Session 1:		Talks are in auditorium 1101 / overflow video broadcast in auditorium 1201	
9:00 AM	Urs Hafeli	Welcome to the 8th Vancouver Nanomedicine Day 2023 – Introduction to Nanomedicines	Pharmaceutical Sciences, UBC
Session 2:			Chair: Shyh-Dar Li
9:15 AM	**Sabrina Leslie**	**Single-Particle and Single-Cell Imaging to Quantitate Biophysical Properties of mRNA Lipid Nanoparticles, and Engineer Improved Vaccines and Therapies**	**Michael Smith Labs, UBC**
9:45 AM	**Miffy Cheng**	**Induction of Bleb Structures in Lipid Nanoparticle Formulations of mRNA Leads to Improved Transfection Potency**	**Biochemistry and Molecular Biology, UBC**
10:00 AM	**Yihao Wang**	**Semiconducting Polymer Dots Directly Stabilized with Serum Albumin: Preparation, Characterization and Cellular Immunolabeling**	**Chemistry, UBC**
10:15 AM		**BREAK**	
Session 3:			Chair: Diana Royce
10:45 AM	**Shirin Kalyan**	**Site Specific Immunomodulators - An Innately Novel Approach for the Prevention and Treatment of Chronic Inflammatory Diseases and Cancer**	**Qu Biologics Inc., Burnaby & UBC**
11:15 AM	**Kefan Song**	**Systemically Administered STING Agonist Drugamer Targeting Dendritic Cells for Cancer Immunotherapy**	**University of Washington, Seattle, USA**
11:30 AM	**Jiamin Wu**	**Self-Assembling Protamine Nanostructures Enable Sublingual Absorption of Proteins**	**Pharmaceutical Sciences, UBC**
11:45 AM	**Diana Royce**	**The NanoMedicines Innovation Network (NMIN) – a Canadian Network of Centres of Excellence (NCE)**	**NMIN**
11:50 AM	**Pieter Cullis**	**What is the Future of Nanomedicines?**	**Biochemistry, UBC**
12:15 PM		**LUNCH BREAK and POSTER SESSION**	

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Dear Participants,

It is my great pleasure to welcome you to the **Vancouver Nanomedicine Day 2023**, now the already 8th meeting of this series. It's truly an honour to being able to attract and see here in person, in our large Pharmaceutical Sciences auditorium (and an overflow auditorium), more than 300 of you who are interested in nanomedicines. After Covid, we on purpose wanted to celebrate the local talents and booming nanomedicine industry here in Vancouver. Despite that, we have a few speakers from Switzerland, Brazil, and the USA. The largest contingent from the United States is from our neighbours in Seattle at the University of Washington. Welcome to all of you!

During Nanomedicine Day, 19 talks and many posters will highlight the discoveries and innovations in nanomedicines that are contributing to global progress in acute, chronic and orphan disease treatment and management. Nanomedicines have allowed us to deliver drugs directly to disease sites, to dramatically improve their efficacy and reduce their toxicity, and to enable gene therapies with the potential to treat most human diseases. Diagnostics and imaging agents based on nanotechnology will help us to detect disease earlier and to more accurately monitor the effectiveness of therapy. In all of these areas, revolutionary developments are ongoing, at enormous speed. The importance of our field has also been acknowledged by the fact that this year's Nobel Prize in Medicine went to Drew Weissman and Katalin Karikó.

Please use this day not only to listen to new things, but also as a chance to network with each other, so that new collaborations can get going between you and the also present clinician-scientists, basic researchers, trainees, research partners, life science and startup biotechnology companies. Maybe even while drinking a beer and eating a pretzel at the end of the day during our reception?

Thank you all for coming and helping us to make this day a success!

Urs Häfeli, Faculty of Pharmaceutical Sciences
University of British Colombia, Vancouver
urs.hafeli@ubc.ca

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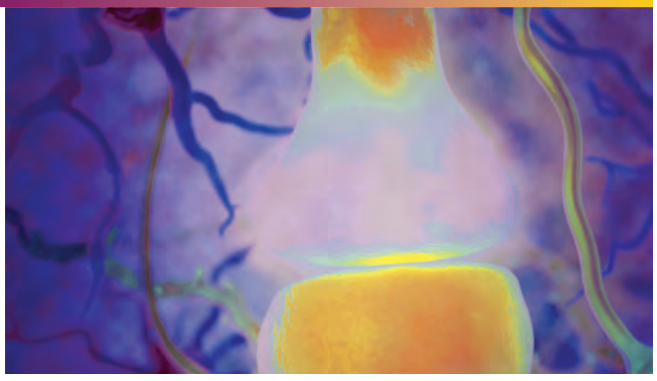

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VANCOUVER
NANOMEDICINE DAY
NMDN
November 15, 2023
Colin Ross
Therapeutic Genome Editing
Sabrina Leslie
Single Cell Imaging and Nanoparticle Engineering
Pieter Cullis
Future of Nanomedicines
Lennart Bohrmann
Aptamer Particles for Cancer Targeting
Shirin Kalyan
Site Specific Immunomodulators
Hannah Safford
mRNA Delivery to the Placenta
Free Food!

TALKS

TALK 1

Single-Molecule and Single-Cell Microscopy of mRNA-Lipid-Nanoparticles: *Applying Nanoscale Physics to Advance Nanomedicines*

Sabrina Leslie

Associate Professor, UBC Department of Physics and Astronomy and Michael Smith Labs

In response to the pandemic, new mRNA-LNP products were developed and injected into billions of human arms. Despite the success of these nanomedicines in suppressing the pandemic at the level of the population, at the level of the individual particle the efficiency of this product is approximately one percent. This presents an important opportunity for microscopists and therapeutic developers to work together to improve these products, as well as to leverage this new mRNA-LNP platform to create a vast array of new nanomedicines ranging from COVID19 vaccines to cancer treatments. In this talk, I will present a quantitative single-particle and single-cell imaging platform called CLiC (Convex Lens-induced Confinement) which we have developed and applied to fill important gaps in understanding and characterization of nanomedicines and thereby help make them better. CLiC imaging enables simultaneous single-particle measurements of multiple properties of mRNA LNPs, such as the distribution of size and mRNA-payload, as well as interaction rates in response to specific biomolecules or solution conditions. Importantly these single-particle measurements can be made in correlation with observations of their dynamic trajectories and interactions within cells. Our overall research, which is highly collaborative with the Cullis lab and members of the Nanomedicine Center for Excellence based at UBC, aims to characterize and understand the mechanisms of action of emerging classes of therapeutics and vaccines, so as to ultimately enable their optimization and come closer to addressing patients' needs. For example, we image individual confined, freely diffusing particles in solution as well as during reagent-exchange, such as in response to a change in solution pH, in order to emulate and explore intracellular dynamics such as endosomal release, but in a controlled setting. Over the long term and in collaboration with health scientists, we are working towards correlating our detailed multi-scale data sets, including single-particle measurements made in vitro as well as in cells and tissues, with clinical results, to create a through-line of understanding of vaccine/drug effectiveness from the microscopic to clinical scale. This talk will begin with our publication in ACS Nano (Kamanzi et al, 2021), share results from two additional manuscripts in submission and preparation, and describe our ongoing collaboration with health scientists to better understand mRNA-LNP vaccines and medicines through new biophysical measurements. In visiting Sweden and establishing new connections after the pandemic, my team and I are very open to new collaborations and finding opportunities for impact together.

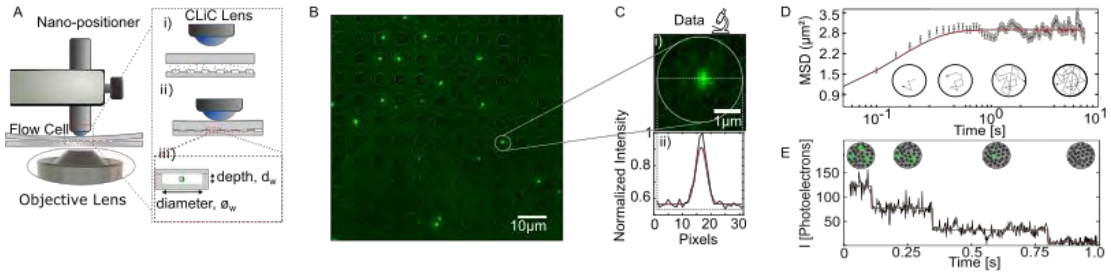
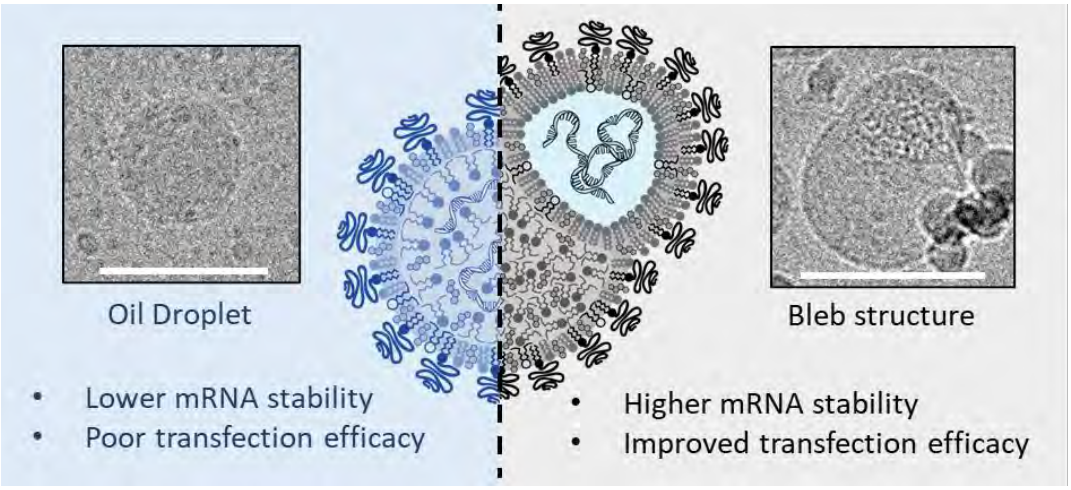


Figure: CLiC single-particle imaging and analysis of Lipid Nanoparticles (LNPs) enables characterization of detailed size and loading distributions of RNA–LNP complexes. A) Compressing the flow-cell confines LNPs for extended study. B) Trapped LNPs are shown in microwells. 100s – 1000s of isolated LNPs are imaged in parallel, a process which can be repeated thousands of times. C) Single-particle tracking is used to obtain LNP trajectories, which are then used to obtain particle diffusivity/ size (D) as well as intensity/RNA payload (E). This information is then used to define LNP size and RNA payload distributions and inform structure-activity relationships of the nanoparticles in conjunction with other dynamic, multi-scale and biological data.

