Cancer vaccines are the one approach to cancer immunotherapy, using tumor cell-associated antigens, to awaken the body's immune system against cancer. Several studies have already shown that Toll-like receptor 7 (TLR7/8) agonist R848 or STING agonist diABZI-loaded nano vaccines can elicit immune responses to achieve the desired effect of cancer treatment[5,6]. In this study, combined STING and TLR 7/8 agonists were encapsulated in the nanocapsules to enhance the immune response to cancer vaccine. To assess the ability of adjuvant-loaded PNC to stimulate bone marrow derived dendritic cells (BMDCs), cytokine secretion and expression of CD80 and CD86 co-stimulatory molecules were measured after 24 h of incubation with various PNC formulations. The results showed that the combination of R848 and diABZI can synergistically enhance the cytokines' expression compare to R848 alone. Consistent with the results obtained from cytokine expression analysis, dual adjuvants loaded NCs can synergistically upregulate co-stimulatory molecules' expression in BMDC. In short, dual adjuvant encapsulated PNC showed their capacity to enhance the immune response.

*Figure 1: A) Synthesis of protein-nanocapsules (PNC) by miniemulsification method. Proteins dissolved in the dispersed phase (water) and surfactant P((E/B)-b-EO) (low HLB surfactant) dissolved in the continues phase (cyclohexane) was emulsified using ultrasonication. The emulsion was heated to obtain PNC in the organic phase. In this step, protein get confined and denatured at the oil water interface. After purification to remove excess of the surfactant, PNC in cyclohexane was transferred to water using SDS as a surfactant (high HLB surfactant), cyclohexane was evaporated at room temperature to obtain PNC exclusively in water and then SDS was removed by centrifugal filtration. Dynamic light scattering (DLS) chromatograms of B) haemoglobin nanocapsules (Hb NC), E) keratin nanocapsules (Ker NC) obtained at 60°. Their corresponding C, F) SEM images and D, G) size distribution obtained from SEM measurements, brown-Hb, green-Ker, CH (cyclohexane), W (water). H) Evaluation of the efficacy of adjuvants loaded Hb NC in inducing cytokine secretion by bone marrow-derived dendritic cells (BMDCs). To achieve this, BMDCs were exposed to varying concentrations (0.1, 0.3, 1, 3, 10, 30, 100 µg/mL) of empty or adjuvant-loaded Hb NC, soluble haemoglobin (sHb), soluble R848 (sR848, 600 ng/mL), soluble diABZI (sdiABZI, 1.1 µg/mL), or LPS (100 ng/mL) for 24 hours. The concentration of soluble adjuvants used for treatment was equivalent to amount present in 100 µg/mL of Hb NC. The supernatants of BMDCs were collected to detect IL-1β, and IFN-β expression (mean ± SD; n=3). All statistical analyses were performed using one-way ANOVA with Turkey's post hoc test. (ns, not statically significant *P ≤ 0.05; **P ≤ 0.01; ****P ≤ 0.0001).*



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OPTIMIZATION OF MIXED MICELLES BASED ON OPPOSITELY CHARGED BLOCK COPOLYMERS BY MACHINE LEARNING FOR APPLICATION IN GENE DELIVERY

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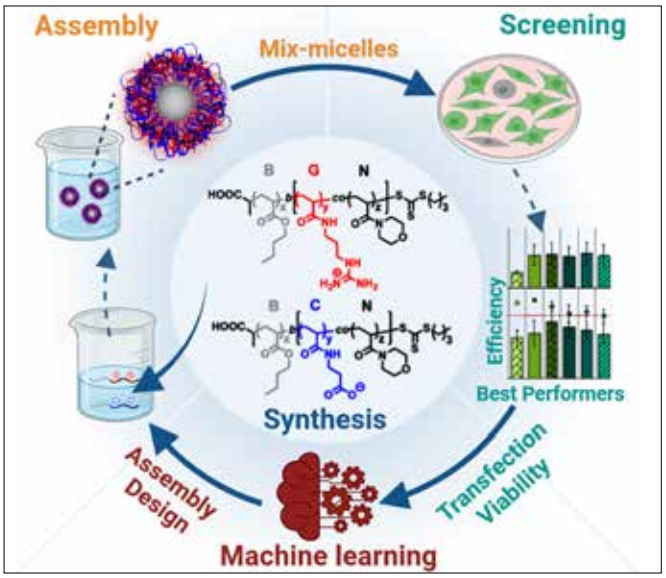

New effective vaccination strategies are recently driving the development of non-viral gene transporters as a new concept with immense potential for gene therapy. However, there are still hurdles to overcome, many of these can be addressed with cationic polymer-based delivery systems due to their durability, reproducibility, large production quantities, versatile composition, and architecture, in particular for pDNA.[1] The combination of hydrophobic and cationic moieties in a block copolymer structure revealed superior efficiency in gene delivery due to their combined impact on cell membranes and nucleic acid interactions. Additionally, stealth and anionic moieties reduce serum interaction and improved biocompatibility.[2] Therefore two sets of diblock terpolymers were synthesized, comprising hydrophobic poly(*n*-butyl acrylate), a copolymer segment made of hydrophilic 4-acryloylmorpholine (NAM), and either the cationic 3-guanidinopropyl acrylamide (GPAm) or the 2-carboxyethyl acrylamide (CEAm), which is negatively charged at neutral conditions. The well-defined cationic diblock terpolymer P(*n*BA)-*b*-P(GPAm-*co*-NAM) (BGN) and anionic diblock terpolymer P(*n*BA)-*b*-P(CEAm-*co*-NAM) (BCN) were assembled at different ratios resulting in the formation of mixed micelles.
Since this approach offers countless possibilities for compositions, the Gaussian-process-based machine learning model was applied to identify an optimal GPAm/CEAm ratio (positive/negative charge ratio) for achieving high transfection efficiency and cell viability with little resource expenses, regarding material and personal resources. After two runs, the model was able to identify an optimal positive/negative charge ratio in a serum-reduced medium.
Further biological investigations approved the superiority of the mixed micelles *vs.* the cationic homopolymer Gua 100 in full growth medium. The results of the best performers underline the success of the incorporation of anionic functionalities into polymeric micelles to optimize their properties e.g., by avoiding strong serum interaction and triggering internalization, retaining cell membrane integrity and viability while still achieving high transfection efficiency.
For the first time machine learning was successfully applied to optimize a complex polymer library to target high transfection efficiency and viability. This highlights the remarkable potential of machine learning for polymer chemistry since it can effectively tackle the enormous number of conceivable combinations for identifying novel and powerful gene transporters.

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CLUSTER DECORATED FUNCTIONAL DNA ORIGAMI BASED BIOSENSOR: TOWARDS SAFE NANO-INNOVATIONS

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The emergence of nanotechnology in the medical field shows the high expectations for new nanotechnology-based health products [1]. The DeDNAed project aims to develop a cutting-edge bioanalytical biosensor platform with advanced sensitivity and versatility using Surface Enhanced Raman Spectroscopy (SERS) as an ultrafast optical analysis method. The platform is based on the assembly and integration of sensing elements (transducer and bioreceptor) using DNA origami [2]. The DNA origami will serve as a "nano-breadboard" to precisely control the position of these elements and thus the sensor architecture at the nanometer scale, enabling highly sensitive SERS measurements.

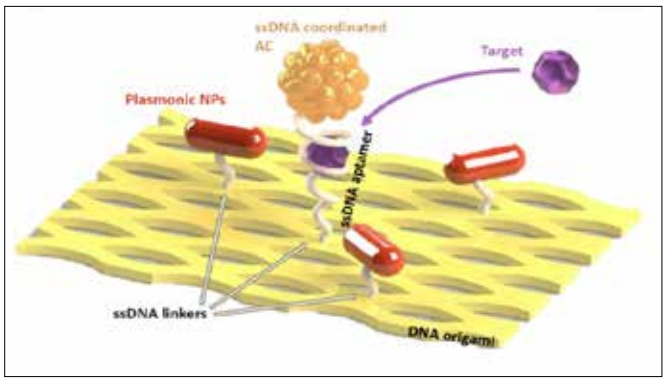

Figure 1. Cluster decorated DNA origami based biosensor.

During the development and innovation process, clarity about the safety surrounding new technologies is one of the most important prerequisites for technology acceptance. Eliminating hazards at the design or planning stage is often easier and cheaper to achieve than making changes later when the hazards become real risks. Thus, throughout the DeDNAed project, we will implement the Safe-by Design (SbD) approach, which refers to identifying the risks and uncertainties concerning humans and the environment at an early phase of the innovation process so as to minimize uncertainties, potential hazard(s) and/or exposure. The SbD approach addresses the safety of the material/product and associated processes throughout the entire life cycle: from the Research and Development (R&D) phase to production, use, recycling and disposal [3].
In the present work we present first approaches on the SbD implementation within an early stage nanoenabled biosensor multicomponent device that will develop new nanoscale components. Some sustainability considerations will be presented as first impressions for a more sustainable development of innovations.

ACKNOWLEDGEMENT:

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IN VIVO APPLICATION OF CRISPR/CAS9 GENE EDITING USING LIPID NANOCARRIERS FOR THERAPEUTIC IMMUNE TARGET IDENTIFICATION IN GLIOBLASTOMA.

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INTRODUCTION

Despite advances in cancer immunotherapy, little progress has been accomplished in its clinical translation for glioblastoma (GBM) therapeutics. Failures are associated with multiple factors including the highly immunosuppressive tumour microenvironment (TME), the reduced number of circulating T cells, tumour heterogeneity, limited drug penetration and the presence of a subset of GBM-stem-like cells (GSCs) that activates different signalling pathways that further contribute to tumour growth and multi-treatment resistance. Increasing evidence stresses the imperativeness of a multitarget approach that acts synergistically to (1) disrupt multiple pathogenic pathways in both GBM and GSC, (2) normalise the defective immunity in the TME and (3) enhance the antitumour immune response. CRISPR/Cas9 gene editing represents a powerful toolbox capable of correcting mutations and deleting pathogenic genes, like oncogenes and tumour-regulatory ones. However, its *in vivo* application cannot take place unless subsidised with an appropriate delivery system.

METHODS

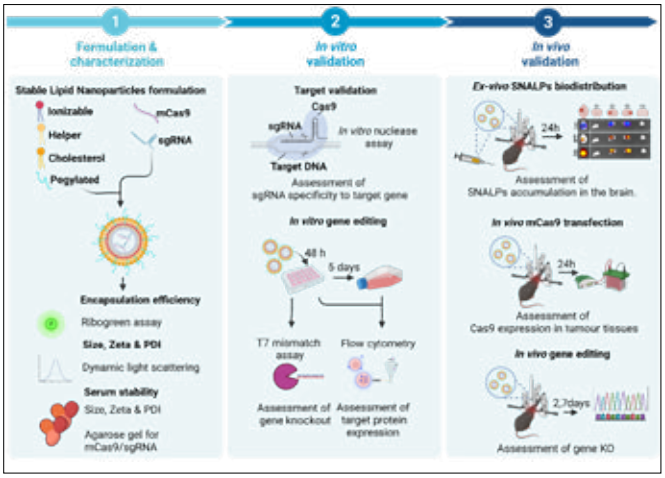
Single guide RNA (sgRNA) against reporter genes (green fluorescent protein (GFP), Luciferase (Luc)) and therapeutic immune targets have been designed and their specificity was validated using *in vitro* nuclease assay. Stable nucleic acid-lipid particles (SNALPs) encapsulating Cas9 mRNA and sgRNA were formulated and characterised utilising dynamic light scattering (DLS). Nucleic acids encapsulation efficiency was measured using Quant-iT™ RiboGreen™ assay. SNALPs stability in serum and nucleases to determine the integrity of Cas9 mRNA and sgRNA was assessed using agarose gel electrophoresis. To understand organ biodistribution profile of SNALPs, mice bearing orthotopic GSC tumours received an intravenous or intracranial injection of SNALPs labelled with far-red dye DiD (SNALPs-DiD). Twenty-four hours later, organs were harvested and imaged for Luc, GFP, or DiD using IVIS Lumina Series III In Vivo Imaging System. Tumours were dissociated into single-cell suspension and analysed by flow cytometry to assess SNALP uptake by cancer and immune cells within the TME. SNALPs ability to mediate Cas9 mRNA delivery to GSC following intracranial administration at 24h post-injection was assessed with Western blot. Finally, gene knockout of two therapeutic genes in healthy brains and GBM and GSCs brain tumour models was evaluated at 2 and 7 days post-intracranial injection using Sanger Sequencing and Inference of CRISPR Edits (ICE) analysis for the identification of CRISPR edits.

RESULTS

sgRNA designed against the different target genes were validated. SNALPs of an average size <150 nm, a neutral surface charge under physiological conditions and high encapsulation efficiency (>80%) of Cas9 mRNA and sgRNA were formulated. SNALPs protected nucleic acids from enzymatic degradation by serum and nucleases. SNALPs induced significant knockout of target genes in GSC with efficiency higher than commercially available transfection reagents. SNALPs accumulated in GSC tumours implanted intracranially in mice after intravenous and intracranial injection as confirmed by optical imaging and flow cytometry. Target genes knockout in tumours was observed at all the time points tested (7d > 2d) following intracranial administration of SNALPs-mCas9 and sgRNA of interest.

CONCLUSIONS

The data suggest that SNALPs are a potent *in vitro* delivery system for CRISPR/Cas9-mediated gene editing of GSCs outperforming commercial transfection reagents. SNALPs accumulated within the TME following both intracranial and intravenous administration highlighting their potency as a gene transfer vehicle for difficult-to-reach tumours such as GBM. SNALPs induced modest to high gene knockout levels in healthy and GBM and GSC models, making it a powerful tool that can be applied in combination with other treatments to induce a synergistic effect against GBM and GSCs. Ongoing work aims to assess the gene editing in GBM and GSCs model following intravenous injection of SNALPs.



LYOPHILIZATION OF MRNA LIPID NANOPARTICLES

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INTRODUCTION

The development of nucleoside-based COVID-19 vaccines has impressively shown the huge potential of lipid nanoparticles (LNPs) as drug delivery system. While the clinical benefit of mRNA coded proteins stands for itself, storage and distribution under frozen conditions remain challenging. As an example, Comirnaty® (BioNTech)[1] can be stored between -90 °C and -60 °C for 12 months, and Spikevax® (Moderna)[2] between -50 °C and -15 °C for 9 months including various tested temperature excursions.
Freeze-drying is one approach to increase storage stability of mRNA-LNPs considering hydrolysis as major degradation pathway of mRNA[3]. First studies have demonstrated improved storage stability of mRNA-LNPs for at least 12 weeks at room temperature[4]. A lyophilized Covid-19 vaccine candidate, ARCT-154 (Arcturus), using self-amplifying mRNA, which can be stored and transported at 2-8 °C, is currently in phase 3 clinical testing[5].
Technical development of mRNA-LNPs has a high material demand and remains expensive due to high costs of ionizable lipids and mRNA. In order to enable formulation and process development of mRNA-LNPs, the establishment of a surrogate is essential.

PURPOSE

The aim of the present project is to establish a suitable surrogate for mRNA-LNP technical development, which can be used for formulation screening and process characterization. The surrogate is designed to enable lyophilization of the formulation in order to improve long-term stability of the LNPs.

MATERIAL AND METHODS

Chemicals
The ionizable lipid SM-102 was obtained from Cayman Chemical (Ann Arbor, Michigan, USA), distearoyl-phosphatidylcholine (DSPC) was provided by Lipoid (Ludwigshafen, Germany). Cholesterol was purchased from Sigma-Aldrich (St. Louis, Missouri, USA), DMG-PEG2000 from Avanti Polar Lipids (Birmingham, Alabama, USA), and sucrose was purchased from Carl Roth (Karlsruhe, Germany). α,α-Trehalose dihydrate was donated by Pfanstiehl (Zug, Switzerland), and Kleptose HPB by Roquette (Geneva, Illinois, USA).
A polynucleotide was used as surrogate for mRNA and will be disclosed on the poster.
Manufacturing
Stock solutions of SM-102, cholesterol, DSPC, and DMG-PEG2000 in ethanol were gently mixed at a molar ratio of 50:37.5:10:2.5. A polynucleotide was dissolved in 50 mM citrate buffer pH 4.0.
Polynucleotide-LNPs (pLNPs) were prepared by T-mixing of the organic and the aqueous phase using peristaltic pumps (Peristaltic Pump P-1, GE Healthcare, Uppsala, Sweden). The parameters of the mixing process are listed in Table 1.

N/P ratio	6
Total Flow Rate (TFR)	10 mL/min
Flow Rate Ratio (FRR)	3

Table 1: Mixing parameters to obtain polynucleotide-LNPs

The pLNPs were dialyzed overnight in 20 mM Tris buffer pH 7.4 using Slide-A-LyzerTM G3 dialysis cassettes (Thermo Fisher Scientific, Rockford, USA).
The pLNPs were formulated to result in defined polynucleotide concentrations by addition of sugar stock solutions. The formulations were filtered through Chromafil 0.20 mm PVDF filters (Faust, Klettgau, Germany).
Lyophilization
300 µL of pLNP solutions were filled into clear 2 mL Fiolax® vials (Schott, Müllheim, Germany) and partly closed with lyo stoppers. Lyophilization was performed on a pilot freeze dryer Epsilon 2-6D (Christ, Osterode, Germany). Samples were frozen at 1 K/min to -40 °C, followed by a primary and a secondary drying cycle at 0.13 mbar at various temperatures. Vials were stoppered and at 750 mbar and capped.
For analytical characterization, lyophilized samples were reconstituted by addition of 300 µL nuclease-free water.
Analytical characterization of mRNA LNPS
Size distribution and polydispersity of mRNA-LNPs were determined by dynamic light scattering (DLS). Zeta potential was measured on a Zetasizer (Malvern Panalytical, Kassel, Germany) using the high concentration cell. Encapsulation efficiency and concentration of the polynucleotide were determined by RiboGreen assay. High-pressure liquid chromatography (HPLC) measurements were performed to verify the lipid concentration6. Residual moisture of the freeze-dried samples was determined by volumetric Karl-Fischer titration.

RESULTS

Different pLNP formulations were freeze dried screening the following formulation and process parameters:
- Formulation buffer/ pH
- Cryoprotectants
- Solid content
- Temperature of primary drying

Formulation and process optimization resulted in pLNPs with a homogenous size distribution and size < 200 nm, as well as a high polynucleotide encapsulation efficiency.

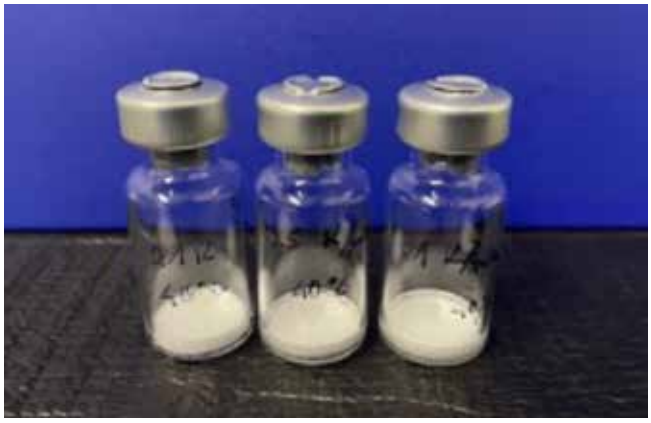

Figure 1: Examples of lyophilized pLNPs
Lyophilized surrogate samples with elegant cake appearance were obtained (Figure 1). First stability studies (2 months, 40 °C) showed a superior stability profile of lyophilized formulations compared to their liquid formulations.

CONCLUSION

We identified a suitable surrogate for mRNA-LNP technical development. The surrogate enables formulation development of both liquid and freeze-dried formulations, and can be used to implement new analytical methods or manufacturing protocols.
The freeze-dried product showed a superior stability profile compared to the liquid formulation at elevated temperatures. Further experiments have to show the stability at room temperature over several months.
The elimination of the cold chain for mRNA-LNPs would ease access to mRNA medication around the world and especially for low- and middle-income countries.

ACKNOWLEDGMENTS

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scale freeze dryer, as well as the support of Lipoid (Ludwigshafen, Germany), Pfanstiehl (Zug, Switzerland), and Roquette (Geneva, Illinois, USA) for providing different chemicals. Furthermore, we thank Schott (Müllheim, Germany) for the kind support with lyo vials.

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ENHANCING THE POWER OF NANOBODIES THROUGH NANOTECHNOLOGY: EXTENDING HALF-LIKE AND REACHING INTRACELLULAR TARGETS.

LUCIA SANJURJO BOUZA

BACKGROUND

Antibody-based therapies have traditionally targeted extracellular or soluble proteins, leaving many potential intracellular oncogenic targets undrugged. Nanobodies (VHH) are promising cancer therapeutics due to their unique features. Their small size (~15 KDa), high stability, easy production, and specificity make them also well-suited for intracellular applications. However, their rapid renal excretion and poor cell penetration have limited their full exploitation for cancer treatment.

OBJECTIVES

We aim to conveniently design nanobody-loaded nanocarriers (VHH-NCs) to collectively improve different drawbacks in the therapeutic application of nanobodies. We expect to extend their circulation time, improve tumor delivery efficiency and achieve cell membrane penetration.

METHODS

VHHs were loaded to control NCs or NCs functionalized with a tumor-penetrating peptide. For a full structural analysis, dynamic light scattering, nanoparticle tracking analysis, and electron microscopy were used. The NCs toxicity was evaluated using resazurin assay in cancer cells and macrophages and their biocompatibility in an *in vivo* assay. The ability of the NCs to deliver VHHs intracellularly was assayed by immunofluorescence and analyzed by flow cytometry and confocal microscopy. Nanobody target engagement was explored by pull-down assays and microscopy in HeLa cells. Finally, biodistribution studies in a lung cancer orthotopic mice model using radiolabeled nanobodies are ongoing.

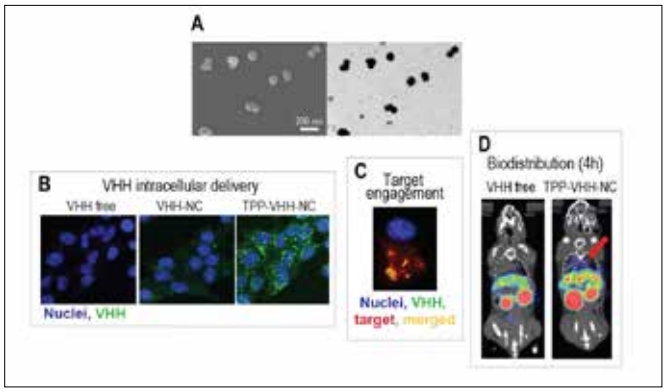
RESULTS

Functionalized NCs that associate VHHs (40-98% association efficiency) were obtained with a particle size of ~90nm, PDI<0.2, and neutral surface charge (figure A). The reproducibility, stability, non-toxic profile, and biocompatibility were confirmed. In uptake studies, the intracellular signal related to the nanobodies was detected when associated with NCs, but not for free nanobodies (figure B). Moreover, target engagement assays revealed that the nanobodies were able to reach their target inside cells (figure C). Finally, preliminary biodistribution studies suggest the capacity of our technology to allow tumor targeting (figure D).

CONCLUSION

We have developed the first targeted nanotherapeutics that allow intracellular delivery of VHHs. Ongoing biodistribution studies will determine the tumor-targeting capacity. Ultimately, this technology will contribute to the expansion of novel nanobody-based immunotherapies toward intracellular antigen targets.

