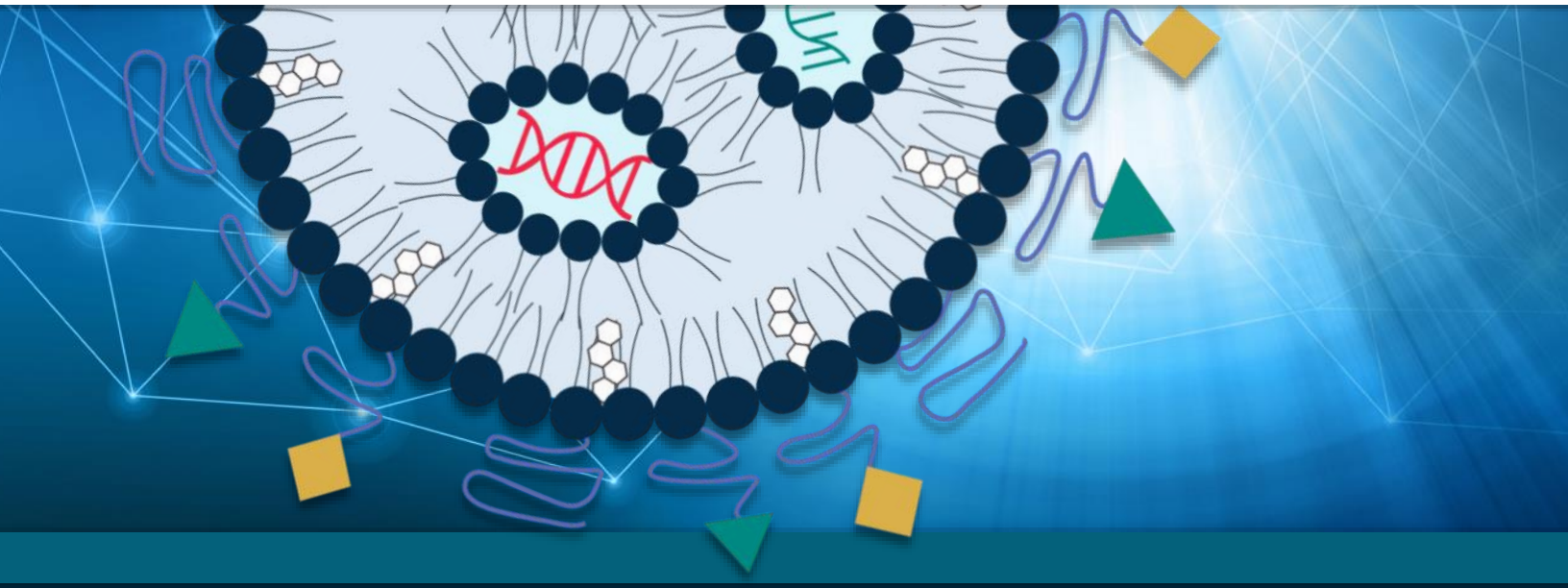
17th LIPOSOME RESEARCH DAYS 2022

University of British Columbia, Vancouver, Canada



Catalyzing the Nanomedicine Revolution

June 12-15, 2022

Life Sciences Centre
2350 Health Sciences Mall
University of British Columbia

THE UNIVERSITY OF BRITISH COLUMBIA

INTRODUCTION & WELCOME

Dear friends and colleagues:

A very warm welcome to beautiful Vancouver and to the University of British Columbia for the 17th Liposome Research Days, 2022!

It is approaching 60 years since Alec Bangham published his ground-breaking paper[1] on enclosed phospholipid structures, later called liposomes. It also marks 22 years since the first Liposome Research Days in Gainsville, FL. The field has flourished during this time with more than 10 liposomal and lipidic nanoparticle products receiving FDA, EMA, and other world-wide approvals for a variety of disease indications. The two most successful liposomal formulations of small molecule drugs have been Doxil®, which achieved blockbuster status with sales of over $1 billion/yr, and Ambisome® with sales in the $0.5 billion range. More recently lipid nanoparticle (LNP) formulations of nucleic acid-based drugs have come to the fore, starting with the FDA approval of Onpattro®, the first siRNA drug, in 2018. Most recently, the massive success of the COVID-19 LNP mRNA vaccines SpikeVax® and Comirnaty® have brought global recognition of the importance of liposomal/LNP technology. With doses in the billions, sales in the 10s of billions and a pivotal role in containing the COVID pandemic, liposomal/LNP delivery systems have achieved greatness. We congratulate all of us who have, through decades of basic and applied research, contributed to this remarkable record.

Our field is now poised to make gene therapy become a reality. We are entering a golden age of new genetic medicines that are enabled by lipid-based delivery systems. New treatments for genetic diseases such as cystic fibrosis, hemophilia, sickle cell anemia and many rare diseases are entering the pipeline. New treatments for chronic diseases such as atherosclerosis and Alzheimer's are on the horizon.

In planning this conference, we have striven to find a balance between acknowledging the contributions of pioneers in the liposome/lipid nanoparticle field and highlighting the ground-breaking discoveries of younger researchers. We have also tried to provide as many opportunities as possible for networking, making new friends, and forming research and commercial partnerships over refreshment breaks, sponsor displays and various social events.

We are so happy that we have the opportunity to meet again and interact in person after the long hiatus of Covid restrictions. Thank you for joining us to celebrate the maturation of lipid-based delivery systems and their revolutionary promise.

Terry Allen and Pieter Cullis
Event organizers

(1) Bangham, A. D.; Standish, M. M.; Watkins, J. C. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965, 13 (1), 238-252. DOI: 10.1016/s0022-2836(65)80093-6.

WiFi: LRD2022 pw: liposome

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University of Alberta

Pieter Cullis
University of British Columbia

Cedric Brimacombe
Polymorphic BioSciences

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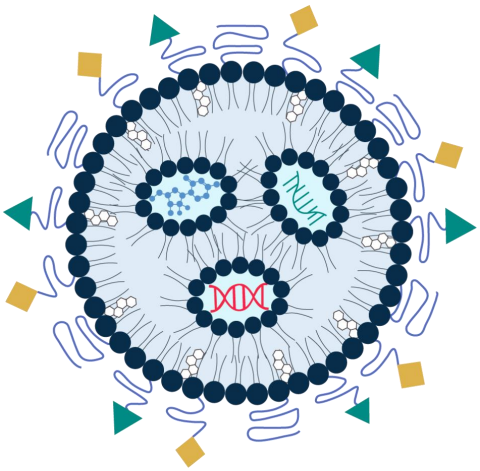


Speaker bios online: nanomedicines.ca/lrd-2022-program/#speakers

COVID protocols & Wifi

UBC requires the use of masks in public areas indoors.

Masks and hand sanitizer are available at the conference registration desk.

COVID testing is widely available at pharmacies in Vancouver:
https://www.bcpharmacy.ca/rapid-tests/list

COVID testing is also available for travelers at the Vancouver airport, but an appointment must be booked ahead of time:
https://bookings.covid-medical.ca/#/customer/yvr

WiFi

LRD2022

pwd liposome

Program by day

SUNDAY, JUNE 12, 2022

INDUSTRY DAY

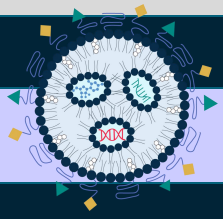
Registration desk will be open **Saturday June 11** 2 pm until 6 pm in the Life Science Institute, lower hallway

10:00 am	**Tea & Coffee | Registration desk opens**
10:15-10:20 am	**Opening Remarks**
10:20–11:00 am	**LNP Systems & the Pharmaceutical Industry** *Clinical opportunities for messenger RNA-LNP pharmaceuticals* **Thomas D. Madden** (Acuitas Therapeutics) 40 min. | **Chair:** Pieter Cullis
11:00 am–12:30 pm	**Industry Session 1: LNPs & Gene Therapies | Chair:** Gert Storm *Therapeutic application of messenger RNA-lipid nanoparticles for in vivo gene editing* **Ying Tam** (Acuitas Therapeutics) 30 min. *Accelerating the development of genomic medicines – insights into lipid nanoparticle delivery & scalable microfluidic manufacturing* **Lloyd Jeffs** (Precision NanoSystems) 30 min. *Tuning lipid nanoparticles for specific applications* **James Heyes** (Genevant) 30 min.
12:30–1:20 pm	**Networking Lunch**
1:20–3:10 pm	**Industry Session 2: LNPs & Gene Therapies | Chair:** Michael Michell *Lipid technology & the race to beat COVID-19* **Steve Burgess** (Avanti) 30 min. *Self-Adjuvanting Lipid Nanoparticle mRNA Vaccine for Solid Tumor Immunotherapy* **Syed Reza** (NOF) 20 min. *Novel Lipid Excipient Development for the Covid-19 mRNA Lipid Nanoparticle Vaccine* **Roger Pak** (Pfizer) 30 min. *Next-generation lipid nanoparticle technologies tailored to a variety of tissues* **Dom Witzigmann** (NanoVation Therapeutics) 30 min.
3:10–3:30 pm	**Networking Break**
3:30-5:30 pm	**Industry Session 3: LNP & Lipid Design & Manufacturing | Chair:** Yvonne Perrie *Advancements in liposomal extrusion technology* **Alex Torres** (Evonik) 30 min. *Phospholipids in vaccines* **Bruce Baretz** (Lipoid) 30 min. *mRNA –vaccines on the fast track – How Polymun contributed to tackling the pandemic* **Andreas Wagner** (Polymun) 30 min. *AmBisome: Continued progress & its role in the pandemic* **Gerard Jensen** (Gilead) 30 min.
5:30–8:00 pm	**Opening Reception**

WiFi: LRD2022 pw: liposome

MONDAY, JUNE 13, 2022

7:00-8:00 am	**Breakfast | Registration desk opens** (8:00 am)
8:00-8:20 am	**Opening Remarks** | Terry Allen
8:20-8:30	**Introductory Remarks: LNPs Rule!** | Pieter Cullis 10 min.
8:30–9:10 am	**Keynote Speaker 1: Lipids, Liposomes & Lipid Nanoparticles: The Past, The Present & The Future** **Pieter Cullis** (UBC | NMIN) 40 min. | **Chair:** Avi Schroeder
9:10 –9:50 am	**Keynote Speaker 2: LNPs & Extra-Hepatic Gene Therapy** | **Chair:** Leaf Huang *Species-agnostic in vivo nanoparticle barcoding* **James Dahlman** (Georgia Tech) 40 min.
9:50–10:20 am	**Networking Break** - Poster viewing
10:20–11:50 am	**Invited Lectures 1: LNP Technology - Part A** | **Chair:** Marcel Bally *Lipid-based nanopreparations for stimuli-sensitive & organelle-specific targeting* **Vladimir Torchilin** (Northeastern U) 30 min. *Multifunctional envelope-type nano device: from controlled intracellular trafficking to clinical application for nanomedicines* **Hideyoshi Harashima** (U Sapporo) 30 min. *Polymer/oil-based nanocapsules vs. LNPs as RNA delivery vehicles* **Maria Jose Alonso** (U Santiago de Compostela) 30 min.
11:50 am–12:50 pm	**Networking Lunch** including mentoring/networking sessions with trainees & senior investigators in rooms 1410 & 1510 LSI
12:50-2:20 pm	**Invited Lectures 2: LNPs & Extra-Hepatic Gene Therapy** | **Chair:** Vladimir Torchilin *On the mechanism of tissue-specific mRNA delivery by selective organ targeting (SORT) lipid nanoparticles (LNPs)* **Dan Siegwart** (UT Southwestern) 30 min. *Boosting intracellular delivery of mRNA therapeutics & its applications* **Gaurav Sahay** (Oregon State U) 30 min. *Development of broadly protective influenza vaccines using nucleoside-modified mRNA* **Norbert Pardi** (U Penn) 30 min.
2:20–3:00 pm	**Networking Break** - Group picture
3:00-4:50 pm	**Invited Lectures 3: LNP Technology & Vaccines** | **Chair:** Maria Alonso *Army Liposome formulation with QS21 (ALFQ): A potent & safe vaccine adjuvant* **Carl Alving** (Walter Reed Army Institute of Research) 30 min. *Optimization of lipid nanoparticles for self-amplifying RNA expression & cellular activation using a design-of-experiment approach* **Anna Blakney** (UBC | NMIN) 30 min. *LNP-formulated saRNA – learning from clinical experience* **Robin Shattock** (Imperial College London) 30 min. *The development of effective medicinal products in Africa by Africa for Africans* **Anne Grobler** (North-West University) 20 min.
4:50–6:00 pm	**Networking Break** - Poster viewing
FREE TIME	
7:00–10:00 pm	**Banquet** Robert H. Lee Alumni Centre, UBC



Speaker bios online: nanomedicines.ca/lrd-2022-program/#speakers

TUESDAY, JUNE 14, 2022

7:00-8:15 am	**Breakfast** | **Registration desk opens** (8:00 am)
8:15-8:30 am	**Opening Remarks**
8:30–9:10 am	**Keynote Speaker 3: LNPs & Immunotherapies** | **Chair:** Phil Felgner *Lipid nanoparticles for overcoming biological barriers to mRNA delivery* **Michael Mitchell** (U Penn) 40 min.
9:10 am–10:00 am	**Lightning Round Presentations** | **Chair:** Cedric Brimacombe **Adjudicated Abstracts** 5 min. each
10:00–10:30 am	**Networking Break** - Poster viewing
10:30 am–12:00 pm	**Invited Lectures 4: LNPs for Gene Therapy of Inheritable Diseases** | **Chair:** Frank Szoka *RNA & lipid nanoparticles for controlling bleeding & thrombosis* **Christian Kastrup** (Medical College of Wisconsin) 30 min. *Overcoming the barrier - Topical gene therapy of skin & lung* **Sarah Hedtrich** (UBC) 30 min. *Optimizing lipid nanoparticles for in vivo therapeutic genome editing* **Colin Ross** (UBC) 30 min.
12:00 am–1:00 pm	**Networking Lunch** including mentoring/networking sessions with trainees & senior investigators in rooms 1410 & 1510 LSI
1:00-2:05 pm	**Invited Phospholipid Research Centre Lectures** | **Chair:** Gert Storm *Introduction to the Phospholipid Research Centre (PRC)* **Gert Storm** (U Utrecht) 5 min. *Translational models to evaluate the performance of lipid based delivery systems* **Jörg Huwyler** (U Basel) 20 min. *Targeted liposomal drug delivery to pediatric sarcomas* **Michele Bernasconi** (U Zurich) 20 min. *Lipid self-assembling nanoparticle for RNA delivery: Toward personalized medicine* **Giuseppe De Rosa** (U Naples Federico II) 20 min.
2:05-3:05 pm	**Invited Lectures 5: LNPs & Cancer Chemotherapy** | **Chair:** Christine Allen *Liposomal Hydroxychloroquine-An example of what can happen to a liposomologist during the COVID pandemic* **Marcel Bally** (UBC | NMIN) 30 min. *Therapeutics based on liposomal drug targeting to well-accessible organs* **Gert Storm** (Utrecht U) 30 min.
3:05-3:45 pm	**Keynote Speaker 4: LNPs & Cancer Chemotherapy** | **Chair:** Thomas Andresen *Nanomedicine quo vadis? Lessons learned as scientist, developer & entrepreneur* **Chezy Barenholtz** (Hebrew University of Jerusalem) 40 min.

Speaker bios online: nanomedicines.ca/lrd-2022-program/#speakers

TUESDAY, JUNE 14, 2022 (continued)	
3:45-4:15 pm	**Networking Break**
4:15–4:55 pm	**Bangham Award Keynote Talk : 100 years of vaccine science** **Phil Felgner** (UC Irvine) 40 min. | **Chair:** Chezy Barenholz
4:55 pm–5:15 pm	**Poster Award Presentations | In Memoriam (Andy Janoff)** **Chairs:** Terry Allen and Pieter Cullis
5:15–5:45 pm	**LRD Business Meeting** **Chairs:** Gert Storm and Terry Allen
FREE TIME	
6:30–10:00 pm	**Speakers' Dinner** Cecil Green House - ***by invitation only***

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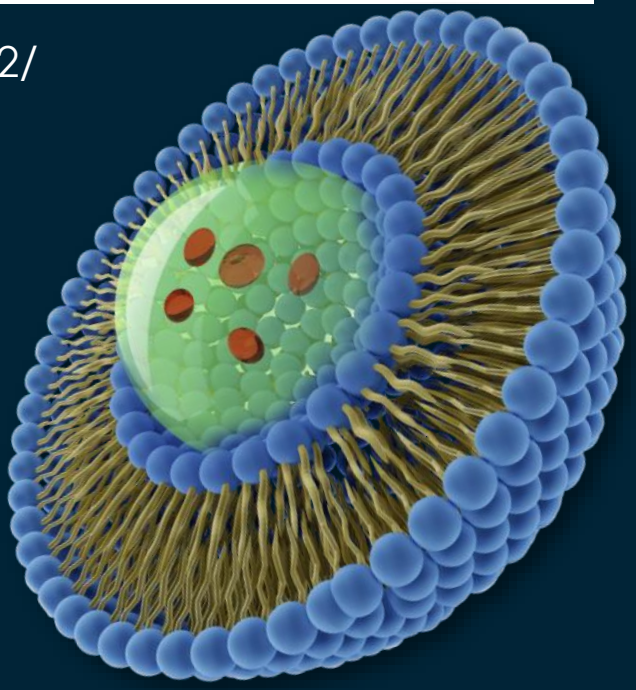
University of British Columbia,Vancouver,Canada

https://www.nanomedicines.ca/LRD-2022/

Student Networking Lunches

Date	Room	Name
June 13	1410 LSI	Avi Schroder & Gang Zheng
June 13	1510 LSI	Maria Jose Alonso & Gerard Jensen
June 14	1410 LSI	Anna Blakney & Christine Allen
June 14	1510 LSI	Gert Storm & Sara Hedrich
June 15	1410 LSI	Pieter Cullis & Yvonne Perrie
June 15	1510 LSI	Chezy Barenholz & Leaf Huang



Program continues on next page...

WEDNESDAY, JUNE 15, 2022

7:00-8:15 am	**Breakfast | Registration desk opens** (8:00 am)
8:15-8:30 am	**Opening Remarks**
8:30–9:10 am	**Keynote Speaker 5: Complexity & Reality: The Case of Thermosensitive Liposomes** **Christine Allen** (U Toronto | NMIN) 40 min. | **Chair:** James Dahlman
9:10 am–10:10 am	**Invited Lectures 6: LNPs & Cancer Chemotherapy** | **Chair:** Sarah Hedtrich *Lipid nanoparticles for cancer immunotherapy* **Shyh-Dar (Star) Li** (UBC | NMIN) 30 min. *Enhancing T cell functionality with nanotechnology in adoptive cell therapy* **Thomas L. Andresen** (Technical University of Denmark) 30 min.
10:10–10:40 am	**Networking Break** - Poster viewing
10:40 am–12:10 pm	**Invited Lectures 7: Lipids, Polymers & Other LNP Components—How can they work together?** | **Chair:** Carl Alving *The PEG-mediated accelerated blood clearance effect in mRNA-LNP evoked T-cell immunity* **Ray Schiffelers** (University Medical Center Utrecht) 30 min. *Porphyrin-lipid nanoparticles: building intrinsic multifunction into LNP* **Gang Zheng** (U Toronto | NMIN) 30 min. *The role of mRNA delivery system and route of administration on vaccine potency* **Yvonne Perrie** (U Strathclyde) 30 min.
12:10–1:10 pm	**Networking Lunch** including mentoring/networking sessions with trainees & senior investigators in rooms 1410 & 1510 LSI
1:10-2:40 pm	**Invited Lectures 8: LNPs & mRNA Therapeutics** | **Chair:** Anna Blakney *The effect of sex & the tumor microenvironment on liposomal cancer treatments* **Avi Schroeder** (Israel Institute of Technology) 30 min. *Liposomal antibiotics induce anticancer immunity by killing the tumor-associated bacteria* **Leaf Huang** (U North Carolina) 30 min. *On the structural characterisation of lipid nanoparticle formulations of nucleic acid* **Jay Kulkarni** (NanoVation Therapeutics) 30 min.
2:40–3:10 pm	**Networking Break** - Poster viewing
3:10–4:50 pm	**Keynote Speakers Panel Discussion: LNP Technology—Past & Future** **Chairs:** Terry Allen and Frank Szoka
4:50–5:00 pm	**Closing Remarks**

Speaker bios online: nanomedicines.ca/lrd-2022-program/#speakers

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Speaker abstracts

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COMPLEXITY AND REALITY: THE CASE OF THERMOSENSITIVE LIPOSOMES

Christine Allen
University of Toronto

8:30 am Wednesday June 15

Over the past few years there have been many articles debating the value of nanomedicines. To date, nanomedicines in oncology have largely resulted in improvements in the toxicity profile of drugs with few leading to enhancements in therapeutic outcomes relative to standard of care. There also continues to be a discrepancy between pre-clinical and clinical success of nanomedicines that may in part be attributed to a prioritization of novelty over innovation. Drug formulation development should be pursued from the outset with a focus on the patients' needs as well as translational feasibility (i.e. scale-up and manufacturing, economic feasibility and competitive landscape). As a case study, I will discuss thermosensitive liposomes, with a specific focus on the pre-clinical development and clinical evaluation of ThermoDox. I will also share studies from our laboratory on the design of new formulations that provide localized delivery of drugs when combined with clinically relevant heating modalities.

POLYMER/OIL-BASED NANOCAPSULES VS. LIPID NANOPARTICLES AS RNA DELIVERY VEHICLES

María J. Alonso
Dept. of Pharmacy & Pharmaceutical Technology & CIMUS Research Institute, University of Santiago de Compostela, Spain

11:20 am Monday June 13

Our laboratory started the development of polymer-based nanocarriers for the delivery of polynucleotides in the late 90's. We first produced PLGA-PEG nanospheres for the delivery of DNA and then, moved to cationic polymer (chitosan, polyarginine and protamine) -based nanocarriers, which were intended for different therapeutic purposes going from ocular drug delivery to vaccination and immunotherapy. The role of the cationic polymers was not only to condense the polynucleotides but also to facilitate their transport across biological surfaces. More recently, in the context of the B-SMART EU project, we developed strategies to deliver RNA to the brain. The nose-to-brain delivery prototype involves the combination of a penetration enhancer (C12-modified-octarginine) and a protective polymer (polyglutamic acid-PEG), whereas the one intended to cross the brain-blood barrier (BBB) through systemic administration is a ligand-functionalized oil-based nanocarrier. Finally, we have also contributed to the knowledge about mRNA vaccination. More precisely, we have developed a series of more than 300 prototypes of polymer/oil-based nanocapsules and lipid nanoparticles. A selected library of prototypes with adequate physicochemical properties was analyzed in terms of their transfection efficacy using model RNA and immunogenicity with a COVID RNA candidate. The overall conclusion was that not only the components but also their structural organization were key for their performance. An immense landscape for the optimization of RNA delivery is now open.

This work was supported by European Union's Horizon 2020 research and innovation program (grant agreement No. 721058), MINECO- PCIN-2017-129/ AEI (EuroNanoMed III), Instituto Salud Carlos III, FEDER Funds, Ref. COV20/00214.

ARMY LIPOSOME FORMULATION WITH QS21 (ALFQ): A POTENT AND SAFE VACCINE ADJUVANT

Carl R. Alving
Emeritus Senior Scientist, Walter Reed Army Institute of Research

3:00 pm Monday June 13

Addition of the triterpenoid saponin QS21 to nano-sized saturated phospholipid liposomes containing >50 mol% cholesterol (when compared to the phospholipids), and also containing monophosphoryl lipid A (MPLA), results in the formation of ALFQ, which contains unilamellar vesicles as large as 30 microns.

This process occurs by a remarkable fusion event in which nanoliposomes are transformed spontaneously into micro-liposomes. When compared with the composition of Adjuvant System 01 (AS01, GSK), a liposomal adjuvant present in two licensed vaccines (Shingrix® and Mosquirix®), ALFQ differs as follows: AS01 contains unsaturated phospholipid (e.g., DOPC) as the bulk lipid, but ALFQ contains DMPC and DMPG; AS01 contains 30 mol% cholesterol, but ALFQ contains >50% cholesterol; AS01 contains 50-100 nm particles but ALFQ is a polydisperse suspension of particles ranging from 50-30,000 nm. Despite polydispersity, nearly all of the ALFQ particles, in terms of total volume and surface area, are pelleted by centrifugation at 12,000 x G; in contrast, none of the nanoliposome particles are visibly pelleted by similar centrifugation.