TALK 2



Induction of Bleb Structures in Lipid Nanoparticle Formulations of mRNA Leads to Improved Transfection Potency

Miffy Hok Yan Cheng[1*"], Jerry Leung[1,2*], Yao Zhang[3], Colton Strong[1,2], Genc Basha[1], Arash Momeni[1], Yihang Chen[1], Eric Jan[1], Amir Abdolahzadeh[4], Xinying Wang[4], Jayesh A. Kulkarni[4], Dominik Witzigmann[4], Pieter R. Cullis[1,4"]

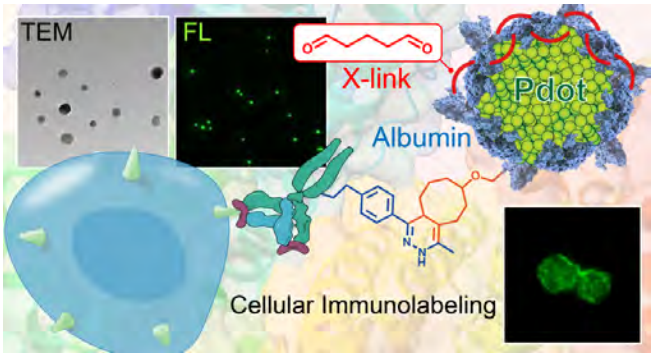
[1]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. [2]Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. [3]School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4. [4]NanoVation Therapeutics Inc., 2405 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 1Z3. * Contributed equally; email: miffy.cheng@ubc.ca

The transfection potency of lipid nanoparticle (LNP) mRNA systems is critically dependent on the ionizable cationic lipid component. LNP mRNA systems composed of optimized ionizable lipids often display distinctive mRNA-rich “bleb” structures. Here, it is shown that such structures can also be induced for LNPs containing nominally less active ionizable lipids by formulating them in the presence of high concentrations of pH 4 buffers such as sodium citrate, leading to improved transfection potencies both in vitro and in vivo. Induction of bleb structure and improved potency is dependent on the type of pH 4 buffer employed, with LNP mRNA systems prepared using 300 mm sodium citrate buffer displaying maximum transfection. The improved transfection potencies of LNP mRNA systems displaying bleb structure can be attributed, at least in part, to enhanced integrity of the encapsulated mRNA. It is concluded that enhanced transfection can be achieved by optimizing formulation parameters to improve mRNA stability and that optimization of ionizable lipids to achieve enhanced potency may well lead to improvements in mRNA integrity through formation of the bleb structure rather than enhanced intracellular delivery.

TALK 3

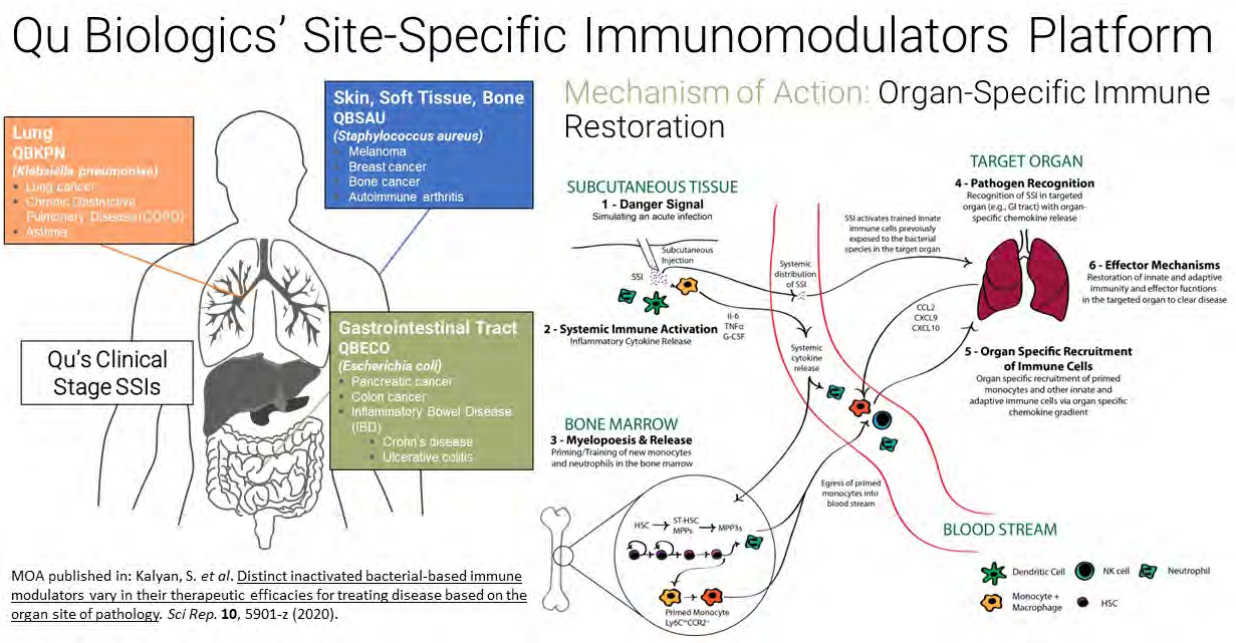
Semiconducting Polymer Dots Directly Stabilized with Serum Albumin: Preparation, Characterization and Cellular Immunolabeling

Yihao Wang†, Rupsa Gupta†, Ghinwa H. Darwish, Jade Poisson, Agnes Szwarczewski, Subin Kim, Christine Traaseth, Zachary M. Hudson, W. Russ Algar*



Semiconducting polymer dots (Pdots) are brightly fluorescent nanoparticles of growing interest for bioanalysis and imaging. A recurring challenge with these materials is obtaining robust physical and colloidal stability and low non-specific binding. Here, we prepared and characterized Pdots with bovine serum albumin (BSA) as the stabilizing agent (BSA-Pdots) instead of a more conventionally used amphiphilic polymer, both without and with crosslinking of the protein using glutaraldehyde (BSA(GA)-Pdots) or disuccinimidyl glutarate. Characterization included fluorescence properties; colloidal stability as a function of pH, ionic strength, and solvent perturbation; shape retention and hardness; and non-specific binding with common assay substrates, fixed cells, and live cells. These properties were contrasted with the same properties for amphiphilic polymer-stabilized Pdots and silica-coated Pdots. On balance, the BSA-stabilized Pdots were similar or more favorable in their properties, with BSA(GA)-Pdots being especially advantageous. Bioconjugation of the BSA-stabilized Pdots was possible using amine-reactive active-ester chemistry, including biotinylation and bioorthogonal functionalization for immunoconjugation via tetrazine-strained-alkene click chemistry. These approaches were used for selective fluorescent labeling of cells based on ligand-receptor and antibody-antigen binding, respectively. Overall, direct BSA stabilization is a very promising strategy for preparing Pdots with improved physical and colloidal stability, reduced non-specific interactions, and utility for in vitro diagnostics and other bioanalyses and imaging.

TALK 4



MOA published in: Kalyan, S. *et al*. Distinct inactivated bacterial-based immune modulators vary in their therapeutic efficacies for treating disease based on the organ site of pathology. *Sci Rep.* **10**, 5901-z (2020).

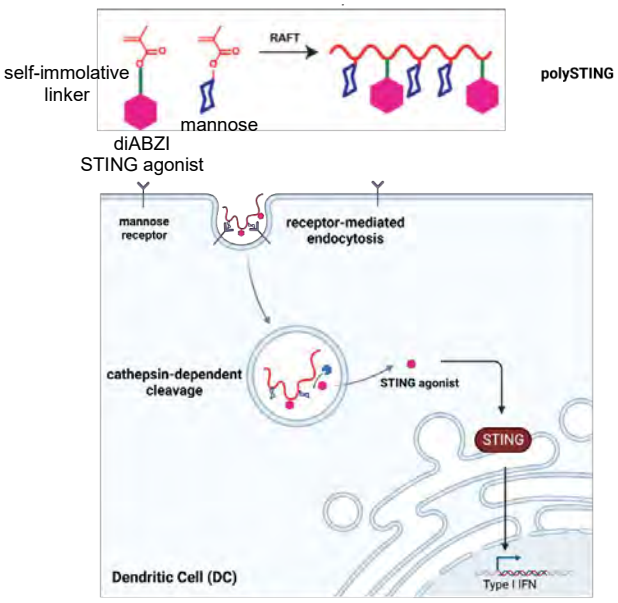
Site Specific Immunomodulators - An Innately Novel Approach for the Prevention and Treatment of Chronic Inflammatory Diseases and Cancer

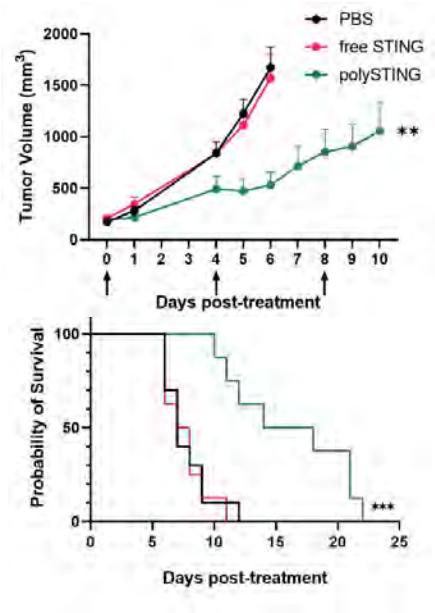
Shirin Kalyan[1*] & Hal Gunn[1] [1]Qu Biologics Inc, Burnaby, BC, Canada. *Email: shirin@qubiologics.com