FIGURE



A VERSATILE FUNCTIONALIZATION PLATFORM FOR LIPOSOMES AND EXTRACELLULAR VESICLES

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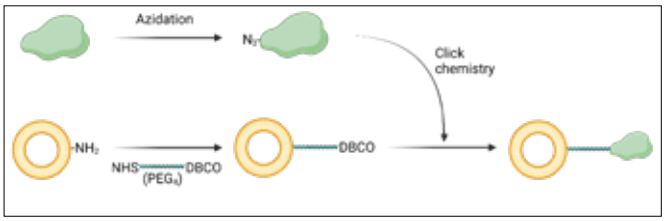
Ligand attachment to the surface of drug delivery vehicles presents a powerful tool to gain control over the targeted transport of a molecule of interest. While a wide variety of drug delivery vehicles are under investigation today, lipid-based formulations such as liposomes are arguably one of the most established forms. Although intrinsically very biocompatible, they still require modification to obtain enhanced stealth and targeting properties. Therefore, strategies to modify the surface of lipid-based carriers are the stepping-stone towards more successful applications.
In this work, we present a surface modification strategy for lipid-based carriers, with an emphasis on the optimization of each step, including characterization and quantification. Liposomes were selected as lipid-based carrier models. The functionalization can be divided into three main steps: azidation of the ligand of interest, conjugation of a short polyethylene glycol (PEG)-linker containing a strained alkyne (in form of a dibenzocyclooctyne (DBCO) moiety) to the carrier surface via *N*-hydroxysuccinimide (NHS)-ester chemistry, and the final click chemistry reaction between the ligand's azide and the carrier's alkyne groups. Each step is followed by quantification and optimization of reaction parameters. Since both the involved NHS-ester chemistry and bio-orthogonal copper-free click chemistry can be performed under physiological conditions, this approach is highly compatible with subsequent *in vitro* and *in vivo* experiments. Once well-established, the strategy will be transferred for example to extracellular vesicles (EVs), which possess a much more complex surface and cannot be functionalized chemically before their generation.

Figure 1: *Schematic illustration of the ligand functionalization of lipid-based self-assembled nanocarriers via strain-promoted alkyne-azide cycloaddition (SPAAC) reaction. (Created with BioRender.com)*



PHOENIX-OITB – A SINGLE ENTRY POINT TO DEVELOP OR UPGRADE INNOVATIVE NANO-PHARMACEUTICALS

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8 Topas Therapeutics GmbH
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Introduction: The PHOENIX Open Innovation Test Bed (PHOENIX-OITB) is focused on overcoming the challenges of production of novel and innovative nanopharmaceuticals (NPs) from lab scale to GMP quality and production, maximising bioavailability, stability and manufacturing to allow their implementation in the medicine field.

PHOENIX-OITB is a non-profit, open and self-sustaining legal entity that works as a Single-Entry-Point (SEP) providing its end-users transparent processes and procedures as well as easy access to services and expertise needed to bridge the gap between the bench and the bedside, i.e., providing them with high quality services, capable of Quality-Efficacy and Safety (QES) evaluation and production of nanopharmaceuticals at large scales meeting the regulatory and GMP requirements.

Methods: The PHOENIX-OITB has been structurally conceived, designed and officially registered under the current on-going H2020 EU-funded project Phoenix (GA n° 953110)[1]. To test the operative capacity of PHOENIX-OITB, five demo-cases of different NP types, manufacturing methods and administration routes will be employed. Additionally, two pro bono demo-cases will be launched and granted to external users to test the services at relevant and operational environment.

Main Results: Process transfer and method development for all five demo-cases are ongoing while GMP production area is being constructed. The Pharmaceutical Quality Management System (PQMS) is being established and all exploitation activities are being managed and can be seen through: https://www.phoenix-oitb.eu/. Furthermore, PHOENIX-OITB Open Call for the granting of two pro bono demo-cases to external end-users has been launched and applications are under evaluation.

Conclusions: PHOENIX-OITB aims to enable the seamless, timely, and cost-friendly transfer of NPs from lab bench to clinical trials by offering a SEP for a consolidated network of facilities, technologies services and expertise covering all needed aspects, from characterisation, testing, verification up to scale-up, GMP compliant manufacturing and regulatory guidance.

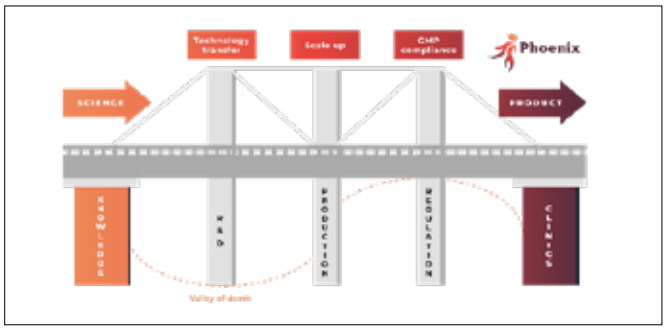

Figure 1: *Phoenix strategy to bridging the innovation valley of death between science and nanopharmaceutical product.*

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[1] Phoenix EU-funded project Grant Agreement No 953110. www.phoenix-oitb.eu. This project has received funding the European Union's Horizon 2020 research and innovation programme.

POSTER-ABSTRACT: A NON-IMMUNOGENETIC PEG DERIVATE: IMPROVING THE EVASION OF THE IMMUNE RESPONSE BY INTRODUCING STERICALLY DEMANDING SIDE CHAINS

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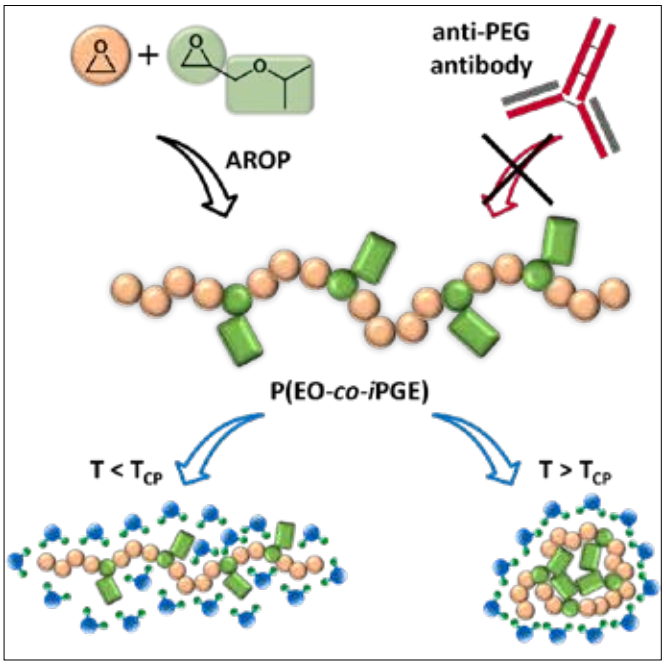
PEGylation is the method of choice for improving the pharmacokinetics and pharmacodynamics of biomolecule therapeutics, due to the unique properties of polyethylene glycol e.g. the stealth effect, water solubility and biocompatibility.[1,2] As a direct consequence, PEG is indispensable for clinical nanomedicine. Nevertheless, a prevalence of up to 72 % for anti-PEG antibodies (APAs) was reported in the general population.[3] The immunogenicity of PEG leads to a growing concern about the safety and benefits of PEGylation and threatens decades of research and application on clinically used PEGylated formulations.[4,5] This results in the mandatory development of alternatives to PEG for medical applications.

The benefits and drawbacks of different polymer classes are frequently discussed as potential alternatives to PEG.[6] In our group, we developed a novel approach of preserving the polyether backbone while randomly incorporating side chains to prevent antibody recognition. This can be achieved *via* living *anionic ring-opening polymerization* (AROP) of ethylene oxide (EO) with glycidyl ethers. The side chains of the glycidyl ethers act as random "point mutations" in the highly regular structure. In a previous study, we demonstrated the reduced immune recognition of rPEGs (randomPEG) containing glycidyl methyl ether (GME).[7] In this work, we want to insert a sterically demanding side chain to improve immune evasion while concurrently reducing the comonomer content. Hence, statistical copolymers of EO and isopropyl glycidyl ether (*i*PGE) were obtained *via* AROP. The resulting P(EO-*co*-*i*PGE) copolymers with *i*PGE ≤ 20 % show significantly reduced affinities against a backbone selective APA in comparison to the GME-rPEGs.

Figure **1***: Random copolymers of EO and iPGE are obtained via AROP. The resulting copolymer inhibits the recognition of anti-PEG antibodies and features a thermoresponsive behavior.*

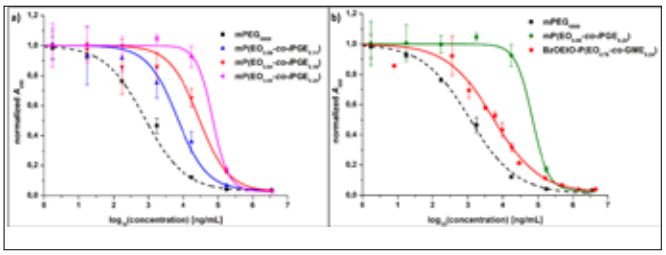


Statistical copolymers of EO and *i*PGE (P(EO-*co*-*i*PGE)) with *i*PGE contents ≤ 20 mol-% and molecular weights up to 6000 g/mol were obtained (Đ < 1.10). Nearly random statistical copolymerization was confirmed *via in situ* ^{1}H-NMR kinetics measurements. Water solubility with a lower critical solution temperature (LCST) was confirmed for all copolymers in PBS buffer. The cloud point temperatures remain above the physiological temperature range.

Cytotoxicity and activation of immune cells were evaluated in murine spleen cells by *fluorescence-activated cell sorting* (FACS). The copolymers up to 16 mol-% *i*PGE display non-toxicity and non-immunogenic behavior in comparison to mPEG. Contrastingly, polymers with 20 mol-% *i*PGE induced cytotoxicity. An increase in necrosis over apoptosis was detected after three hours, while most cells were necrotic or late apoptotic/necrotic after 24 hours. We hypothesize that the amphiphilicity of the copolymer can lead to membrane defects beyond a certain threshold for the *i*PGE content.

The affinity of a backbone selective anti-PEG antibody was determined towards copolymers by competitive *enzyme-linked immunosorbent assays* (ELISA). The APA affinity against the 20 mol% *i*PGE formulation is decreased by 88-fold. This is a three times higher reduction compared to predictions *via* statistical calculations. The non-proportional decrease in immune recognition indicates a pronounced effect for the sterically demanding isopropyl side chain in contrast to GME.

Figure 2: Normalized absorption plotted against the logarithmic polymer concentration for the competitive anti-PEG antibody ELISA measurement. a) Affinity of a backbone selective APA against the mP(EO-co-iPGE) copolymers. b) Comparison of the steric influence on the APA binding.



For proof-of-concept bioconjugation, post-polymerization reactions were implemented to convert the hydroxy end group into a maleimide group. Cysteine-selective mono conjugation of maleimide functional mP(EO-*co*-*i*PGE)-Mal to bovine serum albumin (BSA) was performed and confirmed by SDS-PAGE and MALDI-TOF mass spectrometry.

The mP(EO-*co*-*i*PGE) copolymers feature a significantly decreased immune response and could potentially be used as a one-for-one replacement for mPEG in terms of PEGylation. However, the hydrophobicity of *i*PGE leads to a limitation for this system concerning biomedical applications.

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DEVELOPMENT AND CHARACTERIZATION OF SYNGENEIC TUMOR MODELS FOR HEPATOCELLULAR CARCINOMA IN IMMUNOCOMPETENT MICE

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INTRODUCTION

Hepatocellular Carcinoma (HCC) accounts for 90% of all primary liver tumors [1]. Cirrhosis, due to chronic organ damage, is characterized by a massive accumulation of scare tissue in the liver and is the most frequent risk factor for HCC [2] [3]. But incidences of HCC are also increasingly observed in patients with metabolic-associated steatohepatitis (MASH) without cirrhosis [4]. Common murine models for HCC are lengthy and tumor load tends to be heterogeneous as tumor induction takes around 20 weeks and less than 50% of mice bear tumors [5].

In this work, we introduce a rapid and easy-to-handle injection model for HCC in cirrhotic and non-cirrhotic livers, which recapitulates histological and molecular key features of HCC in patients [6].

RESULTS AND DISCUSSION

For the non-cirrhotic model, mice were intrasplenically injected with Dt81-Hepa 1-6 tumor cells (HCC cells), while for the cirrhotic model, mice were gavaged with profibrogenic CCl_4 for 6 weeks prior tumor cell inoculation. After 4 weeks, inoculated mice developed tumors exclusively in their livers (**Fig. 1a**). Interestingly, livers of the cirrhotic group had a significantly higher tumor load as indicated by higher liver weights (2.5-fold), higher Alpha-fetoprotein (AFP) sera levels and morphometric readouts of liver sections compared to non-cirrhotic mice (**Fig. 1b&c**). RNA-Seq analysis of HCC cells used in this work, revealed that HCC hub genes (AFP, MCM3, SPATS2, NT5DC2, MCM6) were significantly upregulated and tumor cells showed a distinct clustering compared to healthy hepatocytes (**Fig. 1d**).

3D multiphoton microscopy and 2D fluorescence microscopy revealed that extracellular markers (ECM), namely collagen 1-3, collagen-1, collagen-4, and fibronectin were significantly overexpressed in tumor tissues as compared to controls. Furthermore, the adjacent liver of the cirrhotic HCC model displayed fibrillar structures

distributed throughout the non-cancerous tissue, suggesting an enhanced desmoplastic reaction of the tumor (**Fig. 2a**) 3D visualization of collagen structures via single harmonic generation (SHG) showed that collagen fibers are more abundant in the cirrhotic liver (**Fig. 2b**). Next, we analyzed the expression of markers for vasculature. All markers, namely CD31 (endothelial cells) and αSMA (pericyte, fibroblasts) were significantly overexpressed in tumor tissues (**Fig. 3**). No significant difference was observed between marker expression in the adjacent liver of HCC and fibrotic HCC.
Finally, we tested the novel HCC gold standard therapy atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) in our model. HCC mice were injected intravenously once weekly with the atezolizumab equivalent anti-hPD-L1 (8 mg/kg) and bevacizumab (5 mg/kg) (AtezoBev), respectively, starting at day 7 (**Fig. 3a**). AtezoBev showed an improved ($p<0.05$) antitumor effect as indicated by lower liver weights and AFP levels in the sera compared to control mice at day 28 (**Fig. 3b**).

CONCLUSION

We present an easy-to-handle murine model for HCC with high relevance for translational research. The model reflects characteristics of human HCC and showed a positive antitumor response to AtezoBev.

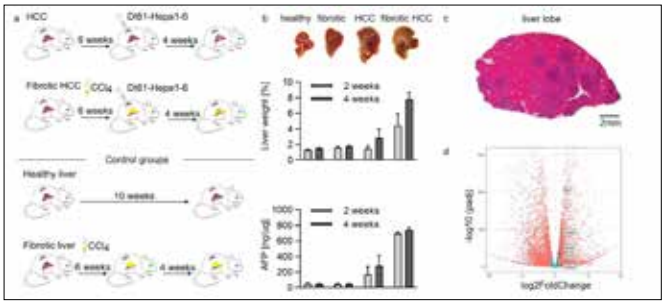

Figure 1. Model development of hepatocellular carcinoma. *a. Schematic representation of animal model development. The HCC model was generated by intrasplenic injection of Dt81-Hepa-1-6 cells. The fibrotic HCC model was generated by CCl_4 administration for 6 weeks and subsequent injection of Dt81-Hepa-1-6 cells. Livers from healthy mice or mice only administrated with CCl_4 were used as controls. b. Liver weight and measured AFP level in plasma mice revealed malignant lesion formation by substantial liver weight increase as well as an increase in AFP in the plasma of tumor-bearing mice. c. H&E staining of whole liver lobes reveal the difference between tumor lesions and the adjacent liver counterpart. d. RNA-Seq analysis of HCC cells used in this work. scale bar= 50 µm.*

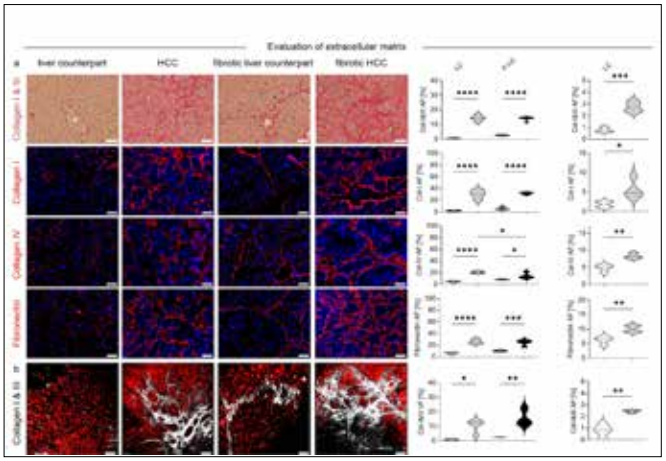

Figure 2. Deposition of extracellular matrix components in tumor and adjacent liver counterparts of HCC and fibrotic HCC model. *a. Representative fluorescence images of staining and analysis of different ECM components reveal that all components are heavily overexpressed in tumor tissue as compared to their respective liver counterpart. In the liver counterpart of fibrotic HCC fiber-like structures are visible which are absent in the liver counterpart of HCC. Together with the respective quantification it indicates an elevated matrix deposition in the former. b. Collagen fibers visualized in 3D via SHG, and quantified volumes confirm overproduction of dense fiber structures in tumors. scale bar = 50 µm, LC= liver counterpart, HCC= Hepatocellular carcinoma, F=Fibrotic, AF: Area fraction, VF: Volume fraction.*

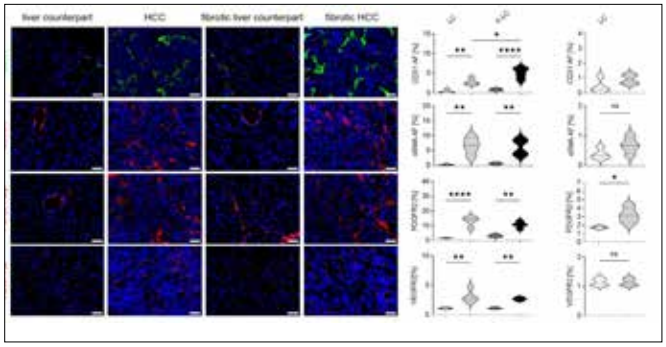

Figure 3. Expression of markers for vasculature and fibroblasts in tumor and adjacent liver counterparts of HCC and fibrotic HCC model. *Representative fluorescence images of staining and analysis of endothelilal cells (CD31) and pericytes (αSMA) revealed that all markers are upregulated in tumor tissues as compared to non-malignant counterpart. scale bar = 50 µm. LC= liver counterpart, HCC= Hepatocellular carcinoma, F=Fibrotic, AF: Area fraction, VF: Volume fraction.*

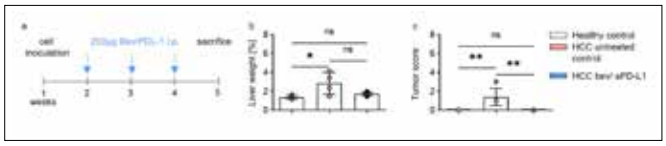

Figure 4: First line tumor-targeted therapy in HCC model. a. *Treatment schedule for checkpoint Bevazizumab/anti-PDL1 therapy on the non-fibrotic HCC model. The treatment resulted in an absence of tumor growth, which is reflected in non-increased liver weight (b) and a tumor score of 0 (c).*

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EXPERIMENTAL APPROACH FOR THE QUANTITATIVE CHARACTERIZATION OF MULTIVALENT LIGAND-RECEPTOR INTERACTIONS OF POLYMERIC NANOPARTICLES WITH TARGET CELLS

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Polymeric nanoparticles have been explored for many years as innovative drug delivery and diagnostic systems, often equipped with complex targeting concepts on their surface to achieve the highest possible target specificity[1]. For this purpose, countless strategies for active targeting have been discussed in literature, relying on both small molecules and peptides as ligands[2]. Common to most of these targeting approaches is that the initial surface contact between ligand-functionalized nanoparticles and target or off-target cells represents a key moment in the concept of cell recognition and is decisive for the subsequent fate of the particles[3]. The characterization of this initial attachment between nanoparticles and the cell surface is therefore of strategic importance for further tailored development approaches. However, the mechanisms of the specific interactions at the level of the individual receptors are still not comprehensively understood and there is a knowledge gap about how many ligands per nanoparticle are involved in the anchoring process and specifically bind to receptors. Mathematical and computational studies predict that adjusting the number of ligands per nanoparticle to the number required for the anchoring process could not only increase the specificity of the particles for their target cells, but also lead to better uptake rates and optimized uptake kinetics[4,5]. Confirming these results in further experimental studies would be of paramount relevance for the development of polymeric nanoparticles. So, despite the technological importance that knowledge of the number of binding ligands could have, there are few studies that shed light on this subject.

The aim of our work is therefore to present an experimental approach to characterize the initial contact of ligand functionalized polymeric nanoparticles with the surface of their target cells and to determine the mean number of simultaneous binding ligands per particle, paying particular emphasis to the complexity of the system (see Fig. 1).

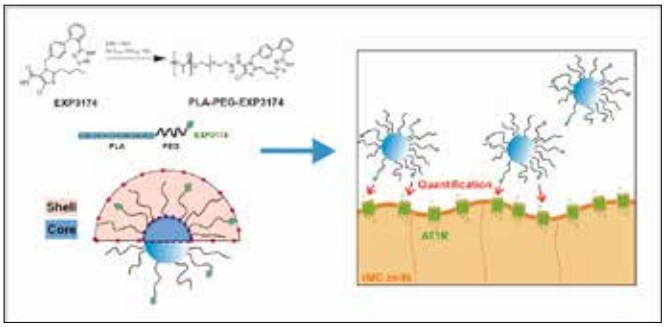

Figure 1. Design concept of the model particle and graphical representation of the research interest

Core-shell nanoparticles composed of PLGA and PLA-PEG copolymer were chosen as a model for our study. This type of nanoparticles shows a high degree of modularity and can be equipped with a variety of ligands tethered on chains of different length[6]. Thus, it allows the construction of complex targeting concepts, which makes it a promising platform and an interesting model. EXP3174, a high-affinity antagonist on the AT1 receptor, was selected as the ligand the nanoparticles were functionalized with. The antagonistic effect of the ligand on the receptor leads to an endocytosis inhibition of the nanoparticles and therefore enables the detection of surface effects in the context of *in vitro* experiments[7].

To experimentally determine the average number of simultaneously binding ligands per nanoparticle, the number of binding ligands was defined as a function of half of the number of target receptors (AT1R) on the cell surface allocated to the number of nanoparticles attached to the cell membrane. This equation can be considered if the applied nanoparticle concentration is equal to their K_i value. An interlocking experimental set-up was established to solve this equation (see Fig. 2, Eq. 1).

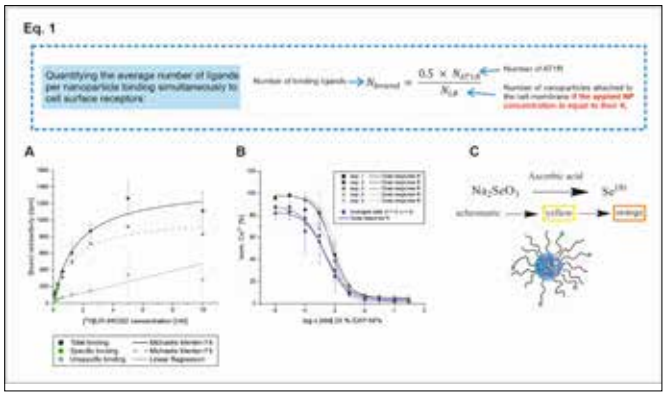

Figure 2. Illustration of the interlocking experimental approach

Rat mesangial cells (rMC) were chosen as a model cell line for the cell surface interaction studies due to their stable expression of the target receptor[8]. The total number of receptors on the cell surface was determined by radiochemical saturation binding assays with [^{3}H]UR-MK292, a tritium labelled angiotensin II derivative[9] (see Fig. 2, A). By measuring the activity of the particles at the target receptor, it was first possible to determine their IC50 value (see Fig. 2, B) and then derive the K_i value of the particles using the Cheng-Prusoff equation. Currently, we are working on the challenge of direct quantification of polymeric nanoparticles in the cell matrix, to determine the number of nanoparticles that are bound to the cell surface when applied in the concentration of their K_i value. To this end, we are testing the approach of quantifying the particles via an ICP-MS tag introduced into the particle core in form of an amorphous selenium dispersion[10] (see Fig. 2, C). Assuming complete binding of the nanoparticles to the cell surface, which previous experiments indicate, a preliminary value for the number of binding ligands could already be calculated. We consider 84 ligands as the lower threshold for the average number of binding ligands per nanoparticle. This preliminary result fits in well with calculations from previously published computational studies[4,5]. We expect to be capable of deriving a final result from the overall view of the experiments.