To date, three experimental vaccines adjuvanted by ALFQ, two malaria vaccines and a SARS-CoV-2 vaccine, have completed phase 1 clinical trials, and have been deemed safe. Potency results are pending. Five additional phase 1 trials with ALFQ adjuvant, three candidate HIV-1 vaccines, a *Campylobacter* diarrhea vaccine, and a universal influenza vaccine with unconjugated peptides as antigens which has shown promising results in a relevant animal model, are approved by WRAIR or other sponsors, and all are in various stages pending initiation.

ENHANCING T CELL FUNCTIONALITY WITH NANOTECHNOLOGY IN ADOPTIVE CELL THERAPY

Thomas L. Andresen
Technical University of Denmark

9:40 am Wednesday June 15

The development of our understanding of the immune system has resulted in many new avenues for nanotechnology including use of liposomes and lipid nanoparticle systems (LNPs) to alter the function of T cells in relation to adoptive T cell therapy. Therapeutically successful adoptive T cell therapy is dependent on effective gene engineering of TCR or CAR targeting proteins, gene editing or other functional modifications to enhance T cell function to overcome barriers to engraftment as well as making the T cells resistant to premature exhaustion. Combing adoptive cell therapies with LNP based vaccines is another avenue for using lipid nanotechnology to enhance therapeutic outcome. Our research has in recent years been focused on various methodology for using lipid nanotechnology to enhance the efficiency of T cell therapy in cancer treatment, which will be presented.

LIPOSOMAL HYDROXYCHLOROQUINE: AN EXAMPLE OF WHAT CAN HAPPEN TO A LIPOSOMOLOGIST DURING THE COVID PANDEMIC

Marcel Bally

Head & Distinguished Scientist, Department of Experimental Therapeutics, BC Cancer

2:05 pm Tuesday June 14

Hydroxychloroquine (HCQ) is an approved anti-malarial drug that has the potential to be repurposed for cancer indications. Preclinical studies found that HCQ can improve the anti-cancer effects of various therapeutic agents by impairing autophagy. These findings are difficult to translate in vivo as reaching an effective concentration of HCQ is challenging. Dose escalation is not sufficient, as HCQ is cardiotoxic. We developed liposomal formulations of HCQ that were capable of increasing the plasma circulation lifetime of HCQ. Pharmacokinetics analysis of plasma samples derived from CD1 mice showed that both formulations engendered ~850-fold increases in total drug exposure over time relative to free drug. An efficacy study carried out with liposomal or free HCQ in combination with the gefitinab (an Epidermal Growth Factor Receptor (EGFR) inhibitor) which induces autophagy, showed that liposomal HCQ provided therapeutic benefits when tested in immunocompromised mice bearing gefitinib-resistant JIMT-1 breast cancer tumor xenografts. Western blot analysis of JIMT-1 tumor tissue harvested from animals at the end of treatment showed that the liposomal HCQ and gefitinib combination augmented inhibition of autophagy in vivo as demonstrated by increased LC3-II and p62/SQSTM1 (p62) protein expression compared to free HCQ combination. Our results suggest that liposomal HCQ modulates autophagy in vivo. But stop the press- the COVID-19 pandemic came and what does one do when it is dictated that cancer research studies need to be put on hold? Well it turns out liposomal HCQ could be repurposed for treatment of SARS CoV2 infections- and when opportunity knocks….But hold the press- Donald Trump has something to say!

NANOMEDICINE QUO VADIS?

Yechezkel (Chezy) Barenholz

The Laboratory of Membrane and Liposome Research, IMRIC, The Hebrew University-Hadassah Medical School

3:05 pm Tuesday June 14

LRD 2022 should be a celebration of the great success of Nanomedicine and nano-drugs as the BioNTech/Pfizer and Moderna mRNA-LNP vaccines saved a large percentage of world population from the COVID-19 pandemic. The success of these vaccines stems from three independent factors: The first one is the sound basic science leading to the development of modified mRNA with improved stability and reduced immunotoxicity; as well as to the parallel development of the LNP based on ionizable cationic lipids and PEG lipids that serve as an efficient delivery system of this mRNA. Upon intramuscular injection the desired mRNA is reaching the immune and muscle cell's cytoplasm. This allowed (without the need to reach cell nuclei) the expression of the protein encoded by the mRNA (Spike protein), leading to trigger of the immune response that protects the vaccine recipient against the infectious virus.

Second factor is the development of a "huge"-(kg)-scale "Good Manufacturing Production" (GMP) of relevant modified mRNAs suitably modified to improve their stability and reduce their immunotoxicity.

The third factor is the development and scale-up of the synthesis at GMP level of unique lipids and lipid compositions which, when combined with the relevant mRNA, are producing the highly efficacious mRNA-LNPs vaccine at a huge-scale of 100th of litters under GMP conditions. The successful combination of these three factors enables the production and the clinical use of billions of efficacious vaccine doses. Therefore, it seems that many of the technological obstacles to the development of nano-liposome and LNP based drug products were overcome.

However, despite this great progress, and the FDA approval of almost 10 nano-drugs, the clinical therapeutic success of nano-drugs and nanomedicine remain limited by biological, and immunological barriers. The reasons are related to the large differences between the desired activity of preventative locally (IM) administered vaccines and therapeutic use of other liposomal/LNP based drug products which in most cases are administered systemically and at much higher levels than the vaccines. It is expected of these drug-products to reach the relevant disease sites. To overcome these obstacles there is a need that the Nanomedicine community will join forces and apply a multidisciplinary- approach based on bio-convergence. Firstly, we must identify the mechanisms that are the basis for barriers to optimal nanomedicine and only then to develop the means to overcome these obstacles, so nano drugs can meet their expectations. This requires focusing on physicochemical, biological, toxicological, immunological, and translational factors and the understanding the crosstalk between these factors.

TARGETED LIPOSOMAL DRUG DELIVERY TO PEDIATRIC SARCOMAS

Michele Bernasconi

Head of Laboratory Research, Department of Pediatric Hematology and Oncology, Insel University Hospital Bern

1:25 pm Tuesday June 14

We are developing liposomes targeted to the tumor site to improve current therapies for pediatric sarcomas. The high relapse rates together with the significant toxicity, generating late side effects, caused by the aggressive chemotherapies needed to fight relapsed tumors, are major complication in pediatric oncology. Peptides and nanobodies with strong affinity for rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children, were selected and tested. In vitro binding of liposomes to RMS could be dramatically increased. The in vivo efficacy is under evaluation in an orthotopic model of RMS.

OPTIMIZATION OF LIPID NANOPARTICLES FOR SELF-AMPLIFYING RNA EXPRESSION & CELLULAR ACTIVATION USING A DESIGN-OF-EXPERIMENT APPROACH

Anna Blakney

Assistant Professor, Michael Smith Laboratories & School of Biomedical Engineering, UBC

3:30 pm Monday June 13

Lipid nanoparticles (LNPs) are the leading technology for RNA delivery, given the success of the Pfizer/BioNTech and Moderna COVID-19 messenger RNA (mRNA) vaccines, and small interfering RNA (siRNA) therapies (patisiran). However, optimization of LNP process parameters and compositions for larger RNA payloads, such as self-amplifying RNA (saRNA), which can have complex secondary structures, have not been performed. Furthermore, the interactions between process parameters, critical quality attributes (CQAs) and function, such as protein expression and cellular activation, are not well understood. Here, we used two iterations of Design of Experiments (DoE) (Definitive Screening Design and Box Behnken Design) to optimize saRNA formulations using the leading, FDA-approved ionizable lipids (MC3, ALC-0315 and SM-102). We observed that PEG is required to preserve the CQAs and that saRNA is more challenging to encapsulate and preserve than mRNA. We identified three formulations to minimize cellular activation, maximize cellular activation or meet a CQA profile while maximizing protein expression. These compositions and parameters may be useful for designing formulations for a range of applications, such as vaccines or protein replacement therapies, for larger RNA cargoes

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LIPID TECHNOLOGY & THE RACE TO BEAT COVID-19

Stephen Burgess
Managing Director, Avanti Polar Lipids

1:20 pm Sunday June 12

Avanti Polar Lipids has supported and been involved in gene therapy research for over 30 years. Through our manufacturing of high-purity lipids for nucleic acid delivery, we have facilitated clinical development of novel vaccines and therapeutics. We will summarize the path that put Avanti at the forefront of the Race to Beat COVID-19.

LIPOSOMES/LIPID NANOPARTICLES RULE!

Pieter Cullis
Department of Biochemistry & Molecular Biology, University of British Columbia

8:20 am Monday June 13

From their beginnings 60 years ago liposomes and lipid nanoparticles are reaching maturity and are changing the world. It is a time for celebration for all of us in the field. In these opening remarks I will salute the pioneers and suggest we all celebrate our massive success!

LIPIDS, LIPOSOMES AND LIPID NANOPARTICLES: THE PAST, THE PRESENT AND THE FUTURE

Pieter Cullis
Department of Biochemistry & Molecular Biology, University of British Columbia

9:10 am Monday June 13

I have been working on lipids, liposomes and lipid nanoparticles for a long, long time. Fifty years, to be exact. In this talk I'll cover some high points from the past, summarize the enormous efforts currently underway that exploit the ability of lipid nanoparticles to enable gene therapies and make some predictions about the future. Exciting times ahead!

SPECIES-AGNOSTIC IN VIVO NANOPARTICLE BARCODING

James E. Dahlman
Georgia Institute of Technology / Emory School of Medicine

9:10 pm Monday June 13

RNA can change the expression of any gene, making these molecules promising drugs. However, whether the drug is comprised of siRNA, mRNA, lncRNA, or another nucleic acid, it is limited by one problem: drug delivery. Chemists design thousands of distinct nanoparticles to deliver DNA or RNA to the desired cell type. However, after nanoparticles are synthesized, their ability to deliver drugs is evaluated using in vitro systems devoid of a liver, kidney, spleen, immune system, pulsatile blood flow, and other selection pressures known to affect nanoparticle delivery in vivo.

Here we describe DNA barcoding platforms to quantify how thousands of nanoparticles deliver nucleic acids in vivo. These systems enable us to quantify how hundreds of chemically distinct nanoparticles

deliver mRNA or siRNA into up to 30 cell types, all in a single animal. To analyze these large in vivo drug delivery datasets, we have also developed an open source bioinformatics pipeline to iteratively evolve nanoparticles that target cells in vivo. Using this high throughput, iterative, in vivo approach, we have identified nanoparticles with tropism to many novel cell types without the use of active targeting ligands.

This work was supported by the National Institutes of Health and DARPA.

LIPID SELF-ASSEMBLING NANOPARTICLE FOR RNA DELIVERY: TOWARD PERSONALIZED MEDICINE

Giuseppe De Rosa

Professor, Department of Pharmacy, University of Naples Federico II

1:45 pm Tuesday June 14

Lipid self-assembling nanoparticles (SANPs) represent a promising platform for RNA delivery. RNA can be loaded before use, thus paving the way for a personalized medicine. Different applications of SANP for RNA delivery will be presented.

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100 YEARS OF VACCINE SCIENCE

Philip Felgner

Professor, University of California, Irvine

4:15 pm Tuesday June 14

The history of vaccination is a testament to the continuity of science, building on decades of knowledge to find truth, develop consensus, and solve problems for the greater good. The origin of vaccination goes back more than 1000 years when variolation - the administration of human pus from smallpox blisters - was practiced to reduce the consequences of smallpox infection. The smallpox virus in the pus is attenuated by the immune response from the donor. In 1798 Edward Jenner recognized that pus from cowpox infected cows can be administered safely to humans to induce protective immunity against smallpox in humans. “Vaccine Farms” were established worldwide to harvest cowpox virus from blisters on the flank of cows, produce enough attenuated virus to vaccinate everyone on earth, and rid the world of smallpox in 1977.

During his lifetime Luis Pasteur developed “Germ Theory” discovering microorganisms that are the cause of numerous infectious diseases. In 1880 Pasteur discovered that attenuated microorganisms could be propagated in the lab and used for vaccination - he predicted that this would rid the world of infectious disease. It wasn’t until finally in 1935 that attenuated Yellow Fever virus vaccine, grown in embryonic chicken eggs was introduced. The next 20 years was the hay day in vaccine development when polio (Sabin/Salk) and 9 other attenuated organism vaccines (Hilleman) were introduced that are still manufactured today in similar antiquated, laborious, and hazardous ways.

In 1993 Maurice Hilleman from Merck threw his support into the field of nucleic acid vaccines calling it, "… one of the most exciting things in modern vaccinology”. Nucleic acid sequences could be administered repeatedly without mounting an immune response against the vector. Manufacturing of plasmid DNA was reproducible and scalable, a modern agile approach to immunize against different encoded proteins and thus protect against a variety of diseases. Much clinical development in nucleic acid immunization was accomplished since then but success never reached the pinnacle it has today. This is largely because of the challenging cancer and viral targets that were prioritized then and because we lacked the spectacular lipid nano particles available today. Through the combined efforts of many. we’re finally at the dawn of this new modality, "… one of the most exciting things in modern vaccinology.”

THE DEVELOPMENT OF EFFECTIVE MEDICINAL PRODUCTS IN AFRICA BY AFRICA FOR AFRICANS

Anne Grobler

North-West University, Pheroid Cluster Incubator NPC, Potchefstroom, South Africa

4:30 pm Monday June 13

More than one-sixth of the world's population are infected with at least one neglected tropical disease (NTD), with an additional two billion people at risk, resulting in more than 185,000 fatalities annually. The WHO lists 18 NTDs that not only affect health, but also the economic prosperity and often the very survival of poor and marginalized communities in regions of Africa, Asia and Latin America, and consequently the achievement of the declared global Sustainable Development Goals (SDGs) is also at risk. Six of the NTDs can be controlled or even eliminated through mass administration of medicines or control of the carrying vector or other effective interventions, but treatment of the other 12 NTDs presents with low efficacy due to drug resistance, short half-life, toxic side effects, and low bioavailability – the development of combat strategies against these 12 diseases in particular requires urgent attention.

Covid-19 vaccine, by demonstrating the effectiveness of vaccine strategies to curb endemic disease spread, has elevated public support for science and created an opportunity for scientists to investigate cures / treatments for infectious diseases in general, including the NTDs. It also points to the safety of nano-delivery systems. Hence we are, for the first time in history, seeing an increase in support for African vaccine manufacturing and transfer of skills by the pharmaceutical industry, with the large Covid-19 vaccine manufacturers setting up modular manufacturing facilities in a number of African countries. Indeed, there is increased interest in developing improved research capabilities, and novel drug delivery systems, in Africa. As illustration, this talk will include some results of (i) preclinical studies of a SARS-CoV-2 vaccine with the potential to be effective, efficient, including for the delta-variant and (ii) the use of a South African developed nano-delivery system in preclinical and clinical studies for various infectious diseases.

The development of vaccines for Africa by Africans has become a favourite slogan of politicians; hopefully this can translate into reality with the strengthening of continental expertise in scientific, regulatory and manufacturing disciplines. The local production of vaccines and medicines that are safe, effective, affordable and accessible will reduce the dependence of this continent on imported medicinal products, it will help to ensure medicinal product security, and will support much needed economic growth in Africa.

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OVERCOMING THE BARRIER - TOPICAL GENE THERAPY OF SKIN AND LUNG

Sarah Hedtrich

Berlin Institute of Health at Charité, Center of Biological Design; Internal Medicine, Infectiology, Respiratory, and Critical-Care-Medicine of the Charité , Universitätsmedizin Berlin; Pharmaceutical Sciences, The University of British Columbia

11:00 am Tuesday June 14

Epithelia such as human skin or human lung are a highly interesting target for the delivery of a great variety of biomacromolecules including biologicals, siRNA, and gene editing tools such as CRISPR. In fact, the main limitation that currently hampers broad clinical translation, especially of gene therapies is the lack of safe and efficient delivery strategies.

As epithelia are the first defense line of the human body, they developed a variety of very effective protective mechanisms. As such, the absorption of molecules across the skin is limited to small and moderately lipophilic substances due to the unique composition and, hence, very tight barrier of the stratum corneum. Lung epithelium instead is covered by a mucus gel which also greatly hampers efficient

penetration of especially biomacromolecules. For both, however, applying drugs topically appears to be the only viable option when targeting the epithelial cells or basal cells of these tissues.

In this presentation, I will discuss the potential of lipid nanoparticles as delivery systems for gene delivery to human epithelia and will allude to the remaining challenges in this field.

MULTIFUNCTIONAL ENVELOPE-TYPE NANO DEVICE FROM CONTROLLED INTRACELLULAR TRAFFICKING TO CLINICAL APPLICATION FOR NANOMEDICINES

Hideyoshi Harashima

Professor, Pharmaceutics; Chair, Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University

10:50 am Monday June 13

We are developing a multifunctional envelope-type nano device (MEND) as a novel non-viral gene delivery system based on a new packaging concept termed “Programmed Packaging”.

Cytosolic delivery: MEND was modified octaarginine (R8) to enhance cellular uptake and GALA peptide was also introduced to enhance endosomal escape. The R8/GALA-MEND can deliver siRNA successfully to dendritic cells (DC) to increase immune response, however, the antitumor activity was not sufficient. Then we introduced newly designed pH-sensitive cationic lipid YSKC12 and YSKC12-MEND can induce remarkable silencing effect in human NK cells as well as DC and T-cells.

In vivo delivery: In order to apply MEND via a systemic administration, we designed a pH-responsive cationic lipid to control biodistribution as well as intracellular trafficking. A newly designed YSK05 can respond to endosomal pH to induce efficient escape from endosome while maintaining neutral surface charge in blood circulation. The YSK-MEND can induce gene silencing in hepatocytes at a dose of 0.06 mg/kg. YSK-lipids were optimized based on chemical library which contains diversified chemical structures of YSK-lipids. The most efficient delivery of siRNA has been achieved by CL4H6 of ED50 at 0.0025 mgsiRNA/kg for gene silencing in hepatocytes in vivo after iv administration. Application of a new pH-sensitive cationic lipids for genome editing will be discussed.

Mitochondrial delivery: We proposed a MITO-Porter, a liposome-based carrier system that introduces macromolecular cargos into mitochondria via membrane fusion manner. An antisense RNA oligonucleotide (ASO) against cytochrome c oxidase subunit II was encapsulated into MITO-Porter to knockdown mitochondrial RNA. MITO-Porter can successfully knockdown the targeted mitochondria-encoded mRNA, protein and membrane potential in HeLa cells. D-arm, a mitochondrial import signal of tRNA to the matrix was chosen as ASO. Mitochondrial gene therapy will also be discussed based on our recent data in mutated human cells.

TUNING LIPID NANOPARTICLES FOR SPECIFIC APPLICATIONS

James Heyes

Chief Scientific Officer, Genevant

12:00 pm Sunday June 12

Lipid Nanoparticles (LNP) are now well established for delivery of nucleic acids (NA) systemically to hepatocytes and for vaccine applications. However, many potential applications for diverse NA modalities exist outside of these areas. LNP with altered biodistribution can be achieved by changing route of administration, and modulating lipid composition accordingly. This presentation will describe the development of specialized LNP for extrahepatocyte use, with examples including compositions targeting the hepatic stellate cell, lung, muscle and CNS.

LIPOSOMAL ANTIBIOTICS INDUCE ANTICANCER IMMUNITY BY KILLING THE TUMOR-ASSOCIATED BACTERIA

Leaf Huang

Fred Eshelman Distinguished Professor, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill

1:40 pm Wednesday June 15

The success of neoantigen cancer vaccine is mostly seen in tumor types with relatively high mutation burden. Neoantigens are rarely found in most of human cancers. On the other hand, many human solid tumors contain abundant intracellular bacteria. We hypothesized that the tumor associated bacteria (TAB) can be the best source for neoantigen if the bacterial antigen can be presented by the tumor cell's antigen presenting mechanism. Mice bearing orthotopic colorectal cancer CT26 were infected by oral gavage of Fusobacteria nucleatum (Fuso). The bacteria could cross the gut epithelium and establish intracellular colonization in the tumor, which caused rapid growth and metastasis of the tumor cells. We have prepared liposomes containing two different antibiotics by the method of active loading. Intravenous injections of liposomal antibiotics effectively killed Fuso in the tumor, but did not affect the gut microbiome population. The death of Fuso triggered a strong T-cell mediated immunity against both infected and uninfected tumor cells which eradicated the tumor in the gut and in the liver metastasis. We hypothesized that strong bacteria epitopes that are shared by the host proteins are the major neoantigens recognized by the host immune system. Using tools of bioinformatics, we have predicted several such epitopes for testing. Synthetic peptides with the predicted sequence could be recognized by the tumor infiltrating T-cells as shown by using a tetramer assay. Thus, targeting TAB for immune recognition might be an alternative and effective approach as a cancer vaccine. Supported by NIH grant CA198999 and Fred Eshelman Distinguished Professorship.

TRANSLATIONAL MODELS TO EVALUATE THE PERFORMANCE OF LIPID-BASED DELIVERY SYSTEMS

Jörg Huwyler

Professor & Head, Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel

1:05 pm Tuesday June 14

The transition from nanoparticle design to in vitro assessment and finally in vivo experiments in higher vertebrates remains a challenge. Phenomena such as protein binding, cellular uptake and intracellular processing are of prime importance since they may have a strong impact on biodistribution and immunogenicity of nanoparticles. Therefore, particle characteristics should be studied in an environment that simulates the situation encountered in biological systems. We will discuss the use of zebrafish (Danio rerio) larvae as a vertebrate screening model to assess the systemic circulation and extravasation of lipid-based drug delivery systems in vivo. To validate this novel approach, monodisperse preparations of fluorescent labelled liposomes with similar size and zeta potential were injected into transgenic zebrafish lines expressing green fluorescent protein in their vasculature. Phosphatidylcholine based lipids differed by fatty acid chain length and saturation. Circulation behaviour and vascular distribution pattern were evaluated qualitatively and semi-quantitatively using image analysis. The circulation patterns in the zebrafish model did correlate with published and experimental pharmacokinetic data from mice and rats. Our findings indicate that the presented translational approach can be used to predict the in vivo performance of lipid based delivery systems.

ACCELERATING THE DEVELOPMENT OF GENOMIC MEDICINES – INSIGHTS INTO LIPID NANOPARTICLE DESIGN AND SCALABLE MICROFLUIDIC MANUFACTURING

Lloyd Jeffs
Senior Director Biopharma Services, Precision NanoSystems

11:30 am Sunday June 12

RNA can be designed and formulated to silence, express, and edit specific genes providing a flexible and powerful approach to preventing and treating diseases. The recent commercialization and widespread distribution of COVID-19 mRNA-LNP vaccines has exemplified the massive potential to rapidly develop and scale-up new genomic medicines to protect from emerging viral threats and treat a wide range of serious diseases with unmet medical needs.

Precision NanoSystems has developed a Genomic Medicine Toolbox for the 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 Precision NanoSystems can accelerate the development of promising RNA-LNP drug candidates for clinical evaluation and commercialization.

AMBISOME®: CONTINUED PROGRESS & ITS ROLE IN THE PANDEMIC

Gerard M. Jensen
Vice President of Operations, Gilead Sciences Inc.

5:00 pm Sunday June 12

We begin with a review of the historical origins of AmBisome® (liposomal amphotericin B injection) in the Southern California 'high shear school' of liposomology that developed a unique platform for liposomal therapeutics with applications to oncology and infectious disease. The formulation and manufacturing principles that co-evolved with the growth in medical application of the drug. Key success factors in manufacturing and scale-up, including the ever tighter needs for aseptic processing quality, and a new parenteral manufacturing facility, will be discussed. Lastly, a mucormycosis fungus epidemic in India, directly related to COVID-19 cases, and the role of AmBisome in that setting, are explored.