Up until our most recent experience with the COVID-19 pandemic, the 20th Century had seen a dramatic decrease in the incidence of acute infection and infection mortality with the advent of antibiotics, vaccines and improved hygiene, but with these gains, we have experienced a concomitant rise in allergies, chronic immune-related disorders and certain cancers, particularly in developed countries. This observation has become popularly known as the "hygiene hypothesis", which postulates that a lack of appropriate training of the immune system by microbial stimulation, especially early in life, is linked to the development of immune pathologies. Trained innate immunity is a recently recognized process that allows innate immune cells to undergo adaptations that increase their efficiencies through a form of non-specific memory that is programmed at the epigenetic and metabolic levels. This "training" can be thought of as being analogous to the training muscle cells get from exercise, which results in epigenetic and metabolic changes at the cellular level that builds muscle memory, resiliency and tone. In the context of innate immunity, this type of training leads to the development of immunological memory, but this immune memory is quite different from the type of memory that is encoded by T cell and B cell receptors that recognize their cognate antigens like a lock and key. In contrast to this highly specific adaptive immune memory, trained innate immunity results in a broader and more comprehensive immune competence that can address a diverse number of challenges. It is this aspect of innate immune training that makes it an attractive therapeutic target to treat and/or prevent the immune dysfunction that is linked with cancer, chronic inflammatory disorders and poor outcomes following infection.

Qu Biologics is developing a first-in class immunotherapy platform, called Site Specific Immunomodulators (SSIs), that is designed to train cells of the innate immune system to respond effectively to infectious and non-infectious challenge and to direct the collective effector functions of innate immunity to specific organ sites to enhance and optimize immune function at the site of disease. In this respect, SSIs can be applied to treat a broad range of solid cancers as well as diseases rooted in immune dysregulation, such as chronic inflammatory disorders. Each organ-specific SSI is derived from an inactivated microbe that is endogenous and common source of infection in the targeted organ (see figure for SSIs in clinical development and the mechanism of action of SSIs). Dr. Kalyan will be presenting the promising data Qu Biologics has acquired to date on the application of SSIs and the two randomized placebo-controlled trials Qu is currently conducting.

TALK 5





Systemically Administered STING Agonist Drugamer Targeting Dendritic Cells for Cancer Immunotherapy

Kefan Song[1], Dinh Chuong Nguyen[2], Simbarashe Jokonya[1], Omeed Yazdani[1], Drew L. Sellers[1], Suzie H. Pun[1,2], Patrick S. Stayton[1,2]

[1]Department of Bioengineering, University of Washington, USA
[2]Molecular Engineering & Sciences Institute, University of Washington, USA
Corresponding authors: spun@uw.edu (S.H.P) and stayton@uw.edu (P.S.S)

The Stimulator of Interferon Genes (STING) pathway is a promising target for cancer immunotherapy. STING activation leads to pro-inflammatory cytokine secretion and exerts an antitumor effect. Despite recent advances, therapies targeting the STING pathway are often limited by routes of administration, insufficient STING activation or off-target toxicity. In many cases, STING agonists need to be delivered through intratumoral injections to achieve moderate STING activation without generating systemic toxicity.

We developed a dendritic cell (DC) - targeted polymer platform (polySTING) to deliver a diamidobenzimidazole (diABZI) STING agonist. PolySTING contains mannose groups, which facilitate DC uptake of STING agonists in the tumor microenvironment (TME). The STING agonist is conjugated through a self-immolative linker for controlled release in the endosomes. When administered intravenously in tumor-bearing mice, polySTING triggered DC activation, promoted $CD8^+$ DC cross-presentation and recruited $CD8^+$ T cells in the TME. Systemic administration of polySTING slowed tumor growth in a B16F10 melanoma model and a 4T1 breast cancer model. We demonstrate that polySTING delivers STING agonists to antigen-presenting cells (APCs) after systemic administration and generates strong anti-tumor efficacy with minimal side effects.

TALK 6

SELF-ASSEMBLING PROTAMINE NANOSTRUCTURES ENABLE SUBLINGUAL ABSORPTION OF PROTEINS

Jiamin Wu, Shyh-Dar Li*

Faculty of Pharmaceutical Science, The University of British Columbia, Canada.

*Corresponding author, E-mail: shyh-dar.li@ubc.ca

Abstract

Therapeutic proteins often required needle-based injections, which compromise medication adherence especially for those with chronic diseases. Sublingual administration provides a simple and non-invasive alternative. Herein, two novel self-assembling protamine nanostructures (lipid-conjugated protamine (micelles) and protamine dimer (nano-rod)) were synthesized to enable sublingual delivery of proteins through simple physical mixing with the payloads. It was found that the protamine nanostructures promoted intracellular delivery of proteins via increased pore formation on the cell surface. Results from *in vitro* models of cell spheroids and human sublingual tissue substitute indicated that the protamine nanostructures enhanced protein penetration through multiple cell layers compared to protamine. The protamine nanostructures were mixed with insulin or semaglutide and sublingually delivered to mice for blood glucose (BG) control. The effects of these sublingual formulations were comparable as the subcutaneous preparations and superior to protamine. In addition to peptide drugs, the protamine nanostructures were shown to enable sublingual absorption of larger proteins with molecular weights from 22 to 150 kDa in mice, including human recombinant growth hormone (rhGH), bovine serum albumin (BSA) and IgG. The protamine nanostructures given sublingually did not induce any measurable toxicities in mice.

TALK 7

NanoMedicines Innovation Network (NMIN): Developing novel nanomedicine-based therapeutics and diagnostics

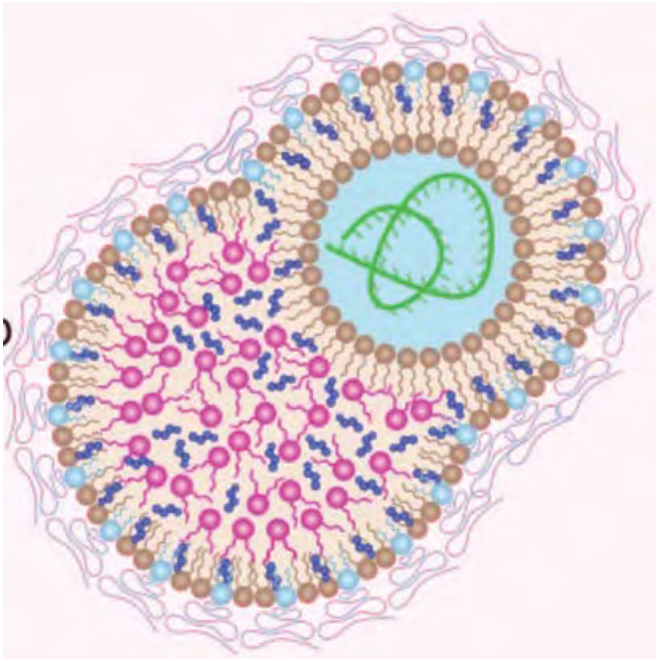
Diana Royce

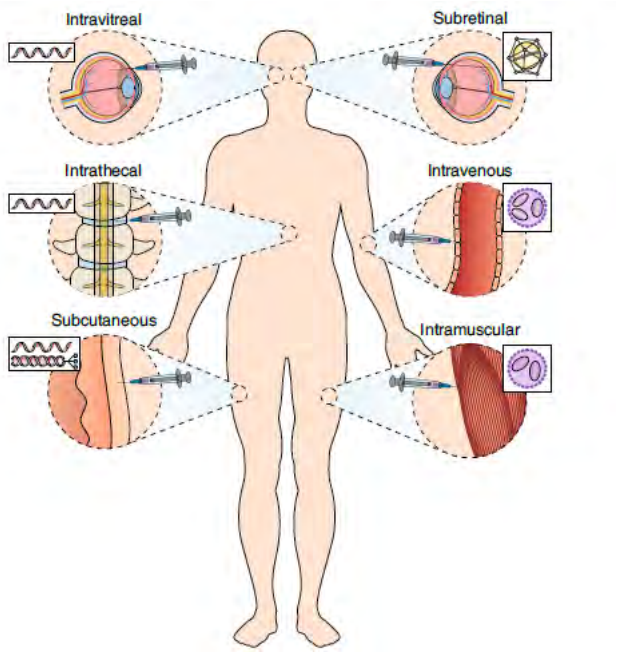
NanoMedicines Innovation Network (NMIN); dianaroyce@nanomedicines.ca

NMIN, the Nanomedicines Innovation Network, is a national research network funded by the Government of Canada through the Networks of Centres of Excellence (NCE) Program, with a mandate to develop novel nanomedicine-based therapeutics and diagnostics that cure high-burden human diseases and detect disease more precisely; to commercialize these products to generate health and economic benefits to Canadians; and to provide advanced training and development opportunities in the next generation to meet the demand for talent within Canada's burgeoning nanomedicines sector.

Between 2019 and 2024, NMIN invested $14.56M in 70 industry partnered research projects, and another $1.7M in nanomedicines-related research and innovation capacity-building initiatives at 10 participating universities across Canada. Over 100 investigators, 250 trainees and 133 partner organizations have resulted in the establishment of six NMIN spin-off companies, the creation of over 100 new jobs; nine patents; and over 825 publications. Legacy initiatives include three research platforms-NanoCore, PharmaCore and an early Health Technology Assessment (eHTA) core facility, and a national database of nanomedicines research expertise.