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SYNTHESIS AND FUNCTIONALIZATION OF POLYETHYLENE GLYCOL (PEG) ISOMERS: REINVENTING A WELL-KNOWN POLYMER

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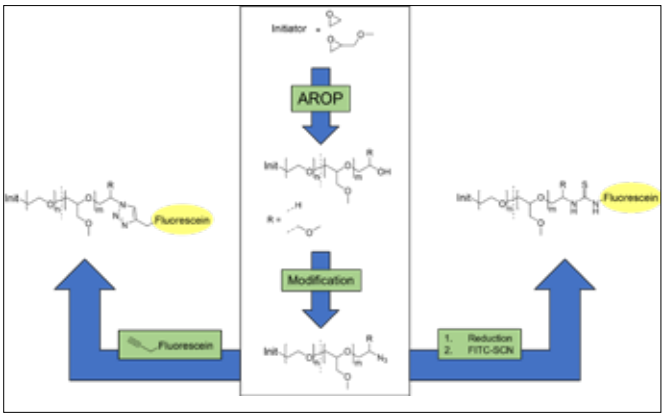
Polyethers represent one of the most important classes of adaptable materials with applications in modern medicine.[1] The most commonly used polyether is polyethylene glycol (PEG) – a linear chain of multiple units of ethylene glycol synthesized via anionic ring-opening polymerization (AROP) of ethylene oxid (EO). This polymer is non-toxic, biocompatible and can be excreted through the human renal system up to a molecular weight of 30 kDa. The functionalization of active pharmaceutical ingredients (APIs) with PEG – the so called "PEGylation" – significantly reduces the immune reaction to said compounds by effectively "hiding" it from antibodies. A corona of PEG molecules surrounding the API inhibits the adsorption of opsonins. Consequently, the circulation time of the PEGylated compound in the bloodstream is greatly increased compared to the non-modified one. This phenomenon is called "stealth effect" and has been utilized in medical science for decades.[2]
However, shortly after the discovery of this effect[3] the existence of anti-PEG antibodies (APAs) was also proven.[4] Therefore, chronic adverse reactions to PEG can appear – ranging from accelerated blood clearance (ABC), negating the stealth effect of the PEGylation, to hypersensitivity in the form of "pseudo-anaphylaxis".[5] A recent publication suggests the occurrence of APAs in well over 90 % of the general population, believed to be caused by overexposure through the vast prevalence of PEG in common household products.[6]
In this work we present a new synthesis of random copolymers derived from EO and glycidyl methyl ether (GME) referred to as "rPEGs". These copolymers are structural isomers of linear PEG chains but distinct themselves by the presence of randomly distributed side chains, each containing one unit of ethylene glycol. So far, the homopolymerization of glycidyl methyl ether and its copolymerization with EO has been proven to be challenging. Copolymers were merely synthesized via less controlled and non-GMP certified polymerization techniques, excluding biomedical applications of these materials.[7]



Scheme 1: Synthesis and functionalization of rPEG copolymers

In contrast, we are able to synthesize rPEG copolymers through classical AROP, eliminating the need for toxic catalysts or additives and therefore enabling the use of these copolymers for various medical applications. Furthermore, the well-established chemistry of PEG was successfully applied for chain end functionalization of the rPEGs. To enable detailed studies of the novel polyether material, fluorescence-dye labeled copolymers were obtained allowing *in vitro* and *in vivo* investigations, thus providing insights into the behavior and performance in biological systems. Consequently, if these copolymers could be used as an alternative to PEG in the PEGylation process the immune response to the functionalized APIs could be significantly reduced or suppressed completely, elevating concerns about hypersensitive reactions.
Meanwhile, the thermoresponsive behavior even at ≥ 50 mol% GME content still shows great similarities to PEG with cloud points varying between 60 °C and 90 °C. These data show the excellent water solubility of rPEGs at body temperature which supports the stealth effect by providing a hydrophilic protective layer around a respective API. While the mentioned properties highlight the benefits of rPEGs, the disadvantage of sensitive immune reactions can potentially be inhibited by these new kinds of polymers, ultimately encouraging the use in human tissue.
Overall, the significant advancement in the synthesis and characterization provides rPEGs with desirable properties such as aqueous solubility and the ability for chain end functionalization, making them promising candidates for various applications in chemistry and medicine.

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MULTICOMPONENT ADJUVANTATION OF ANTIGEN-BASED NANOCAPSULES USING SITE-DIRECTED CLICK CHEMISTRY CROSSLINKING FOR THE TREATMENT OF MELANOMA

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Nanocarrier-based antigen delivery is a promising vaccination approach in the context of tumor therapy. Formulating polymeric nanocapsules (NCs) out of tumor antigens in combination with vaccine adjuvants enables efficient targeting and maturation of dendritic cells (DCs), essential prerequisites for the induction of vigorous cellular immune responses. Aim of the present study was the synthesis of polymeric protein nanocapsules composed exclusively of vaccine antigens and encapsulated with combinations of adjuvants, as well as the evaluation of their potential to induce antigen-specific immune responses.

NCs consisting of ovalbumin (OVA) were bioorthogonally crosslinked by copper-free azide-alkyne click chemistry using the inverse miniemulsion technique. This method ensures integrity and processability of crosslinked antigens leading to effective epitope presentation by dendritic cells. Our polymeric nanocapsules with a spherical morphology were efficiently ingested by DCs. In addition, a combination of the vaccine adjuvants Resiquimod (TLR7/8 agonist) and diABZI (STING agonist) was encapsulated to efficiently trigger strong DC activation analyzed by costimulatory surface marker expression and the secretion of pro-inflammatory cytokines *in vitro* and *in vivo*. The induction of robust antigen-specific T cell proliferation was observed in DC-T cell co-cultures and further demonstrated *in vivo*. Additionally, the high biocompatibility, the effective cure of B16 tumors as well as the immune memory esthablishment elicited by our OVA-NC-based vaccine was demonstrated in different mouse models.

In conclusion, multiadjuvant-functionalized protein nanocapsules are a promising delivery vehicle for the simultaneous transport of antigens and vaccine adjuvants to dendritic cells promoting T cell-based anti-tumoral immune responses. This novel anti-tumor vaccination strategy avoids the use of structural compounds, increases the antigen load of DCs, bears the potential to overcome tolerance and to induce vigorous antigen-specific anti-cancer immunity.

POLYMERIC MICELLAR PLATFORM WITH CONTROLLED RELEASE KINETICS FOR TAXANE AND CORTICOSTEROID CANCER COMBINATION THERAPY

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Introduction: Nanomedicine-based combination therapy has demonstrated great potential in cancer.[1] Paclitaxel (PTX), a widely used taxane chemotherapeutic, is present in some nanoformulations with indications in fibrotic cancers. Fibrotic tumours are notorious for having abnormalities which impede nanomedicine penetration and hinder its efficacy.[2] To tackle these obstacles, strategies such as tuning nanocarrier size and using tumour microenvironment remodelling agents such as dexamethasone (DEX), a potent corticosteroid, have shown promise. Here, we aimed for a nanoparticle size- and drug release rate-tuneable formulation for combination therapy via co-encapsulating PTX with DEX. To this end, we employed mPEG-*b*-p(HPMAm-Bz) block copolymers, which we recently showed high polymerization versatility for, to prepare coloaded micelles.[3] We further expanded by co-encapsulating two other pairs of taxane and corticosteroid. Finally, we assessed the association between different structural and physicochemical properties of the loaded drugs and their retention in the micelles.

Methods: We synthesized block copolymers with three different hydrophobic chain length and used them to prepare micelles. Size and polydispersity index (PDI) of the micelles were measured by DLS. Drug encapsulation efficiency (EE) and release were evaluated by HPLC.

Results: Copolymers of three different sizes (small, medium, and large) were synthesized and used to prepare empty as well as PTX and DEX coloaded micelles. The size of the micelles showed a positive trend as the MW of the polymers increased and both drugs were efficiently loaded into them. Release studies revealed higher drug retention in micelles from larger polymers, and a faster release of DEX as compared to PTX in all the cases. Furthermore, two other taxane-corticosteroid pairs, namely pairs of docetaxel (DTX) with prednisolone (PRD) and cabazitaxel (CTX) with ciclesonide (CIC) were efficiently coloaded into medium-sized micelles. Finally, amongst different structural and physicochemical properties of the loaded drugs, hydrophobicity and molecular weight associated best with their retention in the micelles.

Conclusion: This micellar platform exhibited high tuneability and versatility for codelivery of taxanes and corticosteroids. The slower release rate of PTX compared to DEX can cause their sequential therapeutic effect which is favourable from a pharmacological standpoint. The earlier-released DEX can potentially already start priming the tumor microenvironment prior to the release of the majority of PTX, to further promote the penetration of the micelles and the chemotherapeutic into the site of action.

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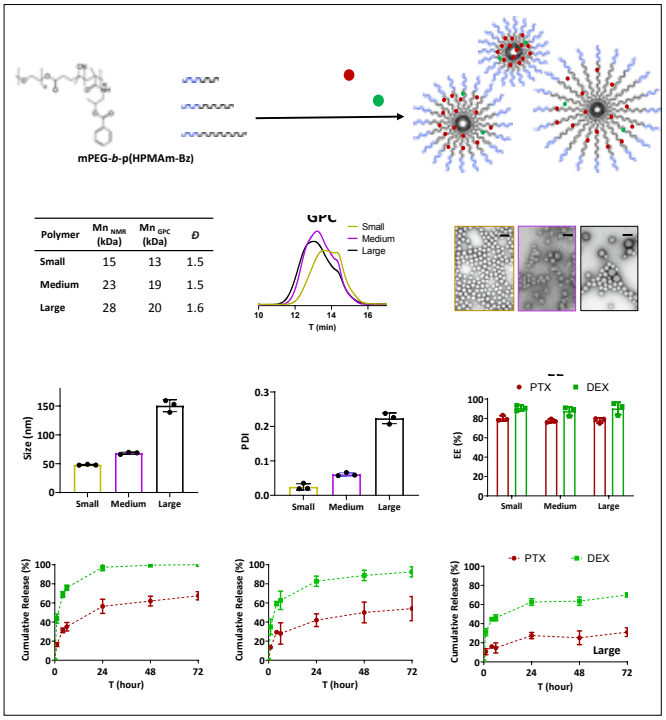
Figure 1. Coloading paclitaxel (PTX) and dexamethasone (DEX) into micelles prepared from copolymers of three different sizes. A) Chemical structure of block copolymer and schematic illustration of PTX and DEX coloaded micelles from small, medium, and large block copolymers. B). Characteristics of small, medium, and large polymers. C)

GPC chromatograms of small, medium and large polymers. D) Transmission electron microscopy (TEM) images of non-loaded micelles prepared from small, medium, and large polymers. E-G) Size (E), polydispersity index (PDI) (F), and drug encapsulation efficiency (EE) (G) of PTX and DEX coloaded micelles prepared from small, medium, and large polymers. H) Drug release under sink condition from micelles prepared from small, medium, and large polymers. Thirty mg of polymer, 7.5 mg of PTX, and 1 mg of DEX were used to prepare 1 mL of micellar dispersion.



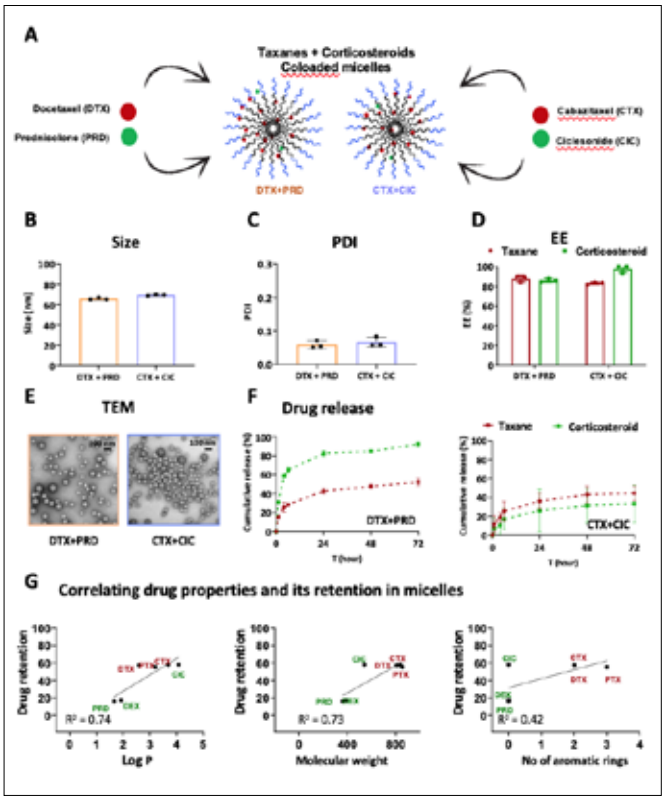


Figure 2. Coloading other taxanes and corticosteroids as well as evaluating the association between different structural and physicochemical properties of the loaded drugs and their retention in the micelles. *Docetaxel (DTX) was coloaded with prednisolone (PRD) and cabazitaxel (CTX) was loaded with ciclesonide (CIC) into micelles prepared from medium block copolymers.* **A)** *Schematic illustration of coloading DTX with PRD and CTX with CIC.* **B-E)** *Size (B), polydispersity index (PDI) (C), drug encapsulation efficiency (EE) (D), and transmission electron microscopy (TEM) images (E) of DTX+PRD and CTX+CIC coloaded micelles.* **F)** *Drug release under sink condition from DTX+PRD and CTX+CIC coloaded micelles.* **G)** *Drug retention in the micelles after 24h under sink condition as a function of log P, molecular weight, and number of aromatic rings. Thirty mg of polymer, 7.5 mg of taxane, and 1 mg of corticosteroid were used to prepare 1 mL of micellar dispersion.*

MULTICOMPONENT SUPRAMOLECULAR PLATFORM FOR THE DESIGN OF GLYCOCONJUGATE ANTITUMOR VACCINES

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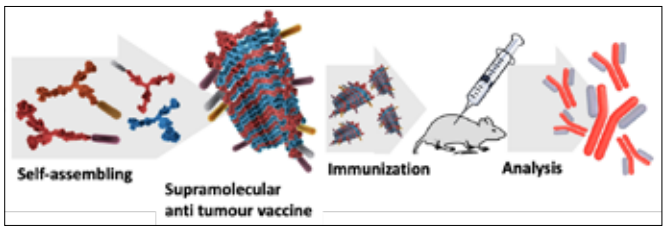
Peptide secondary structures can be harnessed to design monomers capable of self-assembling into nano-scaled supramolecular structures in aqueous media.[1,2] Decorating the surface with immunogenic molecular patterns results in pathogen-mimicking entities and potential vaccine candidates.[3] In the context of antitumor vaccines, the challenge is to overcome self-tolerance mechanisms to enforce an immune response against endogenous, tumor-associated glycopeptide motifs.[4] For this purpose, co-stimulation of B cells with T helper cells is mandatory, which we aim to achieve by co-presentation of different epitopes and immunostimulant agents on the surface of multicomponent supramolecular polymers. The use of thermoset supramolecular hydrogels as a vaccine depot also allows for sustained immune stimulation and could be an alternative to the adjuvants required in conventional vaccination strategies.



B-cell epitopes derived from either breast tumour-associated MUC or melanoma-associated CSPG4 surface proteins and co-presented multivalently with “universal” immunostimulatory T-helper cell epitopes. Mucin 1 (MUC1) is known to undergo O-glycosylation changes during tumourgenesis, making it an excellent tumour-associated target for immunotherapy. Fully synthetic 22 amino acid MUC1-derived glycopeptides bearing sialylated STN tumour associated carbohydrate antigens are therefore being used.[5-7] Chondroitin sulfate proteoglycan 4 (CSPG4) is a surface proteoglycan that has been observed to be highly expressed on tumour cells, whereas expression on healthy tissue is limited, making it a highly potential target for the difficult-to-threat melanoma.[9-10] Future investigations will also be based on fully synthetic derivatives of this marker. T-cell stimulation is achieved by incorporating a small fragment of highly immunogenic tetanus toxin (p30). In addition, an imidazoquinoline, a potent TLR7/8 agonist,[11] has been synthesized as an immunomodulator. Mannose moieties can be attached to the surface of the nanorods to further recruit and stimulate macrophages as accessory cells. These epitopes were conjugated to supramolecular monomers and mixed in aqueous solution to yield polymeric vaccines. High antibody titers of the IgG type were observed in all mice. Furthermore, FACS analysis confirmed the high binding affinity of the generated antibodies to T47D tumor cells.

These results support the potential of this modular supramolecular platform approach for the development of antitumor vaccines.

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PROTEOMICS-GUIDED INTRACELLULAR TRAFFICKING ANALYSIS REVEALS TIME-DEPENDENT PROTEIN CORONA CHANGES AND THE INTRACELLULAR PATHWAY

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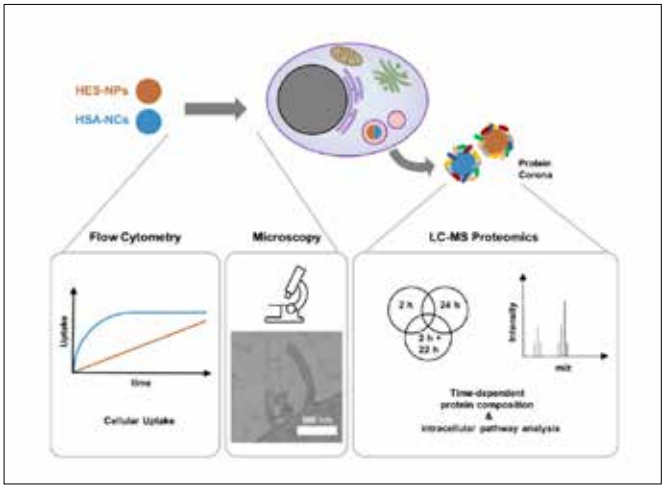
Graphical Abstract



Upon the contact of a nanocarrier with a biomolecule-containing fluid, the biomolecules adsorb spontaneously to the nanocarriers´ surface and lower the surface free energy [1]. Ultimately, the formation of this biomolecular corona influences the nanocarriers' properties, their cellular interaction, pharmacokinetics, tissue penetration, biodistribution, and the release of active pharmaceutical ingredients [2]. The biomolecular corona, also described as protein corona when investigating adsorbed proteins, forms in both extracellular protein-containing fluids and within the cell [1, 3].

The intracellular protein corona remains poorly investigated within the field of nanotechnology-biology (nano-bio) interactions. To deeply understand the intracellular protein corona formation and dynamics, we established a workflow (figure 1a) to isolate the intracellular protein corona of different nanoparticles - magnetic hydroxyethyl starch nanoparticles (HESNPs, figure 1b) and magnetic human serum albumin nanocapsules (HSANCs, figure 1c). Here, the corona formation after different time points (continuous uptake: 2 h, 24 h and discontinuous uptake = replacement of nanocarrier containing media by nanocarrier-free media after 2 h: 2+22 h) of uptake in the murine dendritic cell line DC2.4 was investigated. This intracellular protein corona defines the direct molecular contact partners of the nanocarrier and is, therefore, a prime target for further drug development.

After cell lysis and hard protein corona desorption from the nanocarriers (figure 1a) we performed label-free quantitative LCMS proteomics to analyze the composition of the intracellular protein corona. Based on the 1.5x enrichment of proteins identified in the protein corona samples compared to pure cell lysate controls without nanocarrier incubation we determined a set of corona proteins, whereas the 20 most abundant proteins are displayed in the heatmaps below (Figure 1d/e).

We correlated our findings to conventional methods for uptake and intracellular trafficking of nanocarriers, such as flow cytometry, transmission electron microscopy (TEM), and confocal microscopy (cLSM). We demonstrated that a comparable slow uptake as measured with flow cytometry for the HESNPs correlated with a time point-depending protein corona. Although the HES-NPs are smaller than HSA-NCs (compare figure 1 b/c) we attributed their slower uptake to the stealth properties of HES described earlier [4, 5]. A faster uptake of the HSANCs correlated with the rather stable protein corona with minor time-dependent changes. Thus, the flow cytometry analysis was in accordance with our proteomic measurements. The microscopic methods visualized the morphology of the uptake and we observed a caveolin-dependent (CAV) uptake and macropinocytosis (figure 1f) for HES-NPs, whereas for the internalization of the HSA-NCs we argued for a phagocytotic mechanism (figure 1g). Furthermore, the conventional microscopy methods reveal insights into the further trafficking of both nanocarriers.

Nevertheless, to support these results we also put the proteomic data in the context of intracellular trafficking, since we performed protein annotation with the bioinformatic tool DAVID (6.8) [6] and a cellular compartment (CC) database (namely GOTERM_CC_FAT) as reference data. Hence, our corona proteins as determined by LC-MS were characterized in the context of cellular locations and therefore could provide a reconstruction of the possibly involved intracellular pathways the nanocarriers were subjected to (figure 1h/i).

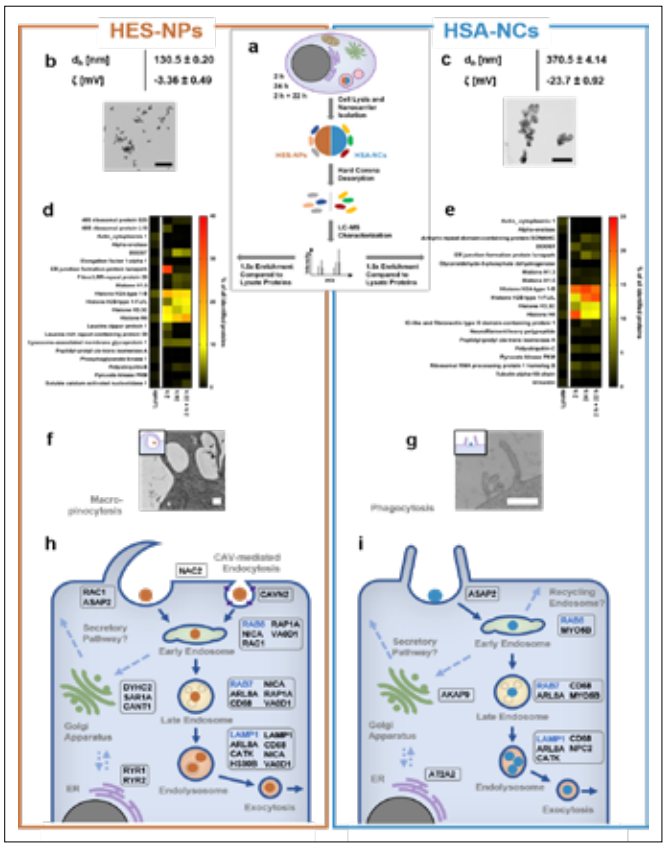


Figure 1: Workflow to analyze the intracellular protein corona after uptake of HES-NPs and HSA-NCs and selection of most important results. Further details in text. In (a) and (b) size is described as dh and zeta-potential as ζ. The scale bars for the TEM micrographs below represent 500 nm. In (f) and (g), the scale bar for the TEM micrographs represent 500 nm. For HES-NPs (g) and HSA-NCs (i), respectively, the LC-MS identified proteins (given with Uniprot ID) were linked to intracellular compartments (as seen in black) and combined with the findings of TEM and cLSM (RAB5, RAB7, and LAMP1; seen in blue) to recreate an intracellular trafficking network.