RNA & LIPID NANOPARTICLES FOR CONTROLLING BLEEDING & THROMBOSIS

Christian Kastrup
Blood Research Institute & Departments of Surgery, Biochemistry, Biomedical Engineering, and Pharmacology and Toxicology, Medical College of Wisconsin

10:30 am Tuesday June 14

Bleeding and thrombotic disorders are common, affecting hundreds of thousands of North Americans, often due to an imbalance between the formation (coagulation) and destruction (fibrinolysis) of blood clots. Modulating fibrinolysis instead of coagulation is an alternate clinical approach for patients with bleeding and thrombotic disorders. However, there are only a small number of medications that influence pro- and anti-fibrinolytic proteins and enzymes, and they are short-acting. We developed a library of siRNA and

lipid nanoparticle (LNP) agents targeting pro- and anti-fibrinolytic proteins to modulate fibrinolysis long-term. Encapsulating siRNA agents in LNPs enables delivery to the liver, where many pro- and anti-fibrinolytic proteins are synthesized. siRNA mediates gene silencing by degrading the target mRNA, resulting in long-term depletion of the corresponding protein in blood plasma for weeks to months. Administering pro- and anti-fibrinolytic siRNA-LNPs effectively modulated fibrinolysis in vivo for weeks to months. In small and large animal models of bleeding and thrombosis, the appropriate siRNA-LNPs corrected bleeding or thrombosis, showing promising therapeutic potential for humans.

ON THE STRUCTURAL CHARACTERIZATION OF LIPID NANOPARTICLE FORMULATIONS OF NUCLEIC ACID

Jayesh A. Kulkarni
NanoVation Therapeutics

2:10 pm Wednesday June 15

Lipid nanoparticles containing short interfering RNA (LNP-siRNA) and optimized ionizable cationic lipids are now clinically validated systems for silencing disease-causing genes in hepatocytes following intravenous administration. However, the mechanism of formation and certain structural features of LNP-siRNA remain obscure. These systems are formed from lipid mixtures (cationic lipid, distearoylphosphatidylcholine, cholesterol, and PEG-lipid) dissolved in ethanol that is rapidly mixed with siRNA in aqueous buffer at pH 4 where the ionizable lipid is positively charged. The resulting dispersion is then dialyzed against a normal saline buffer to remove residual ethanol and raise the pH to 7.4 (above the pKa of the cationic lipid) to produce the finished LNP-siRNA systems. Here we provide cryogenic transmission electron microscopy and X-ray evidence that the complexes formed between siRNA and ionizable lipid at pH 4 correspond to tightly packed bilayer structures with siRNA sandwiched between closely apposed monolayers. Further, it was shown that ionizable lipid not complexed to siRNA promotes formation of very small vesicular structures at pH 4 that coalesce to form larger LNP structures with amorphous electron dense cores at pH 7.4. A mechanism of formation of LNP-siRNA systems is proposed whereby siRNA is first sandwiched between closely apposed lipid monolayers at pH 4 and subsequently trapped in these structures as the pH is raised to 7.4, whereas ionizable lipid not interacting with siRNA moves from bilayer structure to adopt an amorphous oil phase located in the center of the LNP as the pH is raised.

LIPID NANOPARTICLES FOR CANCER IMMUNOTHERAPY

Shyh-Dar (Star) Li
Angiotech Professor in Drug Delivery, Faculty of Pharmaceutical Sciences, The University of British Columbia

9:10 am Wednesday June 15

The current standard of care for peritoneal metastases of cancer includes cytoreductive surgery and chemotherapy, but the treatment response varies among patients with a high recurrence rate. My lab explores using lipid nanoparticles (LNPs) to regionally activate the immune system to promote immune clearance of peritoneal metastases. It was demonstrated that cationic LNPs displayed increased peritoneal retention compared to the neutral and anionic LNPs. R848, a TLR7/8 agonist, delivered by the cationic LNPs showed increased accumulation in the peritoneal fluid and peritoneal immune cells, but delayed absorption to the systemic circulation compared to free R848. This led to elevated levels of IFN-

alpha in the peritoneal fluid but not plasma in the LNP-R848 group. Mice with intraperitoneal metastases of CT26 colon cancer cells were first treated with oxaliplatin followed by LNP-R848, and 60-70% of the mice were cured, compared to a 30% cure rate when treated with oxaliplatin + free R848, and 0% cure rate when treated with oxaliplatin + PBS. These cured mice became immunized against the same tumor in a tumor re-challenge study. Re-exposure of tumor antigens to their splenocytes induced CD4 and CD8 proliferation and secretion of IFN-gamma. The results confirmed that the therapy with LNP-R848 induced specific antitumor immunity in the mice, leading to immune clearance and cure of the disease.

CLINICAL OPPORTUNITIES FOR MESSENGER RNA-LNP PHARMACEUTICALS

Thomas D. Madden
President & CEO, Acuitas Therapeutics

10:20 am Sunday June 12

The global success of COVID-19 mRNA-LNP vaccines has validated the clinical utility of this new pharmaceutical modality. In addition, the unique versatility of this platform may allow us to address currently intractable diseases. This presentation will focus on novel mRNA-LNP vaccines and therapeutics currently beginning clinical development or on the immediate clinical horizon.

LIPID NANOPARTICLES FOR OVERCOMING BIOLOGICAL BARRIERS TO MRNA DELIVERY

Michael Mitchell
Department of Bioengineering at the University of Pennsylvania

8:30 am Tuesday June 14

Significant advances have been made in the development of messenger RNA (mRNA) therapeutics for vaccine, protein replacement, and gene editing applications. However, these therapeutics must overcome numerous obstacles to be successful, including rapid in vivo degradation, poor uptake into target cells, required nuclear entry, and potential in vivo toxicity in healthy cells and tissues. In this talk, I will discuss our efforts towards the development of new lipid nanoparticles (LNPs) that enable the delivery of mRNA to target cells and tissues in vivo. I will describe new therapeutic strategies utilizing these LNPs for (i) mRNA CAR T cell engineering for cancer immunotherapy, and (ii) in utero mRNA delivery for treating disease before birth.

NOVEL LIPID EXCIPIENT DEVELOPMENT FOR THE COVID-19 MRNA LIPID NANOPARTICLE VACCINE

Roger Pak
Pfizer, Inc., BioTherapeutics Pharmaceutical Research and Development

2:20 pm Sunday June 12

Novel lipid excipients were used to encapsulate RNA within lipid nanoparticles forming the delivery technology behind the Covid-19 vaccine developed by Pfizer and BioNTech called Comirnaty®. Multiple challenges were faced during their development and these challenges along with success factors will be discussed in the presentation.

DEVELOPMENT OF BROADLY PROTECTIVE INFLUENZA VACCINES USING NUCLEOSIDE-MODIFIED MRNA

Norbert Pardi
Assistant Professor, Department of Microbiology, University of Pennsylvania

1:50 pm Monday June 13

Influenza virus is one of the most important human pathogen. The influenza mortality is estimated to be approximately 650,000 per year worldwide, in addition, occasional global pandemics can infect up to 20-40% of the world's population. Licensed influenza virus vaccines require annual reformulation and readministration due to poor IgG longevity and lack of neutralization of related viruses. Development of a universal influenza virus vaccine with the potential to elicit long-lasting, broadly cross-reactive immune responses is necessary for reducing influenza virus prevalence. We have utilized lipid nanoparticle-encapsulated, nucleoside-modified mRNA vaccines to deliver a combination of influenza A group 1 or influenza B antigens to induce strong immune responses with substantial breadth and potency in a murine model. A single immunization with 50 ng of combined influenza A group 1 or combined influenza B vaccines induced protective immune responses against a broad panel of group 1 or influenza B viruses, respectively. These findings support the advancement of nucleoside-modified mRNA-lipid nanoparticle vaccines expressing multiple antigens as universal influenza virus vaccine candidates.

THE ROLE OF MRNA DELIVERY SYSTEM AND ROUTE OF ADMINISTRATION ON VACCINE POTENCY

Yvonne Perrie
Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde

11:40 am Wednesday June 15

The efficacy of RNA-based vaccines has been recently demonstrated, leading to the use of mRNA based COVID-19 vaccines delivered using lipid nanoparticles. To investigate the impact of different nanoparticle delivery platforms and administration routes on RNA-vaccine potency, we investigated the immunogenicity of a self-amplifying mRNA encoding the rabies virus glycoprotein encapsulated in different nanoparticle platforms (solid lipid nanoparticles (SLNs), polymeric nanoparticles (PNPs) and lipid nanoparticles (LNPs)). These were administered via three different routes: intramuscular, intradermal and intranasal. Our studies in a mouse model show that the immunogenicity of our four different saRNA vaccine formulations after intramuscular or intradermal administration was initially comparable; however, ionizable LNPs gave higher long-term IgG responses. The clearance of all 4 of the nanoparticle formulations from the intramuscular or intradermal administration site was similar. In contrast, immune responses generated after intranasal were low and coupled with rapid clearance for the administration site, irrespective of the formulation. These results demonstrate that both the administration route and delivery system format dictate self-amplifying RNA vaccine efficacy.

SELF-ADJUVANTING LIPID NANOPARTICLE MRNA VACCINE FOR SOLID TUMOR IMMUNOTHERAPY

Syed Reza
Drug delivery consultant, NOF Corporation

11:40 pm Sunday June 12

Nucleic acid vaccines, DNA and messenger RNA (mRNA), have emerged as promising modalities for infectious disease and for cancer immunotherapy due, in part, to shortened manufacturing cycles and high potency. Currently, there is limited understanding of the mechanisms of antigen presentation and induction of specific T-Cell responses critical to long-term immunity. Lipid nanoparticles (LNP) composed of ionizable lipids are important components of such vaccines as they can convey and present the nucleic

acid effectively to the immune system. Further improvements in LNP carriers require a reduction in the 1) systemic toxicity, 2) improved endosomal escape, 3) potent and T-cell specific adjuvanting function and 4) targeting to specific antigen presenting cells.

Previously we have reported that lipid nanoparticles composed of COATSOME® SS Series can deliver pDNA or mRNA in mice to liver, solid tumors, and other organs via the IV route and achieve high levels of expression. We also evaluated the safety of the lipids in mice where doses of up to 175 mg/kg were well tolerated.

After subcutaneous administration, the LNPs containing an SS-EC, COATSOME® SS Series with vitamin E scaffolds, elicited a higher gene expression activity in comparison with the other LNPs composed of the SS lipids with different hydrophobic scaffolds. Immunization with the SS-EC-LNPs encapsulating mRNA that encodes ovalbumin (OVA, a model antigen) induced both humoral and CTL responses. These findings suggest LNP composed of SS-EC lipid can be effective delivery systems for mRNA vaccines .

OPTIMIZING LIPID NANOPARTICLES FOR IN VIVO THERAPEUTIC GENOME EDITING

Colin Ross

Associate Professor, Faculty of Pharmaceutical Sciences, UBC; Scientist, B.C. Children's Hospital Research Institute

11:30 am Tuesday June 14

Genetic diseases are a leading cause of death and disability in Canada with immense economic and societal burdens. Gene therapy has emerged as a means to effectively treat genetic diseases; however, current gene therapies are limited by their high manufacturing costs, the inability to re-dose, and the safety concerns of some viral vectors. CRISPR genome editing is a new therapeutic approach that aims to directly repair the underlying disease-causing mutations. Conventional CRISPR methods are limited as in vivo therapeutics because they introduce DNA breaks and cause frequent off-target edits. Newer base editors and prime editors overcome the limitations traditional CRISPR genome editing methods because they do not introduce DNA breaks. However, the delivery of genome editors to affected tissues remains a challenge. Viral vectors, such as AAV, are unsuitable because their long expression (years) increases the probability of unintended edits. In contrast, the transient expression (hours-days) of RNA encoding genome editors via nanoparticles is well suited for genome editing, and unlike viral vectors, nanoparticles can be readministered. However, nanoparticle delivery of complex genome editing cargos (large mRNA + small gRNA) remains a challenge, especially to extra-hepatic target tissues such as muscle. To address this, we are developing new ways to safely deliver these new editors using lipid nanoparticles. To efficiently measure the in vivo effectiveness of genome editor delivery via LNPs, we have developed transgenic mice that carry mutations in reporter genes. Precise gene repair of these mutations produce a functional enzyme that emits light (luminescence) that sensitive imagers can detect to precisely measure the location and extent of gene editing in living animals. We have made progress in our goal towards efficient and safe in vivo genome editing that we will share.

BOOSTING INTRACELLULAR DELIVERY OF MRNA THERAPEUTICS AND ITS APPLICATIONS

Gaurav Sahay

Associate Professor, Department of Pharmaceutical Sciences, Oregon State University

1:20 pm Monday June 13

The field of nanomedicine is moving from an age of renaissance towards industrial revolution. In part due to the transformational impact of lipid nanoparticle (LNP) enabled mRNA vaccines against SARS-CoV2. Our lab has worked extensively onto understanding LNP design, structure, and its impact on intracellular

delivery of mRNA. Endosomal sequestration of LNPs remains a formidable barrier to intracellular delivery. Structure-activity analysis of cholesterol analogues reveals that incorporation of C-24 alkyl phytosterols into LNPs (eLNPs) causes 200-fold improvement in gene transfection and the length of alkyl tail, flexibility of sterol ring and polarity due to -OH group is required to maintain high transfection. Cryo-TEM displays a polyhedral shape for eLNPs compared to spherical LNPs, while x-ray scattering shows little disparity in internal structure. eLNPs exhibit higher cellular uptake and retention, potentially leading to a steady release from the endosomes over time. 3D single-particle tracking shows enhanced intracellular diffusivity of eLNPs relative to LNPs, suggesting eLNP traffic to productive pathways for escape. Based on these findings we designed next generation LNPs for deliver mRNA for extrahepatic gene delivery and editing i.e., for the treatment of cystic fibrosis, retinal degeneration, and COVID-19 therapeutics. I will also discuss our recent data on delivery of LNPs delivered mRNA in non-human primate eye. Our findings emphasize the need for greater insights into surface topology and structural properties of nanoparticles, and their subcellular interactions. Next generation LNPs that enable tissue and cell-type specific delivery of genes and genome editors can revolutionize modern medicine.

THE PEG-MEDIATED ACCELERATED BLOOD CLEARANCE EFFECT IN MRNA-LNP EVOKED T-CELL IMMUNITY

Raymond M. Schiffelers

CDL Research, University Medical Center Utrecht, Utrecht, The Netherlands

10:40 am Wednesday June 15

Lipid nanoparticles (LNPs) are currently at the forefront for the delivery of nucleic acid therapeutics, as exemplified by patisiran and the COVID-19 vaccines. We made a library of lipid nanoparticles loaded with mRNA and measured physicochemical characteristics. In vivo tissue distribution was determined after intravenous administration in mice using fluorescent RNA payloads and correlated to functional tissue distribution determined by reporter protein expression. Finally, therapeutic immunogenic mRNA payloads were used to examine the magnitude of an antigen-specific T cell response. From these data a model was built to correlate LNP composition to efficacy. Based on the model a suboptimal and optimal composition was established to validate the model. These compositions were tested for antitumor activity, which demonstrated strong antitumor activity of the optimal LNP composition and validated the model. Crucial cell types for activity were macrophages, monocytes and dendritic cells that likely acted as antigen presenting cells. In addition, Interferon I signaling was crucial for CD8 antigen-specific T cells. Upon repeated injection anti-PEG antibodies were formed that did not preclude induction of a specific T cell response.

This work was supported by grant 16169 TORNADO of the Netherlands Organization for Scientific Research.

THE EFFECT OF SEX & THE TUMOR MICROENVIRONMENT ON LIPOSOMAL CANCER TREATMENTS

Avi Schroeder

Associate Professor of Chemical Engineering, Technion – Israel Institute of Technology

1:10 pm Wednesday June 15

Medicine is taking its first steps towards patient-specific cancer care. Nanoparticles have many potential benefits for treating cancer, including the ability to transport complex molecular cargoes including siRNA and protein, as well as targeting to specific cell populations.

The talk will discuss 'barcoded nanoparticles' that target sites of cancer where they perform a programmed therapeutic task. Specifically, liposomes that diagnose the tumor and metastasis for their sensitivity to different medications, providing patient-specific drug activity information that can be used to improve the medication choice.

The talk will also describe how liposomes can be used for degrading the pancreatic stroma to allow subsequent drug penetration into pancreatic adenocarcinoma, and how nanoparticle' biodistribution and anti-cancer efficacy is impacted by patient' sex and more specifically, the menstrual cycle.

The evolution of drug delivery systems into synthetic cells, programmed nanoparticles that have an autonomous capacity to synthesize diagnostic and therapeutic proteins inside the body, and their promise for treating cancer and immunotherapy, will be discussed.

LNP FORMULATED SARNA – LEARNING FROM CLINICAL EXPERIENCE

Robin Shattok
Imperial College London

4:00 pm Monday June 13

Lipid nanoparticle (LNP) encapsulated self-amplifying RNA (saRNA) is a novel technology with significant, and previously untested, potential for translation into human use in the development of novel drugs and vaccines. Vaccines against COVID-19 require production technology that is highly scalable to meet global demand. Vaccines developed using encapsulated saRNA have unique features, which include low dose administration and a readily modifiable antigenic domain making it possible to formulate vaccines rapidly. As part of the global effort to develop novel SARS-CoV-2 vaccines we took an saRNA vaccine from an early design concept through to clinical evaluation. Early preclinical evaluation demonstrated potent anti-SARS-CoV-2 immunogenicity in small animals even at ultra-low doses (0.01µg) and efficacy against infection in guinea pig challenge studies. However, while LNP formulated saRNA was seen to be safe and well tolerated, human responses to SARS- CoV-2 were significantly lower than those predicted by small animal models, failing to provide seroconversion in 100% of participants. Retrospective analysis of the predictive nature of different animal models and improved understanding species specific differences in innate regulation of saRNA expression are providing critical insight to improved vector design, LNP formulation and development of better preclinical models. This presentation will provide learnt experience form our clinical development program with a view to future improvements and challenges for LNP formulated saRNA.

ON THE MECHANISM OF TISSUE-SPECIFIC MRNA DELIVERY BY SELECTIVE ORGAN TARGETING (SORT) LIPID NANOPARTICLES (LNPS)

Daniel J. Siegwart
Southwestern Medical Center, Dept. of Biochemistry, Simmons Comprehensive Cancer Center, The University of Texas

12:50 pm Monday June 13

Lipid nanoparticles (LNPs) are a clinically mature technology for the delivery of genetic medicines but have limited therapeutic applications due to liver accumulation. Recently, our laboratory developed selective organ targeting (SORT) nanoparticles that expand the therapeutic applications of genetic medicines by enabling delivery of messenger RNA (mRNA) and gene editing systems to non-liver tissues. SORT nanoparticles include a supplemental SORT molecule whose chemical structure determines the LNP's tissue-specific activity. To understand how SORT nanoparticles surpass the delivery barrier of liver

hepatocyte accumulation, we studied the mechanistic factors which define their organ-targeting properties. We discovered that the chemical nature of the added SORT molecule controlled biodistribution, global/apparent pKa, and plasma protein interactions of SORT nanoparticles. Additionally, we provide evidence for an endogenous targeting mechanism whereby organ targeting occurs via 1) desorption of poly (ethylene glycol) lipids from the LNP surface, 2) binding of distinct proteins to the nanoparticle surface because of recognition of exposed SORT molecules, and 3) subsequent interactions between surface-bound proteins and cognate receptors highly expressed in specific tissues. 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.

THERAPEUTICS BASED ON NANOPARTICULATE DRUG TARGETING TO WELL-ACCESSIBLE ORGANS

Gert Storm

Utrecht University, University of Twente, National University of Singapore

2:35 pm Tuesday June 13

In the healthy body situation, the overwhelming majority of systemically administered nanoparticles will end up in the liver and the spleen. Other organs and tissues are not or hardly reached due to the presence of the vascular endothelium barrier. Then only local administration would be an option, though this is only possible in a limited number of cases. These excellent targeting opportunities are known for decades, but nanoparticle-based therapeutic products for treating patients suffering from pathologies associated with these well-accessible organs are still largely lacking, with the recent Onpattro product targeting siRNA to hepatocytes (i.v. administration) and Covid-19 mRNA vaccins (i.m. administration) being recent exceptions. In this talk, I will present our ongoing efforts to exploit the spontaneous passive behaviour of nanoparticles for the development of new therapeutics. Topics will include: targeting to the eye to treat eye inflammation (1), targeting to liver to treat fatty liver (2) and targeting to the spleen to treat cancer (3).

Ongoing collaborations exist with:

1. Prof. Dr. Tina Wong and Dr. Chee Wai Wong (Singapore National Eye Centre (SNEC), Singapore Eye Research Institute (SERI)) and Dr. Bart Metselaar (Liposoma BV, Amsterdam Science Park, and Laurentia Holding, Naarden, The Netherlands))
2. Dr. Ruchi Bansal and Prof. Dr Jai Prakash (Advanced Organ Boengineering and Therapeutics, University of Twente (UT)) and Dr. Jiong-Wei Wang, Dr. Giorgia Pastorin and Prof Dr. Lee Chuen Neng (Surgery, Pharmacy, National University of Singapore) (NUS)) and Dr. Ruchi Bansal and Prof. Dr Jai Prakash (Advanced Organ Boengineering and Therapeutics, University of Twente (UT))
3. Dr. Joke den Haan, Prof. Dr. Yvette van Kooyk, PhD Maarten K Nijen Twilhaar (Molecular Cell Biology and Immunology, Amsterdam University Medical Center (Amsterdam UMC) and PhD Lucas Czenter and Dr. Cornelis van Nostrum (Pharmaceutics, Utrecht University) (UU))

THERAPEUTIC APPLICATION OF MRNA-LIPID NANOPARTICLES FOR IN VIVO GENE EDITING

Ying Tam

Chief Scientific Officer, Acuitas Therapeutics

11:00 am Sunday June 12

Acuitas' lipid nanoparticle systems (LNP) are enabling clinical development of a range of messenger RNA (mRNA)-based therapeutic approaches including genome editing. The identification and development of editing systems such as CRISPR/Cas9, zinc-finger nucleases and TALENs that precisely edit cellular

DNA sequences to stably correct mutated genes and deactivate disease-causing genes has led to a renaissance in gene therapy. The challenge of safe and efficient delivery of editing systems to target cells in the body can be addressed using non-viral LNP-mediated delivery of mRNA encoding the editing enzymes. LNP are able to accommodate the larger nucleic acid payloads often required for editing systems compared to alternative delivery approaches and may also provide potential safety benefits, by avoiding risks associated with viral delivery systems and prolonged enzyme expression to minimize the likelihood of editing at non-target sites. Data demonstrating the ability of intravenously administered mRNA LNP to mediate efficient in vivo editing and exert a pharmacodynamic effect at well tolerated doses in rodents and non-human primates will be presented.

LIPID-BASED NANOPREPARATIONS FOR STIMULI-SENSITIVE AND ORGANELLE-SPECIFIC TARGETING

Vladimir P. Torchilin

University Distinguished Professor, Pharmaceutical Sciences; Director, Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University

10:20 am Monday June 13

The efficacy of new generation lipid-based drug delivery systems, such as liposomes, lipid-core micelles, and solid-lipid nanoparticles can be significantly enhanced by providing them with the ability not only to specifically target certain organs or tissues or even individual cells, but also respond in desired way to local physiological stimuli, such as pH, temperature, redox conditions, and overexpression of certain enzymes, but also penetrate cells and deliver active agents to and into individual intracellular organelles. Such multifunctional preparations can be co-loaded with multiple drug combinations as well as combinations of various RNAs with drugs. We have developed several types of liposomes and lipid-core polymeric micelles based on PEG-phospholipid or PEI-phospholipid conjugates, which can firmly bind non -modified or reversibly-modified siRNA and be co-loaded into chemotherapeutic agents. In experiments with cancer cell monolayers, cancer cell 3D spheroids, and in tumor-bearing animals, it was shown that such nanopreparations significantly down-regulate target proteins in cancer cells, enhance drug activity, and reverse multidrug resistance.

To specifically unload such nanopreparations inside target tissues and even in individual cell compartments, we made them sensitive to local stimuli, such as lowered pH, hypoxia, or locally overexpressed matrix metalloproteases. Using pH-, hypoxia-, or MMP2-sensitive bonds between different components of lipid-based nanopreparations co-loaded with siRNA and drugs, we were able to make the systems specifically delivering biologically active agents in required areas as well as to and into individual cell organelles, which resulted in significantly improved therapeutic response.

ADVANCEMENTS IN LIPOSOMAL EXTRUSION TECHNOLOGY

Alex Torres

Global Product Manager, LIPEX® Product Line, Evonik Canada Inc. University

3:30 pm Sunday June 12

The LIPEX® extruder was first introduced to the market in 1985, and quickly became the industry-standard technology for producing liposomal formulations with a uniform particle size population. While proven and effective, the extrusion technology has not seen any major changes or improvements in the extruder design throughout its lifetime.