TALK 8





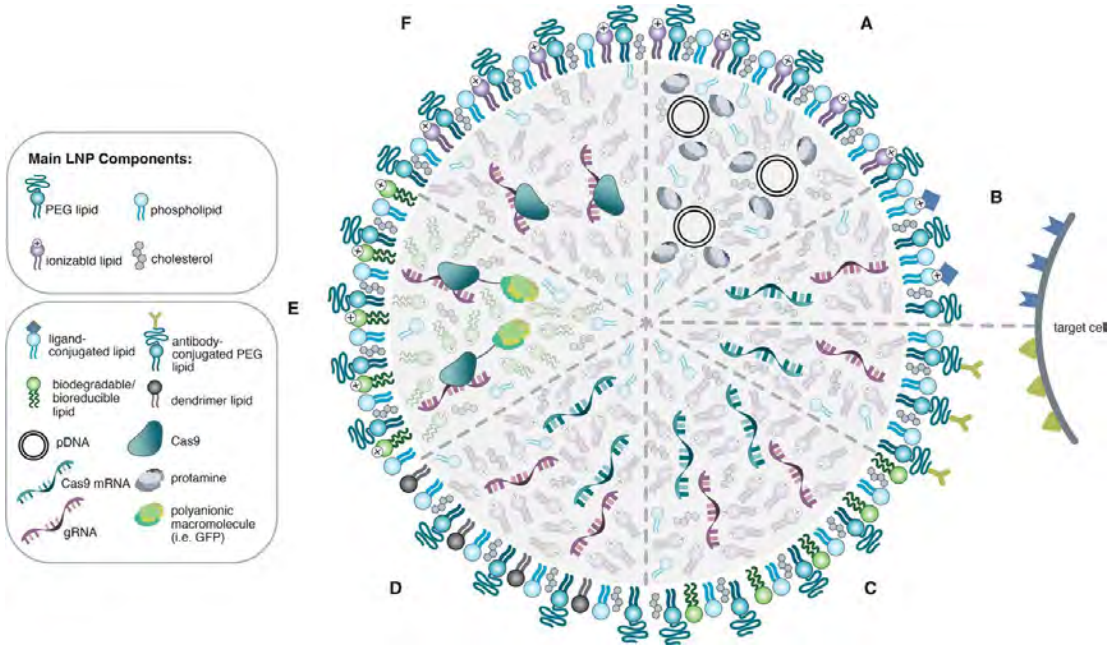
The Future of NanoMedicines

Pieter Cullis

Department of Biochemistry and Molecular Biology, University of British Columbia

Nanomedicines are the medicines of the future. The need is so clear. We need delivery systems to deliver small molecules more accurately to sites of disease and we need delivery systems to deliver large molecules, such as nucleic acid-based drugs for gene therapies to target tissues in the body. Amazingly, these objectives are starting to be realized. While we have not yet achieved specific delivery of small molecules to disease sites such as tumours, the ways of achieving this are becoming clearer with many clinically approved nanomedicines to treat cancer and other diseases. In the case of RNA, we now have clinically approved systems for delivery of siRNA to target tissues in the liver and delivery of mRNA to immune cells for vaccine applications. These have had huge global impact. But this is nothing compared to the future impact as we develop gene therapies for most human diseases. This is the most exciting time to develop new pharmaceuticals in history. The nanomedicines of the future will eliminate many of the currently accepted norms-development times of a couple of months instead of 15 years, costs dramatically reduced from the $1B that is often required at the present and drugs that are highly personalized as opposed to the “one size fits all” model. Revolutionary times indeed!

TALK 9



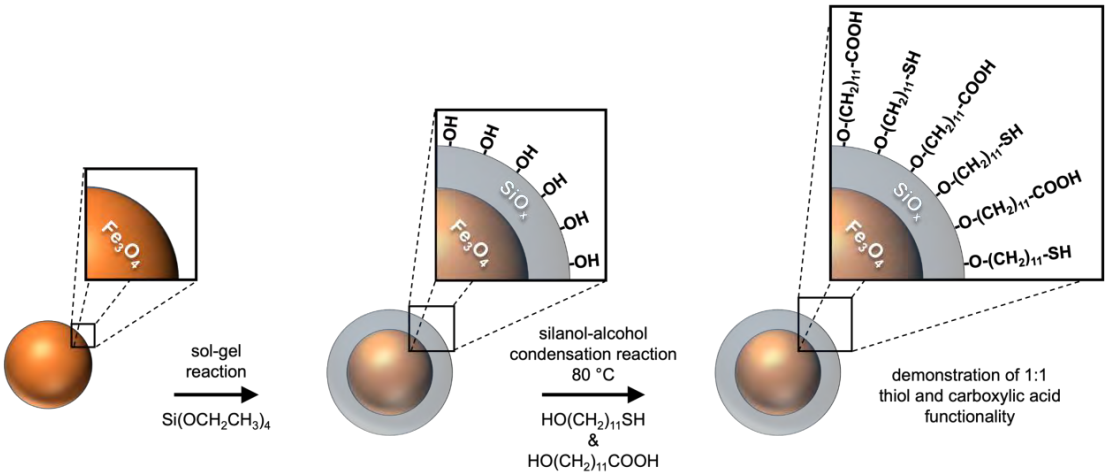
Nanomedicines for Gene Therapy: Optimization of Lipid Nanoparticles for Therapeutic Genome Editing

Colin Ross

University of British Columbia, Faculty of Pharmaceutical Sciences, Vancouver, BC;
colin.ross@ubc.ca

Genetic diseases are a leading cause of death and disability, with immense economic and societal burdens. While individually rare, >7,000 genetic diseases affect 1 in 12 Canadians and impact the lives of ~500,000 children in Canada. Our team is optimizing state-of-the-art CRISPR-based 'base' and 'prime' genome editors and their delivery to treat genetic diseases by directly repairing the underlying disease-causing mutations. The transient expression of RNA delivered via nanoparticles is well suited for this task. However, nanoparticle-mediated delivery of complex gene editing cargo remains a challenge, especially to extra-hepatic tissues. We have developed a new mouse model (LumA) in which to efficiently evaluate in vivo genome editing. We are evaluating novel nanoparticle formulations and genome editor formulations for improved safety and effectiveness of *in vivo* therapeutic genome editing.

TALK 10



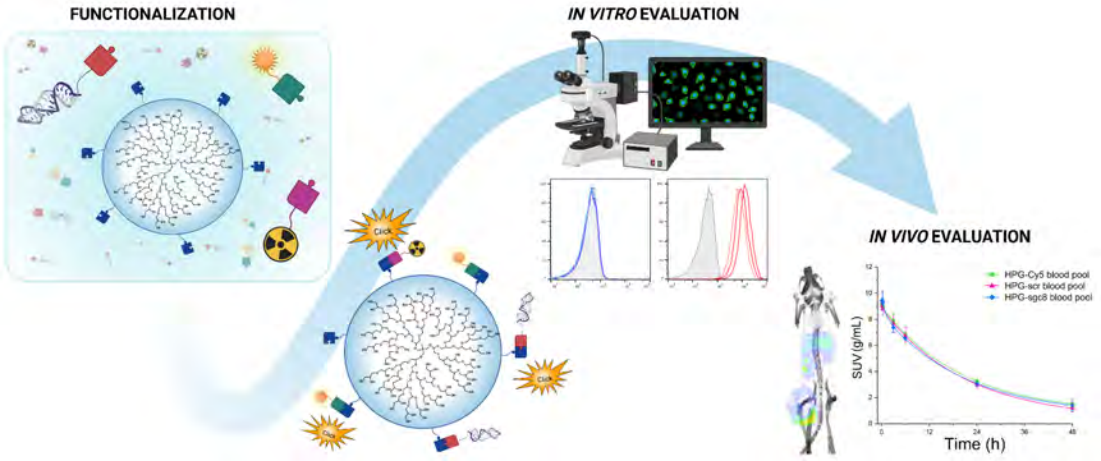
Dual Functionalization of Iron Oxide Nanoparticles

Melissa J. Radford[1], Henry J.H. Kang[1], Audrey K. Taylor[1], Rana F. Ali[1], Stephanie Sonea[1], Byron D. Gates[1]

[1]Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

In this study, we present a novel and efficient method for the surface modification of silica-coated iron oxide nanoparticles. Through a one-pot silanol-alcohol condensation reaction assisted by microwave radiation, we successfully achieved dual functionalization of these nanoparticles, allowing for the simultaneous attachment of two distinct surface chemistries in precise and tunable ratios. Our approach offers significant advantages over traditional functionalization methods, particularly in overcoming challenges associated with introducing functionalities using silanes, which are prone to intermolecular reactions and the formation of multiple layers on the particles. By utilizing this innovative technique, we customized the surface chemistry of silica-coated iron oxide nanoparticles, loading them with thiol and carboxylic acid terminal functional groups in ratios of 1:0, 3:1, 1:1, and 0:1, respectively. Thiol groups provide widespread utility for applications such as attaching biological recognition elements, while carboxylic acid terminal groups offer versatility in attaching various species to surfaces, making them valuable for preparing biosensors and other functionalized nanoparticles. Through comprehensive chemical and spectroscopic analyses, we confirmed and quantified the successful formation of covalently bound molecules with thiol and carboxylic acid terminal groups in well-defined ratios. Moreover, our microwave-assisted, one-pot method significantly reduced processing time compared to convective heating methods, making it a promising approach for tailoring nanoparticle surface properties for a wide range of applications.