In sum, we demonstrated the evolution of intracellular protein corona. The protein corona differed within the different timepoints for the HESNPs with a slow uptake but less for the HSANCs with a rapid uptake. Furthermore, we selectively identified proteins of interest for intracellular trafficking. These proteins served as an effective "fingerprint" and allowed for a more detailed intracellular pathway reconstruction than the conventional methods. Proteomic-driven or complemented investigations improve, therefore, the research on the uptake and intracellular trafficking of nanocarriers. This strategy will prove beneficial when investigating altered intracellular routes through nanomaterial modification and targeting.

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GOLD-COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR CARDIOVASCULAR APPLICATIONS

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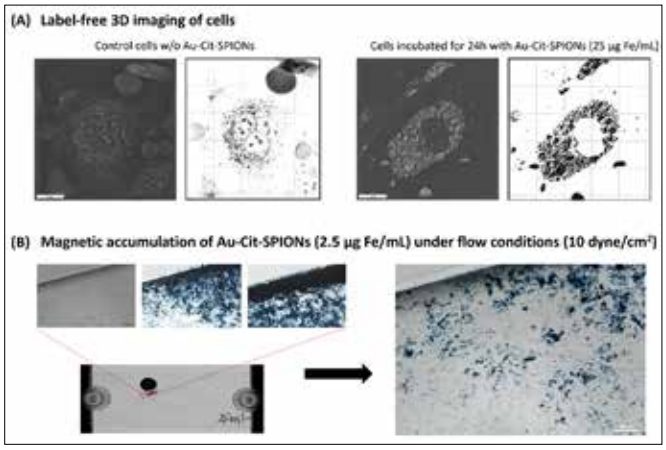

Figure 1. Interactions of Au-Cit-SPIONs with human endothelial cells. *(A) Figure illustrating the internalization of Au-Cit-SPIONs by HUVECs; (B) Magnetic accumulation of AU-Cit-SPIONs under flow conditions in vitro.*

Background: Owing to their magnetic properties, superparamagnetic iron oxide nanoparticles (SPIONs) can provide both enhanced target accumulation, disease treatment and diagnosis. Our project aims at the development of SPIONs for potential cardiovascular applications.

Methods: Gold-coated SPIONs (Au-Cit-SPIONs) by a two-step method. Firstly, citrate-stabilized SPIONs (Cit-SPIONs) were produced using an alkaline precipitation followed by a surface stabilization with citrate ions. After a washing procedure, these particles were coated with a gold layer by directly precipitating gold onto the SPION surface once again followed by a surface stabilization using citrate. Various synthesis parameters have been investigated to achieve stable particles with the desired sizes. The resulting particles were analyzed in terms of hydrodynamic size, ζ-potential, magnetic susceptibility as well as crystal structure.

To investigate the biocompatibility of produced nanoparticles, human umbilical vascular endothelial cells (HUVECs), primary human fibroblasts or Jurkat cells were incubated with Au-Cit-SPIONs and their growth was monitored for 48 h using flow cytometry. The magnetic accumulation of Au-Cit-SPIONs under flow conditions was investigated using peristaltic pump and bifurcating-channel slides (Ibidi®), and their uptake by HUVECs was monitored using live holotomography system (NanoLive). The effects of Au-Cit-SPIONs on angiogenesis were investigated using Matrigel tube formation assay.

Results: The resulting Au-Cit-SPIONs had hydrodynamic diameter of 120 - 150 nm, a narrow polydispersity index of 0.19 and a ζpotential of -50 to -60 mV at pH 7. The gold layer led to a slight decrease in magnetic susceptibility to around 90% of the value of Cit-SPIONs while turning the color of the particles from brown to deeply red. This indicates the formation of nanosized gold on the SPION surface which was additionally confirmed by a crystal structure analysis (XRD).

In the flow cytometric analyses, Au-Cit-SPIONs were well tolerated by Jurkat cells up to 100 µg Fe/mL and the viability of primary human fibroblasts remained above 80% up to the highest tested concentration of 75 µg Fe/mL. The particles were avidly internalized by HUVECs (Fig. 1A), but were toxic above the concentration of 25 µg Fe/mL. Under arterial-like flow conditions *in vitro*, time-dependent, strong accumulation of Au-Cit-SPIONs under flow conditions was observed (Fig. 1B) already at the concentration of 2.5 µg Fe/mL,

leading to endothelial cell death and detachment in the magnetically targeted area after 6-24h. Moreover, Au-Cit-SPIONs induced disruption of tubular networks in the Matrigel-based angiogenesis assay.

Conclusions: In conclusion, the magnetic properties of the developed Au-Cit-SPIONs enable their magnetic accumulation and, potentially, targeting of drugs even under arterial-like flow conditions. In contrast to good biocompatibility with fibroblasts and Jurkat cells, they showed a rapid internalization and pronounced toxicity in endothelial cells, indicating their potential to inhibit neovascularization processes either in atherosclerotic plaques, or in solid tumors.

Funding: This work was supported by the ERA-Net project "MAGNA" (Grant number: 01DJ21004).

MICROFLUIDIC DEVICE FOR THE INVESTIGATION OF NANOPARTICLE DYNAMICS IN THE HEALTHY AND DISEASED STATE OF THE GASTROINTESTINAL TRACT

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INTRODUCTION

The gastrointestinal tract (GIT) is a complex system that poses a challenge to the oral delivery of nanoparticles. The different fluid compositions, pH values, and characteristics of each part of the GIT (i.e., stomach, small intestine, and colon) can vary extensively. Commonly used bulk techniques to assess the fate of nanoparticles through the GIT have been mostly optimized for testing drug compounds' solubility or stability rather than the dynamic behavior of nanoparticles, such as aggregation or enzyme corona formation [1]. Furthermore, little is known about how GIT pathologies may affect nanoparticle stability and physicochemical properties after oral administration. For example, it has been reported that normal pH values, osmolality, and lecithin concentration in the GIT can dramatically change when patients suffer from inflammatory bowel disease (IBD) [2]. Miniaturization of a system where the GIT is mimicked can offer a high surface-to-volume ratio, the possibility of automatization, and a reduced need for valuable chemicals (like enzymes). Cell-free microfluidic devices that mimic drug compound digestion through the GIT have been previously reported [3], [4]. However, these devices have not yet been adapted for nanoparticle studies.

The aim of this work is to develop a microfluidic device in which gastrointestinal media (simulating both the healthy and IBD states) can be mixed with nanoparticles to study how their composition impacts the stability, aggregation, and protein corona formation of inorganic nanoparticles in different parts of the GIT.

METHODS:

Iron oxide nanoparticles were synthesized by flame spray pyrolysis and coated with a silica layer in a single step [5]. They were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR).

To select a micromixer for the development of the microfluidic device, four commercially available micromixers were tested for their efficiency to combine and distribute two convergent flow streams to create a uniform mixture. For this purpose, fluorescein dextran MW 10kDa (FITC) was supplied in one flow stream inlet, and water was supplied in another inlet at an equal flow rate. Finally, the relative mixing index (RMI) was calculated in the fluorescent images by tracing a line through a cross-section of the channel with a 20 pixels width (corresponding to 22 nm) and 190 nm in length. Then, the average of the 20 pixels in the first dimension was calculated. The RMI was computed according to the following equation:

Where

= standard deviation of pixel fluorescent intensity

= standard deviation of pixel fluorescent intensity in the unmixed state

The fluorophore intensity throughout the chips at different inlet flow rates (10, 40, and 80 µL/min) was analyzed. The fluorescent images obtained were analyzed using a Python code available at https://github.com/yaelsuarez/mixing_quantification. Media simulating a broad range of inflammatory conditions in Crohn's disease and ulcerative colitis were developed based on literature. The long herringbone micromixer was utilized to study how pH, soluble protein content, bile acid, and lecithin concentrations of healthy and IBD-simulating media affect nanoparticle stability, aggregation, and protein corona formation. These GI conditions were systematically varied using a design-of-experiments approach. The stability and aggregation of nanoparticles were characterized by dynamic light scattering and small angle X-ray scattering, while the protein corona was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [6].

RESULTS AND DISCUSSION

This work presents advances in developing a microfluidic device aiming to investigate nanoparticle dynamics in the gastrointestinal tract. Iron oxide nanoparticles coated with silica were characterized by XRD and showed a maghemite crystalline phase (Figure 1a). FTIR and TEM also confirmed the presence of the silica layer coating the iron oxide nanoparticles (Figure 1 b, c). A pearl-chain micromixer, two types of herringbone micromixers, and a serpentine micromixer were tested for their mixing efficiency. Mixing of FITC was quantified based on pixel intensity in a cross-section of the last part of the microfluidic channel. The long herringbone micromixer was selected for further development since it exhibited the best mixing efficiency (Figure 2) with an RMI >96% at all the flow rates tested. IBD-simulated media were developed based on the literature, and the effects of the media composition on nanoparticle aggregation behavior and protein corona formation were investigated using the herringbone micromixer to simulate each part (stomach, small intestine, and colon) of the GIT.

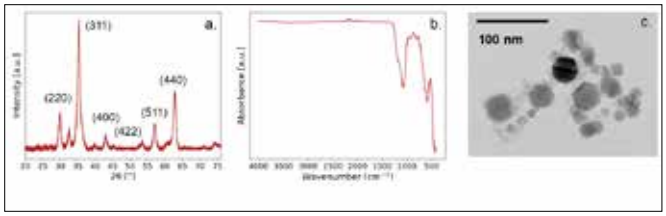

Figure 1. XRD pattern (a), FTIR spectrum (b), and TEM image (c) of flame-made SiO_2-coated γ-Fe_2O_3 nanoparticles.

CONCLUSION

Silica-coated iron oxide nanoparticles were produced in a single step by flame spray pyrolysis. The most efficient micromixer was selected to continue the development of a microfluidic device that allows the systematic study of the different parameters affecting the stability of nanoparticles through each stage of the GIT (stomach, small intestine, and colon) in a healthy or diseased state in an automated and controlled manner.

ACKNOWLEDGMENTS

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COMPARATIVE STUDY OF ADJUVANTS AND THEIR SYNERGISTIC POTENTIAL FOR THE STIMULATION OF DENDRITIC CELLS (DC) AND LIVER NON-PARENCHYMAL CELLS

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Resident liver NPCs (non-parenchymal cells), including Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC), and hepatic dendritic cells (DC), by default induce T-cell tolerance. This inherent T-cell tolerance promoting activity limits the efficacy of intrahepatic anti-tumor responses. Nevertheless, suitable adjuvants can reprogram liver NPC to a T effector cell inducing phenotype.
The project aims to enhance the anti-tumor efficacy of nano-vaccines by employing the most effective adjuvant combinations that activate both antigen-presenting dendritic cells (DC) and liver NPCs to improve the outcome of (liver) tumor therapy. Therefore, an *in vitro* adjuvant screening was conducted comprising Toll-Like Receptors (TLR) and Stimulator of Interferon Genes (STING) agonists to study the activation of DC and NPC subpopulations. Promising candidates were selected and the effects of adjuvant combinations were tested for synergistic effects.
Bone marrow-derived (BM)DC were differentiated via incubation of murine bone marrow cells with either GM-CSF or FLT3L for 7 days. Murine non-parenchymal liver cells were isolated by liver perfusion. Cells were treated with different adjuvant concentrations overnight. The expression of activation markers (CD80, CD86, MHCII) and the secretion of pro-inflammatory cytokines was examined using flow cytometry. Proliferation assays with $CD8^+$ T cells were conducted to evaluate the antigen presenting and T cell stimulatory activity on T cells.

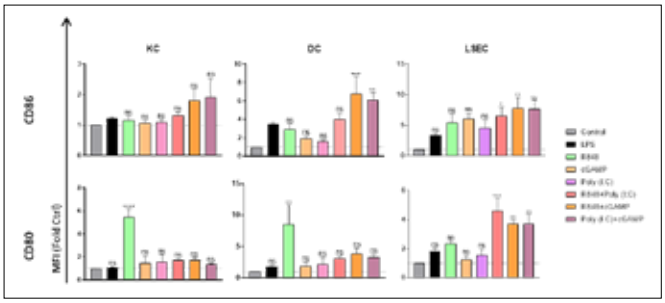

*Figure 1. Adjuvants enhance CD80 and CD86 expression of liver non-parenchymal cells. Liver NPC were incubated with 5 µg/ml R848, Poly (I:C) or cGAMP. On the next day, expression of CD80 and CD86 by LSEC, Kupffer cells and DC was assessed by flow cytometric analysis. Graphs denote the fluorescence intensities (MFI) (mean±SEM of 3-4 experiments) of marker expression. Statistical differences versus *Ctrl are indicated (one way ANOVA, Tukey test). *p<0.05, **p<0.01, ***p<0.001, ****<0.0001.*

The results revealed that combinations of the TLR7/8 ligand resiquimod (R848) or the TLR3 agonist polyinosinic:polycytidylic acid (Poly (I:C)) in combination with the STING agonist cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) yielded the strongest stimulatory activity on either type of liver NPC (**Figure 1**) and BMDC.
In a second set of studies, different unmethylated CpG containing DNA oligodeoxynucleotides (ODN), known to act as TLR9 agonists, were compared regarding their adjuvant activity. The study identified optimal performers for the different cell types, with CpG ODNSL03 constituting the most potent stimulator for both liver NPC and BMDC (**Figure 2**).

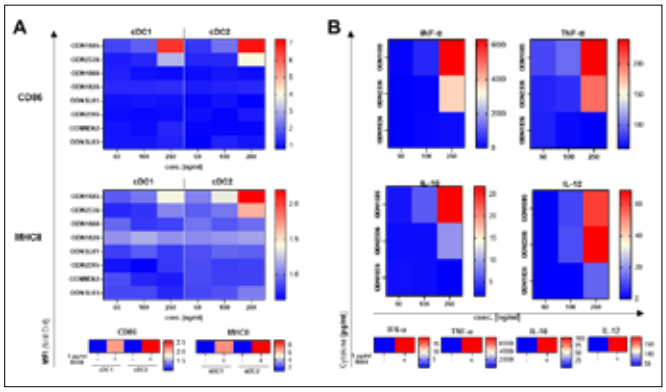

Figure 2. *CpG oligos enhance MHCII and CD86 expression and proinflammatory cytokine release of FLT3L differentiated BMDC. (***A***) BMDC were incubated with different concentrations of CpG oligos (50, 100 or 250 ng/ml) or the TLR7/8 ligand R848 (1 µg/ml). On the next day, expression of MHCII and CD86 by cDC1/2 waas assessed by flow cytometric analysis. Graphs denote the mean fluorescence intensities (***MFI***) (mean±SEM of* **4** *experiments) of marker expression. (***B***) BMDC were incubated over night with CpG oligos. Cytokine concentrations of culture supernatants were determined by CBA (mean±SEM of* **3** *experiments).*

The next step of this project is to formulate the best performing stimulators together with antigen mRNA into nanoparticle formulations carrying a trimannose moiety on their surface to actively co-target DC and liver NPC and therefore improve the efficacy of anti-cancer therapeutic vaccines.

INTELLIGENT SINGLE-ATOM NANOZYMES FOR EFFECTIVE AND SAFE THERAPY OF INFLAMMATORY DISEASES IN PREGNANCY

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Gestational inflammation is pivotal for an uncomplicated pregnancy. Nevertheless, aberrant inflammation can cause obstetric complications, which can be detrimental for the maternal and fetal health. Large body of evidence demonstrates a significant contribution of the placenta in the development of many pregnancy disorders. Current therapies to treat inflammatory diseases during pregnancy consist of conventional NSAIDs and antibiotics, which often have not been tested in pregnancy clinical trials for efficacy and safety. There is increasing concern that conventional anti-inflammatory treatments can be drivers of obstetric complications, such as preterm births and miscarriages or induce adverse health effects in later life.

On this ground, we aim to develop safe and effective nanotherapeutics to treat gestational inflammatory diseases. We will engineer nanoparticles bearing enzymatic activities (nanozymes) with anti-oxidant properties. Specifically, single atom nanozymes (SAzymes) will be fabricated in order to mimic the active center of natural anti-oxidant enzymes, which improves the substrate affinity and catalytic activity compared to classical nanozymes. Metal-organic frameworks (MOFs) will be used as host-scaffold for the adsorption of active element atoms (e.g., Mn, Cu) and thus conferring reactive oxygen species (ROS)-scavenging activity. Their safety and efficacy in resolving inflammation will be investigated in advanced human placenta models. We further want to endow our particles with a micro-environment responsive antimicrobial surface, which will allow the SAzymes to exert a stealth or positively charged surface in the absence or presence of bacterial infection, respectively. A comprehensive multi-endpoint toxicological assessment will be performed using *in vitro* placental co-cultures, *ex vivo* placenta perfusion, placental explant cultures and a microphysiological placenta-embryo chip model to examine the SAzyme safety profile.

Our first SAzyme product is a Porous Coordination Network 224 (PCN224) nanomaterial with encapsulated Mn^{2+} (Mn@PCN224) displaying Superoxide Dismutase (SOD)-activity. Preliminary toxicity studies demonstrated that Mn@APCN224 did not compromise trophoblast or endothelial cell viability. Furthermore, Mn@PCN224 did not impair the barrier integrity in *in vitro* trophoblast-endothelial cell co-cultures.

These preliminary results suggest a high cytocompatibility profile of SAzymes *in vitro*. Further research is ongoing to investigate sublethal and long-term effects of Mn@PCN224 on placenta functionality along with the assessment of cell uptake, accumulation and translocation patterns across the feto-placental interface. Ultimately, the anti-inflammatory and antimicrobial performance will be elucidated as soon as the responsive SAzymes are available.

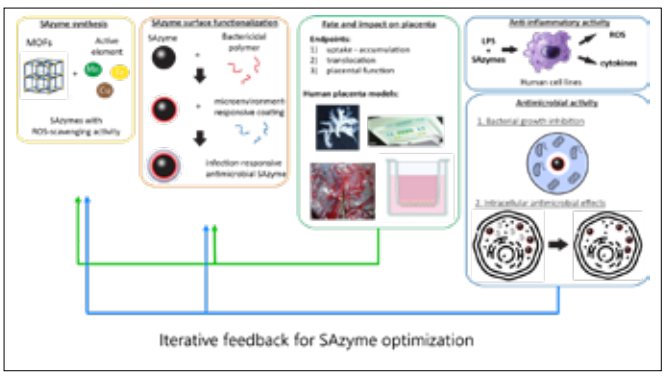

Figure 1: *Project Overview, composed of 3 pillars: 1) Intelligent SAzyme synthesis (SAzyme synthesis and surface functionalization) to enable the dual antibacterial and anti-inflammatory activity, 2) Safety assessment (fate and impact of SAzymes on placenta), 3) Therapeutic potential assessment (anti-inflammatory and antimicrobial activity of SAzymes).*

Acknowledgments:
This research has received funding from the Swiss National Science Foundation (Grant no. IZLCZ0_206059).

SELF-ASSEMBLING NASAL GEL FOR ENHANCED DELIVERY OF GHRELIN TO THE CENTRAL NERVOUS SYSTEM FOR AMYOTROPHIC LATERAL SCLEROSIS THERAPY

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the loss of motor function. Current available treatments for ALS are limited to increased survival, but do not necessarily improve the patients' quality of life. Targeting neurogenesis niches is currently being looked at as a potential therapy based on the hypothesis that repair of the neural system can restore the conditions in the central nervous system. It is also related to the theory that during neurogenesis, the obtained neuronal cells can migrate and integrate with mature neuronal circuit. Moreover, alteration in the neurogenic process is also reported in different neurodegenerative diseases, despite the distinct causes. Ghrelin, an orexigenic hormone produced in the stomach, has been widely reported to possess the ability to induce neurogenesis and promote neuroprotection. For that reason, ghrelin is hypothesised as a promising new candidate for the treatment of ALS. In the circulation, ghrelin exists in two forms, namely unacylated and acylated ghrelin. The acylation of ghrelin in the Ser3 position is necessary for its binding to the receptor for it to exert its activity. However, the active form ghrelin is rapidly deacylated in normal physiological condition with half-life of 9-11 minutes. Specific organ targeting can be the answer to this problem. Nose-to-brain delivery offers an attractive option for direct access of ghrelin to the brain, avoiding systemic degradation and undesirable side effects. This project focusses on preparation and testing of nasal gels for nose-to-brain delivery of ghrelin.

METHODS

This study aims to evaluate the efficacy of nasal gels to deliver ghrelin to enhance its brain bioavailability. A previously reported self-healing gel based on glutamine amide and benzaldehyde was utilised to formulate smart hydrogel with shear-thinning property suitable for intranasal administration of ghrelin. Ghrelin stability in gels was assessed with reversed-phase high performance liquid chromatography (RP-HPLC) and enzyme-linked immunosorbent assay (ELISA). The brain uptake and distribution profile of ghrelin were then studied using healthy C57Bl6 mice. The treatment groups included: 1) intravenous ghrelin (Ghr i.v.); 2) intranasal ghrelin solution in carboxymethylcellulose CMC (Ghr-CMC); and 3) intranasal ghrelin glutamine amide hydrogel (Ghr-HG). Animals were sacrificed at 5, 10 and 30 minutes post administration and brain lysates of whole and coronal brain sections were analysed for total and active ghrelin content using ELISA. Lastly, the bioactivity of ghrelin was assessed through measuring pAMPK/AMPK expression ratio by Western blotting.

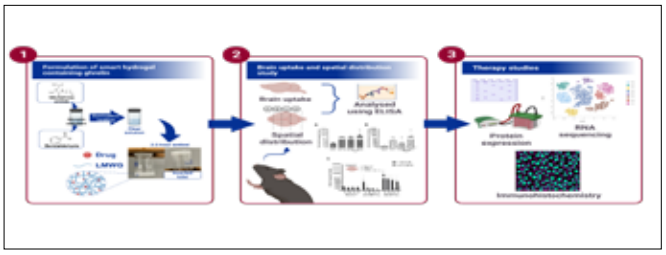
RESULTS

Smart shear-thinning hydrogel formulated with glutamine amide derivative containing ghrelin was successfully prepared in this study. Ghrelin remained stable after the formulation process. From the brain uptake study, intranasal administration of Ghr-CMC and Ghr-HG showed higher total ghrelin levels in the brain after 10 minutes compared to i.v. administration. Highest amounts per tissue weight were found in the frontal brain suggesting nose-to-brain transport pathway involvement in brain uptake. Ghrelin (bio)activity was confirmed by the higher pAMPK/AMPK ratio obtained compared to untreated controls.

CONCLUSION

Biologically active ghrelin was successfully delivered to the brain using nasal gels in a rapid manner within 10 min of instillation. This opens new opportunities for testing ghrelin nasal gels for treatment of a range of neurodegenerative diseases including frontotemporal dementia (FTD) and ALS.

Graphical abstract



PROTEIN CORONA STUDY OF TUNABLE LIPID BILAYER COATED NANOPARTICLES

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INTRODUCTION

In recent years, nanoparticles have garnered significant attention for a wide range of applications, including disease treatment, imaging delivery agents, and vaccines. When these particles enter the bloodstream, proteins attach to their surface creating what is known as protein corona. This corona plays a crucial role in determining the particle's fate, influencing whether it reaches its desired destination or is recognized as a foreign entity by the immune system [1]. To overcome this challenge, several materials have been explored as coatings to shield nanoparticles from immune cells. One promising class of coating molecules to achieve a shielding coating are phospholipids. These types of molecules offer numerous advantages, including biocompatibility due to their resemblance to cell membranes, anti-fouling properties that reduce protein adsorption, and the ability to easily modify the lipid bilayer to optimize the interactions with cells. However, studies have shown that liposomes complement activation can vary depending on the charge [2] and specific head group of the phospholipids[3]the electrostatic properties and activity to complement of two anionic vesicles modified with a carboxylic acid derivative or a conventional acidic phospholipid were compared. Electrophoretic mobility measurements indicated that the negative zeta potential and the electrostatic interactivity of these two anionic vesicles were equal at pH 7.4. However, the infusion of vesicles containing acidic phospholipid induced significant complement activation, while vesicles containing the carboxylic acid derivative failed to activate complement. These results indicate that the negative charge on the surface of vesicles is not critical for the activation complement, suggesting that complement activation is specific to the structure of acidic groups. This finding is likely to be important to the design of anionic biointerfaces and may support the promising medical applications of this anionic vesicle modified with a carboxylic acid derivative. © 2008 Elsevier B.V. All rights reserved.","author":[{"dropping-particle":"","family":"Sou","given":"Keitaro","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Tsuchida","given":"Eishun","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Biochimica et Biophysica Acta - Biomembranes","id":"ITEM-1","issue":"4","issued":{"date-parts":[["2008","4"]]},"page":"1035-1041","title":"Electrostatic interactions and complement activation on the surface of phospholipid vesicle containing acidic lipids: Effect of the structure of acidic groups","type":"article-journal","volume":"1778"},"uris":["http://www.mendeley.com/documents/?uuid=ee34cdc8-9f05-3e7a-b73d-521551bd4055"]}],"mendeley":{"formattedCitation":"[3]","plainTextFormattedCitation":"[3]","previouslyFormattedCitation":"[3]"},"properties":{"noteIndex":0},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}.