A major drawback of current extruders and filter membrane holders is the limited area of the filter utilized for extrusion, which causes filter membranes to clog and rapidly foul. This effect is further compounded by a subsequent increase in extrusion pressure that can impact the quality and performance of the final drug product. Frequent and complex filter membrane change during manufacturing is often required to overcome these challenges.

Through 3D computer-aided design, Evonik has now developed an improved membrane support technology to maximize the filter area utilization, significantly reducing back-pressure and filter clogging. This advancement in liposomal extrusion technology addresses process efficiency and product quality, leading to more robust manufacturing processes.

PHOSPHOLIPIDS IN VACCINES

TBD

Phospholipid Research Center

4:00 pm Sunday June 12

Since decades, vaccines and adjuvants using lipid carriers are explored for human and veterinary use. Examples of adjuvants are MF59 and ISCOMs (immune stimulating complexes) as alternative to aluminium salts. Recently, for vaccination against shingles the product Shingrix® of GSK was introduced. This vaccine uses liposomes containing QS21 an immunostimulating saponin as adjuvant. COVID-19 vaccines of Moderna (Spikevax®) and BioNTech/Pfizer (Comirnaty®) use LNPs (Lipid Nano Particles) to deliver mRNA to dendritic cells. Alternatively, liposomes/ISCOMs containing saponins are the adjuvant in the Novavax vaccine for COVID-19 (Novaxavid®). In these vaccines and adjuvants phospholipids are essential excipients.

In this seminar, an overview on the use of phospholipids and their role in vaccines is provided. The phospholipids are discussed with respect to quality and production requirements.

MRNA-VACCINES ON THE FAST TRACK – HOW POLYMUN CONTRIBUTED TO TACKLING THE PANDEMIC

Andreas Wagner

Head, Liposome Technology, Polymun Scientific

4:30 pm Sunday June 12

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. Data will be presented, which describe hurdles and solutions throughout these processes.

NEXT-GENERATION LIPID NANOPARTICLE TECHNOLOGIES TAILORED TO A VARIETY OF TISSUES

Dominik Witzigmann
NanoVation Therapeutics

2:45 pm Sunday June 12

We are at the most exciting point in human history with respect to the launch and development of nucleic acid therapeutics. In our lifetimes, we will see major diseases including rare genetic, infectious, autoimmune, malignant, and age-related disorders become treatable or even cured. The major issue we currently face is access to state-of-the-art delivery technologies to target diseased tissue safely and effectively. To enable the delivery of nucleic acids to a variety of (extra)hepatic tissues, we have developed a one-stop-shop lipid nanoparticle (LNP) toolbox. NanoVation`s technology portfolio encompasses methods of synthesizing lipids, specialty (ionizable) lipids, LNP compositions, surface-modifying lipids such as PEG-alternatives, as well as mRNA modifications. Our flagship technology, the long-circulating LNP (lcLNP™) platform, enables functional nucleic acid delivery to extrahepatic tissues such as bone marrow, tumors, or skin following intravenous injection. NanoVation`s expertise, platform technologies and services enable partners to rapidly develop their life-changing gene therapies.

This work was supported by an amazing scientific and operational team.

PORPHYRIN-LIPID NANOPARTICLES: BUILDING INTRINSIC MULTIFUNCTION INTO LNP

Gang Zheng
Professor & Canada Research Chair in Cancer Nanomedicine, University of Toronto; Associate Research Director, Princess Margaret Cancer Center

11:10 am Wednesday June 15

Porphyrins are light-absorbing molecules clinically used for photodynamic therapy and fluorescence imaging. Conjugating porphyrin to lysophospholipid forms porphyrin-lipid, a building block molecule that self-assembles into a liposome-like nanoparticle called porphysome. These simple porphyrin-lipid building blocks impart inherent multifunctionality to LNP for unparalleled theranostic utility (e.g., photothermal, photoacoustic, photodynamic, fluorescence, PET, MRI, and drug delivery). Using the same building blocks, we also created a large family of porphyrin-lipid nanoparticles with different sizes, shapes, and functions. Replacing porphyrin-lipid with other dye-lipid analogues such as texaphyrin-lipid resulted in nanotexaphyrins, a new class of radiotheranostic nanomedicine capable of alpha, beta and Auger electron therapies. The simple, intrinsically theranostic nature of the porphyrin-lipid nanoparticles epitomizes a “one -for-all” nanomedicine design paradigm and confers exciting clinical promise.

This work was supported by TFRI PPG, CIHR, OICR CATA, CCSRI, NMIN, CFI, Canada Research Chair Programs, and Princess Margaret Cancer Centre.



Liposomal/LNP Formulation of Drugs

Polymun has unique know-how and technology for the development and manufacturing of liposomal as well as LNP formulations.

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
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- Clinical & Regulatory Support

CLINICAL & REGULATORY SUPPORT
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- Pre-/Clinical Development Concepts
- Organisation of Clinical Studies
- Legal Representative
- Requests for Scientific Advice

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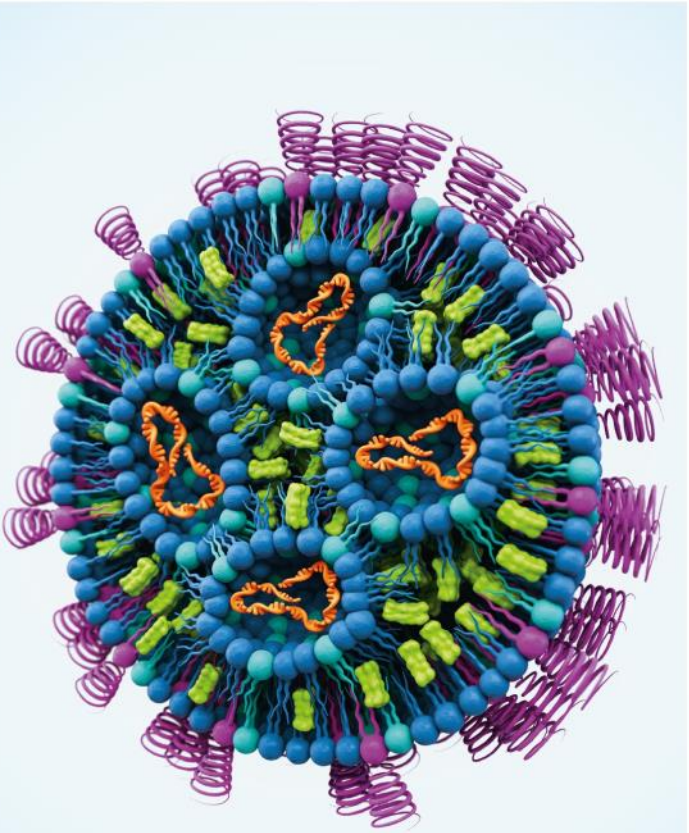
Leading the lipid revolution



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Alex Torres, Global Product Manager LIPEX®
June 12, 2022 | Industry Session 3: LNP & Lipid Design & Manufacturing

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1

SELF-AMPLIFYING RNA TECHNOLOGY FOR *IN SITU* PRODUCTION OF THERAPEUTIC ANTIBODIES

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The synergistic advancements in RNA biology and nanotechnology have driven the evolution of RNA as a disruptive therapeutic technology. Recent approval of mRNA vaccines against COVID-19 in 2020, demonstrated the effectiveness and clinical potential of this platform. Self-amplifying (saRNA) RNA is a novel next-generation RNA technology (derived from alphavirus) which is capable of self-replicating upon delivery into cytosol. saRNA demonstrates several advantages over conventional mRNA therapeutics, such as low dose requirement, and substantially increased levels and duration of protein expression. To date, saRNA is well explored as a vaccine candidate and the robust potential of saRNA could be expanded far beyond vaccines. It could be explored for *in situ* production of therapeutic proteins like therapeutic antibodies which could substantially reduce the cost and make therapeutic antibodies highly impactful and easily accessible and affordable. However, the saRNA molecular design must be well optimised to unleash the complete potential of saRNA technology for producing therapeutic antibodies. The current research work is focused at exploring different molecular designs of saRNA to produce various antibody formats, such as full-length antibody, nanobody, heavy-chain-antibody and provides detailed insight on how the molecular design of saRNA could impact the overall efficacy of therapeutic saRNA.

This work is supported by NSERC, CIHR and UBC start-up funds.

2

MICROFLUIDIC PRODUCTION AND RADIOLABELING OF siRNA LOADED LIPIDOID-POLYMER HYBRID NANOPARTICLES

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Gene-therapy is an evolving field with an enormous potential for treating a variety of diseases. Genetic inhibition is often achieved through RNA interference (RNAi) pathways using small interfering RNA (siRNA). Safe and effective delivery of these duplex nucleic acid structures presents a challenge. A promising class of nanoparticles, lipid-polymer hybrid systems (LPNs), are highly effective for the delivery of siRNAs, while reducing toxicity and offering controlled release; however, traditional batch production approaches lack size control and scalability. Microfluidic mixing techniques have emerged to generate nanoparticles with high controllability, low polydispersity and continuous production of uniform nanoparticles. However, current microfluidic prototyping approaches often utilize expensive materials and machinery. On the contrary, cost-effective polymeric thiol-ene based microfluidic chips offer several advantages such as versatility, biocompatibility, solvent resistance, and mechanical strength. We demonstrate a one-step microfluidic method to synthesize siRNA loaded LPNs, particularly with a high degree of size modulation (50 nm up to 150 nm). In addition to size investigation, we show a radiolabeling method for biodistribution studies, with efficient ^{111}In labelling. We are utilizing this to study the effect of LPN size on the pharmacokinetics of pulmonary delivered lipidoid-polymeric nanoparticles generated using thiol-ene microfluidic chips with SPECT/CT imaging. Our goal is to study the effect of LPN size on siRNA delivery to the lungs for the treatment of different inflammatory and infectious diseases.

LIPID NANOPARTICLES FOR DELIVERY OF SIRNA AND CHEMOTHERAPEUTIC PRODRUGS IN PROSTATE CANCER MODELS

3

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One of the biggest challenges for siRNA-based therapeutics is intracellular delivery, which can be achieved by encapsulating siRNA in Lipid Nanoparticles (LNPs). In this study, we demonstrate the therapeutic efficacy of LNPs loaded with siRNA targeting the androgen receptor (siAR) and taxane prodrugs in different prostate cancer (PCa) models. In addition, given that LNPs are cleared from circulation by the reticuloendothelial system (RES) in the liver and spleen, we explored whether transiently blocking the RES could improve LNP tumor accumulation. The used LNP formulation induced significant knockdown of the AR, both at the mRNA and protein level *in vitro*. In AR-sensitive cell lines, this knockdown resulted in significantly reduced cell viability at low siRNA concentrations (0.1 µg/ml). LNPs loaded with siAR and docetaxel/cabazitaxel derivates further decreased cell viability in these cell lines. As expected, in a mouse PCa xenograft model, LNPs accumulated predominantly in liver and spleen. Quantification of both LNP lipids and siRNA revealed only limited tumor accumulation after intravenous administration to tumor-bearing mice. This could not be improved by pretreatment with the RES blocker dextran sulfate, despite a largely shifted biodistribution pattern towards lungs and spleen. Alternative strategies to enhance tumor accumulation are therefore topic of future investigation.

This research was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (proEVLifeCycle, grant No 860303).

IN VIVO DIRECTED EVOLUTION OF IONIZABLE POLYESTER AND POLYETHER RNA-TRANSPORTERS BASED LIPID NANOPARTICLES (IPORT LNPS) BEYOND THE HEPATOCYTES

4

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RNA drugs have reached an inflection point and are now paving the way for a new wave of precision therapies. The design of non-hepatocyte RNA delivery systems without targeting ligands, however, remains a challenge. Here we used organocatalysis for controlled ring-opening polymerization to engineer ionizable Polyester and Polyether RNA-Transporters Based lipid Nanoparticles refered to as iPORT LNPs. Encouraged by their attractive profiles that include chemical structures with a high degree of flexibility, high tunability and adaptable functionality, we manipulated polymer architecture and adopted it in LNP co-assembly system to access the required physicochemical properties needed to enhance *in vivo* stability and endosomal escape.

In vivo screening of > 200 iPORT LNPs elicited mRNA functional delivery in lungs, spleen, and liver in a programmable manner. Interestingly, structure-activity relationships revealed that primary monomeric and initiator structures are key parameters for eliciting *in vivo* functional RNA delivery. Specifically, we show that monomer side-chains, side-chain location and monomer stereochemistry can be exploited for controlling functional mRNA delivery efficiency and tropism. Additionally, iPORT LNPs were shown to induce potent therapeutic effect in multiple disease models in mice. Overall, iPORT LNPs may expand the chemical space of evolvable lipopolymers/lipo-oligomers based LNPs with diverse, researcher-defined chemical repertoires.

5

ON THE MECHANISM OF TISSUE-SELECTIVE GENE TRANSFECTION BY LIPID-BASED CARRIERS

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The era of nucleic acid nanomedicine has arrived with the FDA approval of Patisiran, an siRNA encapsulated within LNP, and recent emergency use of mRNA vaccines against COVID-19, which are mRNA loaded LNP. These breakthroughs in the field of non-viral gene delivery have attracted substantial interest worldwide and demonstrate the potential of non-viral systems such as LNPs for delivery of new drugs in the future. A next step is to use these nanoparticle systems for targeting tissues other than the liver, and significant research efforts, such as development of new materials and extensive screenings, are being conducted. However, there are a lack of mechanistic studies in this area. Here, we aim to elucidate the mechanisms driving differences in gene expression of delivered nucleic acids by comparing two types of LNPs which have different tissue-tropism of pDNA delivery, one being liver-selective and the other spleen-selective. First, gene expression and biodistribution of each LNP was measured after the injection via tail vein into mice. We observed little difference in the biodistribution of the two LNPs despite the 100~1000-fold difference in gene expression. Next, we quantified the amount of pDNA and mRNA in each tissue both intracellulary and in the nucleus by qRT-PCR in order to evaluate the intracellular processes, such as nuclear delivery, transcription and translation. These results showed more than 100-fold difference in the translation step while there were little differences in nuclear delivery of pDNA or the amount of mRNA expression between two LNP deliveries. These quantitative analysis indicate that biodistribution is not sufficient for evaluation of functional gene delivery, and it is necessary to determine where the LNP transfection affects its biological process influencing protein expression such as translational process on the cell.

6

EXPRESSION OF EXOGENOUS PROTEINS IN DONOR PLATELETS TREATED WITH LIPID NANOPARTICLES

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Platelet transfusions are an integral treatment for managing bleeding and thrombocytopenia. Given the role of platelets in hemostasis and disease, enhanced platelets loaded with exogenous therapeutic protein could improve donor platelets as cell therapy for a variety of clinical indications. Although anucleate, mature platelets synthesize protein *de novo* during circulation and storage, making them amenable to mRNA gene therapy; however, there remains to be an effective transfection technique. Advancements in lipid nanoparticle (LNP) technology has enabled leading COVID vaccines and is an efficient method to deliver nucleic acids into target cells. Here we describe a transfection technique to express exogenous protein in donor platelets *ex vivo* using mRNA lipid nanoparticles. Transfected platelets maintain minimal levels of basal platelet activation, while maintaining coagulation capabilities and agonist responsiveness. Further optimization of this technology can lead to the development of more effective platelet therapies.

A NEW STRATEGY FOR MULTIPLE SCLEROSIS TREATMENT WITH AUTOANTIGEN-MODIFIED LIPOSOMES

7

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Current treatments for multiple sclerosis (MS) are chemotherapeutics which cause harsh side effects; thus, the development of a curative treatment is urgently required. Here we show an autoreactive immune cell-targetable approach using autoantigen-modified liposomes for the treatment of MS.

In these studies, experimental autoimmune encephalomyelitis (EAE) induced by autoantigenic myelin oligodendrocyte glycoprotein (MOG) or myelin proteolipid protein (PLP) peptide were used as models of primary progressive MS or relapsing-remitting MS, respectively, and MOG-modified liposomes encapsulating doxorubicin (DOX) (MOG-LipDOX) and PLP-modified liposomes encapsulating DOX (PLP-LipDOX) were used as the therapeutic drugs. Our results showed that progression of encephalomyelitis symptoms of MOG-immunized, or PLP- immunized mice was significantly suppressed by intravenous injection of MOG-LipDOX, or PLP-LipDOX, respectively, whereas DOX, non-modified liposomes encapsulating DOX (Cont-LipDOX), and autoantigen-modified liposomes without DOX did not show an effect. FACS analysis revealed that the number of both MOG-recognizable CD4+ T cells and effector Th17 cells in the spleen were reduced after MOG-LipDOX treatment, and that regulatory Treg cell abundance was concomitantly increased. These findings suggest that the use of autoantigen-modified liposomes holds promise as a therapeutic approach for the cure of MS.

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DEVELOPING A MULTIMODAL SINGLE-CELL RESOLUTION SYSTEM TO STUDY *IN VIVO* MRNA-LNP DELIVERY FOR CANCER IMMUNOTHERAPY

8

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A wide variety of lipid nanoparticle (LNP) functional screens have contributed to our understanding of the impact of their formulation composition on the biological activity of mRNA-LNPs. Currently, LNP formulations are typically screened in cell culture (*in vitro* or *ex vivo*) but this approach falls short in reliably predicting *in vivo* mRNA delivery. By contrast, *in vivo* studies are more reliable at predicting tissue and organ-specific LNP targeting but are expensive, time- and effort-consuming. Additionally, fluorescent or radio-labelled mRNA-LNPs often fail to provide high-dimensional single cell resolution, usually only revealing biodistribution of the LNPs at the organ or tissue level.

Employing flow cytometry has been used to study LNP cell specific targeting and *in vivo* cellular responses to nanoparticles. However, our studies using mass cytometry (CyTOF) revealed that LNPs can modify expression profiles of surface proteins in transfected cells, thus altering antibody binding characteristics and complicating accurate cell type classification schemes.

To mitigate these issues with standard mRNA-LNP screening approaches, we are developing a multimodal high-throughput *in vivo* screening approach in pre-clinical mouse cancer models. This pipeline will facilitate the identification of LNPs with defined immune cell targeting and regulatory potential, and accelerate the discovery of clinically relevant LNPs, capable of preferentially transfecting key immune cell subsets for cancer immunotherapy.

This work was supported by the NanoMedicines Innovation Network (NMIN).

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MIR-193A-BASED LNP DRUG TREATMENT FOR ACUTE MYELOID LEUKEMIA

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Acute Myeloid Leukemia (AML) is a blood cancer with a 5-year survival rate of 21% in Canada. We recently highlighted the potential of microRNAs (miRNA) encapsulated with LNPs to target specific mutations in leukemia *in vivo*. Our preliminary data underscored the therapeutic potential of miR-193a, a tumor-suppressive miRNA downregulated in AML patient cells, whose engineered overexpression delayed leukemogenesis in a preclinical murine AML model. The aim of this study is to investigate LNP-based approaches to restore miR-193a expression for the treatment of AML.

InteRNA Technologies developed INT-1B3, a proprietary LNP formulation of miR-193a-3p and the first of its kind as a miRNA-based therapeutic that is currently under investigation for solid tumor patients in a Phase I trial. To study the systemic delivery of synthetic miR-193a in AML cells, we tested INT-1B3 for its anti-leukemic effect in preclinical AML models. *In vivo* experiments showed an enrichment of miR-193a-3p in bone marrow at 48h after a single i.v. dose of INT-1B3 in mice that fully developed AML. Target engagement analysis revealed the downregulation of c-kit (a marker elevated in AML) in leukemic cells and modulation of the T and B cell activation pathways. In a preclinical AML immunocompetent mouse model, continuous i.v. treatment with INT-1B3 delayed leukemogenesis and increased overall survival rates compared to the controls. This data supports the future use of miRNA-LNP based drugs to treat AML and hopefully a preclinical rationale to initiate a Phase I study in BC.

This work was supported by Michael Smith Health Research BC.

Selected for oral presentation

BRINGING THE MRNA TECHNOLOGY TO THE ACTIVATED HEPATIC STELLATE CELLS *IN VIVO;* A NEW CONCEPT AND A PROMISING POTENTIAL

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Liver fibrosis is a chronic life-terminating disease with limited therapeutic modalities. The activated Hepatic Stellate Cells (aHSCs) are a fundamental player in the development of hepatic fibrogenesis. In the current work, we developed a novel platform based on lipid nanoparticles (LNPs) for the robust and selective mRNA delivery to aHSCs *in vivo* following systemic administration to mice undergoing liver fibrosis. A library of diverse pH-sensitive lipids was screened *in vitro* and *in vivo* to identify the top-performing candidates. The composition and physico-chemical properties of the LNPs were tweaked to control their *in vivo* performance. The robustness of mRNA delivery and the biosafety of LNPs were also investigated. We identified a promising pH-sensitive lipid with a high affinity to aHSCs. The composition and physico-chemical characteristics of the developed LNPs dramatically affected their *in vivo* selectivity, transfection efficiency, and mRNA delivery efficiency to aHSCs. Consequently, the optimum conditions for a ligand-free targeting of aHSCs were recognized for the first time. Furthermore, the robustness of mRNA functional delivery was confirmed upon recruiting diverse mRNAs. The developed LNPs demonstrated a high biosafety at high mRNA doses. The findings of our study resulted in the development of the first mRNA delivery platform to aHSCs, with a high potential for the gene therapy of liver fibrosis. Moreover, a new concept for ligand-free targeting of other hepatic cell populations beyond the hepatocytes was introduced. This work was supported by a *Grant-in-Aid* from Japan Society for the Promotion of Science (21F21105).

NANOPARTICLES ACCUMULATE IN THE FEMALE REPRODUCTIVE SYSTEM DURING OVULATION AFFECTING CANCER TREATMENT AND FERTILITY

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Throughout the female menstrual cycle, physiological changes occur that affect the biodistribution of nanoparticles within the reproductive system. We demonstrate a 2-fold increase in nanoparticle accumulation in murine ovaries and uterus during ovulation, compared to the nonovulatory stage, following intravenous administration. This biodistribution pattern had positive or negative effects when drug-loaded nanoparticles, sized 100 nm or smaller, were used to treat different cancers. For example, treating ovarian cancer with nanomedicines during mouse ovulation resulted in higher drug accumulation in the ovaries, improving therapeutic efficacy. Conversely, treating breast cancer during ovulation, led to reduced therapeutic efficacy, due to enhanced nanoparticle accumulation in the reproductive system rather than at the tumor site. Moreover, chemotherapeutic nanoparticles administered during ovulation increased ovarian toxicity and decreased fertility compared to the free drug. The menstrual cycle should be accounted for when designing and implementing nanomedicines for females.

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A NOVEL RNA LIPID NANOPARTICLE PLATFORM: GENE-EDITED CAR T CELLS FOR OFF-THE-SHELF CANCER THERAPY

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The expression of chimeric antigen receptor (CAR) on T cells turns a patient's own cells into cell-based therapies to combat various cancers. Despite high response rates, evidence suggests an increasing need for complex genetic engineering, particularly for allogeneic (off-the-shelf) CAR T cell therapy. A promising new approach for T cell engineering is the use of RNAs to express gene editing nucleases and therapeutic proteins, currently primarily delivered using electroporation. However, the sequential electrical pulses for multi-step gene engineering often leads to severe losses in cell viability making this approach inefficient.

Herein, we report on a novel and gentle method for CAR T cell engineering through LNP mediated RNA delivery. The RNA-LNPs were produced in less than 5 minutes using microfluidic nanoprecipitation. We show that LNPs are highly efficient at CRISPR-Cas9 knockouts (80 ± 8%) and at expressing the CAR protein (91 ± 5%) in primary human T cells. All the while, LNPs maintain high cell viability (>90%). We knocked out clinically relevant targets such as the T cell receptor (TCR) and CD52 for allogeneic CAR T cell therapy, then expressed an anti-CD19 CAR construct. The resulting gene-edited CAR T cells were co-cultured with leukemia cells, showing highly efficient target-specific killing, whereas gene editing itself had no negative impact on therapeutic potential.

This work exemplifies the power of LNPs for complex CAR T cell engineering. Our optimized manufacturing workflow allows for rapid research and development for cell therapy advancement, while the scalable microfluidic technology enables the smooth translation from benchtop to the clinic.