TALK 11



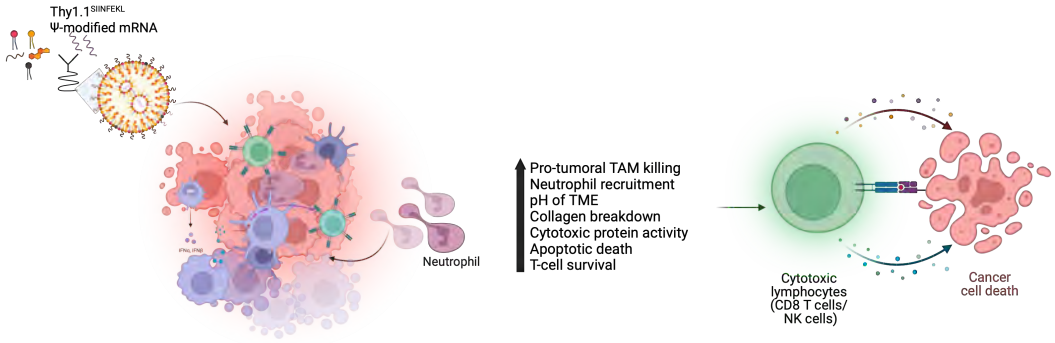
Aptamer Conjugated Nanoparticles for Cancer Targeting: In Vitro and In Vivo Evaluation

Lennart Bohrmann [1,2], Tobias Burghardt [1], Cristina Rodríguez-Rodríguez [1], Matthias M. Herth [3,4], Katayoun Saatchi [1], Urs O. Häfeli [1,2]

[1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada; [2]Department of Pharmacy, Faculty of Health and Medicinal Sciences, University of Copenhagen, Copenhagen Denmark; [3]Department of Drug Design and Pharmacology, Faculty of Health and Medicinal Sciences, University of Copenhagen, Copenhagen Denmark; [4]Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Copenhagen,Denmark

Despite tremendous efforts in nanomedicine research, only minute fractions of the administered dose reach to solid tumors A potential strategy to improve drug delivery and diagnostics in cancer is active targeting of nanomedicines to cancer-specific antigens. Aptamers posses several advantages over other targeting agents, such as high specificity and affinity, chemical stability, low production cost and low immunogenicity. The aim of this project was to functionalize high molecular weight hyperbranched polyglycerol (HPG) with the PTK7 specific aptamer sgc8, fluorescent reporters and ^{111}In-DTPA *via* IEDDA based click chemistry. Binding of the nanoprobe against a range of cell lines was evaluated using flow cytometry and confocal microscopy. High affinity of the nanoprobe against triple negative breast cancer derived MDA-MB-431 cells as well as epidermoid carcinoma derived A431 cells was observed. Furthermore, conjugation to HPG significantly improved the plasma stability of sgc8, a typical limitation of aptamers. Despite preferential binding of sgc8 conjugated HPG, control experiments with a scrambled oligonucleotide sequence revealed non-specific binding of ssDNA conjugated HPG. While aptamers have been proposed as promising alternatives to antibodies more than three decades ago, these results highlight some of the challenges that still prevent their broader clinical application. *In vivo* SPECT/CT imaging in a preclinical tumor model was used to quantitatively assess tumor uptake and pharmacokinetic profile of the nanoprobe. The EPR effect was identified as the prevailing tumor uptake mechanism of the nanoprobe potentially due to heterogenous perfusion of the tumors.

TALK 12



Comprehensive mRNA-LNP Characterization using Single-Cell Techniques Reveals Tumour Microenvironment Reprogramming for Cancer Immunotherapy

Abishek Wadhwa[1], Danielle L. Krebs[1], Jessica A.F.D. Silva[1], Maunish Barvalia[1], Genc Basha[2], Parneet Sekhon[1], Rein Verbeke[2], Karen Chan[2], Norbert Pardi[3], Dominik Witzigmann[2], Pieter R. Cullis[2], Kenneth W. Harder[1*]

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Cancer is a leading cause of death in Canada and the rest of the world. New efficacious and safe mRNA-LNP-based therapeutics exhibiting site-specific tumour targeting to enhance pre-existing but inefficient tumour immune responses, will be instrumental in fighting cancers.

Tumour-resident myeloid cells such as tumour-associated macrophages (TAMs) constitute up to 50% of cell mass within most solid tumours and harness several mechanisms that contribute to an immunosuppressive tumour microenvironment (TME).

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. We characterized a novel formulation (Thy1.1^{SIINFEKL} mRNA-LNP2) with significant (p=0.0004) preferential targeting of hard-to-transfect TAMs, depletion of which was hypothesized to impact tumour growth kinetics.

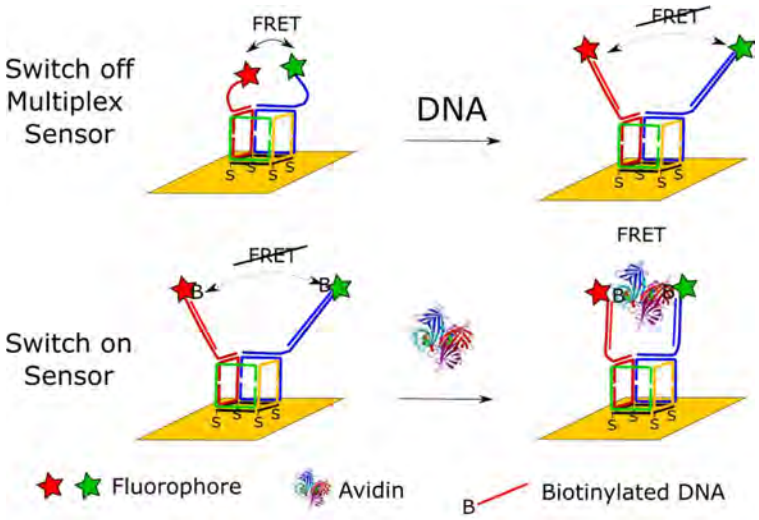
Resolving TAMs into pro-tumoral TAMs and anti-tumoral TAMs revealed that LNP2 preferentially depleted pro-tumoral macrophages (p<0.0001). A dramatic increase in the proportion of mature neutrophils was also observed in the LNP2 experimental group (p=0.002), accompanied by our observation of yellow pus formation at the tumour site, suggesting cell death and inflamed tumours. Mice treated with Thy1.1^{SIINFEKL} mRNA-LNP2 exhibited a significant delay in tumour growth and increased survival (p=0.0012), as compared to mice treated with Thy1.1^{SIINFEKL} mRNA-LNP1 (control LNP) and vehicle controls, while no significant differences could be observed in tumour growth between mice treated with control LNP and vehicle controls. Our comprehensive characterization involved antibody-based single-cell techniques - flow cytometry, mass cytometry, and CITE-seq for high-resolution analyses. These multimodal findings, including the transcriptional analysis of tumour-associated immune cells after LNP treatment, suggest that the mechanism of the anti-tumour effect is mediated by cytotoxic protein- and apoptosis-related pathways likely involving CD8 T cell and/or NK cell activation.

Significant mRNA-LNP mediated TME reprogramming is an attractive strategy to hamper TAM-driven pro-tumorigenic processes and holds strong cancer immunotherapeutic potential.

Acknowledgements

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

TALK 13



DNA Nano – Cubes as Highly Tunable Scaffold for Biosensors Engineering

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Minimalistic wireframe DNA structures are of particular interest due to ease of preparation, high functionality, and reduced cost of needed DNA strands. Recently such structures were presented as cargo delivery vehicles to the cells[1] as well as cell membrane protein mimics[2]. Here we present the use of DNA nano – cubes, pioneered by Sleiman[3] et al., as the scaffold to engineer and distribute DNA probes on the electrode surface for bio – molecules detection.

Thanks to precise dimensions of the DNA structure, we can space out DNA probes 10 nm apart with one donor and one acceptor fluorophore probe on each cube. In this arrangement the probes are close enough for energy transfer (FRET) to occur, but far enough so that changing the conformation of one strand does not affect the other. We prepared a multiplex sensor that detects two different DNA sequences simultaneously and independently using two different signals. The binding event is detected by FRET signal disappearance. We can identify which sequences are present by observing increase in signal of respective fluorophores. Moreover, simple modification to introduce biotinylated DNA, allowed us to successfully capture avidin, used here as a model dimerizing protein. Dimerized DNA strands produced an increase of up 100% in FRET signal. We have also demonstrated that the structures are released from the electrode at negative potentials, which could be used as targeted cellular delivery vehicles. We have shown that precise arrangement of DNA probes on nano – cubes allow us to perform complex sensing mechanisms that otherwise would be impossible using random probes distribution.

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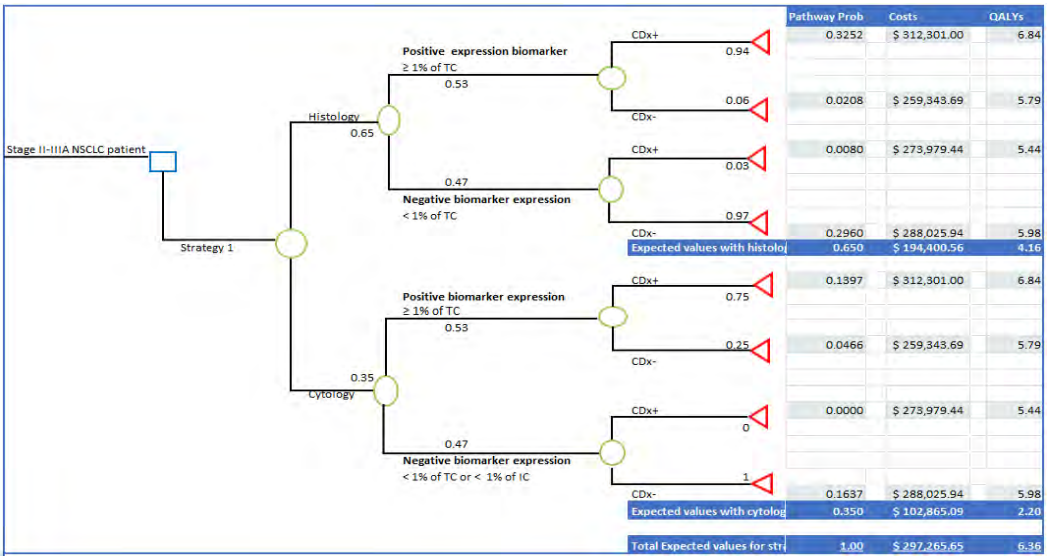
TALK 14



Innovation Headroom for a More Accurate PD-L1 Companion Diagnostic in Early-Stage Non-Small Cell Lung Cancer

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Programmed cell death ligand 1 (PD-L1) inhibitors like atezolizumab have transformed the treatment of non-small cell lung cancer (NSCLC) in recent years. A number of PD-L1 companion diagnostics (CDx) are currently used to guide treatment for NSCLC, with the standard of care (SoC) for early-stage NSCLC involving the use of atezolizumab as an adjuvant therapy for patients with elevated PD-L1 expression (tumour proportion score (TPS)>1%). However, existing PD-L1 CDx kits have limited accuracy, with a 6% false negative rate (FNR) in histology specimens and an estimated 25% FNR in cytology specimens, which are the only available specimen in 30-40% of cases. Emerging nanotechnologies offer the promise of PD-L1 expression assays that are more accurate than the SoC [1].