To unravel the underlying causes of these differences, our study focuses on coating silica nanoparticles with various lipid bilayer compositions: neutral, positively charged, or negatively charged. Through a thorough investigation of the resulting protein corona, we aim to gain a deeper understanding of these variations.

METHODS

Silica nanoparticles were coated with the Solvent-Assisted Lipid Bilayer method and were subsequently hydrodynamically characterized using dynamic light scattering (DLS) and ζ-potential measurements.

For the protein corona studies, both the coated and uncoated nanoparticles were incubated with 60% human serum for 20 min at 37°C. This was followed by 3 washes with cold PBS and centrifugations at 13000 RPM for 40 min at 4°C to remove any unbound proteins. The particles were then loaded into different acrylamide gels, allowing for protein separation based on their molecular weight. Different staining techniques were applied depending on the specific experiment. For visualization of distinct protein profiles, the gel was stained with silver nitrate, which binds irreversibly to the proteins. In the case of the proteomics assay, Coomassie blue staining was employed. Desired bands were digested and subsequently analyzed using mass spectrometry to identify the predominant proteins.

RESULTS AND DISCUSSION

Different coatings were considered including zwitterionic, negatively charged and positively charged lipids, for the nanoparticles. The hydrodynamic diameter of the coated nanoparticles slightly increased to 144nm compared to the uncoated silica nanoparticles measuring at 135nm. This size increasement suggests the presence of lipids surrounding the nanoparticles. The ζ-potential exhibited changes based on the charge of the phospholipids. In the case of DOPG, a negatively charged phospholipid, the nanoparticles were more negative compared to the uncoated particles.

When comparing the protein profiles of the coated nanoparticles, significant distinctions are seen between DOPG, DOTAP and DOPC:DOPS (80:20). Mass spectrometry analysis was conducted on these distinct protein bands. Immune system-related proteins, such as IgM and factor C3, were found in all the coated samples. In the case of DOPG, histidine-rich glycoprotein was the most predominant protein, which has been reported to work as a dysopsonin when present in the hard corona surrounding particles [4]or hard corona, may influence the biological and pharmacological features of nanotheranostics by altering their cell-interaction selectivity and macrophage clearance. With the goal of identifying specific corona-effectors, we investigated how the capture of amorphous silica nanoparticles (SiO2-NPs; Ø = 26 nm; zeta potential = −18.3 mV.

CONCLUSIONS

We have successfully coated silica nanoparticles with diverse phospholipids coatings which has given the opportunity to test the dif-

ferent protein corona. With these promising initial findings we can confirm the tunability of the coating to obtain different corona profiles. Our future studies are directed to determine which output this different protein coronas will have in *in vivo* conditions.

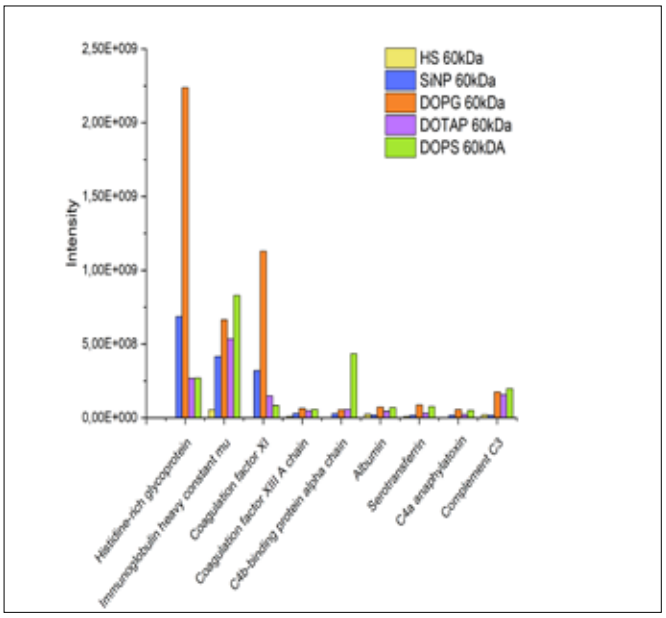

Figure 1. *Proteomics analysis of the 60 kDa band of silica nanoparticles uncoated and coated with DOPG, DOTAP, DOPC:DOPS 80:20 (DOPS) and human serum control (HS)*

ACKNOWLEDGEMENTS

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THERANOSTIC BIMODAL LIPID-BASED NANOMEDICINES FOR EFFECTIVE CANCER TREATMENT

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INTRODUCTION

Drug resistance and tumour heterogeneity pose substantial barriers to successful cancer treatment. Early and accurate cancer detection is crucial to maximise the effectiveness of therapies. This has led to the emergence of theranostics, an approach that combines diagnosis and treatment strategies. Theranostics enables the simultaneous detection of specific targets, drug distribution tracking, and therapeutic response evaluation, ultimately leading to the development of personalised medicine [1, 2]. Lipid-based nanoparticles (LNPs) possess characteristics that make them suitable for theranostic nanomedicines. However, the applicability of LNPs with encapsulated hydrophobic small molecule drugs, often highly effective therapeutics, is challenging. Firstly, the interaction with the lipid bilayer can potentially disrupt the integrity of the lipid membrane, thereby affecting the stability of liposomes and their capacity to encapsulate drugs. Secondly, LNPs administrated *in vivo* can undergo dilution processes and interact with biological serum proteins, leading to a gradual separation of a drug until it reaches the target tissue [3]. Therefore, this study aimed to develop highly stable theranostic LNPs (TNPs) with the incorporated hydrophobic active pharmaceutical ingredient (API) ellipticine (Elli@TNPs) for real-time tracking using magnetic resonance imaging (MRI). Ellipticine and its derivatives have garnered significant interest for clinical applications due to their remarkable efficacy against various cancer types, minimal toxic side effects, and absence of haematological toxicity. [4].

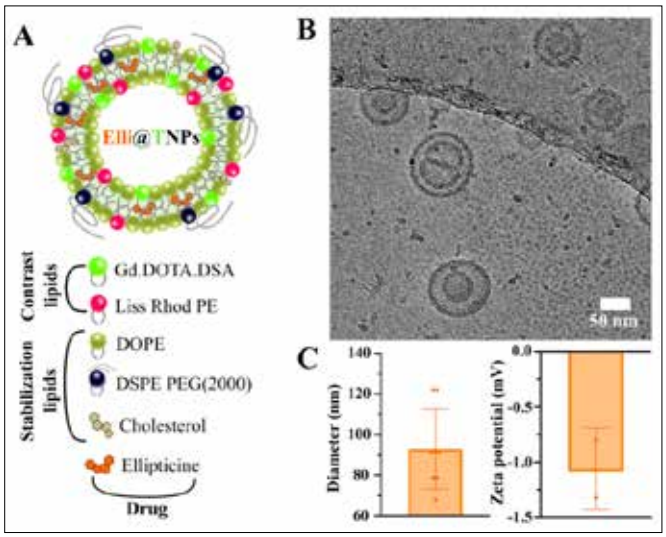

Fig. 1. Theranostic bimodal lipid-based nanomedicines. A) Schematic drawing and composition of Elli@TNPs . The Elli@TNPs shell contains Gd.DOTA.DSA enabling MRI imaging, and Liss Rhod PE for fluorescence imaging. B) Cryo-TEM micrograph of TNPs. C) Size distribution obtained by DLS and zeta potential of Elli@TNPs.

MATERIALS AND METHODS

The injection method was used to prepare TNPs and to encapsulate the therapeutical compound ellipticine. Lipids were dissolved in organic solutions (ethanol or chloroform) and mixed with ellipticine dissolved in ethanol in the molar ratio 1:3 (lipids:ellipticine). The final composition of TNPs is depicted in Fig. 1A. The mixture was slowly injected into the 15 mM NaCl with 2 mM HEPES at 65 °C with gentle stirring. Subsequently, the Elli@TNPs were extruded through 80, 100 and 200 nm filters to achieve uniform size with PDI

≤ 0.1 and underwent quality assessment for size and zeta potential. The cytotoxicity of Elli@TNPs was evaluated using an MTT assay on the MDA-MB-231 breast cancer cell line, and cellular uptake was examined using confocal scanning laser microscopy. Furthermore, Elli@TNPs were utilised for treating the CT26 cell line-induced tumor xenografts. A dosage of 100 µl (1×90 mg ellipticine/kg) was administered intravenously on the 8th day post tumor induction.

RESULTS AND DISCUSSION

In this study, we successfully developed theranostic Elli@TNPs with exceptional properties, including an encapsulation efficiency of approximately 40% and a sustained drug release profile. The optimised injection method combined with extrusion resulted in uniform nanoparticles with a size of 93.0 ± 18.4 nm (PDI 0.07 ± 0.02) and a neutral zeta potential (Fig. 1C). Cryo-transmission electron microscopy (cryo-TEM) confirmed the size of the nanoparticles, which was consistent with the measurements obtained by dynamic light scattering (DLS). Cryo-TEM revealed the presence of multilamellar vesicles with bilayered structures (Fig. 1B). Moreover, long-term DLS measurements over a span of 6 months at room temperature and 4 °C demonstrated excellent storage stability, as there were no changes in size indicating nanoparticles aggregation.

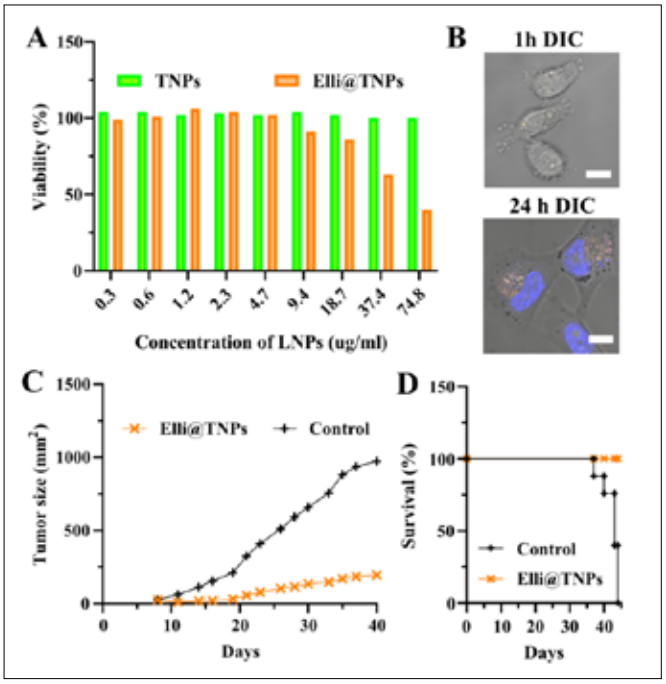

Fig. 2. Elli@TNPs exhibit substantial therapeutic activity. A) Cytotoxicity of Elli@TNPs compared to empty TNPs. B) Cellular uptake of Elli@TNPs after 24 hours (scale bar 10 um; yellow, ellipticine, pink, empty TNPs and blue, cell nuclei). C) Spider plots of tumour growth up to 40 days (8 mice per group). D) Kaplan–Meier survival curves, revealing significant improvements in therapeutic outcomes for animals treated with Elli@TNPs.

The biocompatibility of Elli@TNPs was evaluated *in vitro* using an MTT cell viability assay after 72 hours of incubation with the MDA-MB-231 cell line (Fig. 2A). Elli@TNPs exhibited a decrease in cell viability at LNPs concentration of 9.4 µg/mL, equivalent to 16 µM ellipticine (where free ellipticine showed only 1% viability at this concentration) with determined EC_{50} 129.5 uM ellipiticine. Empty TNPs did not display any toxicity at any dose level, indicating that the observed toxicities in the case of Elli@TNPs were attributed to the release of the API. Confocal scanning laser microscopy confirmed the release of the majority of the API within 24 hours. Ellipticine was detected in the nuclei, where it induced DNA strand breaks and resulted in cellular toxicity [5]. Based on the successful characterisation of Elli@TNPs as effective nanomedicines, we conducted *in vivo* maximum tolerated dose (MTD) tests, followed by treatment on mouse models with CT26 cell line-induced tumours (Fig. 2C). Elli@TNPs exhibited modest activity in this aggressive cancer model compared to the control group. Tumour size and weight in the Elli@TNPs group remained stable for 20 days, slightly increasing afterwards but not exceeding 200 mm^2. Furthermore, treated mice survived beyond 40 days, whereas the first death in the control group occurred after 32 days (Fig. 2D). The treatment is still ongoing.

CONCLUSION

We present a novel nanomedicine strategy that provides a promising theranostic approach for potential treatments across different cancer types. Based on *in vitro* and *in vivo* results, our Elli@TNPs nanomedicine demonstrates excellent biophysical properties, including stable size up to 100 nm with a low PDI below 0.1 and a neutral charge. Furthermore, Elli@TNPs exhibit encouraging biological properties with low cytotoxicity and show effectiveness in treating CT26 tumours. These findings offer potential for translation into clinical therapies.

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A BIOMIMETIC DUAL-DRUG LOADED LIPID NANOCARRIER ENHANCES APOPTOSOME ASSEMBLY FOR CANCER THERAPY

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Introduction: Apoptosis is the natural programmed cell death process, which is responsible for abnormal cell clearance. However, many cancer cells develop various mechanisms to escape apoptosis through interrupting apoptosome assembly, which is a key step to initiate apoptosis. This promotes tumorigenesis and drug resistance, and thus, poses a great challenge in cancer treatment. Herein, a biomimetic lipid nanocarrier, co-loaded with a pro-apoptotic protein Cytochrome C (Cyt C) and a glycolysis inhibitor lonidamine (LND) was developed, to promote apoptosome formation and the subsequent cell apoptosis. We incorporated cardiolipin, a natural lipid found in mitochondrial inner membrane in the liposomal formulation, to achieve high loading capability of Cyt C, due to its natural high complexation affinity. The lipid formulation was co-loaded with LND to modulate the metabolic activity within cancer cells and sensitize the cells to Cyt C-induced apoptosis (**Figure 1**). We further conjugated a tumor homing peptide, LinTT1, on the nanovesicle, to increase tumor accumulation and the efficacy of pro-apoptosis cancer therapy.

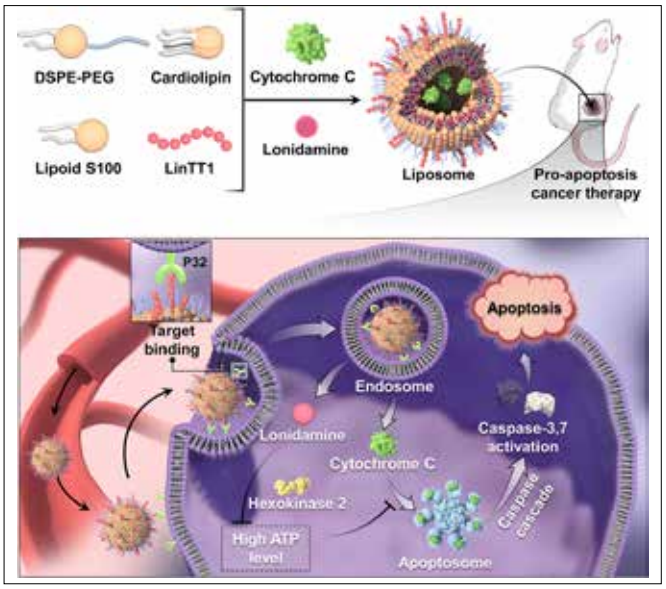

Figure 1 Overall scheme showing the design of the lipid nanocarrier and the pro-apoptotic mechanism.

Methods: The cardiolipin-containing biomimicking lipid nanovesicles co-loaded with Cyt C and LND was were fabricated by a standard lipid-hydration method. The formulations were characterized, and the loading conditions were optimized. Afterwards, the anti-tumor and pro-apoptotic effects of the formulation with or without LinTT1 were evaluated in breast cancer cells, 4T1 and MBA-MB-231. Particularly, apoptotic marker proteins (Apaf-1, Cyt C) involved in the apoptosome formation was characterized to validate our hypothesis and reveal the mechanisms of pro-apoptotic effects. Furthermore, the synergetic anti-tumor effect of drug combination *in vivo* was also investigated a 4T1 breast cancer-bearing mouse model.
Results: The introduction of cardiolipin in the liposomal formulation enhanced Cyt C loading and delivery, while LND reduced the intracellular ATP levels and promoted the cytoplasmic Cyt C binding with Apaf-1, leading to enhanced apoptosome formation and the activation of effector Caspase 3/7 in the downstream of the intrinsic apoptosis pathway (**Figure 2**). The combination of Cyt C and LND demonstrated significant anti-tumor efficacy by improving tumor apoptosis levels (**Figure 2**). Besides, the LinTT1 tumor homing peptide modification further improved the therapeutic effects due to enhanced liposome uptake and accumulation in the tumor site (**Figure 3**). Overall, the liposomal formulations developed here with a synergistic Cyt C and LND delivery capability provide new insights into cancer therapy targeting apoptosome formation, and thus, making them promising candidates for cancer pro-apoptosis cancer therapy.

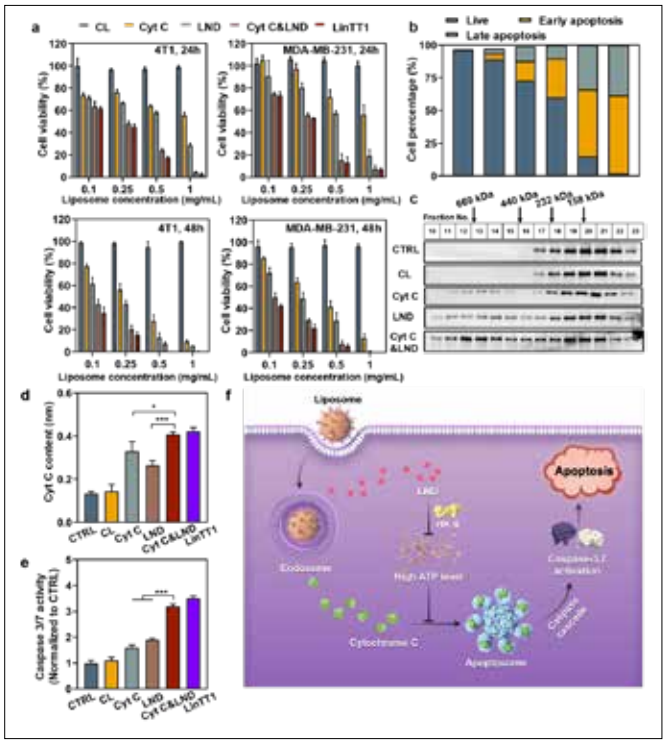

Figure 2. In vitro anti-tumor efficacy of all liposome formulations. (a) The cytotoxicity of different liposomes treated 4T1 and MDA-MB-231 cells after 24 and 48 h incubation at different concentrations. (b) Apoptosis level of different liposomes treated 4T1 cells (0.25 mg/mL, 48 h) based on annexin V-FITC/PI staining. (c) Apoptosome level of different liposomes treated 4T1 cell (0.25 mg/mL, 24h) by Apaf-1 Western Blot analysis.(d) Cyt C content per 10000 cells (nmol) of different liposomes treated 4T1 cell (0.25 mg/mL, 24h). (e) Caspase 3/7 activity of different liposomes treated 4T1 cell (0.25 mg/mL, 24h). (f) Scheme of mechanism on Cyt C and LND combination in apoptosis. Results are shown as mean ± SD, n=3.

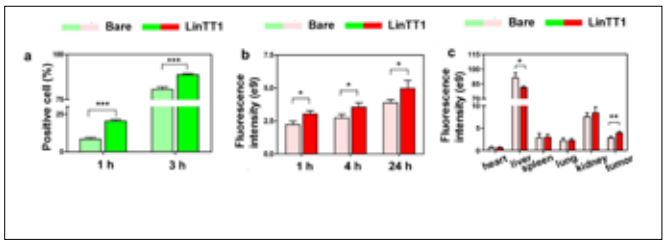

Figure 3. Tumor homing ability of LinTT1 liposomes in vitro and in vivo. (a) Quantitative analysis of 4T1 cellular uptake by flow cytometry. (b) In vivo fluorescence of bare and LinTT1 lipo biodistribution in 4T1 tumor bearing mice quantitatively. (c) Ex vivo fluorescence of organs (heart, live, spleen, lung, kidney) and tumors after 24 h injection quantitatively. Results are shown as mean ± SD, n=3.

Acknowledgements: This work was supported by grants from the Academy of Finland, the Sigrid Jusélius Foundation, UMCG Research Funds, the Chinese Scholarship Council, the National Key Research and Development Program of China, and the GuangCi Professorship Program of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. In addition, the travel expenses for attending CLINAM were partially covered by the Travel Award sponsored by the open access journal published by MDPI.

POLYSARCOSINE-FUNCTIONALIZED MRNA LIPID NANOPARTICLES TAILORED FOR IMMUNOTHERAPY

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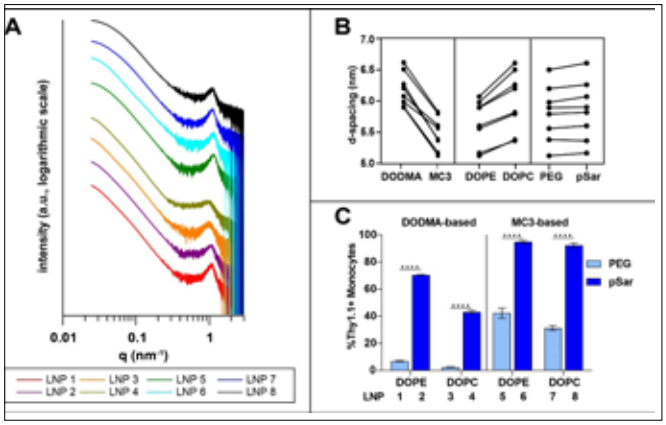
The Covid-19 pandemic led to rapid clinical testing and subsequent approval of the first two mRNA-based drug products. These lipid nanoparticles (LNPs) are designed to deliver various types of mRNA for therapeutic purposes and induce an antigen specific immune response. They have shown promising results, not only with the success during the pandemic, but for a wide range of therapeutic applications. To achieve optimal mRNA delivery, lipid formulations must fulfill specific properties, such as protection of nucleic acids against degradation, ensuring extended circulation in the bloodstream to reach the intended destination and successful release of mRNA in the targeted cells or organs of interest. While the basic concept behind approved LNP formulations for nucleic acid delivery remains consistent, the choice of the actual lipid composition can vary considerably. The influence of lipid composition on structural characteristics and its implication on *in vitro* and *in vivo* efficacy still remain unclear. Gaining deeper understanding of these relationships can prove immensely valuable for the rational development of LNPs and the implementation of quality control measures. LNPs were prepared by a single-step protocol which enables use of the particles without further treatment (dialysis, tangential flow filtration). We compared two well-established ionizable lipids, namely DODMA and MC3, in combination with two helper lipids, DOPE and DOPC, and two polymer-grafted lipids, either with polysarcosine (pSar) or polyethylene glycol (PEG). In addition to standard physicochemical characterization (size, zeta potential, RNA accessibility) small-angle X-ray scattering (SAXS) was used to analyze the structure of the LNPs (Fig. 1A). SAXS can be used as a powerful tool to gain information on internal structure and lamellarity as well as overall particle properties (Fig. 1B). To assess biological activity, we performed transfection and cell binding assays in human peripheral blood mononuclear cells (hPBMCs) using Thy1.1 reporter mRNA and Cy5-labeled mRNA, respectively.
Our results demonstrated that SAXS analysis provides a sensitive method to determine the impact of the respective lipids on the LNP structure. This detailed understanding of the molecular organization of the particles enabled us to derive refined correlations with their potency, surpassing the conventional approach of determining pK_a values. pSar was successfully applied for particle manufacturing, resulting in particles with similar internal structural parameters to those achieved using PEG as the stealth component. In contrast, the pSar- grafted LNPs tended to show more irregular and rough surfaces compared to their PEG counterparts. Furthermore, pSar formulations showed enhanced *in vitro* activity (Fig.1C), highlighting their potential as an alternative to PEG, the gold standard, which is hypothesized to be the cause of several adverse effects after administration.