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A NOVEL LIPOSOMAL IRINOTECAN (CPT-11) FORMULATION WITH POTENTIAL TO TREAT COLORECTAL CANCER

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Irinotecan (CPT-11) is a potent topoisomerase I inhibitor that is currently used in first and second-line chemotherapy treatments for multiple cancers. Onivyde is the first clinically approved liposomal CPT-11 but offers limited therapeutic response in treating metastatic colorectal cancer (mCRC) and its use is associated with a high incidence rate of severe GI toxicity. Our lab previously described a liposomal formulation of CPT-11: Irinophore C™ (IrC™), which demonstrated improved efficacy in over twenty different xenografts models. Importantly, IrC™ exhibited significantly reduced GI toxicity in a validated rat model when compared to free CPT-11. This has the potential to enable the use of liposomal CPT-11 for treatment of mCRC. The method used to prepare IrC™ relied on copper, a metal ion that can complex with CPT-11. However, more than 75% of the Cu(II) ion used to prepared IrC™ was lost from the liposome as the drug was encapsulated. Here we present that an alternative encapsulation method where there is little loss of encapsulated Cu(II). The new formulation, referred to as Irinosome High-C, exhibits an increased loading capacity for CPT-11, where a maximum drug-to-lipid ratio of 0.8 (mol/mol) can be achieved. The resulting formulation exhibits improved CPT-11 retention as determined by *in vitro* and *in vivo* assays. Cryo-electron microscope analysis of the formulation revealed optical differences between the Irinosome High-C and IrC™. The new formulation is substantially more efficacious in vitro against CT26 and MC38 cell lines compared to the free drug. This novel liposomal CPT-11 may prove superior to Onivyde in treating mCRC.This work is supported Canadian Institutes of Health Research (CIHR), Nanomedicine Innovation Network (NMIN) and Cuprous Pharmaceuticals Inc.

Selected for oral presentation

CURCUMIN INCREASES ENCAPSULATION OF SN-38 AND LOWERS POLYDISPERSITY IN POLYMERIC NANOPARTICLES

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The anti-cancer drug CPT11 is converted to SN-38 in the body, but only 2-8% of the CPT-11 administered to a patient is converted to SN-38, which is 100-1000 times more potent. Use of pure SN-38 is limited because it is virtually insoluble in water and is inactive above pH 6, making it a prime candidate for encapsulation. However, it is very difficult to encapsulate SN-38. By combining SN-38 with another drug, there is potential to increase its efficacy and encapsulation efficiency.

Using a liquid-gas two-phase microfluidic reactor, we manufactured particles co-encapsulating SN-38 and curcumin in amphiphilic polymer PCL-PEO. We characterized these particles for drug content, size, and polydispersity, and used our optimized formulations to perform cytotoxicity studies and study release kinetics. Our data demonstrate the utility of co-encapsulation of SN38 in nanoparticles.

Particles without curcumin had a ~6% encapsulation efficiency of SN-38, while particles with a combination of curcumin and SN-38 had an encapsulation efficiency of ~12%. Particle size remained constant at approximately 50 nm up to a ratio of 10:1 of curcumin:SN-38. Particle polydispersity decreased with increased curcumin, from a polydispersity of 0.34 with SN-38 alone to a polydispersity of 0.07 at a ratio 100:1 curcumin:SN38.

This work was supported by the PoND CREATE grant and the University of Victoria

RHENIUM LIPOSOME (^{186}RNL) IN A PHASE 1/2A DOSE ESCALATION TRIAL FOR GLIOBLASTOMA. BENEFITS OF THE LIPOSOME CARRIER FOR INTRATUMORAL SOLID TUMOR THERAPY

16

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Background:^{186}RNL via image-guided direct intratumoral convection enhanced delivery (CED) allows targeted, beta-particle 2 mm pathlength radiation achieving a wide, favorable therapeutic index. The liposome carrier allows the ^{186}Re to move through the tumor while it also enhances retention within the tumor with this therapy not requiring drug release from the liposome. The 9% gamma photon allows imaging of the precise distribution achieved by the liposomes within the tumor. **Methods**: A dose escalation trial to determine the maximum tolerated dose of ^{186}RNL in patients with recurrent glioma treated with a single convection enhanced delivery (CED) administration of ^{186}RNL. **Results**: 21 patients across 8 cohorts received 1.0-22.3 mCi in a volume of 0.6-8.80mL. The maximum CED rate ranged from 5-20µl/min with 1-4 catheters/patient. Mean absorbed radiation dose to the tumor was 255 Gy (8.9-740Gy) while exposure outside the brain and white matter was negligible. No dose limiting toxicities have occurred with only one grade 3 adverse event. In this preliminary Phase I/2a data, beneficial effects with increased survival were observed when doses > than 100 Gy dose were delivered to >80% of a tumor. **Conclusion:** This novel liposome carried convective radiotherapeutic approach promises to be useful for a large variety of tumors in which local control is difficult or where all other therapies are exhausted, ineffective or too toxic. Advancement to Phase 2b/3 pivotal trial is planned. Liposomes provided a significant controlled delivery advantage for this local radionuclide therapy.

17 IMPROVED TUMOR ANTIGEN DELIVERY AND ANTITUMOR IMMUNITY VIA AN INNOVATIVE LIPOSOMAL IMMUNE BOOSTER

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Currently, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) serve as the standard of care for peritoneal carcinomatosis, however recurrence still results in high mortality due to incomplete remission. To improve the therapeutic outcome, we developed a liposomal formulation that can enhance intraperitoneal delivery of a toll-like receptor agonist, resiquimod (R848), through a cell penetrating peptide (CPP) modification. The CPP-R848 showed prolonged peritoneal retention by 36-fold in peritoneal fluid and 5.3-fold in peritoneal cells compared to our previous cationic formulation (DSTAP-R848) without spiking the plasma concentration. CPP-liposomes facilitated model antigen uptake by dendritic cells (DCs) by 4-fold compared to the DSTAP-liposomes. CPP-R848 induced a 1.5-fold increase in interferon α (IFN-α) in peritoneal fluid compared to DSTAP-R848 without altering plasma levels. In a preliminary efficacy study with mice intraperitoneally inoculated with CT26 colon cancer cells, CPP-R848 in combination with oxaliplatin, an immunogenic cell death inducer that is used in HIPEC, greatly inhibited tumor progression in the peritoneal cavity. Consequently, the survival study indicated that 80% of mice treated with this combination became free of tumor 27 days after treatment and remained tumor free for >100 days (n=5). Taken together, CPP-R848 is an innovative and promising immunotherapeutic drug for tumors that have spread in the peritoneal cavity.

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18 AN ASSESSMENT OF DIETHYLDITHIOCARBAMATE (DDC) MEDIATED RELEASE OF CX5461 FROM COPPER CONTAINING LIPOSOMES

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CX5461 was developed as an RNA polymerase I inhibitor and is currently being evaluated in patients with acute myelocytic leukemia and breast cancer. The low aqueous solubility of CX5461 has been addressed through use of low (<4.0) pH buffers and this solution is not ideal. Our research has described a method to enhance solubility of CX5461 through use of metal binding. CX5461 was added to the outside of the copper-containing liposomes and encapsulated in the liposome through copper binding. Unlike previous anticancer drug formulations, the slow dissociation of CX5461 from liposomes could not be changed through simple changes in liposomal lipid composition. We hypothesized that the rate limiting step for CX5461 release was likely dissociation of copper from the CX5461. To assess this hypothesis, we studied how the addition of DDC, a strong copper binder, could enhance the release of CX5461 from liposomes. DDC was thought to serve as a competitive ligand that would bind copper stronger than CX5461. In vitro release studies were completed with Cu(CX5461) liposomal formulations where the liposomes were composed of DSPC/Chol or DMPC/Chol (55:45 mol/mol). At indicated time points and as a function of temperature, aliquots were removed, liposome-associated drug was separated, and analyzed for CX5461, lipid, and copper contents. The results indicated that addition of DDC did not increase, but decreased, drug release rates. Surprisingly, these studies also demonstrated that copper binding to CX5461, destabilized the drug. For this reason, further study will be performed to investigate the chemical stability and biological activity of CX5461 when it binds to different transition metals.

UNPRECEDENTED LONG CIRCULATING LIPID NANOPARTICLES ENABLED BY HIGH-DENSITY GD-DTPA-BIS(STEARYLAMIDE) FOR ENHANCED TUMOR MRI

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Porphysome (PS) nanoparticles are composed of porphyrin-conjugated lipids which allow them to perform a variety of diagnostic and therapeutic applications. We recently designed a PS formulation integrating diethylenetriaminepentaacetic acid gadolinium (III) bis(stearylamide) (Gd-DTPA$_{BSA}$) lipids into the PS lipid membrane which provide them with additional magnetic resonance imaging (MRI) contrast agent functionality. This design of Gd-DTPA$_{BSA}$ integrated PSs (Gd-dPS$_{BSA}$) has resulted in exceptional serum stability and T_1 and T_2 relaxivities of 13mM^{-1}s^{-1} and 19mM^{-1}s^{-1}, respectively. *In vivo* studies in mice showed Gd-dPS$_{BSA}$ having significant enhanced retention in blood circulation with a half-life of 13.6 hours and high tumor accumulation in select models with quantitative biodistribution showing up to 19.5% injected dose per gram in the tumor 72 hours post-injection. Additionally, Gd-dPS$_{BSA}$ displayed excellent MRI tumor enhancement over 24, 48 and 72 hours with T-$_1$ relaxation time percent changes from baseline of 35.8%, 38.2% and 38.3%, respectively. These results indicate the high potential of Gd-dPS$_{BSA}$ as a novel contrast agent and for other diagnostic and therapeutic functions using Gd-DTPA$_{BSA}$ lipids.

This work was supported by CIHR.

PREPARATION OF LIPOSOMES CONTAINING COPPER DIETHYLDITHIOCARBAMATE FOR TUMOR TREATMENT VIA DUAL CENTRIFUGATION

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Copper diethyldithiocarbamate (Cu(DDC)2) is a complex formed when two molecules of diethyldithiocarbamate (DDC-) chelate a single Cu2+-ion. Although the drug has a potent toxic effect on cancer cells, its insolubility in aqueous solution limits its usefulness as a treatment option. However, this limitation can be circumvented by incorporating the drug into a nanoparticulate formulation. Therefore, the preparation of liposomes loaded with Cu(DDC)2 for cancer treatment is being studied in detail by our research group.

Here we report a novel preparation of Cu(DDC)2-liposomes by dual centrifugation. The dual centrifuge utilizes shear forces and an extensive homogenization process and enables the sterile preparation of CuSO4-filled liposomes. Subsequently, the copper-liposomes are converted into Cu(DDC)2-liposomes by remote loading with DDC-.

After sample purification, UV-Vis measurements were used to determine the concentration of the entrapped drug in the final liposomes. In addition, various liposomal properties such as hydrodynamic diameter, homogeneity and drug to lipid ratio were investigated and compared with Cu(DDC)2-liposomes prepared by extrusion. The stability of the liposomes is being determined at different storing temperatures in ongoing studies.

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LIPOSOMAL FORMULATION OF A DISULFIRAM METABOLITE THAT ACTIVATES ANTI-CANCER IMMUNITY THROUGH IMMUNOGENIC CELL DEATH

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Despite increasing clinical success of immune checkpoint inhibition (ICI) in solid tumours, only a fraction of patients respond to treatment. An immunosuppressive microenvironment often underlies this resistance, and recent clinical evidence supports the use of ICI with activators of immunogenic cell death (ICD) to stimulate adjuvant signaling and improve antitumour immune response. Disulfiram is an alcohol aversion drug that has been studied against cancer for decades, and recent reports have shown its potential to activate ICD. We have previously described a liposomal formulation of copper diethyldithiocarbamate ($Cu(DDC)_2$), the active anti-cancer form of disulfiram. Here, we describe an improved formulation, which relies on the copper ionophore ability of diethyldithiocarbamate (DDC), a metabolite of disulfiram, to shuttle $Cu(DDC)_2$ into the liposome. The novel formulation was efficacious in an immunocompetent 4T1 tumour model, and triggered expression of damage-associated molecular patterns (DAMPs) in CT26 murine colon carcinoma cells. A vaccination model of ICD resulted in 50% tumour-free animals as compared to 10% in the untreated group following vaccination with cells treated with the formulation. We have generated a new formulation of Cu $(DDC)_2$ that is more suitable for injection into animals, and have provided further evidence to support the immune activating role of $Cu(DDC)_2$ in the first *in vivo* validation of ICD with $Cu(DDC)_2$ using the gold-standard vaccination model. This research was funded by CIHR (153132), CCSRI (705290), and NMIN.

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IN VITRO AND *IN VIVO* VALIDATION OF A NEW HYBRID MOLECULE LOADED IN LIPOSOMES FOR MELANOMA MANAGEMENT

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A new hybrid molecule (HM), combining derivatives of tyrosine and triazene, was recently synthesized showing high specificity towards tyrosinase, which is overexpressed in melanoma, and cytotoxic properties towards tumour cells.

To enhance the anti-melanoma effect, HM was efficiently loaded in long blood circulating liposomes (LIP HM). In a syngeneic subcutaneous melanoma model, LIP HM reduced tumour progression to a much greater extent than HM in the free form, with concomitant increased caspase activity, an apoptosis marker, and decreased tyrosinase activity in tumour sites. In a syngeneic metastatic melanoma model, a superior anti-tumour effect was also observed, with markedly reduced lung metastases compared to control treatments including free HM and temozolomide. Biodistribution studies using ^{111}In-labelled LIP HM demonstrated that liposomes were able to target tumor sites.

Overall, the newly HM nano-formulation appears as a potent therapeutic strategy against melanoma.

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Selected for oral presentation

RESIQUIMOD DELIVERED BY CATIONIC LIPOSOMES ENHANCED ANTITUMOR IMMUNITY IN PERITONEAL METASTASES

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Colorectal metastases in peritoneum are currently treated by cytoreductive surgery and hyperthermic intraperitoneal chemotherapeutics. This standard of care is associated with high morbidity, mortality, and recurrence rates. To augment the existing therapies, we developed a liposome-based delivery system containing 1,2-stearoyl-3-trimethylammonium-propane (DSTAP), a cationic lipid, to localize a toll-like receptor agonist, resiquimod (R848), in the peritoneal cavity (PerC) for enhancing antitumor immunity in the PerC. The liposomes delivered intraperitoneally prolonged peritoneal retention of R848 by 14-fold while retarding its systemic absorption, leading to a 5-fold decreased peak plasma concentration compared to free R848 in mice. The DSTAP-liposomes were found in ~40% of the dendritic cells in the PerC. DSTAP-R848 significantly increased interferon α (IFN-α) in the peritoneal fluid by 2-fold compared to free R848, without eliciting systemic induction. Combined with oxaliplatin, a cytotoxic agent inducing immunogenic cell death, DSTAP-R848 effectively inhibited the progression of CT26 murine colorectal tumor in the PerC, whereas a mild inhibitory effect was observed in the combination with free R848. Additionally, the combination of oxaliplatin and DSTAP-R848 significantly increased infiltration of CD8+ T cells in the PerC compared to oxaliplatin combined with free R848, indicating the enhanced antitumor immunity. The results demonstrate that DSTAP-R848 can potentiate existing therapy augmentation for treating peritoneal colorectal metastases via localized immune activation. This work was supported by the Nanomedicine Innovation Network, Canadian Institutes of Health Research, the Natural Science and Engineering Research Council in Canada, the Canadian Cancer Society, and the National Organization for Rare Disorders.

TARGETED LIPOSOMAL DRUG DELIVERY TO PEDIATRIC SARCOMAS

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Pediatric sarcomas account for about 15% of pediatric cancers, with high relapse rates and extremely poor prognosis. The aggressive chemotherapies needed to fight relapsed tumors have a significant toxicity generating late side effects, a major complication in pediatric oncology. Liposomal formulations can decrease systemic side effects by passive accumulation through the enhanced permeation and retention (EPR) effect. We investigated the possibility to further increase local drug concentration, and to overcome the clinical limitations of the EPR effect, by targeting liposomes to the tumor site. We selected ligands (peptides, nanobodies) with strong affinity for rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children. These were used to formulate liposomes (egg sphingomyelin, cholesterol, $C_{16}PEG_{2000}$-ceramide (PEGC), and either DSPE-PEG_{2000} (DPEG) or peptide-labeled DPEG) loaded with vincristine (VCR). Initially, in a subcutaneous xenograft mouse model of RMS, we could observe higher accumulation of liposomal VCR in tumors and increased circulation time compared to free VCR. However, targeting of liposomes with a furin-binding peptide (TmR) did not further increase VCR accumulation in tumors, nor the therapeutic effect. We next selected peptides with greater affinity to RMS (F3), and nanobodies targeting FGFR4, which is highly specific for RMS. *In vitro* binding of liposomes to RMS could be dramatically increased by these ligands. The *in vivo* efficacy is under evaluation in an orthotopic model of RMS.

This work was supported by the Phospholipid Research Center, Heidelberg, Germany.

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A LOW DOSE OF LIPOSOMAL GP100 ANTIGEN COMBINED WITH CPG-ODN IMPROVED PD-1 BLOCKADE IMMUNOTHERAPY IN MELANOMA MODEL

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Lack of pre-existing tumor infiltrated T cells resulting in resistance to programmed cell death protein 1 (PD-1) blockade therapies can be solved by combining with anti-cancer vaccines and CpG-ODN in increasing T cell expansion and infiltration. Therefore, we prepared an ex vivo dendritic cell-based (DC) vaccine pulsed with a low dose of either liposomal or non-liposomal gp100 antigen (2.8 µg) plus CpG-ODN (800 ng) formulations and evaluated its anti-tumor activity in combination with anti-PD-1 therapy. Our results showed a combination of liposomal peptide plus CpG-ODN pulsed DC with anti-PD-1 antibody was more efficacious, as evidenced by a significant increase in T_{eff}/T_{reg} TILs with a marked fourfold elevation of IFN-γ expression level in the tumor site of treated mice which reversed resistance to PD-1 blockade in a CD8 T cell-dependent manner. Furthermore, this combination also led to a remarkable tumor remission and prolonged survival rate in melanoma-bearing mice compared to non-liposomal peptide plus CpG-ODN or single-treated liposomal peptide formulations. Our results provide essential insights to devise combining regimens to improve the efficacy of immune checkpoint blockers even by a low dose of peptide and CpG-ODN.

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ON THE IMPACT OF PHOSPHOROTHIOATE MODIFICATIONS ON LIPID NANOPARTICLE MORPHOLOGY

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Advancements in nucleic acid therapeutics have allowed for broader potential treatment of diseases at the genetic level. However, nucleic acids are prone to degradation by serum endonucleases and clearance through the immune system. To address these barriers, nucleic acids include chemical modifications to improve stability or modulate immune responses. Lipid nanoparticles (LNP) have enabled increased potency of these therapeutics by protection through encapsulation and improving their intracellular delivery. An assumption thus far is that non-specific ionic interactions drive LNP formation and chemical modifications to the nucleic acid backbone do not impact LNP delivery. Here, we demonstrate that chemical modifications do impact LNP morphology substantially, and phosphorothioate modifications produce stronger interactions with ionizable amino lipids, resulting in enhanced entrapment. This work represents a major first step towards a greater understanding of the interaction between the lipid components and nucleic acids within an LNP. We use a 15-mer antisense oligonucleotide (ASO) against *c-myc* as our nucleic acid in the form of unmodified phosphodiester (PO-ASO) and complete phosphorothioate (full-PS-ASO). Through cryogenic transmission electron microscopy (cryo-TEM) and measuring encapsulation efficiency, we have found that LNP formulations of PO-ASOs result in decreased encapsulation as the amount of cationic lipid is increased; however, LNPs formulated with full PS-ASOs maintain high levels of encapsulation regardless of composition, where differences in morphology can be observed. This work is supported by the Canadian Institutes of Health Research, NanoMedicines Innovation Network, and UBC Genome Science and Technology program.

SIMULTANEOUS, SINGLE-PARTICLE MEASUREMENTS OF SIZE AND LOADING GIVE INSIGHTS INTO THE STRUCTURE OF DRUG-DELIVERY NANOPARTICLES

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Albert Kamanzi,[1] Yifei Gu,[1] Radin Tahvildari,[2] Zachary Friedenberger,[2] Xingqi Zhu,[2] Romain Berti,[1] Marty Kurylowicz,[2] Dominik Witzigmann,[3] Jayesh A. Kulkarni,[3] Jerry Leung,[3] John Andersson,[4] Andreas Dahlin,[4] Fredrik Höök,[5] Mark Sutton,[2] Pieter R. Cullis,[3] and Sabrina Leslie*[,1]

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Nanoparticles are promising delivery platforms that can be used to deliver a wide range of drugs. However, the low drug delivery efficiency is constraining the clinical translation of nanoparticle platforms. To optimize the efficiency, understanding therapeutic properties as functions of biophysical parameters is essential. Current existing tools make an average measurement across a heterogeneous population, obscuring potentially valuable information. In this work, we develop and apply a method for characterizing nanoparticles with single-particle resolution. We use CLiC (Convex Lens-induced Confinement) microscopy to isolate and quantify the diffusive trajectories and fluorescent intensities of individual nanoparticles in microwells for long periods of time. First, we benchmark measurements of fluorescent polystyrene nanoparticles against prior data to validate our approach. Second, we apply our method to investigate the size and loading properties of lipid nanoparticle (LNP) containing silencing RNA (siRNA) as a function of lipid formulation, solution pH, and drug-loading. Using the correlation between the intensity and size measurements, we gain insight about LNP structure and how the siRNA is distributed in the LNP. Beyond introducing an analytic for size and loading, this work allows for future studies of dynamics with single-particle resolution, such as LNP fusion and drug-release kinetics.

TOPOLOGICAL CONTROL AND ULTRA-HYDRATION: DESIGN OF A NOVEL CRYOPRESERVATIVE FOR HIGHLY EFFICIENT PROTEIN AND LIPID NANOPARTICLE STABILIZATION

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Stabilization and preservation during cryo-storage of Lipid nanoparticles (LNP) is paramount to maintain their activity and to reduce the cost of these precious products. Thus, highly optimized lyo- and cryo-preservation agents are vital to prevent damage, aggregation, and loss of therapeutic activity. Macro-molecular stabilizing agents have been known for their ability to protect precious biological compounds during storage and transport; however, there are no established design criteria for developing these agents.

We have designed and synthesized a set of novel ultra-hydrating polymers (UHPs) with a hydration number in the range of 14 to 27 water molecules per monomer whereby the hydration depends on the polymer topology and chemical nature. The hydration of UHP is close to or higher than trehalose, the gold standard for ultra-hydrating molecules. UHPs exhibit low intrinsic viscosity and our data suggests that they are highly effective in stabilizing and preserving the activity of mRNA encapsulated into LNPs during freezing at -70 °C and lyophilization. UHPs protected the mRNA-LNP from cryo-damage as evident from their particle size, polydispersity (PDI), and encapsulation efficiency. The addition of UHP outperformed all other tested cyro-preservatives. Finally, the UHP preserved mRNA-LNP also showed excellent activity after cryo-storage as demonstrated by transfection in HeLa cells. Building on these results, in vivo evaluation is ongoing in order to further understand the preservation capabilities of this new class of cryoprotecting polymers. The novel UHPs were also tested to be highly effective in the protection of therapeutic proteins.

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VISUALIZING PH-INDUCED LIPID NANOPARTICLE DYNAMICS PROVIDE A BETTER UNDERSTANDING OF THEIR FORMATION AND DRUG DELIVERY MECHANISM

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In recent years, Ionizable Lipid nanoparticles (LNPs) have proven to be ideal drug delivery vehicles for RNA nanomedicines. This is especially true for LNP formulations containing ionizable cationic lipids, which get protonated/de-protonated as a function of buffer pH. These dynamic changes in the LNP structure are essential to their drug delivery mechanisms, through allowing them to: 1) encapsulate negatively charged nucleic acid drugs during particle formation (at low pH, ~4); 2) remain neutral during delivery and circulation (at physiological pH, ~7.4); and 3) interact with maturing endosomes in the target cell (at low pH, ~5), allowing their cargo to be delivered. A detailed understanding of these processes is necessary to further optimize their performance. In this work, we use Convex Lens-induced Confinement (CLiC) microscopy, in combination with Förster resonance energy transfer (FRET) measurements, to study LNP dynamics as a function of increasing buffer pH. Our results show the effect of varying buffer ionic strength, LNP formulations and drug loading ratios on LNP dynamics. Next, we apply our measurements to study LNP interactions with model endosomes, as a function of decreasing buffer pH. These measurements provide a new method for characterizing potential drug candidates, hence allowing for a better understanding of their drug release in target cells, by connecting the LNP dynamics to their physiological performance.