To assess the potential value of a new PD-L1 CDx to payers, the objective of this study was to conduct a headroom analysis by comparing a hypothetical perfect PD-L1 assay (100% sensitivity and specificity) with the SoC using a decision-tree based cost-effectiveness analysis. Relative to the SoC, a perfect PD-L1 CDx would have a cost-effectiveness of US$49,000 per quality-adjusted life-year (QALY) gained if the price of the new CDx was the same as current test kits (~$70), which is well below the $100,000/QALY threshold used in the United States. At this threshold, the maximum reimbursable price for a perfect PD-L1 CDx would be ~US$4,000 per patient (including the cost of new lab equipment). While a new test is unlikely to have perfect accuracy, this suggests that significant innovation headroom exists for a more accurate CDx for this indication.

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TALK 15

A novel liposomal irinotecan formulation is a potent immunogenic cell death inducer- Should it be developed for use in patients with colorectal cancer?

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Background: Irinotecan (CPT-11) is a topoisomerase I inhibitor that is currently used in first and second-line chemotherapy treatments for multiple cancers. The use of CPT-11 has been associated with side effects like gastrointestinal (GI) toxicity which limits its use in metastatic colorectal cancer (mCRC).[1] It is possible that an appropriately designed liposomal formulation of CPT-11 may reduce this GI toxicity. More importantly, the liposomal formulation may be ideally suited for use in combination with immunotherapeutic, specifically immune checkpoint inhibitors (ICIs). CPT-11 can induce a cell death pathway that leads to what is referred to as immunogenic cell death (ICD), an effect that may best be obtained using a drug delivery system such as liposomes.[2] The ICD effect should enhance response rate when using immunotherapy treatments. A novel liposomal formulation of CPT-11 (referred to as Irinosome High C) is described. This formulation relies on Cu(II) ions to complex CPT-11 and control its release from the liposome. It is hypothesized that this formulation will enhance the therapeutic effects of ICIs through the induction of ICD. If ICD is induced, the treated apoptotic cells should exert a cancer vaccine like effect *in vivo.*

Method: Murine and human colorectal cancer cell lines will be treated with Irinosome High C *in vitro* and the treated cell culture media harvested and analyzed to assess the change of HMGB-1 levels (using an established commercial ELISA assay kit). ICD inducing effect can be assessed by measuring the release of damage associated molecular patterns (DAMPs) such as high mobility group box-1 (HMGB-1) when cells are exposed to drugs. The ability of Irinosome High C to induce ICD was also evaluated in a whole cell vaccination study. CT26 and MC38 cells were treated with Irinosome High C *ex vivo* and inoculated into syngeneic mice. The mice were then challenged with parent (untreated) CT26 and MC38 cells and monitored for tumor growth.

Results: Irinosome High-C is better at stimulating the release of HMGB-1 in the tested drug (CPT-11) concentrations compared to free drug. The formulation can induce a vaccine-like effect *in vivo* in mice inoculated with CT26 and MC38 cells. When challenged with parent CT26 and MC38 cells, mice that were pre-inoculated with Irinosome High C treated CT26 and MC38 cells tumors growth was mitigated.

Conclusion: This novel liposomal CPT-11 formulation Irinosome High C is a robust ICD inducer against both murine colorectal cancer cell lines and human cancer cell lines. This formulation thus may possess therapeutic potential to enhance immunotherapy treatment outcome for mCRC. Future studies will be assessing whether the TVN effect that is unique to the liposomal formulations contributes to the activity of immunotherapeutics.

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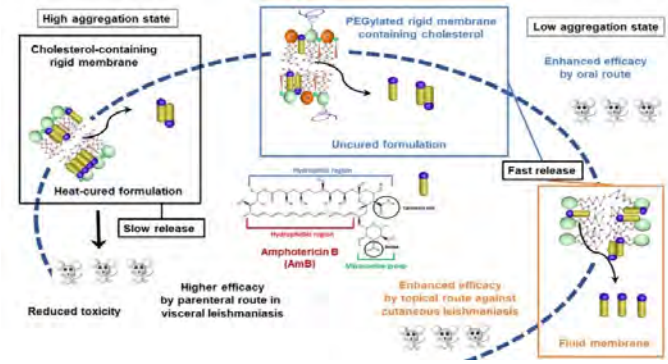
TALK 16



DEVELOPMENT OF PEGYLATED LIPOSOMAL AMPHOTERICIN B FOR ENHANCED TREATMENT OF CUTANEOUS LEISHMANIASIS THROUGH PARENTERAL AND ORAL ADMINISTRATION

Guilherme S. Ramos[1], Virgínia M.R. Vallejos[1], Gabriel S.M. Borges[2], Raquel M. Almeida[3], Izabela M. Alves[2], Marta M.G. Aguiar[2], Christian Fernandes[2], Pedro P.G. Guimarães[1], Ricardo T. Fujiwara[3], Philippe M. Loiseau[4], Lucas A.M. Ferreira[2], Frédéric Frézard[1].

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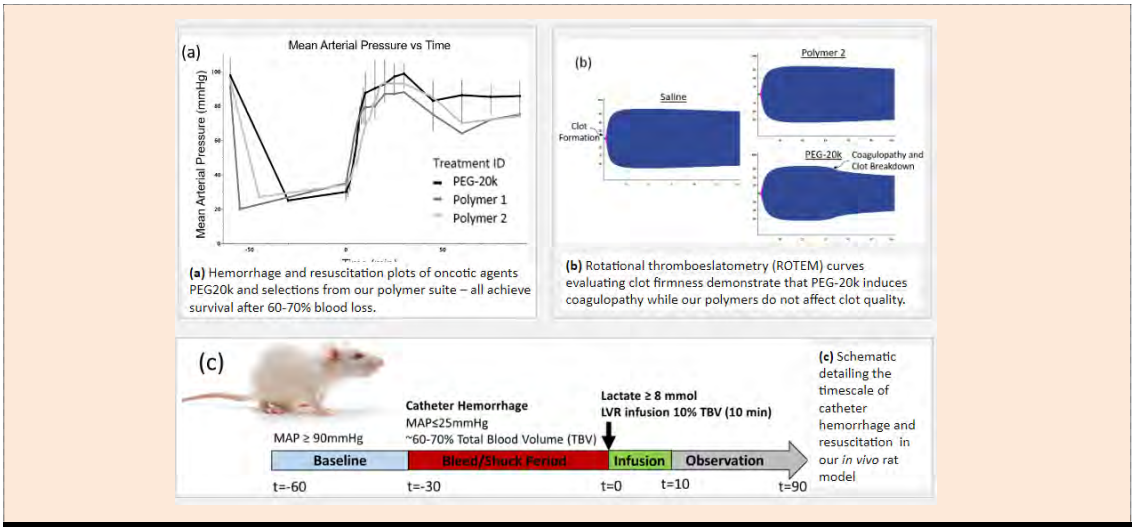
Liposomal amphotericin B (AmB), including the renowned AmBisome®, stands out as the most effective and safer therapeutic option for visceral leishmaniasis (VL). However, its clinical efficacy faces limitations in the context of cutaneous leishmaniasis (CL) and HIV/VL co-infection. This study aimed to formulate AmB in PEGylated liposomes, introducing a novel approach, and subsequently compare its therapeutic efficacy with AmBisome® using a murine model of CL. The liposomal formulations of AmB, whether conventional or PEGylated, prepared according to an innovative process [1], underwent thorough characterization for particle size, morphology, drug encapsulation efficiency, and aggregation state. Vesicles with diameters of 100-130 nm and low polydispersity were achieved, incorporating over 95% of AmB under low aggregation state. A first comparative study with AmBisome® involved administering seven doses of the formulations to *Leishmania amazonensis*-infected BALB/c mice by intraperitoneal (IP) or intravenous (IV) routes every 4 days (5 mg/kg of AmB). The PEGylated AmB formulation significantly outperformed conventional liposomal AmB and AmBisome®, demonstrating higher levels of reduction of the lesion size growth and the parasite load in the murine model of CL. Our study extended to a 10-dose treatment regimen at 2-day intervals, where the PEGylated formulation given IP, but also by oral route, consistently achieved significant reductions in lesion size growth and parasite load, comparable to IP-administered AmBisome®. Evaluation of the markers of renal and hepatic functions revealed a notable advantage of the PEGylated formulation given orally, compared to AmBisome® (IP) and PEG-LAmB (IP) that showed significantly increased urea levels. This groundbreaking study highlights, for the first time, the potential of PEGylated liposomal AmB in promoting a safer and more efficacious treatment of experimental CL through both parenteral and oral administration. The PEGylated AmB formulation, in addition to be easy to prepare and scale-up, emerges as a promising drug candidate for CL treatment (Work supported by the Brazilian agencies FAPEMIG, CNPq and FINEP).

[1] *Frézard et al. (2021) BR1020210068205, Patent application.*

TALK 17



Engineering Polymers for Resuscitation of Trauma Patients

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Hemorrhagic shock is the leading cause of death across civilian and military populations, with 150,000 trauma-related deaths annually in the United States alone[1,2]. Patient rescue is achieved by resuscitation, which reintroduces fluid into the bloodstream to prevent hypovolemia and to recover blood pressure and tissue oxygenation. Traditional resuscitants operate on the principle of aggressive fluid replacement, a strategy which can interfere with coagulation, is impractical in the prehospital setting[3]. However, recent efforts have demonstrated the powerful oncotic resuscitation profiles of high molecular weight, hydrophilic polymers such as PEG-20k[4]. We are developing a suite of novel oncotic polymers for emergency resuscitation applications and investigating the effects of polymer composition, architecture, and branching density upon oncotic profiles. Our research aims to develop next-generation low-volume resuscitants for the treatment of traumatic injury.