Figure 1. **A:** *SAXS pattern for each investigated formulation. Patterns are vertically shifted for better visualization. All curves display similar features dominated by a single broad peak, resulting from lipid-RNA stacks.* **B:** *Comparison of d-spacing (repeating unit length) derived from SAXS analysis of the derived peaks depending on their lipid composition. Investigated pairs were generated with formulations only differing in one lipid component. Systematic differences depending on lipid composition could be observed.* **C:** *Transfection efficacy of all PEG-grafted LNPs versus pSar-grafted counterparts shown as %Thy1.1+ Monocytes. Data are presented as mean ± S.D., analyzed by a two-way ANOVA with Šidák's multiple comparison test, ****P < 0.0001, n= 3 technical replicates per LNP formulation.*



TARGETED REGULATION OF CERAMIDE SYNTHESIS AMELIORATES NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of chronic liver disease caused by excessive fat accumulation in the liver, with a prevalence of up to 40% in the United States and in Singapore. NAFLD can develop into a more severe form, non-alcoholic steatohepatitis (NASH), characterized with liver inflammation and fibrosis, and ultimately cirrhosis and liver cancer. Currently, efficacious drugs reversing the various forms of this disease are not yet available. Ceramides, a class of sphingolipids have been associated with NASH development in clinical studies, however, their pathogenic contribution to NASH remains largely unexplored. In this study, we identified a ceramide synthesis pathway that is highly upregulated in a cohort of NASH patients and several clinically relevant NASH animal models. Employing the DLin-MC3-based lipid nanoparticles (LNP) for siRNA delivery, we achieved effective knockdown of ceramide synthesis enzymes in the liver and lowered both hepatic and circulating ceramides in animals. Intravenous administration

of LNP-siRNAs (weekly for four weeks) remarkably improved animal lipid profiles and ameliorated NASH disease progression, including steatosis (hepatic lipid accumulation), inflammation and fibrosis. Apart from biochemical and histological evidence, the therapeutic efficacy was also confirmed by our recently developed myeloperoxidase-responsive T_1 and T_2 switchable magnetic resonance imaging (MRI) approach. To conclude, this proof-of-concept study demonstrates the feasibility of LNP siRNA system for the treatment of metabolic diseases.

This work was supported by the Singapore Ministry of Education MOE AcRF Tier 1 grant (NUHSRO/2021/113/T1/Seed-Sep/06).

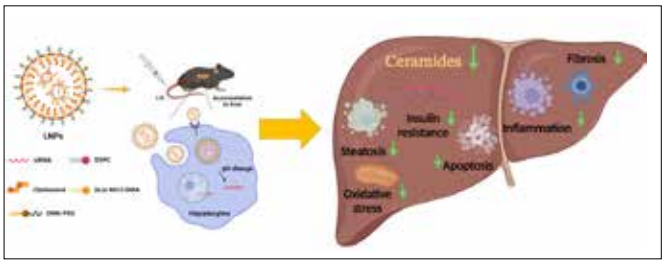

Fig 1. *Lowering hepatic ceramides via LNP mediated siRNA knockdown prevents NASH progression.*

ASSESSING BRAIN TARGETING EFFICIENCY OF IONISABLE LIPID NANOPARTICLES ENCAPSULATING CAS9 MRNA/GGFP FOLLOWING DIFFERENT ROUTES OF ADMINISTRATION IN MICE

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BACKGROUND

Treatment of neurological disorders with modern medical and surgical approaches remains difficult. Gene therapy, allowing the delivery of genetic materials that encodes potential therapeutic molecules, represents an attractive option. The treatment of brain diseases with gene therapy requires the gene editing tool to be delivered efficiently to the central nervous system. In this study, we explored the efficiency of different delivery routes namely intravenous (*i.v.)*, intra-cranial (*i.c.*) and intra-nasal (*i.n*.) to deliver stable nucleic acid-lipid particles (SNALPs) containing gene-editing tools namely Cas9 mRNA and sgRNA encoding for GFP as a reporter protein. We hypothesise that SNALPs can reach the brain and perform gene-editing to different extents depending on the administration route. Intranasal administration (*i.n*.) offers an attractive and non-invasive way to access the brain circumventing the blood–brain barrier. Successful delivery of gene-editing tools to the brain offers a great opportunity for therapeutic target validation and nucleic acids therapeutics delivery to improve treatment options of a range of neurodegenerative diseases. In this study, we utilised Rosa26-Cas9 knock-in mice, expressing GFP, to study brain distribution and gene-editing efficiency of SNALPs after *i.v.; i.c.* and *i.n.* routes of administration.

METHODS

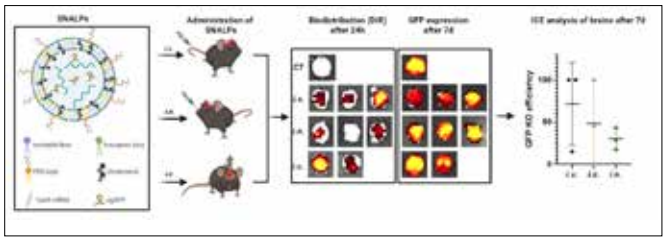
Single guide RNA (sgRNA) against GFP has been designed and validated by *in vitro* nuclease assay. SNALPs were formulated and characterised using dynamic light scattering. Encapsulation efficiency of nucleic acids (NA) was measured by RiboGreen™ assay. SNALPs were incubated in serum to assess their ability to protect NA from degradation. Rosa26-Cas9 knock-in mice were *i.v.*; *i.n.* or *i.c.* administered with SNALPs to test *in vivo* gene-editing (GFP knockout) efficiency. SNALPs were given as three doses of 0.64 mg/kg sgGFP following *i.v.* and *i.n.* or a single dose of 0.25 mg/kg sgGFP following *i.c.*. knockout efficiency was assessed after 7 days using Sanger Sequencing and Inference of CRISPR Edits (ICE) analysis. *In vivo* biodistribution of DiR labelled SNALPs (SNALPs-DiR) was assessed at 24h post-administration using IVIS Lumina Series III.

RESULTS

Serum-stable SNALPs produced were 130-140 nm in diameter with ~90% nucleic acid loading efficiency. SNALPs could reach and stay in brain for up to 24h following *i.v.*; *i.n.* and *i.c.* administration. Decreasing GFP expression (around 50% after *i.v.* and *i.c.* and 20% following *i.n.*) was confirmed by optical imaging. Despite small number of mice used, ICE analysis confirmed GFP knockout in mice brain. Additional studies are currently taking place to increase mice numbers.

CONCLUSION

Results confirmed efficient gene-knockout achieved by SNALPs in Rosa26-Cas9 knock-in mice expressing GFP following different routes of administrations in the following order *i.v.= i.c.> i.n.* Each of the administration routes have their pros and cons. The next stages of the project involve assessing gene-editing efficiency in wildtype mice and replacing GFP as a model target with therapeutic target genes implicated in Motor Neuron Disease pathology.



Scheme 1. Biodistribution & GFP knockout of formulated DiR-SNALPs in mice brain after i.v.; i.n. and i.c. administration.

DEVELOPING NUCLEIC ACID-BASED THERAPIES TARGETING IMMUNE CHECKPOINTS IN GLIOBLASTOMA MICROENVIRONMENT USING LIPID NANOPARTICLES

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BACKGROUND/ AIMS

Immunotherapy strategies have revolutionised the treatment of many cancers, increasing the hope for Glioblastoma Multiforme (GBM) therapy. Among the most viable strategies is the use of nucleic acid (NA)-based therapies to manipulate the expression of immunecheckpoints (ICPs) in GBM microenvironment. ICPs act as gatekeepers of the immune system by enhancing (positive ICP) or inhibiting (negative ICP) immune responses. Lipid nanoparticles (LNPs) represent the most clinically advanced non-viral vector for the delivery of NAs. Our aim is to develop LNPs capable of delivering NA therapies that target synergetic ICPs in GBM microenvironment to elicit an immune response against tumour cells.

METHODS

OX40 ligand mRNA (mOX40L) was chosen as a positive ICP target based on its proven efficacy. Stable nucleic acid-lipid particles (SNALPs) were formulated using 5 different ionisable lipids (DLin-MC3-DMA, DLin-KC2-DMA, ALC-0315, SM-102, C12-200) and 5 dif-

ferent PEG-lipids (C14:0-PEG2000, DMG-PEG2000, C16-PEG2000, C18:0-PEG2000 PE, or C18:1-PEG2000 PE) using the ethanol injection method. Particle size, polydispersity, and surface charge were characterised using dynamic light scattering. Encapsulation efficiency (EE%) was measured using RiboGreen Assay. *In vitro* transfection and uptake studies were performed to compare SNALPs efficiency in delivering mRNA using flow cytometry. Fluorescence microscopy was used to assess the cellular uptake of SNALPs. Cytotoxicity was assessed by MTT assay. Experiments were performed in GBM (stem) cell lines: GL261 and BL6 cells.

RESULTS

All SNALPs formulations showed acceptable physicochemical characteristics. Particles size < 200 nm, PDI < 0.23 and %EE between 82% to 99%. MC3 and C12-200 ionisable lipids exhibited the highest transfection efficiencies in GL261 and BL6 when coupled with C16- and DMG-PEG 2000, respectively. BL6 cells appeared more susceptible to SNALPs toxicity while C12-200 exhibited higher toxicity than other ionisable lipids. Uptake studies and fluorescence images revealed a dose- and time-dependant pattern of SNALPs cellular capture which in some cases but not all correlated with transfection efficiency pattern.

CONCLUSION

GL261 cells were harder to transfect than BL6 cells. SNALPs outperformed the commercial reagents, lipofectamine, especially in stem cells. The order of transfection efficiency obtained with PEG-lipids was C16-PEG2000 > C14:0-PEG2000 > DMG-PEG2000 > C18:1-PEG2000 > C18:0-PEG2000 PE in GL261 and DMG-PEG2000 > C16-PEG2000 > C14:0-PEG2000 > C18:1-PEG2000 > C18:0-PEG2000 PE in BL6. The order of transfection efficiency obtained with ionisable lipids was MC3 > KC2 > C12-200 > SM-102 > ALC-0315 in GL261 and C12-200 > KC2 > ALC-0315 > SM-102 > MC3 in BL6.
MC3/ C16-PEG2000 and C12-200/ DMG-PEG 2000 exhibited highest transfection efficiencies in GL261 and BL6 respectively. Due to toxicity observed with C12-200, MC3/ C16-PEG2000 was the transfection system of choice for future studies.

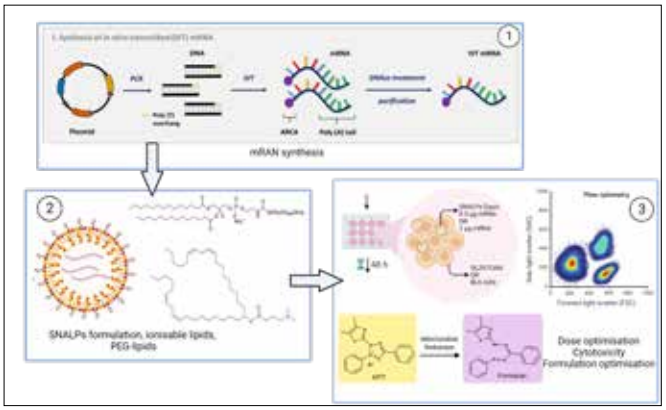

Figure 1 Schematic representation of workflow for optimising and testing different SNALP formulations for mRNA delivery in GBM (stem) cells.

COMPARATIVE STUDY OF ADJUVANTS AND THEIR SYNERGISTIC POTENTIAL FOR THE STIMULATION OF DENDRITIC CELLS (DC) AND LIVER NON-PARENCHYMAL CELLS

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Resident liver NPCs (non-parenchymal cells), including Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC), and hepatic dendritic cells (DC), by default induce T-cell tolerance. This inherent T-cell tolerance promoting activity limits the efficacy of intrahepatic anti-tumor responses. Nevertheless, suitable adjuvants can reprogram liver NPC to a T effector cell inducing phenotype.
The project aims to enhance the anti-tumor efficacy of nano-vaccines by employing the most effective adjuvant combinations that activate both antigen-presenting dendritic cells (DC) and liver NPCs to improve the outcome of (liver) tumor therapy. Therefore, an *in vitro* adjuvant screening was conducted comprising Toll-Like Receptors (TLR) and Stimulator of Interferon Genes (STING) agonists to study the activation of DC and NPC subpopulations. Promising candidates were selected and the effects of adjuvant combinations were tested for synergistic effects.
Bone marrow-derived (BM)DC were differentiated via incubation of murine bone marrow cells with either GM-CSF or FLT3L for 7 days. Murine non-parenchymal liver cells were isolated by liver perfusion. Cells were treated with different adjuvant concentrations overnight. The expression of activation markers (CD80, CD86, MHCII) and the secretion of pro-inflammatory cytokines was examined using flow cytometry. Proliferation assays with $CD8^+$ T cells were conducted to evaluate the antigen presenting and T cell stimulatory activity on T cells.

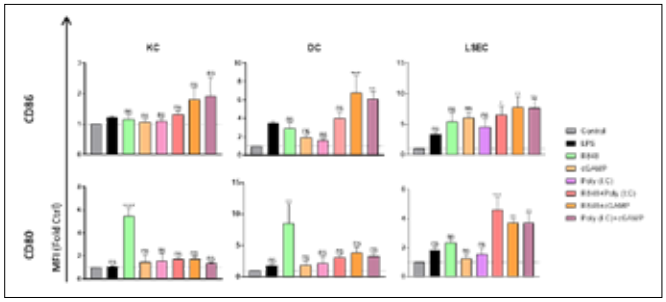
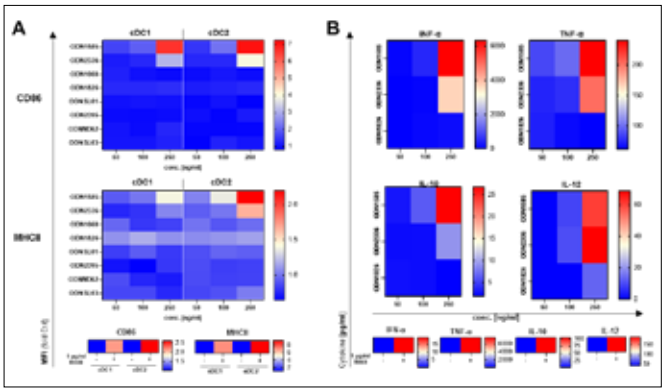


*Figure 1. Adjuvants enhance CD80 and CD86 expression of liver non-parenchymal cells. Liver NPC were incubated with 5 µg/ml R848, Poly (I:C) or cGAMP. On the next day, expression of CD80 and CD86 by LSEC, Kupffer cells and DC was assessed by flow cytometric analysis. Graphs denote the fluorescence intensities (MFI) (mean±SEM of 3-4 experiments) of marker expression. Statistical differences versus *Ctrl are indicated (one way ANOVA, Tukey test). *p<0.05, **p<0.01, ***p<0.001, ****<0.0001.*

The results revealed that combinations of the TLR7/8 ligand resiquimod (R848) or the TLR3 agonist polyinosinic:polycytidylic acid (Poly (I:C)) in combination with the STING agonist cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) yielded the strongest stimulatory activity on either type of liver NPC (**Figure 1**) and BMDC.
In a second set of studies, different unmethylated CpG containing DNA oligodeoxynucleotides (ODN), known to act as TLR9 agonists, were compared regarding their adjuvant activity. The study identified optimal performers for the different cell types, with CpG ODNSL03 constituting the most potent stimulator for both liver NPC and BMDC (**Figure 2**).

Figure 2. *CpG oligos enhance MHCII and CD86 expression and proinflammatory cytokine release of FLT3L differentiated BMDC. (**A**) BMDC*

*were incubated with different concentrations of CpG oligos (50, 100 or 250 ng/ml) or the TLR7/8 ligand R848 (1 µg/ml). On the next day, expression of MHCII and CD86 by cDC1/2 was assessed by flow cytometric analysis. Graphs denote the mean fluorescence intensities (***MFI***) (mean±SEM of* ***4*** *experiments) of marker expression. (***B***) BMDC were incubated over night with CpG oligos. Cytokine concentrations of culture supernatants were determined by CBA (mean±SEM of* ***3*** *experiments).*



The next step of this project is to formulate the best performing stimulators together with antigen mRNA into nanoparticle formulations carrying a trimannose moiety on their surface to actively co-target DC and liver NPC and therefore improve the efficacy of anti-cancer therapeutic vaccines.

PRECISE AMPK ACTIVATOR DELIVERY AND ANTIBODY CONJUGATION VIA NOVEL CORE-SHELL POLYMER BRUSH FOR ENHANCED COMBINED IMMUNOTHERAPY

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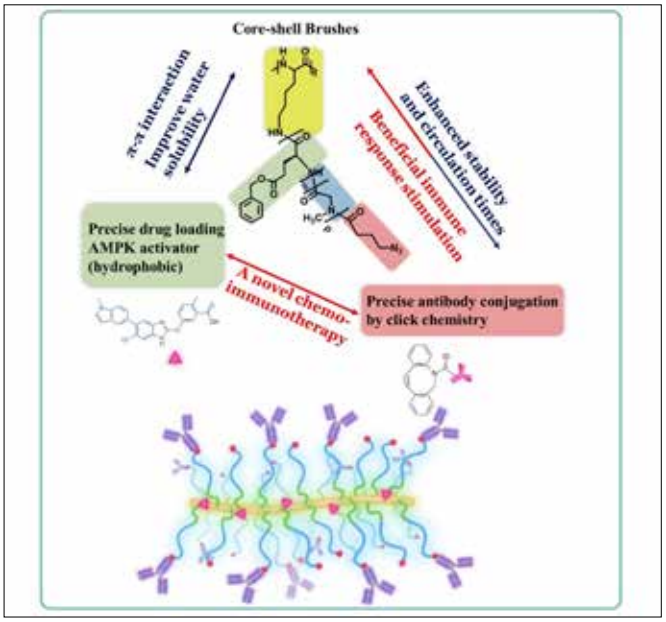

Fig. 1 Design of polysarcosine-based core-shell brush for precise AMPK activator loading through π-π interaction and antibody conjugation by click chemistry.

BACKGROUND

Combining monoclonal antibodies and small molecule drugs has shown promise as an effective approach for cancer therapeutics. However, challenges persist in optimizing the activities of monoclonal antibodies and ensuring precise delivery of small molecule drugs to tumor cells. In this study, we introduce a novel core-shell brush based on polypept(o)ides, designed to achieve precise delivery of AMP-activated protein kinase (AMPK) activators and efficient antibody conjugation.

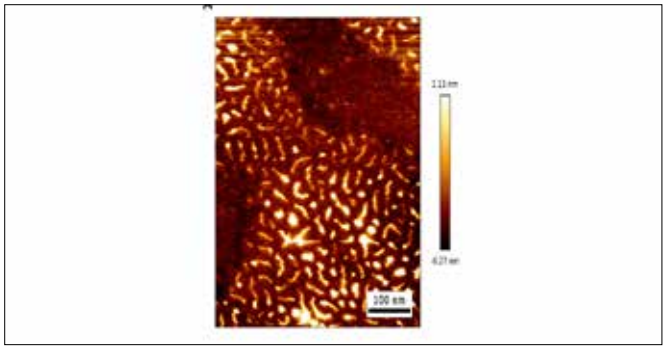

Fig. 2 AFM images of core-shell brushes.

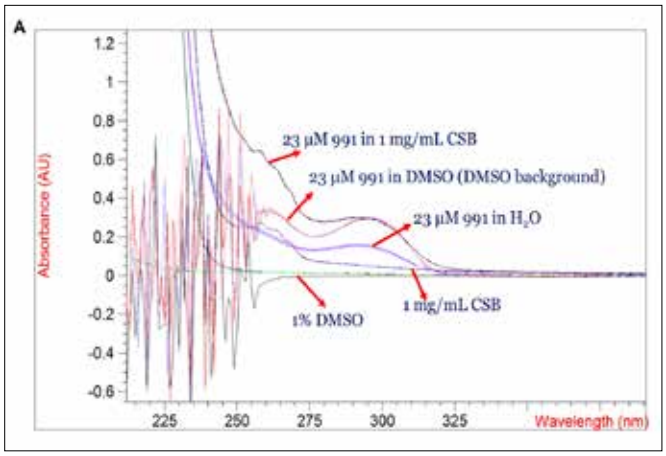

Fig. 3 Uv-vis spectroscopy for the concentration measurements of brushes and drugs.

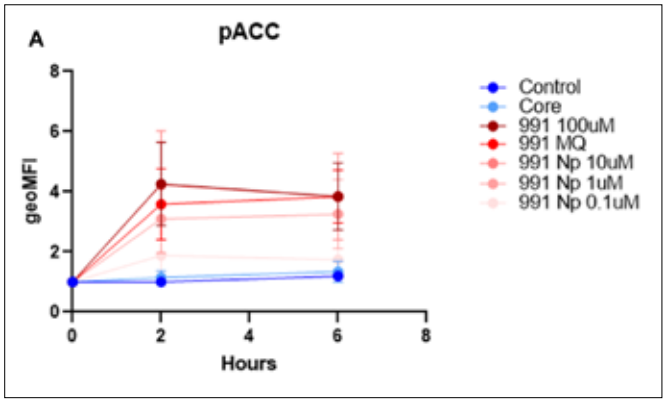

Fig. 4 The pACC expression measurement showed pAMPK could be activated by core-brush loaded 991.

AIMS

AMPK activators have demonstrated synergistic antitumor effects when combined with antibodies in tumor models. Nevertheless, the clinical application of activators is limited by their strong hydrophobicity and delivery methods. To address this, we focus on designing and synthesizing a core-shell brush based on polysarcosine to facilitate the precise delivery of a potential drug, Compound 991, into the benzyl core through π-π interactions. This approach aims to enhance Compound 991's water solubility and enable its co-delivery alongside conjugated antibodies.

METHODS

We employed a "grafting-from" strategy, using poly($_L$-lysine) as a backbone to graft poly(γ-benzyl-$_L$-glutamic acid) and polysarcosine

as side chains (pLys$_m$-*g*-pGlu(OBn)$_n$-*g*-pSar$_p$(N$_3$)). The structure of the core-shell brush was characterized using ^{1}H NMR spectroscopy and gel permeation chromatography (GPC). Diffusion-ordered spectroscopy (DOSY), UV-vis spectrophotometry, Single-angle dynamic light scattering (DLS), and Multi-angle DLS experiments were conducted to validate and quantify the drug loading efficiency. Additionally, atomic force microscopy (AFM) was used to study the brush's morphology. Phosphorylated acetyl-CoA carboxylase (pACC) expression was measured to analyze the impact of Compound 991 on pAMPK.