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SCREENING FOR OPTIMAL LIPOSOME PREPARATION CONDITIONS BY USING DUAL CENTRIFUGATION

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Dual Centrifugation (DC) is an in-vial homogenization technique for the preparation of liposomes with high *encapsulation efficiencies (EE)* for water soluble drugs of over 50%. The technique is based on the additional rotation of vials filled with a viscous lipid-buffer mixture during conventional centrifugation. Liposome sizes and size distributions as well as *EE*-values strongly depend on the viscosity of the lipid/ buffer mixture and thus from the lipid concentration. Furthermore, the lipid-concentration also influences the lamellarities since the typically used lipid concentrations are higher than needed for the formation of densely packed small unilamellar vesicles (SUV). Instead, the excess of lipid needed for establishing sufficient viscosity results in the formation of small multilamellar vesicles (SMV).To speed up liposome screening utilizing DC-homogenisation, time-resolved fluorescence spectroscopy (TRF) was introduced to determine entrapped and non-entrapped calcein without removing the non-entrapped marker from the liposomes. As a further advantage, *EE* (TRF) and size/size distribution (DLS) can be determined from the same cuvette. To rapidly determine lamellarity of DC-prepared liposomes, a screening method based on quenching a membrane-bound fluorescence marker was developed and validated by cryo-TEM.

A rapid method to screen liposome preparation conditions was developed, allowing the preparation and complete characterisation of up to 40 liposomal formulations within one working day.

Selected for oral presentation

HOW TO ANALYZE AND QUANTIFY LIPID COMPOUNDS AND HYDROPHOBIC DRUGS SIMULTANEOUSLY BY HPLC-DAD-CAD

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Liposomes have proven to be attractive carriers for hydrophobic drugs to enhance their solubility and improve their therapeutic applications. For formulation development and quality control, a precise quantification of both drug and liposomal excipients is required by regulatory authorities. To address this need, a simple and time-saving method for their concurrent analysis was established using high performance liquid chromatography (HPLC). As most commonly used phospholipids cannot be detected spectrophotometrically, a diode array detector (DAD) in combination with a charged aerosol detector (CAD) was used. This combined detection system enables the analysis of a broad range of liposomal formulations.

The method was validated with cholesterol, POPC, DOPC and DSPC as representative widely used liposomal excipients. The wide detection range of these excipients (10-1000 µg/mL) enabled the analysis of samples with different drug to lipid ratios. Low limits of detection (≤ 1.8 µg/mL) and limits of quantification (≤ 5.9 µg/mL) were achieved for all analytes. With regards to multiple-component formulations, the possibility to quantify mixtures of the tested excipients was also demonstrated. Finally, the applicability of the method was demonstrated with mitotane liposomes which are currently in development as a potential novel treatment for adrenocortical carcinoma .

ON THE INFLUENCE OF IONIC STRENGTH TOWARDS THE FORMATION OF LIPID NANOPARTICLES

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Lipid nanoparticle (LNP) formulations of nucleic acids have shown incredible clinical utility for therapeutic and vaccine applications. Such formulations are typically composed of ionizable cationic lipids, phospholipids, cholesterol, and poly-ethyleneglycol (PEG)-lipids, where rapid mixing of lipids with an acidic (pH 4) aqueous phase followed by pH neutralization produces the final LNP with an electron-dense oil phase as the particle core. However, conflicting observations have been reported on the particle morphology immediately following rapid mixing before pH neutralization, with either a combination of electron-dense and liposomal structures, or the formation of almost entirely electron-dense particles. Here, we use Förster resonance energy transfer (FRET), cryogenic transmission electron microscopy, small angle X-ray scattering, and dynamic light scattering to show that buffer composition and ionic strength play a major role in modulating particle formation. In particular, increasing buffer ionic strength at pH 4 induces particle fusion to form multilamellar structures that encapsulate macromolecular payloads and are similar in size to particles formed via a pH-dependent fusion process. These studies help advance the understanding of LNP formation and morphology and can be applied to further inform formulation practices.

Selected for oral presentation

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INVESTIGATING THE COMPOSITION OF LIPID NANOPARTICLES UPON LOADING BY LC-MSMS

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Lipid nanoparticle (LNP) drug delivery systems have taken on a leading role in the nanomedicine field, and especially so during the COVID 19 pandemic, as the Comirnaty (Pfizer/BioNTech) and Spikevax (Moderna) vaccines are comprised of mRNA delivery nanoparticles. With this increased interest in liposomes, the research is being pushed toward more complex lipid nanoparticles that combine drug delivery with diagnostics (theranostics), active targeting, multimodal therapeutics, targeted drug release and so on. With that in mind, what is lacking in this area are better analytical methods to investigate these carriers, and further, a description of how loaded liposomes differ from their “empty” counterparts. In this work, we present a quantitative liquid chromatography-tandem mass spectroscopy (LCMSMS) method used to analyse various LNP compositions with different cargos (RNA and DNA, hydrophilic and hydrophobic drugs) to determine the relative lipid composition before and after drug loading. Phospholipids, ionizable lipids, PEGylated lipids, triglycerides and cholesterol will be the LNP building blocks to quantified with this method. Different formulation methods will also be explored in order to draw conclusions about the self-assembly of LNPs.

This work was supported by the NanoMedicines Innovation Network (NMIN).

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ANTI-PEG ANTIBODIES COMPROMISE THE INTEGRITY OF PEGYLATED LIPID-BASED NANOPARTICLES VIA COMPLEMENT

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PEGylation of lipid-based nanoparticles and other nanocarriers is widely used to increase their stability and plasma half-life. However, either pre-existing or de novo formed anti-PEG antibodies can induce hypersensitivity reactions and accelerated blood clearance through binding to the nanoparticle surfaces, leading to activation of the complement system. In this study, we investigated the consequences and mechanisms of complement activation by anti-PEG antibodies interacting with different types of PEGylated lipid-based nanoparticles. By using both liposomes and LNPs, we demonstrate that complement activation triggered by anti-PEG antibodies can compromise the bilayer/surface integrity, leading to premature drug release. Anti-PEG antibodies also can induce deposition of complement fragments onto the surface of PEGylated lipid-based nanoparticles and induce the release of fluid phase complement activation products. We identified a major role for the classical complement pathway in the early activation events leading to the activation of C3. Our data also confirms the essential role of amplification of C3 activation by alternative pathway components in the lysis of liposomes. Finally, the levels of pre-existing anti-PEG IgM antibodies in plasma of healthy donors correlated with the degree of complement activation (fixation and lysis) induced upon exposure to PEGylated liposomes and mRNA LNPs. Taken together, anti-PEG antibodies trigger complement activation by PEGylated lipid-based nanoparticles, which can potentially compromise their integrity, leading to premature drug release or cargo exposure to serum proteins. This was supported by EU Horizon 2020 Grant No. 825828.

CONTINUOUS FLOW MANUFACTURING OF LIPOSOMAL DISPERSIONS, FROM FORMATION TO IN-LINE QUALITY CONTROL

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Liposomal drug products have maintained a frontrunner position among FDA/EMA-approved nanomedicines. Yet, conventional industrial-scale methods for liposome manufacturing suffer from inefficiency and scalability issues. To address such drawbacks, continuous flow manufacturing (CFM) has emerged as a viable alternative. Nevertheless, CFM is predominantly focused on the initial liposome formation step, while production steps later in the manufacturing procedure are less often addressed. Here, we designed a CFM set-up that integrates the entire manufacturing procedure of liposomal nanomedicines. We identified lipid concentration, total flow rate, and residence time of the liposomes in high ethanol environment as the critical parameters that enabled us to control liposome particle size between 80 and 150 nm while the set-up was running. Residual ethanol was washed away using in-line ultrafiltration. We explored the in-line measurement of both the liposome particle size and residual ethanol content. We conclude that a fully integrated CFM set-up is feasible and can facilitate the industrial-scale production of liposomal drug products for clinical trials as well as for the market.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the Research Training Group 2375 “Tumor-targeted Drug Delivery" grant 331065168.

COMPARISON OF MIXING TECHNOLOGY FOR PARTICLE QUALITY AND SCALE UP

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Lipid nanoparticles (LNPs) are a proven platform for delivery of nucleic acids such as mRNA. Historically, LNPs were initially made using opposing flow of nucleic acid in buffer, against an ethanolic lipid solution, through a tee shaped mixer. Later, alternative technologies were developed such as microfluidics, which claim increased control, better particle size and polydispersity, as well as scaleability.

Here we show data comparing microfluidic technology for making mRNA LNP, to the tee mixer regularly used at Genevant Sciences. A comparison of particle size, encapsulation efficiency and polydispersity being the main readout. A variety of flow rates normallized to linear velocity, and flow ratios of nucleic acid to lipid were attempted using a control lipid composition.

The scalability of each technique was determined based on flow rates of industry standard microfluidic technology compared to scale up parameters typically used with tee mixers, increasing flow rates and internal tee diameters.

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IMPROVING STABILITY OF LYOPHILIZED MRNA LNPS AT 2-8°C AND 25°C

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Lipid nanoparticles (LNPs) are a proven platform for delivery of nucleic acids such as mRNA. Long term storage and distribution of mRNA LNPs prior to administration is a challenge due to the requirement of frozen cold chain. While siRNA LNPs can be stored at 2-8°C, mRNA is a much larger and less stable molecule, requiring mRNA LNPs to be stored frozen in order to maintain formulated mRNA integrity. We have developed a stable lyophilized format of mRNA LNPs with 2-8°C storage that has significant benefits for distribution and ease of use. Short term storage at 25°C is possible, however challenges remain with long term stability at 25°C.

There are multiple facets of mRNA LNP drug product stability including particle size, mRNA concentration, encapsulation efficiency, and mRNA integrity. Lyoprotectant buffers have been developed that enable lyophilization of mRNA LNPs. Lyophilized mRNA LNPs have been demonstrated to have good particle stability properties, however mRNA stability remains stability limiting at 25°C. This work outlines the challenges of stabilizing lyophilized mRNA LNPs with 2-8°C and 25°C storage, and demonstrates that improvements to mRNA stability can be achieved with stabilizing additives.

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UNDERSTANDING THE 3D STRUCTURE OF ZEIN PROTEIN AND ZEIN PROTEIN NANOPARTICLE FORMULATIONS IN SILICO TO OPTIMIZE DRUG LOADING AND DELIVERY

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Nanoparticles are being developed as drug carriers to improve in vivo stability and localization. One such formulation, the Zein nanoparticle system, consists of the Zein protein extracted from the maze plant. This system shows tremendous prospect due to its low cost, excellent biocompatibility, and GRAS FDA approval[1]. In-silico 3D modeling can assist in evaluating drug-carrier interactions to help predict its encapsulation efficiency which can further triage the drugs and enhance the delivery platform. The lack of a validated in-silico models of Zein has proven challenging for selecting drugs for further testing[1]. To tackle this problem, we generated several models for our Zein system utilising energy-based prediction algorithms instead of homology-based methods due to its poor homology with well characterized protein structures. Some methods we have used to create our models include Rosetta, AlphaFold, RaptorX etc. We then validated our models utilising the QMEAN energy and Ramachandran plot analysis. Interaction fingerprints against a predetermined list of anti-cancer drugs will then be generated with our Zein models. These will be used to determine the types of interactions the drugs will have with the carrier. The list of drugs and their predicted interactions will then be tested in-vitro and a weighted analysis of the chem-descriptors of the drugs will be performed. This experiment will determine whether in-silico models can be used to reliably correlate drug-carrier interaction and encapsulation efficiencies in our system. If successful, this can be used to triage drugs for encapsulation experiments and create novel therapies using Zein based nanoparticle systems.

LASER TRIGGERED RELEASE OF ANTITUMOR DOXORUBICIN FROM LIPID NANOPARTICLES CONTAINING GOLD NANOPARTICLES

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With the aim to introduce an efficient triggered release of antitumor drug chemotherapy, namely Doxorubicin (Dox), we have developed Lipid nanoparticles (LNPs) containing Gold Nanoparticles (GNPs) that can be stimulated by laser irradiation, resulting in local release of the cargo contained within the LNPs. The mechanism is based on the absorbed light by the GNPs causing photothermal effects on a lipid membrane, i.e., a phase transition in the lipids of the cell membrane or denaturation of the glycoproteins, resulting in local transient pores and the release of the cargo. It is anticipated that the encapsulation of Dox in LNPs reduces delivery to non-target tissues, reducing potential side effects of the drug, and allowing higher doses to be administered. We hypothesize that combining these novel hybrid Dox loaded LNP/GNPs with a laser irradiation triggering release of the payload will significantly increase the therapeutic index of the drug and allow spatially precise triggered release. Here, we present preliminary in vitro results of laser triggered release of Dox on breast cancer cell lines incubated with LNP/GNP/Dox and irradiated by either a pulsed nanosecond or a femtosecond laser. Incubation time, laser characteristics and irradiation parameters have been investigated and optimized. Results indicate that Dox release is increased by at least a factor of 10 when compare with LNP/Dox. Discussion on further developments of the laser triggered release optimization and translation to in vivo animal model will be presented.

This work was supported by the Nanomedicine Innovation Network (NMIN).

APPLICATION OF EARLY HEALTH TECHNOLOGY ASSESSMENTS TO NANOMEDICINE: A REVIEW OF CONCEPTUAL FRAMEWORKS

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Key challenges in the successful clinical translation and commercialization of nanomedicine technologies include attracting investors and partners, and securing reimbursement of a product once market access has been granted. Early Health Technology Assessment (eHTA) can be used to maximize the value proposition at an early stage by using health economics modelling and stakeholder preference studies to manage risks and identify unmet need. Given that applications of eHTA have mostly centered around medical devices, it is unclear how best to adapt them to nanomedicine, often involving platform technologies, the treatment of rare diseases, and complex care pathways. The objective of this study was to summarize existing literature on eHTA frameworks (understood as *conceptual eHTA models providing a systematic approach* to guide early evidence generation and demonstrate value to industrial partners and other stakeholders at an early stage of development), and to explore the applicability of these frameworks to nanomedicine. Using a *rapid review methodology*, we identified all relevant studies in English, French and Spanish from PubMed/MEDLINE and EMBASE until February 2022. We only included frameworks relevant to the pre-clinical and early clinical (Phase I) stages of development. From 737 reviewed abstracts, 40 publications were selected for inclusion. Key data points extracted include the eHTA methods, intended use cases and users, the technologies being studied, and their stages of development. This review will offer guidance in assessing clinical needs, clinical pathway positioning, market sizing, and identifying cost-effective target product profiles for nanomedicine products at an early stage of development.

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REMDESIVIR LIPID COMPLEX: A NEW FORMULATION FOR A COMPOUND WITH POOR WATER SOLUBILITY

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Remdesivir (Veklury®, GS-5734) is a broad-spectrum antiviral drug that exhibits potent activity against several viral families including *Arenaviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Orthomyxoviridae*, and *Pneumoviridae*, via inhibition of the viral RNA polymerase. It was approved or authorized for emergency use to treat COVID-19 in multiple countries. The original formulation of the poorly water-soluble remdesivir contains the solubilizing excipient cyclodextrin. Amid concerns about supply chain constraints during the early phases of the pandemic, the challenge of obtaining large quantities of cyclodextrin for manufacturing millions of vials of remdesivir was not at first certain. Hence, there was a need to create an alternative remdesivir formulation. Here, we present our work on developing a new formulation of remdesivir, the Remdesivir Lipid Complex (RDV-LC). To manufacture this phospholipid-based formulation, the active ingredient remdesivir was mixed into an organic mixture consisting of soy phosphatidylcholine (soy PC) and distearoyl phosphatidylglycerol (DSPG) dissolved in methanol and chloroform. This mixture was tray-dried and the resulting intermediate paste was then hydrated in a sucrose-acetate buffer. To produce the liposomes, the solution was processed by high-shear homogenization. Finally, the lipid complex solution was filled into vials and lyophilized. We have observed an acceptable shelf-life with the freeze-dried RDV-LC based on chemical and colloidal stability profiles under various storage conditions. This new lipid-based formulation also demonstrated comparable metabolite profile to the cyclodextrin-based formulation in pharmacokinetic studies. Our work presented here is a unique demonstration of using liposomal technology for formulating drugs of limited water solubility and achieved a formulation comparable to the commercialized cyclodextrin alternative.

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DEVELOPING STUDIES OF CELL INTERACTIONS OF CELL CLUSTERS IN MICROGELS USING A MICROFLUIDIC DEVICE

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Understanding intercellular interactions are crucial in areas ranging from biofilm formation, tissue engineering and cancer therapy. While these interactions are being deeply studied in large groups of cells, less is known on the small cell cluster interactions (a group of less than 10 cells). Given the heterogeneity among cells in a population, studies of small cell cluster of cells by flow cytometry are promising for teasing out the roles of subpopulations. Small cell cluster interactions are simple models of the immune response to infective cells. Here we will present a microfluidic screening system which was successfully tested and established to measure the interactions between acute myeloid leukemia cells and macrophages in a high throughout fashion. The hydrogel carrier was modified to obtain desired chemical and mechanical properties, and the cell clusters were encapsulated in microgel with a controlled number of cells.

Furthermore, cell clusters within the microgel showed the capability of taking up different nanoparticles including lipid nanoparticles, and exhibited expression of GFP mRNA. Notably, the phagocytosis of the cell clusters within the microgels were also studied by applying enhancing agents. The correlations between apoptosis and phagocytosis were examined.

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THE ROLE OF PRE-FORMATION INTANGIBLE ASSETS IN ENDOWING SCIENCE-BASED UNIVERSITY SPIN-OFFS

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Scientist-entrepreneurs can play formative roles in commercializing lab-based scientific inventions through the formation of well-endowed university spin-offs. Through case study analysis of three science-based university spin-offs of varying uncertainty within a biotechnology innovation ecosystem, we explore the interplay between pre-formation intangible assets (research excellence, patents, and international networks) and pre-formation entrepreneurial capabilities (technology-market matching, claiming and protecting the invention, mentoring, and strategic timing of firm formation) of scientist-entrepreneurs and how that interplay impacts firm performance (operationalized through product commercialization, survival and revenue ten years after firm formation). We find evidence of the mobilization of pre-formation intangible assets by academic scientists and how this impacts firm performance. A theory-driven model is developed depicting the role of pre-formation intangible assets in endowing science-based university spin-offs for success. Recommendations are provided for academic scientists, policymakers and university leadership to better enable the development and deployment of pre-formation intangible assets and entrepreneurial capabilities.

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OPTIMIZED PHOTOACTIVATABLE LIPID NANOPARTICLES ENABLE RED LIGHT TRIGGERED DRUG RELEASE

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Encapsulation of small molecule drugs in long-circulating LNPs can reduce toxic side effects and enhance accumulation at tumor sites. A fundamental problem, however, is the slow release of encapsulated drugs from these liposomal systems at the disease site resulting in limited therapeutic benefit. Here we demonstrate that incorporation of UV-A or red-light photoswitchable-phosphatidylcholine analogs (AzoPC and redAzoPC) in conventional LNPs generate photoactivatable LNPs (paLNPs) having comparable structural integrity, drug loading capacity, and size distribution to the parent DSPC-cholesterol liposomes. In vitro it is shown that upon irradiation, ~70% drug is released which induces cytotoxic effects in human cancer cells. In vivo studies in zebrafish embryos confirm that paLNPs have similar pharmacokinetic properties as the clinically approved LNPs with added benefits of light-induced drug release based on *trans*-to-*cis* azobenzene isomerization.

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TARGETED THERANOSTIC ^{111}IN/LU-NANOTEXAPHYRIN FOR SPECT IMAGING AND PHOTODYNAMIC THERAPY

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Theranostic nanoparticles aim to integrate diagnostic imaging and therapy to facilitate image-guided treatment protocols. Herein, we present a theranostic nanotexaphyrin for prostate-specific membrane antigen (PSMA)-targeted radionuclide imaging and focal photodynamic therapy (PDT) accomplished through the chelation of metal isotopes (In, Lu). To realize nanotexaphyrin's theranostic properties we developed a rapid and robust ^{111}In/Lu-nanotexaphyrin radiolabeling method using a microfluidic system that achieved a high radiochemical yield (>90%). The optimized metallated nanotexaphyrin displayed excellent chemical, photo, and colloidal stabilities, potent singlet oxygen generation and favorable plasma circulation half-life in vivo ($t_{1/2}$ = 6.6 h). Biodistribution, including tumour accumulation was characterized by NIR fluorescence, SPECT/CT imaging and gamma counting. Inclusion of the PSMA targeting ligand enabled preferential accumulation of ^{111}In/Lu-nanotexaphyrin in PSMA-positive prostate tumours (3.0 ± 0.3 %ID/g) at 48 h. In combination with light irradiation, the PSMA targeting nanotexaphyrin showed a potent PDT effect and successfully inhibited PSMA+ tumour growth in a subcutaneous xenograft model. This study is the first demonstration of the inherent metal chelation-driven theranostic capabilities of texaphyrin nanoparticles, which in combination with PSMA targeting enabled prostate cancer imaging and therapy.

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HALF-LIPOSOMES: LIPID MONOLAYER-COATED PERFLUOROCARBON NANODROPLETS

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Superheated sub-micrometer liquid droplets made of low-boiling perfluorocarbons (PFCs, e.g., perfluoropentane, b.p. 29°C, and perfluorobutane, b.p. -2°C) and stabilized with a lipid shell can maintain liquid state at physiological temperatures. These nanodroplet (ND) particles can be converted to gas bubbles upon the action of focused ultrasound; resulting bubbles rapidly compress and expand in the ultrasound field, with localized energy deposition. Following intravenous administration, this acoustic droplet vaporization phenomenon can be used to suppress tumor growth. Here we describe preparation, characterization and in vivo testing of PFC NDs.

NDs were prepared by repeated Nuclepore filtration or amalgamation of C_5F_{12}, C_6F_{14} or high-boiling C_8F_{18} in the aqueous saline medium that contained micellar DSPC, PEG stearate and DSTAP, with good recovery of PFC. Lipid dye DiI was used as a tag for lipid quantification. Unincorporated lipid was removed from NDs by centrifugation. Resulting ND size (as assessed by NTA and DLS) was in the 200-400 nm range, depending on the shell composition and preparation technique. Upon storage at 4°C, NDs demonstrated no change of mean particle size and moderate reduction of concentration for over four months. PFC quantification was achieved by NMR spectroscopy. Lipid monolayer shell structure was confirmed by cryo-TEM. Focused ultrasound tumor treatments (twice a week, 1 MHz, 6 MPa) of the subcutaneously grown MC38 murine tumors following intravenous administration of C_5F_{12} NDs resulted in the significant reduction of the rate of tumor growth.

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Selected for oral presentation

SONODYNAMICALLY SENSITIVE LIPOSOMES FOR CERVICAL CANCER TREATMENT

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The aim of the project is to develop ultrasound-sensitive liposomes loaded with sonodynamically activated drug (sonosensitizer) and subsequent *in vitro* research of the effect of high frequency ultrasound-triggered liposomal release of sonosensitizer into target cells leading to an enhancement of the antitumor sonodynamic therapy (SDT). The SDT is based on the administration of a sonosensitizer, which is activated by ultrasound, and the subsequent cytotoxic effect. Our goals are to develop carriers in which the best possible ratio between the therapeutic and harmful effect of the drug are achieved, and to construct a unique sonication vessel to provide highly controllable characteristics of ultrasound field within the vessel. For the liposome generation, the DPPC lipids (Avanti Polar Lipids, USA) and the Avanti Polar Extrusion method was used. During these experiments, we observed a strong liposomal size dependence on the number of passages through the extruder membrane. For a following step of purification, the liposome extruder purification method was used, and liposomes were visualized by atomic force microscopy. We next used 3D printing technology to construct a body of a unique sonication vessel with a silicone membrane at its bottom for cell adherence prior to liposome administration. Compared to the commonly used laboratory plastic (e.g. multiwell plates, Petri dish) our vessel enables to minimize the unwanted interactions of ultrasound with the plastic. Toxicity studies of several materials used for 3D printing were evaluated by MTT assays of cells incubated with samples of these materials. The adherence of HeLa cells to silicone membrane was determined using transmitted light microscopy. This newly developed system will serve as an advanced method for preparation of liposomes used in targeted cervical cancer treatment.