Through analysis of our suite of oncotic polymers, we are now able to understand the relationship between polymer architecture/composition and desirable oncotic properties for resuscitation. Through our in vivo model, we have demonstrated that a 10% blood volume dose of oncotic polymer recovers mean arterial pressure (MAP) and achieves resuscitation and survival after severe hemorrhages in which 60-70% blood volume was lost (Figure 1A). Our oncotic polymers are competitive with PEG-20k, meaning that resuscitation is achieved with dramatic improvement over the current standard of care. Furthermore, we demonstrate for the first time an oncotic polymer capable of achieving resuscitation without interfering with coagulation, an imperative but historically ignored criterion for trauma therapies5 (Figure 1B). The performance of our polymers is evaluated in a rat model of lethal hemorrhagic shock (Figure 1C).

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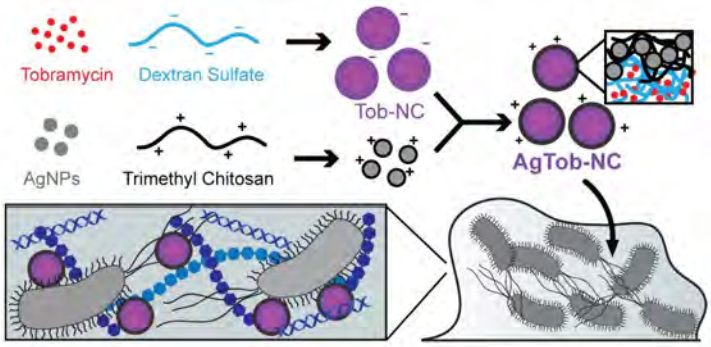

Directing the Biointerface of Polyelectrolyte Nanocomplexes to Treat Bacterial Biofilm Infections

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A majority of bacterial infections are present as biofilms: bacteria encapsulated within a dense matrix of polysaccharides, DNA, and proteins. Biofilms increase virulence and prevent effective antibiotic delivery, leading to infections with up to 1000x antibiotic tolerance.[1] New strategies to enhance the activities of already approved antibiotics such as tobramycin (Tob) are therefore needed to combat biofilm infections, such as the *P. aeruginosa* lung infections associated with cystic fibrosis. The Finbloom lab leverages bioinspired approaches to develop nanomaterials with directed microbe-material biointerfaces for enhanced antibiotic delivery and efficacy.

We recently developed polyelectrolyte nanocomplexes (NCs) for the codelivery of the synergistic antimicrobials silver nanoparticles and tobramycin.[2] AgTob-NCs were optimized with diameters of 200 nm and high Tob/Ag loading efficiencies of ~75% and >95%, respectively. AgTob-NCs with negative or positive charges were formulated to evaluate how surface chemistry influenced NC biofilm navigation and drug delivery. Positively charged NCs had broader biofilm distribution and resulted in near-complete eradication of biofilms *in vitro*. In mouse models of lung infection, positively charged NCs reduced bacterial burden by 1.5 log-fold, improved survival outcomes to 80% when compared to 37% for Tob alone, and led to an overall enhancement in lung health.

Current research efforts are focused on developing dynamic Tob-loaded NCs inspired by neutrophil extracellular traps (NETs). NET-inspired NCs have pH-responsive properties to increase positive charge and swell in size when exposed to infection-associated acidic microenvironments in order to capture bacteria and trigger drug release. These studies provide insight in designing nanocomplexes to overcome drug delivery barriers. By directing microbe-material biointerfaces through design of charge, size, and dynamic behaviours, we are working to enhance antibiotic delivery and treat bacterial infections.

[1] Flemming, H.-C. *et al*. Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* **14**, 563–575 (2016).
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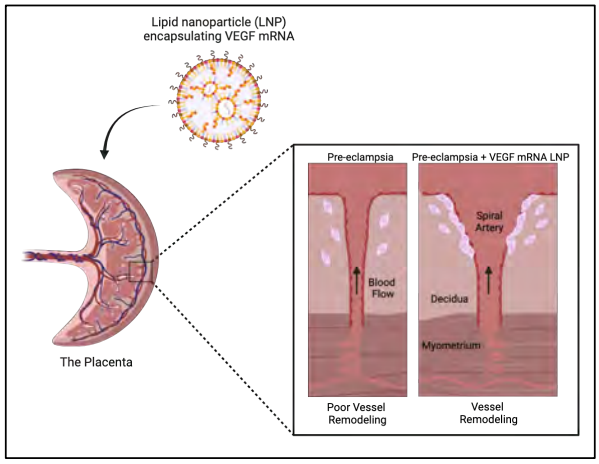
TALK 19



Ionizable Lipid Nanoparticles for In Vivo mRNA Delivery to the Placenta

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Pre-eclampsia is a placental disorder characterized by insufficient blood flow to the placenta and is a leading cause of maternal and neonatal morbidity and mortality worldwide. Despite the global prevalence of pre-eclampsia, there are currently no curative treatment options that address the underlying placental vascular dysfunction.[1] Therefore, therapies that improve vasodilation in the placenta during pre-eclampsia have the potential to benefit both maternal and fetal health. Lipid nanoparticle (LNP)-mediated delivery of pro-angiogenic mRNA, such as vascular endothelial growth factor (VEGF), holds great promise as a therapeutic to treat pre-eclampsia. However, while LNPs are the most clinically advanced non-viral platform for mRNA delivery, they have been minimally investigated for placental disorders due to the challenges associated with achieving extrahepatic mRNA delivery. Since LNPs primarily deliver their cargo to the liver upon systemic administration, we can achieve extrahepatic delivery of LNPs by altering either the chemical structure of the ionizable lipid or the relative molar ratios of the lipid excipients.

To facilitate LNP-mediated mRNA delivery of pro-angiogenic factors to the placenta, we engineered placenta-tropic LNPs through a two-phase screening approach. First, we synthesized a library a novel ionizable lipids to formulate LNPs and identified a lead ionizable lipid candidate that enabled selective, extrahepatic *in vivo* mRNA delivery to the placenta compared to benchmark, liver-tropic LNP formulations. We then utilized the principles of orthogonal design of experiments (DOE) to engineer libraries of LNPs with varied excipient molar ratios to identify lead excipient compositions for enhanced *in vivo* mRNA delivery to the placenta. Through *in vitro* and *in vivo* screening, we identified an optimized LNP formulation that demonstrated potent *in vivo* mRNA delivery to the placenta and reduced mRNA delivery to the maternal liver when compared to an LNP formulated with a standard excipient molar ratio. Within the placental microenvironment, the optimized LNP formulation facilitated mRNA delivery to trophoblasts, endothelial cells and immune cells—three key cell types in the placenta that are involved in the pathophysiology of pre-eclampsia. Finally, delivery of the placenta-tropic LNP formulation encapsulating VEGF mRNA facilitated placental vasodilation, demonstrating the potential of mRNA LNPs for protein replacement therapy during pregnancy to treat placental disorders.

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VANCOUVER
NANOMEDICINE DAY
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Colin Ross
Therapeutic Genome Editing
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Future of Nanomedicines
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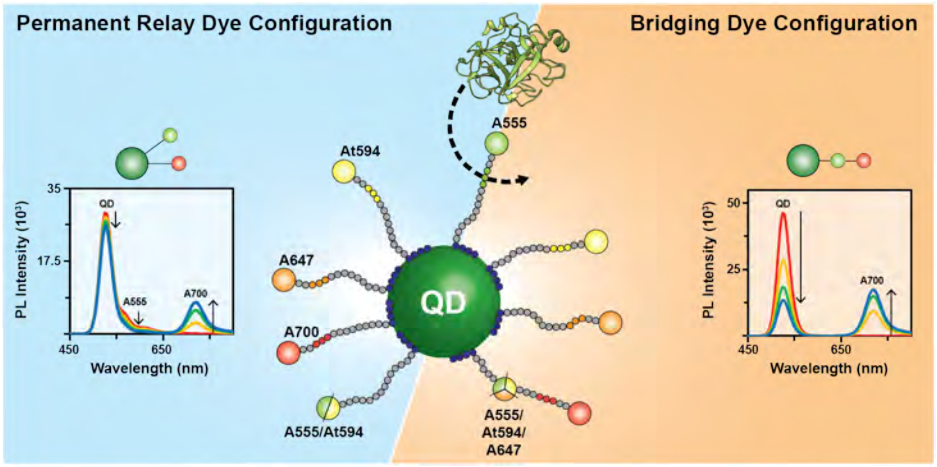
Lennart Bohrmann
Aptamer Particles for Cancer Targeting
Shirin Kalyan
Site Specific Immunomodulators
Hannah Safford
mRNA Delivery to the Placenta
Free Food!