RESULTS/CONCLUSIONS

The results from ^{1}H NMR spectroscopy and GPC confirmed the successful synthesis of the core-shell brush. DOSY, UV-vis, and single-angle DLS experiments demonstrated that Compound 991 effectively loaded into the benzyl core through π-π interactions, significantly improving its water solubility. Multi-angle DLS experiments precisely calculated the number of loaded 991 molecules per brush by measuring the molecular weight of the core-shell brush before and after drug loading, supported by AFM morphology results. The pACC expression test indicated a strong dosage dependency of Compound 991 loaded by core-shell brushes.

In conclusion, we have developed a novel core-shell brush based on polysarcosine, which offers a potent cancer chemo-immunotherapy platform with precise control over the loading number of AMPK activators per brush. In the next phase of this project, we will investigate the precise conjugation of antibodies and explore immune response stimulation *in vivo* through co-delivery. Our findings hold significant potential for advancing cancer therapeutics by optimizing the combined immunotherapy approach.

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GLUCOSYLATED HYBRID TIO$_2$/POLYMER NANOMATERIALS FOR ACTIVELY TARGETED SONODYNAMIC THERAPY OF CANCER

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Traditional anti-cancer treatments like chemotherapy can harm healthy cells that are rapidly dividing, such as hair follicles, the lining of the gut, and blood cells.[1] This can lead to severe side effects, which may reduce the administration of the treatments or interrupt them altogether, resulting in a loss of therapeutic efficacy.[2] To address this issue, researchers are exploring advanced personalized therapeutic strategies like targeted nanomedicines, immunotherapy, and combination therapies that may be more precise and have fewer side effects.[3,4]

Sono-dynamic therapy (SDT) is an enhanced form of photodynamic therapy (PDT)[5] that uses ultrasound waves to activate a sono-sensitive molecule or nanomaterial (sonosensitizer), which generates reactive oxygen species (ROS) in the tumor microenvironment and kills cancer cells.[6] SDT offers the advantage of deeper tissue penetration and the ability to target specific regions of the body with minimal damage to healthy tissues.[7]

Recently, our research group developed a new family of hybrid nanomaterials made of amorphous titanium dioxide (aTiO$_2$) and poly(ethylene)-b-poly(propylene) block copolymers (PEO-PPO) with fine-tunable size and surface.[8,9] The aTiO$_2$ matrix makes the NPs sono-responsive, while amphiphilic PEO-PPO copolymers modify the NP surface with PEO blocks, which increases their physical stability in suspension and reduces their elimination by phagocytic cells.[10] In addition, the surface of the NPs can be modified with ligands that actively target receptors overexpressed in specific cancer cells.[11] Finally, hydrophobic PPO domains in the NP core enable the encapsulation of hydrophobic cargos.[8,9] In the current work, we report the efficacy of actively targeted SDT of a sarcoma overexpressing glucose transporters (GLUTs) with glucosylated hybrid aTiO$_2$/PEO-PPO nanomaterials in cell cultures and a murine model. This study shows how the novel actively targeted ceramic/polymer sonosensitizer nanomaterial can treat drug-resistant rhabdomyosarcoma efficiently. Our synthetic procedure enables us to regulate the size of the nanoparticles, ranging from 30 to 300 nm, and modify their surface with glucose moieties to actively target tumors that overexpress glucose transporters **(Fig 1a)**. Our experimentation with 2D and 3D cell cultures unveiled that the glucosylated hybrid nanoparticles exhibited significantly higher sonodynamic efficacy compared to the unmodified ones **(Fig 1b,c)**. Furthermore, we discovered that by fine-tuning the size and surface features of the nanoparticles, we could boost tumor accumulation by ten times compared to off-target organs, such as the liver, in a murine in-vivo model **(Fig1 d)**. Finally, our treatment of rhabdomyosarcoma-bearing mice with 50-nm glucosylated nanoparticles resulted in a dramatic increase in animal survival and a remarkable decrease in tumor volume by 100 times compared to treatment with only ultrasound or nanoparticles **(Fig1 e,f)**. Overall, our findings confirm the therapeutic potential of this SDT platform and pave the way for the development of combination therapies.

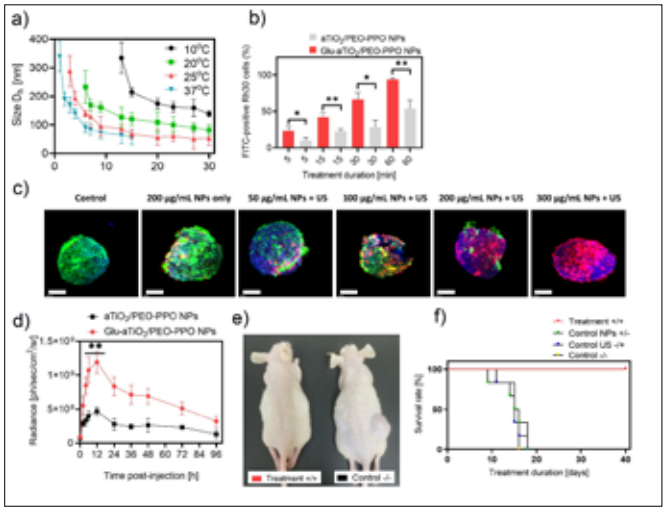

Figure 1. (a) *Hydrodynamic diameter (Dh) of NPs prepared with Ti(IV) oxo-organo complexes aged at different temperatures.* **(b)** *Fluorescence intensity in Rh30 cell cytosol after exposure to the NPs, as measured by ImageStream® flow cytometry* **(c)** *Representative LSFM micrographs of Rh30 cell 3D spheroids exposed to glu-aTiO$_2$/PEO-PPO NPs and irradiated or not with ultrasound at an intensity of 1.2 W/cm^2 and frequency of 1.0 MHz for 3 min. NPs are stained with RITC (red), cell nuclei are stained with 4,6-diamidino-2-phenylindole (DAPI, blue), and viable cells are stained with calcein AM (green) (n = 4).* **(d)** *Fluorescence radiance intensity profiles in the tumor region of interest (ROI) analysis (n = 6).* **(e)** *Representative micrograph of treated and control Hsd:Athymic Nude-Foxn1nu + mice with a subcutaneous Rh30 xenograft on day 14.* **(f)** *Kaplan-Meier survival plot of Rh30 tumor-bearing mice treated thrice a week with 8 mg/kg of 1 mg/mL of NPs, followed by US irradiation with relevant control groups. The US frequency is 1.0 MHz, and the intensity is 1.2 W/cm^2, and 2- or 5-min irradiation cycle. Untreated mice (-/-) and mice treated only with NPs (+/-) or US (-/+) are used as control (n = 6).*

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EXPRESSION OF CYTOKINES IN PBMC AND SPIKE PROTEIN CODING MRNA IN VARIOUS TISSUES OF THE PIG AFTER COMIRNATY VACCINATION: POTENTIAL MECHANISMS OF LONG TERM ADVERSE EVENTS

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INTRODUCTION

A small, but not negligible percentage of people immunized with mRNA-containing liposomal (LNP-mRNA) vaccine developed allergy-like symptoms shortly after vaccination occasionally leading to severe reactions or even death. It mimicked hypersensitivity type reactions (HSRs) to i.v.-administered nanomedicines, called complement (C) activation-related pseudoallergy (CARPA). In a previous set of studies, we investigated CARPA-like reactions after administration of Comirnaty (CMT), an LNP-mRNA type vaccine using our naturally hypersensitive pig model. We were able to show that CMT administration induced HSRs showing all characteristic properties of CARPA.

The time elapsed since mass vaccination campaign revealed a decent number of people with long-term adverse events. Its mechanism is yet unknown but the prolonged presence of spike protein (SP) coding mRNA or SP per se in cells and tissues are suspected. In the present studies, we followed the expression of COVID-related cytokines as well as the presence of SP-coding mRNA following CMT administration in our pig CARPA model.

Materials and Methods

Pigs: Domestic pigs (20-25 kg) were sedated with ketamine/xylazine (10 and 2 mg/kg, respectively) and anesthetized by isoflurane (2-3%) in O_2 flow. In spontaneously ventilating animals, the pulmonary arterial pressure (PAP) was measured using a Swan-Ganz catheter introduced into the pulmonary artery via the right external jugular vein, while systemic arterial pressure (SAP) and heart rate (HR) were measured in the femoral artery. The left femoral vein was cannulated for blood sampling. Test agents were injected in bolus (~30 sec) via the left external jugular vein. Hemodynamic changes and ECG were continuously monitored using an AD Instruments (ADI) PowerLab System. Mean PAP, SAP, HR and ECG data were evaluated by the ADI LabChart software.

Blood sampling: Blood samples of 2 ml, each were collected from the pigs before (time 0), and at pre-determined time points (1-3-5-10-30 min) after the injection. Samples were collected into K_3-EDTA blood tubes, of which samples for TXB2 analysis were containing indomethacin. Aliquots of 100 µl blood were drawn into tubes with K_3-EDTA for haematological analysis, performed by an Abacus (Diatron) analyser. Blood was centrifuged at 1500 rpm for 10 min at 4 °C, and plasma was stored at -80 °C until analysis.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

PBMC and tissue sample analysis: After administration of CMT, serial blood samples were taken to measure blood cell changes, cytokine gene transcription in peripheral blood mononuclear cells (PBMC) and blood levels of inflammatory cytokines, using qPCR and ELISA. At the end of the study tissue samples were taken from multiple organs (incl. heart and kidney) for histological and SP-coding mRNA sequence analysis.

Test item: Repeated doses of 5x of the human dose of CMT vaccine were given three-times as an i.v. or i.m. bolus injection.

RESULTS

In 10 of 15 pigs acute changes were followed. Similarly, to previous findings a transient increase in PAP, accompanied by TXA2 release and other hemodynamic and blood cell changes as SAP elevation, granulocytosis, lymphopenia, and thrombocytopenia were observed. Three pigs developed anaphylactic shock that required resuscitation. Repeated dosing had variable outcome, with or without tachyphylaxis. In some cases, skin flush was also observed.

In PBMC COVID-related cytokine levels (IL1RA, CXCL10, TNFα) mostly elevated, but individual variations were rather high. SP-coding mRNA expression could also be observed in cardiac and renal tissue 6 h after the 1st CMT injection with similarly high variability.

CONCLUSIONS

This study investigated the short and long term immune reactive properties of Comirnaty (CMT), an LNP-mRNA type vaccine. CMT administration induced HSRs showing all characteristic properties of CARPA. In addition, COVID-related cytokine and SP-coding mRNA expression could be observed. This phenomenon may be a contributing factor to the long term events after HSR to CMT and potentially other mRNA vaccines.

FINANCIAL SUPPORT

This study was supported by the European Union Horizon 2020 projects 825828 “Expert” and 952520 “Biosafety”, as well as by the National Research, Development and Innovation Office of Hungary under the Investment in the Future funding scheme (2020-1.1.6-JÖVŐ-2021-00013) and Semmelweis University Grant (STIA-KFI-2022).

EVALUATING FORMULATION AND PROCESS PARAMETERS FOR LYOPHILISATION OF PI ELECTRON STABILIZED POLYMERIC MICELLES

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INTRODUCTION:

Long-term stability of nanoparticles is a key challenge in nanomedicine clinical translation.(1) This has been exemplified with the recent surge of mRNA-containing lipid nanoparticles, which require deep freeze temperatures during storage and shipment, thereby limiting worldwide accessibility and significantly raising distribution costs.(2) Freeze-drying or lyophilisation is a commonly employed technology to increase shelf-life of pharmaceutical drug products under more feasible storage conditions. However, this process induces various stresses that can result in nanoparticle instability, such as aggregation and cargo leakage.(3) Depending on the nanoparticle type, optimal formulation and process requirements for efficient freeze-drying differ. Moreover, while there are protocols established for lipid-based nanoparticles, the research on polymeric micelles, particularly non-crosslinked, is still limited. In this work, we aim to investigate the key formulation and process parameters for the freeze-drying of paclitaxel (PTX)-loaded [(mPEG-*b*-p(HPMAm-Bz)]-based π–π interaction stabilized polymeric micelles, which have exhibited promising preclinical performance and are currently under upscaling evaluation.(5) To do so, we first assessed the impact of different formulation excipients on physicochemical properties and drug retention during freeze-drying. Secondly, we investigated the influence of different freezing and drying settings on the formulation properties.

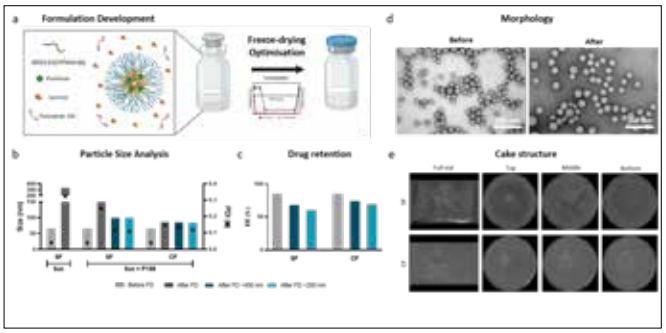

Figure (1): Formulation and process development of paclitaxel-loaded polymeric micelles for stabilization upon freeze-drying. a) Schematic overview of the study. The optimised formulation of paclitaxel (PTX)-loaded [(mPEG-b-p(HPMAm-Bz)]-based polymeric micelles contains 8% (w/v) sucrose (Suc) and 1% (w/v) poloxamer 188 (P188). b-c) Size (hydrodynamic diameter), polydispersity (PDI) and PTX content analysis in terms of encapsulation efficiency (EE) before and after freeze-drying (FD), in the presence of Suc, and with and without P188. Two freezing methods were used: snap freezing (SF) or controlled shelf freezing (CF). Sequential filtration steps have been implemented to omit the effect of aggregated micelles. d) Transmission electron microscopy images of PTX-loaded micelles before and after freeze-drying using the CF method. e) Non-invasive micro-computed tomography (μ-CT) scans of the lyophilized cake structure after freeze-drying after either SF or CF.

METHODS:

PTX-loaded [(mPEG-*b*-p(HPMAm-Bz)]-based micelles were prepared using the nanoprecipitation method. The micelles were characterized by measuring their hydrodynamic diameter (size), polydispersity index (PDI), morphology and PTX encapsulation efficiency (EE). To prepare the freeze-dried formulations, various cryoprotectants and surfactants were added to the micelles, followed by freeze-drying. The effect of process parameters (freezing method and drying temperature) was also examined. The lyophilized cake structure was visualized using micro-computed tomography (μ-CT). After reconstitution, the properties of the micelles in terms of size, PDI, EE, morphology and drug-release profile were compared to their freshly prepared counterparts.

RESULTS:

Our data showed that while the use of sucrose as a cryoprotectant was able to preserve formulation properties during freeze-thaw cycles, it alone was insufficient to stabilize PTX-loaded polymeric micelles during freeze-drying. However, the addition of surfactants, such as poloxamer 188 (P188) (Figure 1a), significantly improved the stability of the formulation in terms of size, PDI, EE, and morphology (Figure 1b-d). These findings therefore suggest that, during lyophilisation, the micelles might tend to accumulate at surfaces and liquid-ice interface, and P188 acted as a cushion for the micelles against stresses that occur during the process, efficiently protecting them from collapsing and aggregating. Additionally, comparison of the impact of two main freezing conditions (snap freezing and controlled shelf freezing) showed that controlled shelf freezing conditions produced a more homogenous cake structure after drying, which also resulted in less aggregation of PTX-loaded micelles in comparison to the snap freezing method (Figure 1e).

CONCLUSION:

Our findings show that relying solely on conventional cryoprotectants did not prevent micelle aggregation upon freeze-drying, and thus the addition of poloxamer 188 was crucial to preserve the properties of the formulation during lyophilisation. Additionally, we showed that the freezing step can significantly affect the homogeneity of the frozen sample and the formulation properties as well, and that this step needs to be controlled. Our work provides valuable insights into formulation and process parameters impacting on the lyophilisation process of non-crosslinked polymeric micelles, overall paving the way towards their pharmaceutical development and manufacturing.

ACKNOWLEDGEMENTS:

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NANOPARTICLE-LOADED MESENCHYMAL STEM CELLS FOR TUMOR-TROPIC DELIVERY OF THERANOSTIC AGENTS

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In the past decade, mesenchymal stem cells (MSCs) have been derived to track down and destroy malignant cells taking advantage of their tumor-tropic property. MSCs are multipotent cells found in all adult tissues and have the ability to self-renew and differentiate into various tissues. MSCs are able to escape the immune system and migrate to sites of pathology including tumors by using specific receptors or ligands to facilitate trafficking. Therefore, due to their tumor-tropic migratory properties, MSCs can be used as delivery systems to transport nanoparticles directly to cancer cells [1]. Various sources of MSCs had been unambiguously proved to migrate, once uploaded with nano-cargo, towards cancer cells *in vitro* and in experimental animals [2, 3]. However, the cargo for such transportation is not that easy to construct. While clinically approved chemotherapeutics can be injected into veins for systemic administration, the same drugs could not be used for MSCs upload, because of drug killing effect. Therefore the cargo itself must be wisely constructed, so the migratory properties of MSCs would not be affected. Many non-toxic bio-friendly nanoparticles had been suggested, including gold nanoparticles, iron oxide nanoparticles (NPs), core-shell quantum dots (QDs), and rear-earth doped upconverting nanoparticles (UCNPs) for cell labeling (diagnostics), however they lack the component which does the killing of cancer cells (therapy). The problem could be solved by chemical attachment of one of the clinically available chemotherapeutic drugs or radionuclides, but then it becomes toxic to MSCs itself and the migratory properties would be affected.

One of the solutions to the above-mentioned problem is the photoactivable compounds – photosensitizers. Photosensitizers (PS) – are a class of organic compounds, which are non-toxic in the dark, but are easily activated by harmless light and the generation of fatal reactive oxygen species begins [4]. Photosensitizers can be attached to the surface of NPs by covalent or non-covalent bonding; without light, no toxic effect would be present. PS is already applied in clinics, however only for superficial lesions due to low penetration of red light through the tissues. NPs could improve the traditional photosensitizers by expanding the gap of light activation into the NIR region, where light loss due to absorption of surrounding molecules is minimal.

In our study we used QDs-PS and UCNPs-PS complexes, to test the MSCs' capability to transport the theranostic cargo toward human breast cancer cells MDA-MB-231. QDs and UCNPs are composed of different chemical elements, but share one common property - the capability to transfer energy of light to PSs [5, 6]. Therefore, both QDs-PS and UCNPs-PS represent theranostic nanoplatforms, combining diagnostics and therapy in one agent. Although QDs and UCNPs had been successfully used to label live cells [7, 8], it is still unknown how would they affect the migratory properties of MSCs toward tumors. Our study aims to investigate the migratory capabilities of skin-derived mesenchymal stem cells once they are uploaded with NPs-PS cargo.

The MSCs were derived from eyelid plastic surgery waste tissues (ethical permit nr. 158200-18/6-1036-548). For migration studies, MSCs were uploaded with QDs-PS or UCNPs-PS for 24 hours to construct MSC-nano-vector. The migration was assessed using Boyden chamber assay (Fig.1). MSCs were placed into the upper level of the chamber with a porous membrane of 8 µm in serum-free medium. MDA-MB-231 cancer cells were cultivated on the bottom level in a serum-free medium for 24h prior experiment to excrete the signals associated with the tumor microenvironment. After 24 hours of co-cultures, non-migrated MSCs were removed from the chamber, while migrated ones were fixed on the other side of the porous membrane, stained with nuclei dye Hoechst, and imaged with laser scanning confocal microscope; magnification 20x. The results indicate that MSCs uploaded with theranostic cargo migrate towards cancer cells, but the migration efficiency is dependent on the type of theranostic complex. QDs-PS complexes do not change the migratory potential of MSCs, while UCNPs-PS complexes increase the migration efficiency almost 2 times.

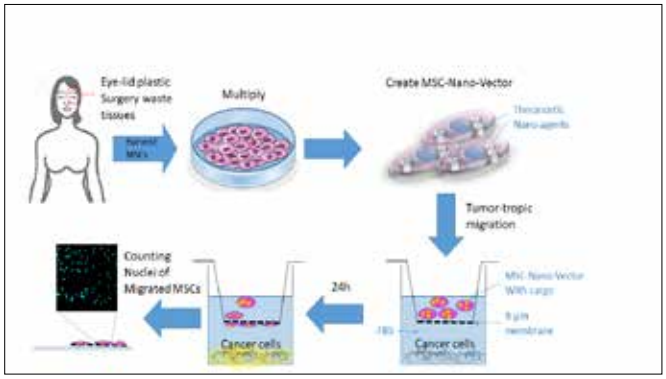

Fig. 1. Schematic representation of experiments with MSCs.

In conclusion, we demonstrated the capability of skin-derived MSCs to transport theranostic nano-agents toward cancer cells. While the migration of MSC-nano-vectors uploaded with QDs-PS was the same as the migration without cargo, the migration efficiency of MSC-nano-vectors uploaded with UCNPs-PS complexes was 2 times increased, indicating that UCNPs itself affects the migratory potential of MSCs in a favorable way. Further studies are needed to prove the migration efficiency in 3D spheroids or experimental animals.

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ANTIMICROBIAL PEPTIDES: LIPID CLUSTERING AND LEAKY FUSION IN PG/PE MODEL VESICLES

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The constantly growing antibiotic resistance emphasises the importance of alternatives to classical antibiotics, such as membrane-active antimicrobial peptides (AMPs). We examine the mechanism of action of the antimicrobial trivalent cyclic hexapeptide cR_3W_3. More precisely, we investigate the effects of the peptide on binary model membranes containing anionic phosphatidylglycerol (PG) and zwitterionic phosphatidylethanolamine (PE). In fact, POPG/POPE model vesicles are supposed to reflect bacterial membranes, but exhibit non-ideal lipid mixing behaviour and a high propensity for vesicle aggregation and fusion.

Isothermal titration calorimetry (ITC) measurements show binding selectivity for negatively charged membranes over zwitterionic membranes. This agrees with the observed selectivity of the peptide for bacteria over mammalian cells [1].

Differential scanning calorimetry (DSC) and Laurdan fluorescence spectroscopy reveal the influence of cR_3W_3 on the thermotropic membrane behaviour, such as lipid chain melting. Finger et al. described DSC-detectable electrostatic lipid clustering in saturated lipid membranes induced by the peptide [2]. As figure 1 illustrates, our findings also enable the detection of electrostatic lipid clustering in unsaturated POPG/POPE lipid membranes.

We use the self-quenching dye Calcein to quantify vesicle membrane leakage induced by the peptide. Figure 2A shows the characteristic, sigmoidal dose-response leakage curve as a function of the peptide concentration. Despite the leakage, the lifetime of the still entrapped calcein in POPG/POPE vesicles remains constant at approximately 0.4 ns (see figure 2B). This indicates an all-or-none leakage behaviour. On closer inspection, leakage turns out to be mainly caused by leaky fusion [3]. Yet, the biological relevance of this mechanism should be assed carefully.

In conclusion, binding of cR_3W_3 to model membranes induces various effects: electrostatic lipid clustering, membrane fusion, vesicle aggregation, and vesicle leakage.

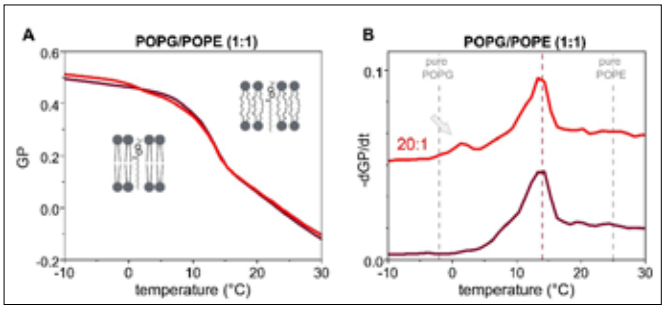

Figure 1: Laurdan fluorescence spectroscopy of POPG/POPE liposomes in the absence (light red) and presence of the peptide (dark red) is used to measure the thermotropic behaviour of the model membrane and to quantify lipid phase transitions. [4] A: The calculated generalized polarisation (GP) reveals the lipid order in the membrane. B: A peak in the derivative of GP indicates a lipid phase transition. The binding of the peptide leads to a small second peak (arrow), indicating demixing of the binary membrane and potentially electrostatic lipid clustering.