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PORPHYRIN-BASED LIPID NANOPARTICLE ENHANCED RNA ENDOSOMAL ESCAPE THROUGH NEAR-INFRARED LIGHT

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Lipid nanoparticle (LNP)-based delivery platforms have been extensively developed and showed broad clinical successes in delivering siRNA, mRNA, and CRISPR gene editing tools. However, current LNPs are still bottlenecked by extremely low RNA endosomal escape efficiencies (e.g. 1–2% for siRNA) after LNP endocytosis, which dramatically impaired the efficacy of RNA. Herein, we developed a porphyrin-based LNP (porphy-LNP), which disrupts endosomal membranes through photoexcitation of porphyrin-lipid, thereby allowing trapped RNAs to release into the cell cytosol. Robust and rapid (within 30 s) siRNA release was confirmed by confocal microscopy following laser irradiation upon porphy-LNP uptake. This dramatic siRNA endosomal escape pattern has been verified in 5 cancer cell lines and lead to over 2-fold increase in siRNA efficacy via *in vitro* luciferase knockdown assay. Disruption of endosomes was also verified by observing mCherry-GAL9 recruitment and imaging LNP-gold subcellular localization by transmission electron microscopy. Overall, porphy-LNP provides a general solution to improve RNA endosomal escape and therapeutic efficacy for current LNP systems, which will broadly and viably benefit LNP-based RNA therapeutics as well as unleash the massive hidden power of RNAs.

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Selected for oral presentation

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LIPOSOMAL DELIVERY OF PORPHYRIN PHOTOSENSITIZER FOR THE ANTICANCER THERAPY

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Photodynamic therapy is an alternative treatment for cancer and other diseases. Anticancer Photodynamic therapy (PDT) is based on the application of a photodynamically active substance (i.e., photosensitizer), its selective accumulation in malignant tissue, and subsequent irradiation with a specific wavelength leading to the activation of the photosensitizer to cause cell death. Although the concept of PDT is dated to 1900, drug delivery modifications in order to enhance the therapeutic effect of these compounds presents an unremitting challenge for clinical applications. As nanoparticle carrier systems tend to accumulate passively in tumor tissues through the leaky tumor vasculature, there is an opportunity for the delivery systems of a nanoparticle size in this therapy. To this end, liposomes present a type of clinically established nanoparticle, consisting of inner aqueous core where hydrophilic molecules can be captured, and lipid bilayers where hydrophobic molecules accumulate. Here, the extrusion method for the formation of 200 nm DPPC liposomes was used, with the varying number of passages through the filters alternating the final zeta-potential. The liposome-extruder purification (LEP) technique was used to purify liposomes from the external photosensitizer. The final concentration of a porphyrin photosensitizer (TMPyP) within liposomes after encapsulation and LEP purification was 0.1 mM. The liposomal size and zeta-potential were determined by Dynamic Light Scattering measurements, and the intracellular localization of free TMPyP and TMPyP encapsulated within liposomes was assessed on HeLa cells using confocal spinning disk imaging. The viability of HeLa cell line after the therapy application was determined via MTT assay. This work was supported by Ministry of Health of the Czech Republic, grant nr.NU21J-03-00062.

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DELIVERY OF IMIQUIMOD TO HEPATOCYTES REDUCES HEPATITIS B VIRUS SURFACE ANTIGEN: AN IMPROVED TREATMENT FOR CHRONIC HEPATITIS B

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Chronic hepatitis B (CHB) is one of the leading causes for severe liver diseases and liver transplantation worldwide. The standard of care for CHB includes interferon-a (IFN-a) to enhance the cell immunity against HBV. However, this therapy is not curative and is not well tolerated by most patients due to its systemic side effect. An alternative approach to enhance the efficacy of CHB treatment is to induce release of endogenous IFN-a locally in the liver. Imiquimod (IMQ), a Toll-Like Receptor (TLR) 7 agonist, stimulates the release of IFN-a and exhibits potent antiviral activity. Here, we demonstrated the use of lipid-based nanoparticles (LNPs) to deliver IMQ to the liver and compared efficacy of two types of liver-targeted LNP formulations including DSPG-liposomes and phospholipid-free small unilamellar vesicles (PFSUVs) targeting the Kupffer cells and the hepatocytes, respectively. PFSUVs selectively delivered IMQ to the hepatocytes and locally increased IFN-a levels in the liver in mice compared to DSPG-liposomes and the standard IFN-a therapy. Finally, in an experimental HBV mouse model, PFSUVs significantly reduced serum levels of HBsAg by 12-, 6.3- and 2.2-fold compared to the untreated, IFN-a, and DSPG-liposome groups, respectively. These results suggest that the hepatocyte-targeted PFSUVs-IMQ may provide safe and enhanced therapy for CHB compared to standard IFN-α.

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CLINICAL EXPERIENCE WITH LIPOSOMAL GLUCOCORTICOID NANOMEDICINES IN SEVERAL INFLAMMATORY AND ONCOLOGY DISEASE INDICATIONS

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Since 2008 we have embarked on a series of clinical studies with PEG-liposomal targeted glucocorticoids with the aim to show an improved efficacy-safety ratio in patients. Trials were performed in patients with rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, kidney failure, eye inflammation, prostate cancer and multiple myeloma with liposomal prednisolone and dexamethasone formulations, mostly given intravenously but in one particular case also by local injection. Pharmacokinetic profiles consistently reveal very long circulation half-lives of around 3 days and a volume of distribution not larger than the plasma volume, which is in sharp contrast with the rapid distribution and clearance normally seen with the free drug. Since in the circulation the glucocorticoids remain encapsulated as inactive phosphate prodrug form, the systemic exposure to free active drug is limited, which is likely the reason why we observe low systemic glucocorticoid-related toxicity in all these studies. Persistent and pronounced therapeutic activity was seen in several but not all disease indications. It is important to discuss for what patients liposomal targeted delivery of glucocorticoids is most relevant, also in view of the many other drugs that now enter the market including biologic therapies. We believe, however, that there still is an important unmet medical need for PEG-liposomal glucocorticoids in specific subpopulations of patients and that further studies are needed to confirm this.

NANOPARTICLE-BASED DELIVERY OF PORPHYRIN PHOTOSENSITISERS WITH SIGNIFICANTLY SHORTER DURATION OF SKIN PHOTOSENSITIVITY TO SUN LIGHT EXPOSURE FOLLOWING ADMINISTRATION

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PORPHYSOMES are porphyrin photosensitiser-containing nanoparticles intended for photodynamic therapy (PDT) of solid tumours. A challenge in the clinical implementation of PORPHYSOMES is their unintended photosensitisation of light exposed tissues such as the skin. Modest accumulation of PORPHYSOMES in the skin may be sufficient to generate photodynamic activity upon prolonged exposure to sun light, resulting in dose-dependent erythema and oedema (i.e., phototoxicities). In this study, the cutaneous photosensitivity of PORPHYSOMES was investigated in nonpigmented rat skin irradiated with simulated solar light at different time intervals post injection. Escalating doses of solar light were tested to determine the minimum erythemal dose (MED) at each time interval post injection. Higher MEDs are desirable as these correspond to longer light exposures before erythema occurs. The MED for PORPHYSOMES (5 mg porphyrin/kg BW, IV) was compared at each time interval with an unencapsulated porphyrin photosensitiser (porfimer sodium, 5 mg/kg BW, IV). The peak concentration of PORPHYSOMES in skin occurred 24 h post injection and cleared with an apparent half-life of ~130 h. The MED for PORPHYSOMES gradually increased from ≪ 80 J (≪ 13 min) at 4 days post injection to 160 J (~26 min) by 8 days. At 12 days post PORPHYSOME injection, no erythemal response was observed at the highest light doses tested. In contrast, porfimer sodium exhibited unchanging MEDs ≪ 80 J (≪ 13 min) from 4 to 12 days post injection. Thus, the 12-day period of skin photosensitivity after POPRHYSOME administration is significantly shorter in duration compared with the unencapsulated porphyrin photosensitiser. This work was supported by TFRI.

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HOW CHOICE OF NANOMEDICINE TRACER CAN RESULT IN CONTRADICTORY BIODISTRIBUTION OUTCOMES

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Fluorophores are commonly utilized as a tracer molecule to study the *in vivo* fate of liposomes. Use of low molar percentage tracer molecule (≤ 1 mole%) and conserved physicochemical properties of liposomes (*e.g.* size, charge, PDI, morphology) are often considered excluding a potential effect related to the fluorophore itself. In this study, we report that choice of lipid-fluorophore conjugate can result in large differences in biodistribution. Unexpected clearance of fluorescently labelled anionic and pegylated liposomes was observed in zebrafish larvae, an emerging model for *in vivo* pre-screening of nanomedicines. Screening studies in zebrafish embryos revealed selective receptor-mediated uptake of lipid-fluorophore, based on (1) fatty acid tail mismatch with liposome bilayer and/or (2) choice of fluorophore. When lipid-fluorophore design was optimized and validated for these properties, formulations demonstrated biodistribution results in agreement with literature. Altogether, this study highlights the critical importance in choice of tracer molecule in biodistribution studies of nanomedicines.

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MECHANISTIC STUDY OF THE HEPATOCYTE-TARGETED DELIVERY BY PHOSPHOLIPID-FREE SMALL UNILAMELLAR VESICLES

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Phospholipid-free small unilamellar vesicles (PFSUVs), are an innovative delivery system composed of 5:1 mol ratio of cholesterol and TWEEN80, with an average diameter of 60 nm and display efficient and predominant delivery to the hepatocytes after intravenous (i.v.) injection. Here, we conducted a series of experiments to elucidate the hepatocyte-targeting mechanism. We first examined the role of serum proteins on the increased cellular uptake of PFSUVs by HepG2 cells. We then analyzed the plasma protein corona adsorbed to PFSUVs and identified subtypes of apolipoproteins were enriched, specifically apolipoprotein AII (ApoA2). The cellular uptake was increased by 1.5-fold when the culture medium was supplemented with apolipoprotein ApoA2, but not ApoC1 and ApoE. Furthermore, uptake of PFSUVs in HepG2 cells was reduced significantly in the presence of BLT-1, an inhibitor for the scavenger receptor B-1 (SR-B1), which is a receptor for ApoA2. These results suggest that upon i.v. delivery, PFSUVs adsorbed serum ApoA2 to the surface, which is targeted by SR-B1 expressed by hepatocytes, triggering receptor-mediated endocytosis or macropinocytosis. Following internalization, PFSUVs were found in the endosomes after 1-2 h post treatment and finally colocalized in the lysosomes in 4 h. The data suggest the important role of ApoA2 and SR-B1 in hepatocyte-targeted delivery of PFSUVs.

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EFFECTS OF INTERNAL TRAPPING AGENT ON DRUG RELEASE KINETICS

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Remote loading techniques using ionic gradients are commonly used for ionizable drug packing into liposomal cores. For such liposomes, factors that contribute to stability include drug:lipid ratio and core morphology – an observation that has been extensively studied in doxorubicin (DOX) liposomes. Herein we show the importance of internal trapping agent selection when optimizing liposomal drug release – particularly for dual drug modalities.

Liposomes with varying internal core conditions were prepared by extrusion and high pH buffer exchange. Ionizable drugs niraparib (NIRB) and DOX were co-loaded into the core of drug-free liposomes through incubation above the phase transition temperature. Purified liposomes were then subject to physiologically relevant conditions to assess *in vitro* release.

Despite sustained release when encapsulated singularly, NIRB and DOX co-loaded using a citrate trapping agent exhibited rapid release over a five-day period. In contrast, liposomes co-loaded and entrapped using triethylammonium sucrose octasulfate (TEA_8SOS) showed virtually no release over the same testing period despite similar drug:lipid ratios. Further investigation by cryo-TEM revealed that TEA_8SOS drastically alters drug loaded core morphology, a physical characteristic that potentially inhibits drug release.

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DEVELOPMENT OF CAPILLARY ELECTROPHORESIS (CE) FOR EVALUATING SELF-AMPLIFYING RNA (SARNA) INTEGRITY AND STABILITY FOR A COVID-19 LIPID NANOPARTICLE (LNP) VACCINE

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A SARS-CoV-2 self-amplifying RNA (saRNA) lipid nanoparticle (LNP) vaccine is being developed using PNI's proprietary ionizable lipid and NanoAssemblr® manufacturing platform. The LNP encapsulated saRNA encodes for a cytoplasmic replicon leading to SARS-CoV-2 spike protein expression at a very low dose *in-vitro* and *in-vivo*. Manufacturing processes were optimized based on formulation activity, stability, scalability, and critical process parameters. Four 50 mg batches of SARS-CoV-2 self-amplifying RNA-LNP-2 were made using our GMP system and processed using tangential flow filtration (TFF). In addition to particle parameters during and after the manufacturing process, the integrity of the encapsulated saRNA is a significant consideration during manufacturing and storage of the product. One method of RNA analysis is capillary electrophoresis (CE). This technique allows for the electrophoretic separation of RNA based on size, resulting in a visual representation of RNA identity, integrity, and purity. Unfortunately, legacy mRNA CE applications are not well suited to saRNA's size, and few alternatives are described within the literature. Consequently, we evaluated saRNA CE applications using the Sciex PA 800 Plus™ system. Parameters such as denaturant and injection voltage were optimized to provide the best resolution. Analytical results from the CE indicate that PNI manufactured saRNA has a main peak integrity of at least 62%, and the LNP manufacturing process does not significantly degrade saRNA. Experiments also looked at saRNA stability, and CE data was correlated to *in vitro* potency of saRNA LNP samples at different time points. Additionally, stability studies suggest that at room temperature, encapsulated RNA degrades at a faster rate than free RNA.

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THE CLINICAL DEVELOPMENT OF A THERAPEUTIC CANCER VACCINE USING LIPID NANOPARTICLES CO-LOADED WITH URIDINE-MODIFIED MRNA AND ALPHA-GALCER

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While the COVID-19 mRNA vaccines have gained global recognition for being generally safe and highly effective, questions remain on the mode of action and inflammatory nature of this novel vaccine platform (Verbeke R. et al. JCR 2021).

In our research group, we investigate how mRNA vaccines interact with innate immune cells upon administration, and how choices in mRNA and lipid particle design influences the immunogenicity and reactogenicity profile. We believe that new fundamental insights in this topic will lead to improved vaccine designs, and could open up new possibilities for other diseases. In addition, we are exploring the use of the natural killer T (NKT) cell activator α-GalCer (αGC) as a smart adjuvant to strengthen uridine-modified mRNA vaccines to elicit conventional T cell responses, while broadening the immune response by also activating NKT- and NK cells (Verbeke R. et al. ACS Nano 2019). We demonstrated that this αGC-adjuvanted mRNA nanovaccine (Galsomes) induced superior antitumor responses compared to other types of mRNA platforms, especially when combined with checkpoint inhibition. Based on the preclinical data, we are currently organizing a phase I clinical trial in lung cancer patients. Up to now, this clinical translation process comprised the fine-tuning of the Galsome composition and the set-up of a GMP-compliant production system. Eventually, we hope that Galsomes using a neo-epitope targeting approach could work as a strategy to increase the rather low (20%) response rate to checkpoint inhibition therapy in lung cancer patients.

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DEVELOPMENT AND ASSESSMENT OF THE NUCLEIC ACID LOADED LIPIDIC NANOPARTICLES AS A VACCINE DELIVERY SYSTEM: *A PROOF-OF-CONCEPT STUDY*

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Lipid nanoparticles (LNPs) have been explored for nucleic acid delivery for gene therapy, cancer immunotherapy and vaccine. Whereas three RNA-loaded LNP products have been approved clinically, DNA -containing LNPs are still in the development stage. We utilized microfluidics to formulate pDNA into LNPs prepared with various cationic lipids, phospholipids and cholesterol. By manipulating the lipid formulation and microfluidic conditions, pDNA-LNPs with different particle sizes, zeta potential and loading efficiency were fabricated. The optimal formulation that showed significant pDNA transfection in the muscle of mice following intramuscular injection (IM) displayed a mean diameter of ~100 nm, a polydispersity index of <0.2, zeta potential of >30 mV, and loading efficiency of >80%.

pDNA encoded with OVA protein will be loaded into the LNPs and IM delivered to mice to test the preventative effect against OVA-cancer. Alternatively, therapeutic effect of this pDNA vaccine will be studied in OVA-tumor-bearing mice.

This work is supported by CHIR.

Selected for oral presentation

IDENTIFYING THE IMMUNE MECHANISMS AND PROTECTIVE CAPACITY OF MESSENGER RNA (MRNA) AND SELF-AMPLIFYING RNA (SARNA) VACCINES DELIVERED WITH LIPID NANOPARTICLES

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The recent approval of effective SARS-Cov-2 vaccines with the RNA platform paves the road to discovery of new therapeutic approaches to many diseases, including bacterial infections and age-related disorders. Self -amplifying RNA vaccines are next generation vaccine platforms, derived from alphaviral genomes. They have not yet been FDA approved, as their larger size and uncharacterized immune sensing complicates their clinical application. However, saRNA protein expression in vivo lasts between 30 to 60 days due to a replicase region that mediates the amplification of the molecule, and they require smaller doses. The currently approved mRNA vaccines elicit protein expression that lasts up to 5 days in vivo, and subunit protein formulations have even faster elimination rate. Depending on the route of administration, size, nanoparticle formulation, modifications, and type of antigens, RNA molecules induce different immune responses, which define the success of these therapies. There is little understanding of what drives immunity and protective capacity of RNA vaccines. Elucidation of saRNA and mRNA innate, and adaptive immune responses could be vital for development of an effective and cheaper vaccine therapies. In this project we are going to compare the length of antigen expression, and the differences in innate and adaptive immune responses different RNA vaccines elicit with different nanoparticle formulations and evaluate their protective capacity in targeting of bacterial infections and chronic diseases.

NON-VIRAL DELIVERY OF MRNA AND SARNA TOWARDS VACCINE AND CELL & GENE THERAPY APPLICATIONS USING A LIPID NANOPARTICLE LIBRARY

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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®). Scarcity of lipids that are suitable for vaccination, cell therapy and protein replacement therapies continue to be a problem in advancing many potential therapeutic/vaccine candidates to the clinic.

Herein, we describe the development of novel ionizable lipids to be used as functional excipient for designing vehicles for nucleic acid therapeutics/vaccines in vivo or ex vivo use in cell therapy applications. We first studied the transfection efficiency (TE) of LNP-based mRNA formulations of these ionizable lipid candidates in primary human T cells. Lipids were then tested in rodents for Influenza, SARS-CoV-2 vaccine applications using self-amplifying RNA (saRNA) encoding H1N1 Influenza antigen or SARS-CoV-2 full length spike protein. We have then evaluated various ionizable lipid candidates for protein replacement applications by administering human Erythropoietin (hEPO) encoded mRNA LNPs intravenously at a dose of 0.5 mg/kg in C57BL6 mice.

We believe that these studies will pave the path to the advancement in development of RNA vaccines, cell-based therapy, and targeted delivery of other nucleic acids for finding a cure for many rare diseases, where treatment options rarely exist.

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SELF-AMPLIFYING MRNAS SUPPORT THE DEVELOPMENT OF NEXT GENERATION VACCINE PLATFORM

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Self-amplifying mRNA (saRNA) has emerged as a newly designed mRNA platform that can replicate itself, allowing substantially lower dose for effective immune response. Herein, we demonstrated the preclinical success of our saRNA-based vaccines encased by lipid nanoparticles (LNPs) that were scaled-up by microfluidic technology. Our preclinical investigations with lead saRNA-based vaccine candidates exhibited the generation of robust humoral and cell-mediated immune response in mouse models. Viral neutralization titres were further analyzed. Finally, protective immunogenic responses of our saRNA-based vaccine candidates were evaluated in a hamster virus challenge model against SARS-CoV-2 virus. Vaccine candidates protected hamsters from weight loss and an increased lung weight following SARS-COV-2 challenge. Vaccinated hamsters had higher levels of SARS-CoV-2 specific IgG antibodies and reduced viral loads. In both animal models, no clinical symptoms and adverse effects were observed.

Overall data provide a clear insight that our NxGen microfluidic technology platform is fully capable of scaling-up large volumes of LNP-based saRNAs vaccines by retaining the Critical Quality Attributes of the drug product. In addition, our studies strongly suggest pre-clinical evidence for exploring the development of saRNA LNP based vaccine platform along with mRNA LNPs.

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COMPARISON OF ADJUVANTICITY OF MONODISPERSED NANOLIPOSOMES CONTAINING MPLA + QS21 AND POLYDISPERSED NANO- + MICROLIPOSOMES (ALFQ)

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Army Liposome Formulation with QS21 (ALFQ), a polydispersed nanoliposome + microliposome suspension containing DMPC and DMPG, 55% Chol, MPLA, and QS21, have been used as a potent adjuvant in four phase 1 clinical vaccine trials, as well as in other vaccine studies. To compare the adjuvanticities of nanoliposomes vs. nano- + microliposomes, each containing the same lipid composition as ALFQ, we created ALFQ lacking QS21 (ALF55) made by the NanoAssemblr^{O} or by the lipid deposition method followed by microfluidization. QS21 was then added to each of the two preparations of ALF55 to create ALFQ. Transmission electron microscopy (TEM) images showed polydispersed size distribution of the liposomes. Female Balb/c mice were immunized 3 times, at weeks 0, 3, and 6, with 10 µg of A244.gp120 Delta 11 (HIV-1 clade AE glycoprotein) antigen with each of the three formulations. The adjuvanticity comparision data suggest that adjuvanted nanoliposomes compared to adjuvanted nano- + microliposomes induced similar immunogenicity of a protein antigen.

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CATIONIC POLYMERS AS RNA VACCINE DELIVERY SYSTEMS

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Vaccine technologies can be defined as being either "classic vaccine platforms" or "next-generation vaccine platforms". Next generation vaccines are created with sequencing information alone, using DNA or RNA as their basis. Self-amplifying RNA (saRNA) is one example of a next-generation vaccine platform. This type of RNA requires a smaller dose than mRNA to induce an immune response, which is hypothesized to reduce side effects and increase vaccine production efficiency. Like other vaccines, it requires a delivery system to prevent degradation and promote cellular uptake. Many delivery systems already exist, including lipid nanoparticles (LNPs) and cationic polymers.

In depth comparisons of LNPs and the cationic polymer pABOL RNA vaccine delivery have shown that LNP delivery led to an overall higher immune response, while pABOL delivery induced approximately 100x higher intramuscular protein expression. Alteration of surface charge has been shown to affect tissue tropism, and immunogenicity of delivery systems. Using biocompatible anionic polymers to neutralize pABOL's high surface charge, we expect to see an increased immunogenicity, while retaining high levels of protein expression. This study shows preliminary data demonstrating incorporation of biocompatible anionic polymers alters surface charge, without affecting protein expression *in vitro.* Follow up studies will include analyzing how polymer incorporation affects *in vivo* tissue tropism and its impacts on vaccine immunogenicity. Due to its cheap manufacturing and scalability, an immunogenic pABOL has potential to be an effective RNA vaccine delivery system.

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REVEALING PATHWAYS INVOLVED WITH THE IMMUNOGENICITY OF RNA-LIPID NANOPARTICLES

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The clinical approval of the two mRNA-based vaccines against the ongoing pandemic of COVID-19 has accelerated the growth of the RNA therapeutics field. This success is possible because of the breakthrough in the field of the drug delivery systems, especially lipid nanoparticles (LNPs), a lipid-based system that has been developed to deliver siRNAs and other nucleic acid cargo.

The ability of any foreign substance to elicit the body's immune response is the "immunogenicity" of the substance. In the context of vaccine development, this is a desirable trait to the extent that it doesn't interfere with the process of antigen presentation. Although it has been appreciated that both the exogenous mRNAs encapsulated and the carrier itself contribute to the overall immunogenicity of the vaccines, the exact mechanisms associated with the immunogenicity of LNPs and LNP-RNAs have not been delineated. Here, we focus on uncovering the underlying mechanisms of LNP sensing and detection in human peripheral blood mononuclear cells (PBMCs) using an approach that combines conventional biochemical techniques (e.g. flow cytometry) and single-cell RNA sequencing (scRNA seq). Specifically, the focus will be on comparing LNP formulations that have been optimized in preclinical models for delivering non-replicating mRNA vs. self-amplifying RNAs (saRNAs). These findings will enable clinically translatable LNP-RNA formulations to be used in RNA vaccines and therapies in the future.

This work is supported by NSERC, CIHR, CFI and UBC start-up funding.

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WHAT HAPPENED TO THE SPHAGETTI / MEATBALL-TYPE STRUCTURES?

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In 1994, at the third LRD Conference, taking place at UBC in Vancouver, we presented new structures formed during interaction of cationic, DC-Chol/DOPE liposomes with plasmid DNA. Visualized by freeze-fracture electron microscopy they resembled bilayer-covered DNA tubules (spaghetti) and liposomal complexes (meatballs).[1] The morphology of these structures was confirmed by negative staining- and cryo-TEM. The total diameter of the spaghetti was measured with 13nm giving 5nm for the inner volume accommodating the supercoiled plasmid DNA.[2]

A molecular level theory was developed showing that the thermodynamic stability of the spaghetti-type structure is lower than of the honeycomb-type structure because of the additional bending energy of the external monolayer of the bilayer mantle.[3] The thread-like mRNA backbone has a diameter of less than 2nm making the bending of a bilayer tube and therefore the formation of the spaghetti-type structure impossible.

To study the interaction of spaghetti/meatball-type structures with cells, cultured human keratinocytes were used as a test model. After short incubation times free spaghetti-type structures were observed intact inside cross-fractured cells as well as at their fracture faces. After a longer incubation time, bigger meatball-type complexes are taken up by endocytosis.[2]

Interestingly the Spaghetti-type structure did not survive the test of time but it shows how molecular forces determine superstructure formation. The densely packed meatballs can be considered as the great-grandfathers of the highly sophisticated LNPs used nowadays in gene delivery.

[1]Sternberg, B., Sorgi, F.L., Huang L. FEBS Lett 356 (1994) 361-366. [2]Sternberg, B. Lasic and Papahadjopoulos (eds.) Med. Applications of Liposomes (1998) Elsevier Science B.V. 395-427. [3]May, S., Ben-Shaul, A. Biophysical Journal, 73, (1997) 2427-2440.

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NON-VIRAL GENE DELIVERY TO THE RETINA

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The human retina is made up of close to 100 different specialized cells that are responsible for processing the light coming in, and outputting an image to the brain via intricate synaptic messengers[1]. This peculiarity calls for a cell-type specific approach to the delivery of corrective genes and gene editors to the eye for the purposes of slowing disease progression or restoring vision altogether. Adeno-associated virus therapy via subretinal injection is the only FDA approved method for delivery of modified genes to the retinal pigmented epithelia cells (LUXTURNA^{TM})[2]. Lipid based nanoparticles can also deliver RNA therapeutics through subretinal injections is restricted to the retinal pigmental epithelial cells[3]. Moreover, the subretinal delivery of viral and non-viral vectors poses higher risk of retinal detachment as well as vitreous and submacular hemorrhage and potential immunogenicity tied to viral carrier[4]. To address this issue we employed a peptide phage display library in order to identify cell-type specific targeting peptides that reach different subsets of cells within the complex retina machinery. We have identified novel peptides that can traverse ocular barriers and reach to selective-cell types like the photoreceptors and other cell-types. Decoration of LNPs with these cell specific peptides enabled delivery mRNA to the neuronal retina beyond the epithelia in rodent and non-human primate retinas. These studies open new avenues for treatment of inherited retinal degeneration through LNP enabled RNA therapeutics.

IMPROVING TRANSFECTION EFFICIENCY AND CYTOTOXICITY IN HEK293 CELLS BY USING NANOPARTICLE DELIVERY SYSTEMS

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In vitro cell models are an invaluable first stage for demonstrating proof-of-principle effective gene repair of pathogenic mutations. These models often rely on transfection of nucleic acids to express genome editing machinery and components. The current "gold-standard" for the delivery of endogenous nucleic acids *in vitro* is Lipofectamine 3000, which has a relatively high transfection rate in select cell types but is limited by cytotoxicity and the inability to be translated into *in vivo* models. The cationic lipid is toxic to cells which prevents it from being a viable strategy for *in vivo* delivery, making the transition into animal models difficult.

Given the limitations of current delivery methods, our **objective was to explore the potential of lipid nanoparticles (LNPs) and polymers to act as effective delivery systems for the transfection of nucleic acids**. We transfected HEK293 cells with tdTomato, a red fluorescent protein, or base editors to correct fluorescent reporters and quantified transfection with flow cytometry. We found that alternative nanoparticle delivery systems were able to achieve significantly higher transfection and correction rates than Lipofectamine 3000 with better rates of survival. The nanoparticle systems were extremely potent compared to Lipofectamine 3000 which makes them promising vehicles for delivering effective therapeutic doses.

The LNPs are promising for addressing both the toxicity of *in vitro* systems and the immunogenicity of using viral vectors *in vivo*. We hope to use these formulations in the future for *in vivo* delivery of gene editing components.

COMBINED THERMOSENSITIVE LIPOSOME AND IMMUNOTHERAPY TREATMENTS PROVIDE SYSTEMIC ANTI-TUMOR EFFECT IN A MURINE MODEL OF RHABDOMYOSARCOMA

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Introduction: Off-target drug distribution plays a key role in chemotherapy treatment failures. One approach to mitigate non-specific drug distribution is the use of triggered drug delivery systems, such as thermosensitive liposomes (TSL). However, such localized treatment strategies can hinder treatment efficacy in metastatic diseases. Our group recently demonstrated that vinorelbine (VRL) encapsulation into TSLs combined with mild hyperthermia (HT) affords improved specific tumor treatment in a murine model of rhabdomyosarcoma (RMS). Here, we combined this bimodal therapy with immunotherapy to enhance its systemic treatment capabilities in a two-tumor murine model of RMS.

Methods: Murine M3-9-M cells were injected subcutaneously into the left and right flanks of male C57BL/6 mice. Animals were treated with ThermoVRL, ThermoVRL + anti-PD-1 mAB, anti-PD-1 mAB, or saline, all in combination with HT (42°C, 25min) localized to one of two tumors.

Results: ThermoVRL treatment effectively inhibited tumor growth in the heated tumor. However, only the addition of the immune checkpoint inhibitior led to an effect in the contralateral, unheated tumor.

Conclusion: The addition of anti-PD-1 treatment to ThermoVRL and HT has potential to provide systemic treatment capabilities to this otherwise localized treatment approach.

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69 MICROWAVE RESPONSIVE POLYMER CAPPED GOLD NANOPARTICLES SYNERGIZES THE EFFECT OF SORAFENIB AND 9

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Cancer is one of the biggest killers because of its heterogeneous nature, high occurrence and mortality. Multiple cellular pathways are affected in cancer concurrently, making its treatment even more challenging. Low concentration of the anti-cancer drugs at the target site and side effects on the normal cells complicates the treatment even further. Multifunctional and stimuli responsive drug loaded nanoparticles are being studied to address these issues. Both endogenous (pH) and exogenous (Laser, ultrasound, light, thermal, microwave) stimuli can be employed for tunable drug release at region of interest. Use of microwave (MW) as a stimulus offers the advantage of hyperthermia and deep tissue penetration which makes it an ideal combination therapy for solid tumors like hepatocellular carcinoma.

We have developed polyvinyl pyrrolidone (PVP) capped sorafenib loaded gold nanoparticle (PSGN) and checked the efficacy of these nanoparticles over multiple cancer cell lines including FLT3 mutated acute myeloid leukemia, hepatocellular carcinoma (HCC), rhabdomyosarcoma etc. This study employs the microwave thermal therapy (MWTT) in combination with PSGN for treatment of HCC. PSGN, 30 nm, with % loading of 13 $\mu g.mL^{-1}$ were synthesized. Characterization was done using UV-Vis, FTIR, NMR, HPLC and TEM. The drug release kinetics of the PSGN with or without MW exposure showed a different release pattern. Cytotoxicity against HepG2 cells showed significantly higher cell death with MWTT than without. Similar results were observed in mice liver tumor model.

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70 LIPID NANOPARTICLE FORMULATIONS OF A TRIPLE ADJUVANT FOR INTRANASAL VACCINES: IN VITRO CYTOTOXICITY AND CELLULAR UPTAKE IS AFFECTED BY LIPID COMPOSITION AND PROPORTIONS

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Mucosal vaccines have shown great promise but still must overcome certain barriers such as local tolerance and antigen stability. A novel triple adjuvant system based on poly(I:C), innate defense regulator peptide IDR-1002 and polyphosphazene (TriAdj) has been formulated as cationic lipid nanoparticles (L-TriAdj) to provide enhanced mucoadhesion and immune stimulation for intranasal vaccines. L-TriAdj comprised of DDAB/DOPE or DDAB/DOPE/DSPC/cholesterol was formulated by self-assembly with anionic TriAdj to generate cationic lipid nanoparticles. Lipid:TriAdj ratio was varied as well as lipid composition and concentration. L-TriAdj cytotoxicity was assessed in RAW 264.7 macrophages by MTT assay at 4h. Cellular uptake was observed using NBD-PE lipid or rhodamine-123 labeled poly(I:C). L-TriAdj mean diameters depended on the ratio of lipid-TriAdj (135-300nm and 37-59mV). Inactivated porcine respiratory and reproductive virus was added to L-TriAdj to a fixed concentration. Addition of antigens increased the mean diameter and decreased the zeta potential. The MTT assay results showed that viability was dependent on L -TriAdj concentration. Significant viability differences were seen in cells treated with DDAB/DOPE/ DSPC/ Chol based L-TriAdj as a function of lipid:TriAdj ratio. Flow cytometry indicated >95% cellular uptake of L-TriAdj at 4hrs. Florescence imaging of cells treated with L-TriAdj or whole vaccine were in agreement with flow cytometry. This work was supported by the SK Agricultural Development Fund, CIHR and the Nanomedicines Innovation Network (NMIN).

PREPARATION OF MULTI-FUNCTIONAL PROTEOLIPOSOMES AND INVESTIGATION OF THEIR EFFECTS ON ERYTHROCYTES

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Sickle cell disease (SCD) is a chronic disease that occurs due to a point mutation in the β chain gene in the hemoglobin A molecule and is usually fatal. The only treatment for SCD is bone marrow transplantation and a new drug delivery system is needed to be developed. In this study, it was aimed to prepare lipid-based carriers to prevent erythrocyte dehydration and oxidation, which begins with the polymerization of erythrocytes and leads to vascular occlusion in SCD.

For this purpose, L-Glutamine encapsulated DOPC-DPPG mixed liposomes were prepared. Characterizations of the drug-loaded liposomes were performed by light scattering and zeta potential measurements, FTIR, differential scanning calorimetry, transmission electron microscope (TEM) and scanning electron microscope. Encapsulation efficiency and release of L-Glutamine was analyzed by chromatography. Liposomes were interacted with erythrocytes and after interaction, results of hemolysis measurements and SEM images showed that the presence of the carriers provide reduced erythrocyte oxidation. Afterwards, Aquaporin, which is a water-channel protein, was integrated into the membranes of liposomes, for the first time in the literature to prevent dehydration of erythrocytes. The characterization of the proteoliposomes were implemented via FTIR, fluorescence microscope and TEM. After the interaction with erythrocytes, it was obtained that carriers provide reduced erythrocyte dehydration. Although additional investigations and optimizations are needed and *in vivo* experiments should be conducted in the future studies, promising results were obtained towards reducing erythrocyte dehydration and oxidative stress using a single carrier. This study is supported by the Hacettepe University, Scientific Research Projects Coordination Unit with the project number THD-2019–18441.

A MAGNETIC SEPARATION METHOD FOR ISOLATING AND CHARACTERIZING THE BIOMOLECULAR CORONA OF LNPS FOR GENE DELIVERY

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Lipid nanoparticle (LNP) formulations are a proven method for the delivery of nucleic acids for gene therapy. Enroute to their target, LNPs interact with biological fluids (i.e. blood), components of which adsorb onto the LNP surface forming a layer called the "biomolecular corona" which affects LNP stability, biodistribution, and tissue targeting. Technical hurdles in corona isolation combined with the fact that corona composition changes in different species and patients have made LNP-cell interactions difficult to predict, and correlation between in vitro and in vivo models is almost absent. Therefore, this project aims to understand and exploit the corona of clinically relevant gene therapeutics to improve their clinical applications. In this method, LNPs were formulated with a superparamagnetic iron oxide core and incubated with human serum. This allowed the separation of LNP-corona complexes from un-bound serum proteins and lipoproteins using magnetic separation. Isolated corona components were then analyzed by mass spectrometry. A species-specific corona was identified on the surface of isolated LNPs which played a key role in triggering specific cellular recognition. This study illustrates the importance of using biologically relevant media for the study of LNP-cell interactions and highlights the need for improved understanding of the correlation between LNP composition, corona formation, and targeting to improve the design, localization, and clinical success of LNP -based gene therapeutics.

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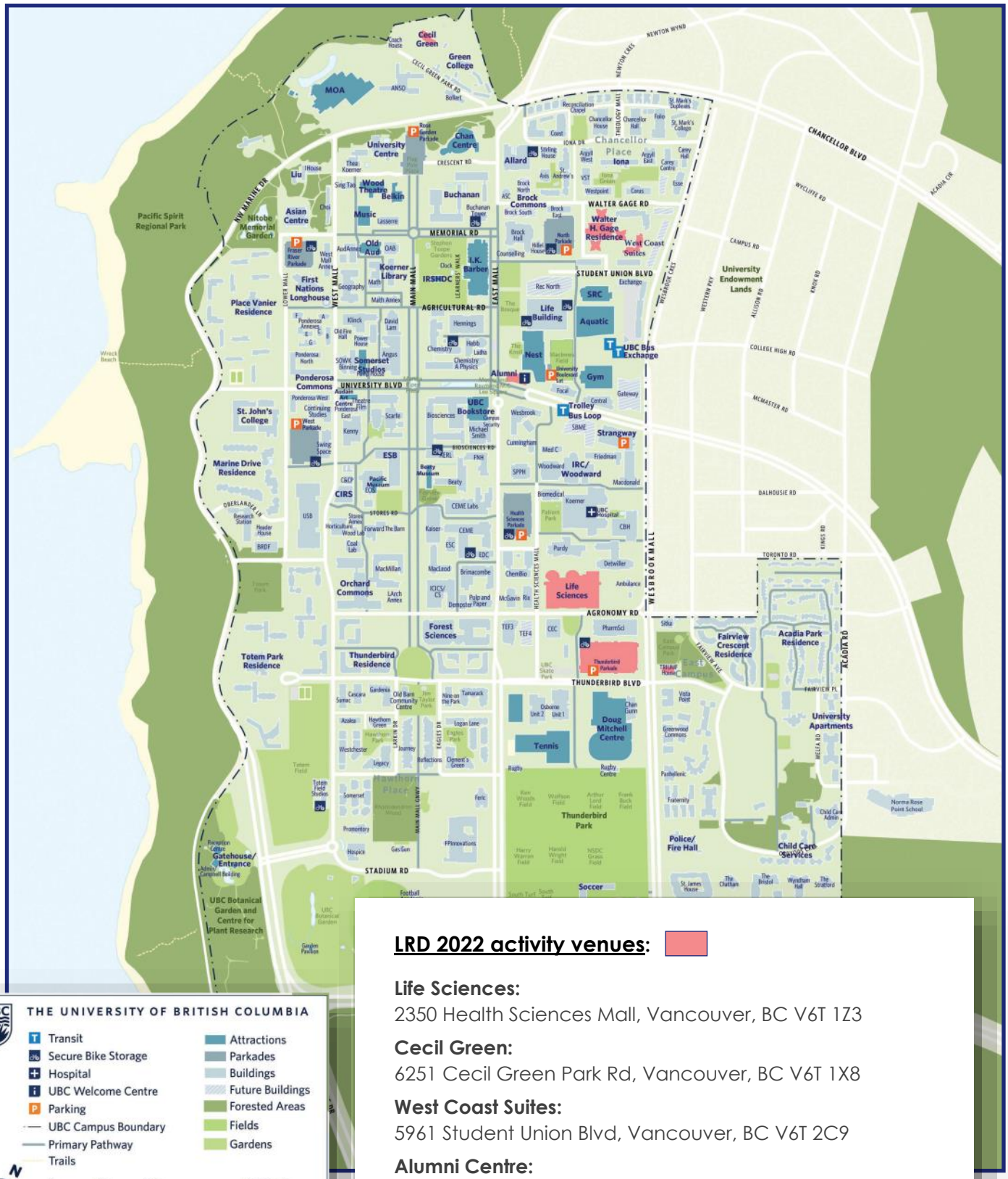
VENUE MAPS



LRD 2022 activity venues:

Life Sciences:
2350 Health Sciences Mall, Vancouver, BC V6T 1Z3

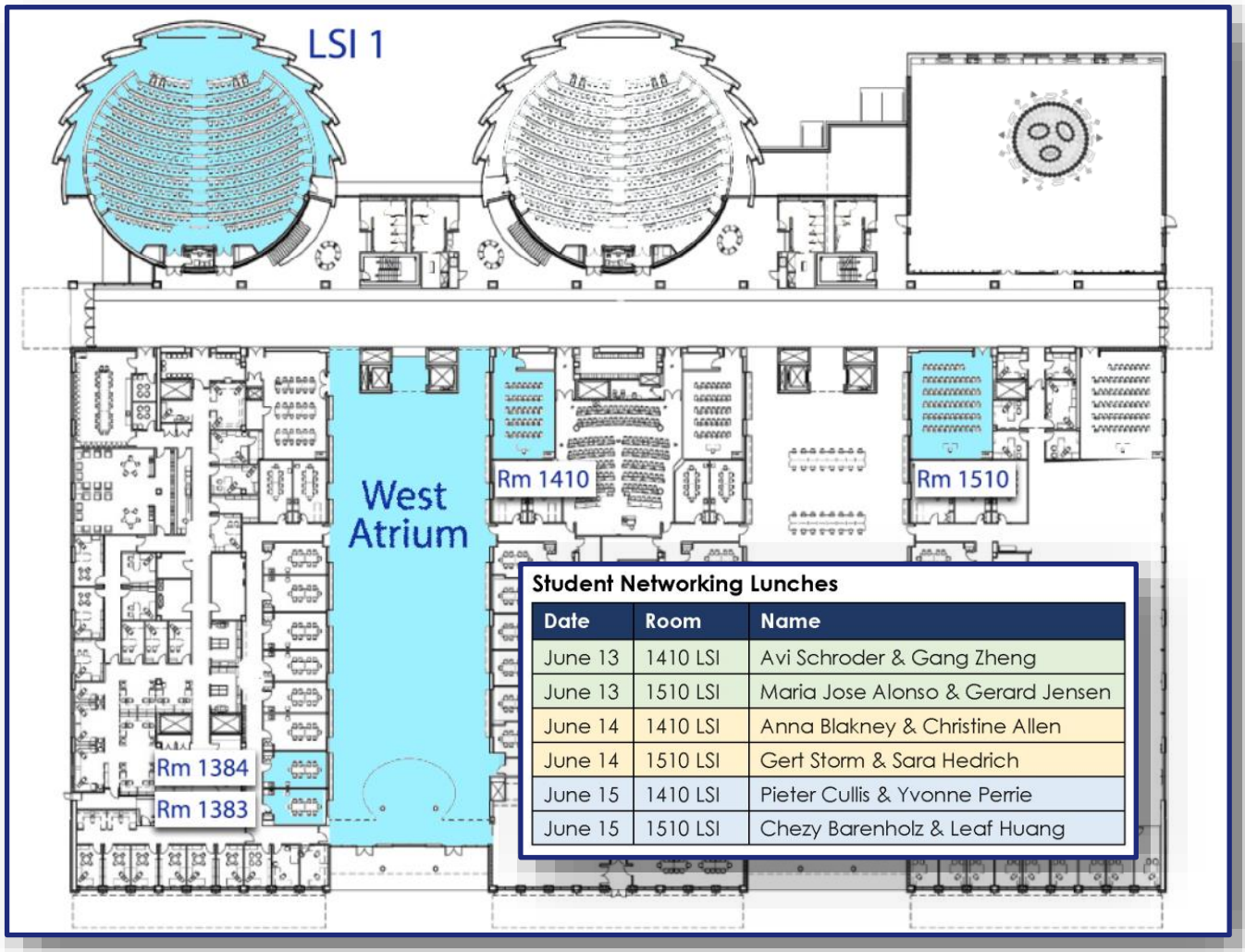
Cecil Green:
6251 Cecil Green Park Rd, Vancouver, BC V6T 1X8

West Coast Suites:
5961 Student Union Blvd, Vancouver, BC V6T 2C9

Alumni Centre:
6163 University Blvd, Vancouver, BC V6T 1Z1

VENUE MAPS

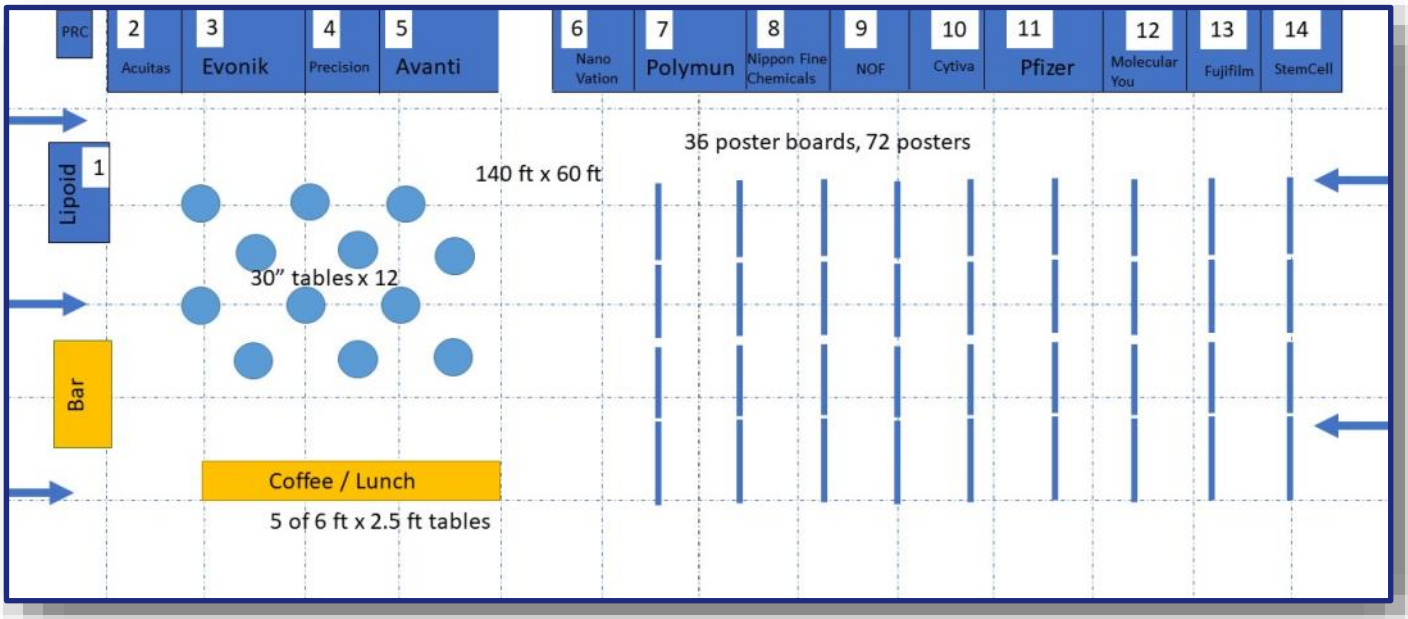
Life Sciences Centre | 2350 Health Sciences Mall | UBC



Student Networking Lunches

Date	Room	Name
June 13	1410 LSI	Avi Schroder & Gang Zheng
June 13	1510 LSI	Maria Jose Alonso & Gerard Jensen
June 14	1410 LSI	Anna Blakney & Christine Allen
June 14	1510 LSI	Gert Storm & Sara Hedrich
June 15	1410 LSI	Pieter Cullis & Yvonne Perrie
June 15	1510 LSI	Chezy Barenholz & Leaf Huang

West Atrium | Life Sciences Centre



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17th LIPOSOME RESEARCH DAYS 2022

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