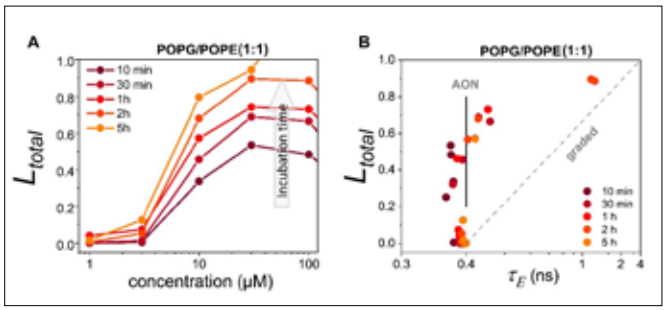

Figure 2: Calcein leakage induced by different concentrations of cR_3W_3 in 30 µM POPG/POPE model vesicles. A: The total leakage as a function of peptide concentration at various incubation times. B: The total leakage as a function of the fluorescence lifetime of the entrapped calcein dye, τ_F. The theoretical behaviour of all-or-none (AON) and graded leakage are marked.

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CONTINUOUS MANUFACTURING OF PEGYLATED LIPOSOMES: TAILORING SIZES FOR DIVERSE CLINICAL APPLICATIONS

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INTRODUCTION:

Liposomes are lipid-based vesicles extensively utilized in pharmaceutical industries for drug delivery. However, large-scale production is impeded by expensive and time-consuming batch methods, leading to batch-to-batch variability, which ultimately influences liposome sizes. This is of utmost importance as liposome size significantly impacts *in-vivo* biodistribution, accumulation, and uptake behavior. To overcome these challenges we developed a continuous flow manufacturing setup to enable large-scale production of liposomes with uniform, reproducible sizes. To achieve this, we employed a Design of Experiments (DoE) approach to identify critical process parameters influencing liposome hydrodynamic diameter (Z-avg) and Polydispersity Index (PDI) to enable the tailoring of liposome sizes to suit diverse clinical applications.

METHODS:

Production of liposomes was achieved using a millifluidic device developed by Sheybanifard *et.al.,(1,2).* The effects of process parameters (lipid concentration, FRR, and TFR) on the physicochemical properties (Z-avg and PDI) of liposomes were investigated using a DoE approach. The formed liposomal samples were diluted 10x with phosphate buffered saline (PBS), then Z-ave and PDI were determined by dynamic light scattering (DLS). A response surface

model was generated to access the relationship between the process parameter and the critical quality attributes. Then a tangential flow filtration (TFF) system was integrated into CFM setup to remove residual ethanol and un-encapsulated drug from the liposomal suspension. The effect of a) inlet flow speed (inlet pressure), b) filter unit outlet diameter (outlet pressure), c) sample dilution, and d) filter unit molecular weight cut-off (MWCO) on the liposome cleaning efficiency were investigated.

RESULTS:

Size and PDI as the main liposome critical quality attributes were significantly affected by lipid concentration and FRR. TFR, on the other hand, had no effect on the physicochemical characteristics of the liposomes. At any given TFR, increasing FRR and higher lipid concentration resulted in larger liposomes with increased polydispersity. More importantly, the design space created by DoE showed that our system can allow for the production of liposomes with broad size variation (80-200nm) and narrow PDI (below 0.15). To optimize the TFF setup in terms of cleaning efficiency, the effect of several parameters was tested. Decreasing the inlet pressure led to higher waste volume fractions, likely due to reduced tendency for membrane clogging. Surprisingly, increasing the outlet pressure didn't significantly improve cleaning, possibly due to increased clogging of the filter pores by pushing the liposomes in. Sample dilution was found to have the strongest impact on cleaning efficiency with waste volume fraction reaching ≈ 50%, resulting in better cleaning.

CONCLUSION:

The effect of key process parameters (lipid concentration, FRR, and TFR) on the physicochemical properties of empty liposomes produced in the millifluidic process was investigated using a DoE approach. The lipid concentration and FRR were identified as the critical process parameters to be controlled in order to achieve the desired liposomal size particles. These results demonstrate the versatility of our CFM to produce nanomedicines for different clinical applications to meet both market and individual needs. For the TFF module in the CFM set-up, a low inlet pressure, small outlet diameter, and high sample dilution maximized cleaning efficacy. In the future, we plan to integrate inline process analytical technologies (PATs) with feedback loops to allow for more accurate control of the manufacturing process and ensure that the final eventual nanomedicine products exactly meets individual patient's therapeutic need.

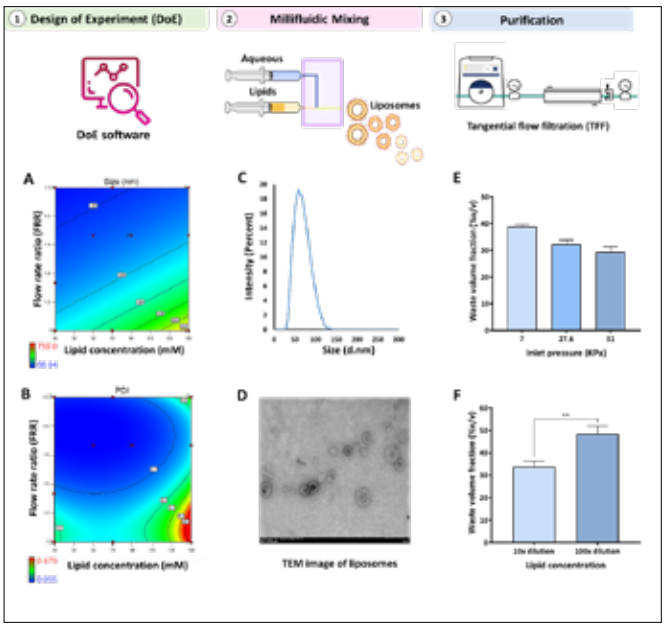

Figure 1. *Outlines liposomal preparation, characterization and purification process. A,B) DoE was used to access the effect of process parameters (lipid concentration, FRR and TFF) on liposome size and PDI. Liposomes size and morphological analysis was measured using C) DLS and D) TEM, respectively. Then the effect of E) inlet pressure and F) sample dilution on the cleaning efficiency of the filtration unit was investigated.*

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ENHANCING TUMOUR-TARGETED DRUG DELIVERY BY DMOG-INDUCED VASCULAR PROMOTION

ASMAA SAID SAYED ELSHAFEI

INTRODUCTION

Effective drug delivery to tumors is one of the most complicated steps in cancer therapy, owing particularly to barriers in the tumour microenvironment (TME), such as aberrant vasculature and excessive stroma[1]. Inducing vascular promotion is a promising strategy for overcoming this challenge with a one-size-fits-all solution. We here used the prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) [2], which promotes tumour blood vessel formation via upregulation of hypoxia inducible factor (HIF) and vascular endothelial growth factor (VEGF) signalling, to enhance tumor-targeted drug delivery.

METHODS

In vitro, 4T1 and MDA-MB-231 triple-negative breast cancer cells were incubated with DMOG for 72 h before collecting the supernatant. This supernatant was used in tube formation and scratching assays to determine the activation of both murine and human vascular endothelial cells. DMOG-induced vascular promotion was verified via the aortic ring assay. The impact of DMOG on collagen-1 deposition in tumour spheroids co-cultured with fibroblasts was investigated using immunofluorescence microscopy. The effects of DMOG treatment on biological barriers, including tumour vascularization and ECM were assessed. Multimodal and multiscale optical imaging tools will be employed to track the accumulation, distribution and penetration of polymeric-based drug delivery systems [3, 4].

RESULTS/DISCUSSION

The supernatant of cancer cells (4T1 and MDA-MB-231 TNBC) treated with DMOG increased the expression of VEGF-A, Ang1, and EPO-R in vascular endothelial cells. The proliferation, tube formation, scratching, and ring assay all significantly visualized the dynamic activation of the endothelial cell after being treated with DMOG and are all indicative of the induction of vascularization. In cancer cells fibroblast heterospheroids immunofluorescence revealed a decrease in total collagen-1 content. DMOG increased tumour vascularization and perfusion *in vivo*, according to ultrasound and immunofluorescence analysis. Interestingly, the latter vascular and tumour microenvironment changes aided in the accumulation, distribution and penetration of polymeric-based drug delivery systems in both tumour models (Fig. 1).

CONCLUSIONS

Our findings show that DMOG-based vascular promotion is a stepping stone in overcoming biological barriers limiting drug delivery. As a result, combining DMOG-induced vascular promotion with (nano-, chemo-, and/or immuno-) advanced therapeutic agents have the potential to significantly enhance accumulation, distribution and penetration thus improving treatment outcomes

Keywords: Drug Delivery, Therapeutic Efficacy, Tumor Microenvironment, Tumor-targeting, Vasculature

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Image/Figure Caption:

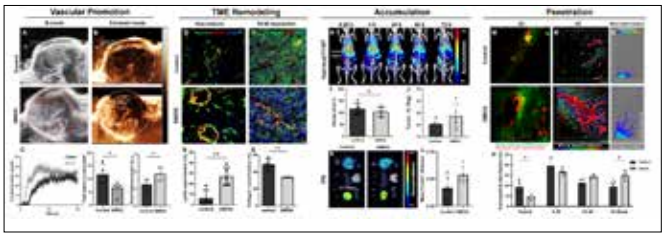


Figure 1: TME remodelling via DMOG treatment enhances the accumulation and penetration of PHPMA polymers.

[A-C] Ultrasound images display that DMOG significantly increases tumour blood vessel perfusion and vascularization. [D-E] DMOG significantly promotes tumour blood vessel functionality [F-G] and reduces collagen 1 deposition. [H-J] 3D µCT-FLT images and [K-L] Ex vivo 2D FRI images show an increase in the accumulation of labelled PHPMA polymers in the group treated with DMOG. [M] 2D fluorescence microscopy images display the distribution of NP around blood vessels. [N] NP-to-vessel distance measurements of 3D reconstruction of tumour vasculature show deep penetration of labelled NPs in the DMOG group. [O-P] The 3D intensity distribution of labelled NP reveal that DMOG increases the homogenous micro-distribution of the NP in tumour tissues.

CLINAM

European Foundation for Clinical Nanomedicine

14/2023

Basel, October 8 – 11

EXHIBITORS

ARDENA

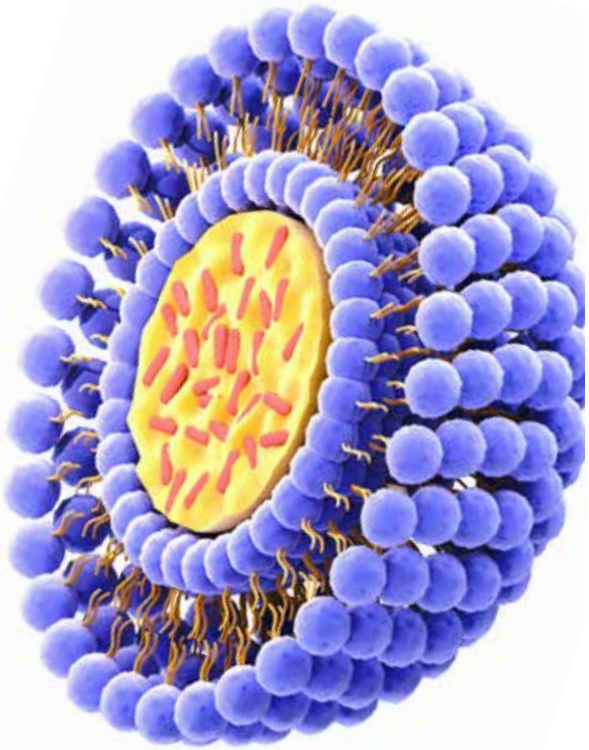
The Nanomedicine Expert

Development, production and analytics of nanoparticles

Development and synthesis of building blocks → Process and analytical development of nanoparticles → GMP manufacturing and release

Nanoparticle Types

- LNPs, liposomes and lipid micelles
- Polymeric nanoparticles
- Metal and metal oxide nanoparticles



Encapsulation of Payloads

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- Peptides and Proteins

Synthesis of building blocks

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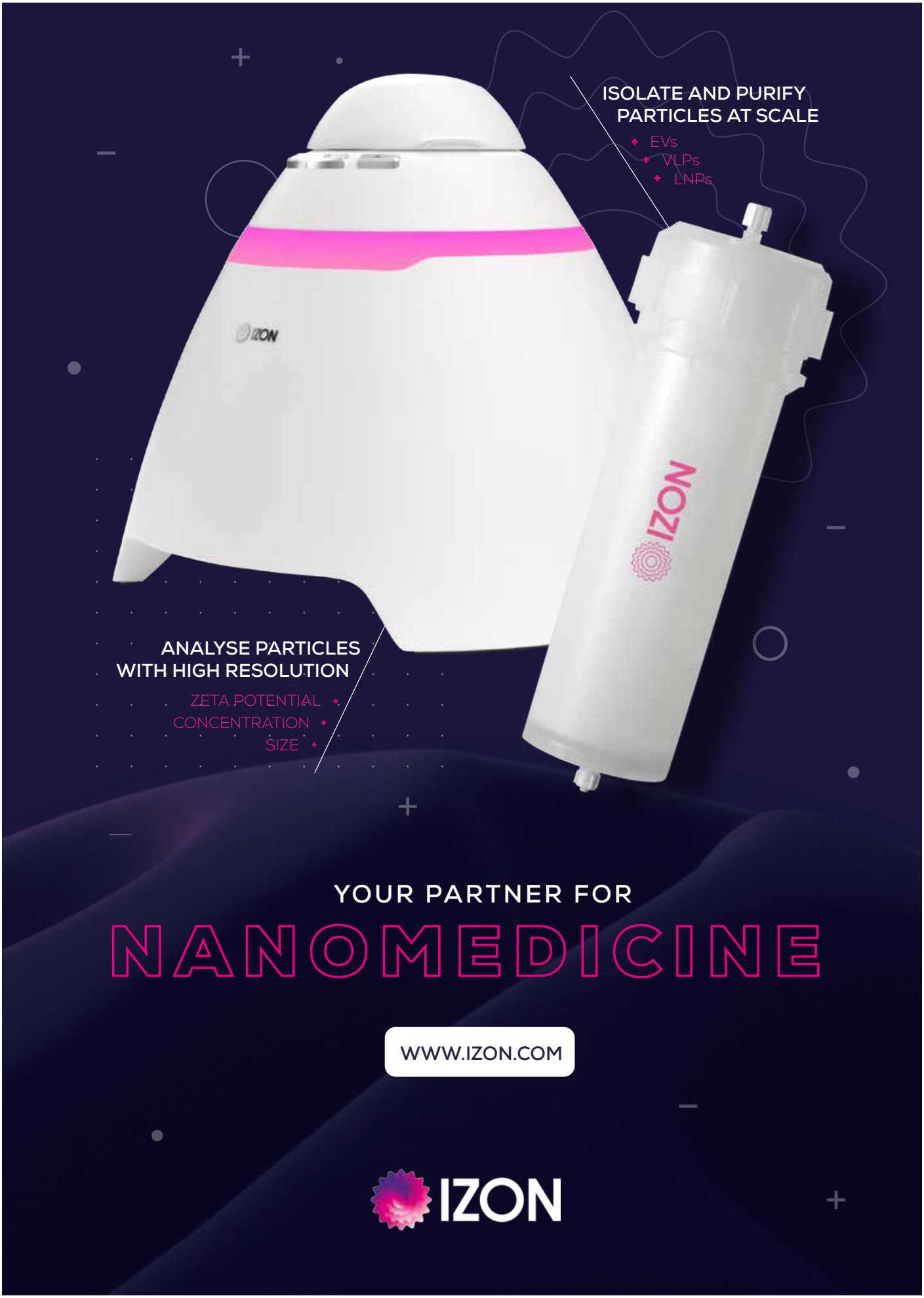
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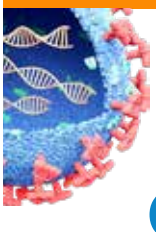

The NanoAnalyzer

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- Two Fluorescent Channels
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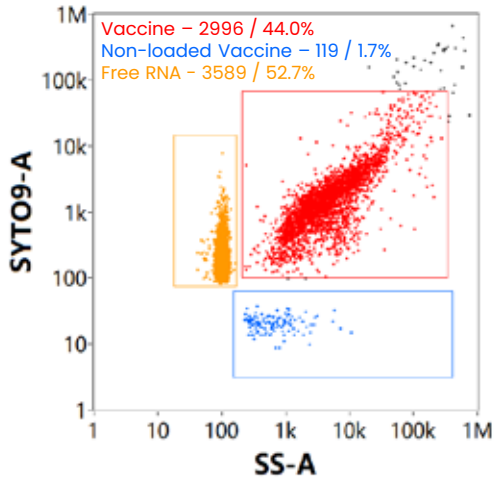

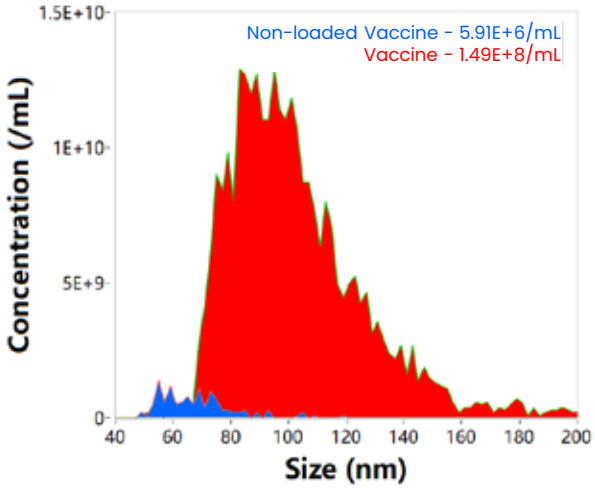

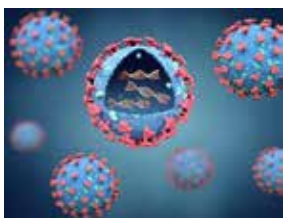

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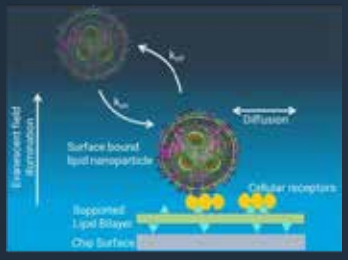
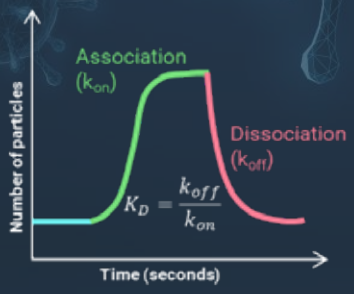
- **Membrane interaction and single-particle tracking of LNPs in real-time without the need for labelling**
- **Size distribution and sub-population discovery**
- **Encapsulation efficiency**
- **Refractive index**
- **Protein corona formation**

Our instrument comes as a microscope stage insert which can integrate with any upright or inverted microscope and allows you to repurpose the existing imaging infrastructure for comprehensive lipid nanoparticle characterization.

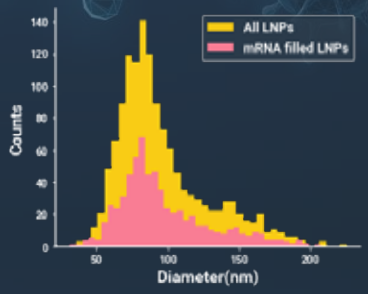
Membrane interactions of LNPs



Ligand-receptor interaction for targeting LNPs



Size and encapsulation efficiency of LNPs



TECHNICAL SPECIFICATIONS

Nanoparticle types	Lipid nanoparticles, Extracellular Vesicles, Liposomes, Viruses, Polymeric and Metal nanoparticles
Nanoparticle Size Range	40 nm – 300 nm diameter in scattering mode
Minimum sample volume required	100 µL
Minimum sample concentration required	10^8 particles/ml
Excitation wavelength	488 nm, other lines can be added as per need
Chip surface	Silicon dioxide (spin-on-glass)
Chip channel height and volume	35 µm, ~ 0.5 µL

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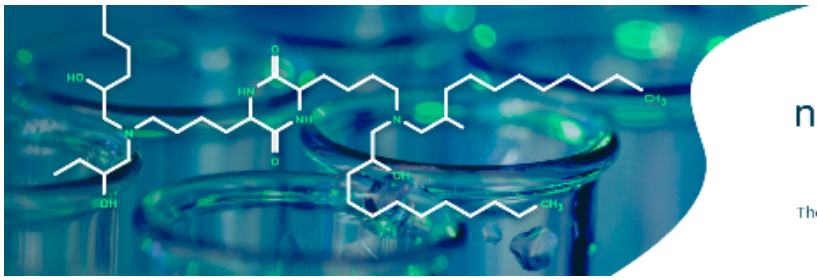

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Polymun offers the development of liposomal/LNP formulations for all kinds of pharmaceutically active ingredients such as mRNA, oligonucleotides, small molecules and proteins as well as vaccine antigens. A broad spectrum of formulation techniques as well as analytical methods have been established for this purpose. Polymun produces GMP material including all necessary documentation for IMPD/IND.

We assist in planning and implementation of clinical trials. Finally, license agreements are offered for the respective substance on an exclusive basis. Contracts can be arranged step by step – proof of concept, in-depth analysis, GMP production, product license – or all in one.

MAIN CHARACTERISTICS OF OUR TECHNOLOGY

FULL SCALABILITY

The injection module is the heart of the liposome/LNP production. The process parameters determine the size of the liposomes/LNPs regardless of the scale. Production of up to 1,000 liters of liposome/LNP batches takes only 3 hours. This large scale can be achieved by using several injection modules in parallel.

ASEPTIC PROCESS

A closed system is used for production. All components can be added via sterile filtration. Subsequent concentration by crossflow filtration is possible as well.

HOMOGENEOUS, UNIFORM VESICLES

All process parameters are controlled precisely. This results in a very narrow size distribution, necessary for reliable targeting and transport characteristics.

SINGLE STEP PROCESS

Liposome/LNP size is adjusted by modulating the process parameters during vesicle formation. No additional downsizing is required.

EXCELLENT BATCH TO BATCH CONSISTENCY

High quality of raw materials and precisely controlled process parameters guarantee excellent reproducibility – essential for pharmaceutical products.

MILD PROCEDURE – STABILITY

The crossflow injection technique is a very mild procedure that allows the processing of sensitive drugs. Together with the high quality of raw materials and narrow size distribution, we achieve long term stability of liposomes/LNPs even at room temperature.

SERVICES

- Formulation Development
- Analytical Method Development
- Process Development
- GMP Production
- Filling
- Clinical & Regulatory Support

CLINICAL & REGULATORY SUPPORT

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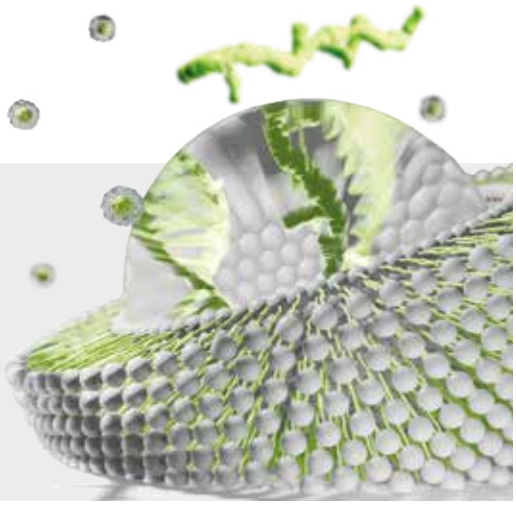

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