POSTERS

#	Presenting Author	Poster Title	Institution / Company
P1	Agnes Szwarczewski	Identifying Candidate Concentric FRET Configurations for Highly Multiplexed Bioimaging and Sensing	UBC, Chemistry
P2	Alina Kunitskaya	Increased T-cell Transfection by mRNA Lipid Nanoparticles for Cell Therapy Manufacturing	UBC, Michael Smith Labs
P3	Behnoush Kermanshahi	Development of Lipid-Based Drug Delivery Systems for Cannabidiol Oral Delivery	Langara College, Applied Research Centre
P4	Christine Traaseth	A Laser Scanning Smartphone-Based Imaging Platform Toward Point-of-Care Diagnostic Testing	UBC, Chemistry
P5	Dinh Chuong Nguyen	STING Agonist Drugamers Enhance Peptide Vaccine Delivery Efficacy of an Endosomolytic Peptide Delivery Platform	University of Washington, Seattle, WA, USA
P6	Emma Kang	mRNA-LNP Transfection of Platelets is Compatible with Current and Alternative Storage Practices	UBC, Pathology & Lab Medicine
P7	Jasmine Bernal Escalante	Development of Bright Antibody Conjugates of Quantum Dots (QDs) Towards the Analysis and Characterization of Extracellular Vesicles (EVs) Through Single-Molecule Fluorescence (SMF) Technology	UBC, Chemistry
P8	Kelly Rees	Development of Dextran-Functionalized Super-Nanoparticle Assemblies for Bioanalysis and Imaging	UBC, Chemistry
P9	Liam O'Keeffe	Proof of Principle Base Editor Correction of Pathogenic Mutations in Lipoprotein Lipase Deficiency In Vitro Disease Model	UBC, Pharmaceutical Sciences
P10	Oluwafemi Obisesan	Synthesis and Characterization of Poly(Epsilon-Caprolactone) Nanoparticles Loaded with Tenofovir Disoproxil Fumarate: A Novel Anti-HIV Treatment Approach	North-West University, Mafkeng, South Africa
P11	Ramya Kannan	Polymer Modified Lipid Nanoparticles (LNPs) for mRNA Delivery: Improved Endosomal Escape and Liver Targeting	UBC, Pharmaceutical Sciences
P12	Robert Cocciardi	Routine and Advanced Characterization of Non-Viral Vectors by Multiple Technologies (DLS + NTA + DSC + SAXS)	Malvern Panalytical, St. Laurent, QC
P13	Seth Keenan	Prototype Smartphone-Based Device for Flow Cytometry	UBC, Chemistry
P14	Tessa Morin	Safety of Genome Editing: Development of a Fluorescent Model System to Investigate Reducing Off-Target Genome Edits by Base Editors	UBC, Pharmaceutical Sciences
P15	Vanessa Chan	Liposomal Resiquimod for the Treatment of Peritoneal Metastasis of Colorectal Cancer	UBC, Pharmaceutical Sciences
P16	Virginia Vallejos	Innovative Liposomal Nanoformulation Co-Incorporating Amphotericin B and Miltefosine for the Therapy of Visceral Leishmaniasis	Federal University of Minas Gerais (UFMG), Brazil
P17	Wenchen Zhao	Lipid Nnanoparticle-Mediated Nucleic Acid Delivery to Mouse Brain via Local Administration	Genevant Sciences Corporation, Vancouver
P18	Zhengyu Chen	Well Characterized Lipid Nanoparticle Library Accelerates Development of Next Generation Genomic Medicines	Precision NanoSystems ULC, Vancouver
P19	Pardis Kazemian	Gene Correction in Humanized Mutant Progranulin Mice for the Treatment of Progranulin-Associated Frontotemporal Dementia	UBC, Medical Genetics
P20	Colton Strong	Genetically Engineered Transfusable Platelets Using mRNA-Lipid Nanoparticles	UBC, Biochemistry and Molecular Biology

POSTER 1



Identifying Candidate Concentric FRET Configurations for Highly Multiplexed Bioimaging and Sensing

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Förster resonance energy transfer (FRET) is a powerful tool for bioanalysis. Concentric FRET (cFRET) is a multifunctional extension of conventional FRET, wherein multiple copies of two or more dye acceptors are assembled onto a quantum dot (QD) donor [1]. A cFRET probe, as a single vector, is able to detect multiple biological targets and potentially track multiple biological pathways [1, 2]. cFRET is thus very promising for bioimaging and sensing; however, a significant challenge is that cFRET probes are presently limited to a maximum of three targets. The utility of cFRET for biological applications would significantly increase if a higher level of multiplexing (e.g., four-plex) was possible.

A criterion for efficient FRET is overlap between the emission spectra of the donor and the absorption spectra of the acceptor. As more red-shifted dyes are added to a cFRET configuration to reach higher levels of multiplexing, the spectral overlap diminishes, making it challenging to obtain functional energy transfer (ET). Here, we present our efforts to address this challenge by exploring two potential configuration designs: (1) incorporation of a bridging dye-to-dye ET pathway on biomolecular probes; and (2) co-assembly of permanently linked blue-shifted dyes to facilitate ET to red-shifted dye labels on biomolecular probes.

With a developed Python code that enables *in silico* simulations of cFRET systems, we designed a library of potential candidate four-plex configurations, built them *in silico*, and simulated their emission spectra. We assessed each design, compared their performance and properties to select the optimal configurations, and evaluated empirical, statistical, and machine learning approaches for data analysis and benchmarking simulated analytical performance. The simulation results are currently guiding construction of a real four-plex cFRET configuration.

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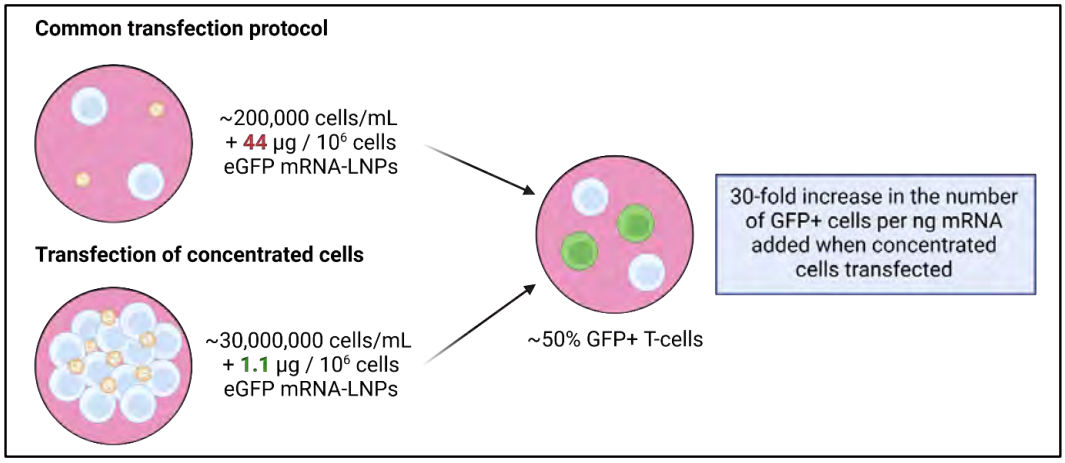
POSTER 2



Created using BioRender.com.

Increased T-cell Transfection by mRNA Lipid Nanoparticles for Cell Therapy Manufacturing

Alina Kunitskaya[1, 2], Sunny Chen[1, 2], Anna K. Blakney[1, 2], James M. Piret[1, 2, 3]

1. Michael Smith Laboratories, 2. School of Biomedical Engineering, 3. Chemical & Biological Engineering, University of British Columbia, Vancouver, Canada; email akunitsk@mail.ubc.ca

The availability of T-cell therapies for patients remains limited due to their complex and costly biomanufacturing. Genetic engineering of T-cells is often achieved using viral vectors, that are costly[1] and have safety concerns[2]. Non-viral alternatives would be preferred but a major barrier to their use is their far lower efficiency of gene delivery (the fraction that transfects cells). We have found that transfection efficiency can be greatly increased by concentrating the cells with the lipid nanoparticles (LNPs), such that they bind cells within a short period of time. Human T-cells were transfected with green fluorescent protein (GFP) mRNA-LNPs using a conventional protocol or under concentrated conditions. We achieved approximately 50% GFP^+ T-cells with both protocols, while using far less mRNA per million concentrated cells. There was a 30-fold increase in the number of GFP+ cells per ng mRNA from the concentrated condition. At 24 hours post-transfection, the cell viability was >70% in both conditions. Overall, the developed process could address a barrier to widespread implementation of LNPs vs. viral vectors for a wide range of cell and gene therapy applications. This could improve treatment safety and reduce manufacturing costs, making these life-saving therapies more widely available to patients.

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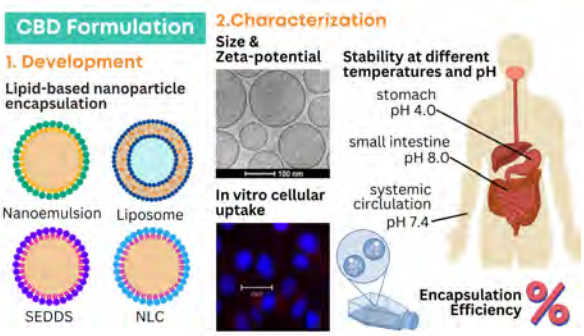
POSTER 3



Development of Lipid-Based Drug Delivery Systems for Cannabidiol Oral Delivery

Behnoush Kermanshahi[1], Gokce Alp[1], Andrea Olaizola[2], Brendan Morris-Reade[1], XuXin Sun[2] , Tom Skrinskas[3], Jessica Kalra[1, 2, 3]

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Among all of the phytocannabinoids, cannabidiol (CBD) is considered to be the most promising due to its potential pharmaceutical properties [1]. However the physicochemical properties of CBD hinders its utility in clinical settings. A key parameter limiting bioavailability of CBD is its poor water solubility. Lipid-based drug delivery systems (LBDDSs) have gained much interest in the past few decades due to their ability to enhance bioavailability of lipophilic drugs, their biocompatibility and high encapsulation efficiency [2]. We have used a variety of LBDDSs to encapsulate CBD towards improving biodistribution, each with their own specific utility. These include: 1. nanostructured lipid carriers (NLC); 2. liposomes; 3. self-emulsifying drug delivery systems (SEDDS); and 4. Nanoemulsions (NE).

Physicochemical properties such as particle size, zeta potential, stability in different physical (temperature) and chemical (pH) conditions, as well as in gastrointestinal fluids were tested. Encapsulation Efficiency and preliminary studies examining cellular uptake were also conducted. All formulations had an average particle size of less than 250 nm with a polydispersity index below 0.3. Encapsulation efficiency for SEDDSs, NEs and liposomes was higher than 70%. All formulations except NLC, maintained stability at, 4°C, and at a pH of 4, 7.4, and 8, except NLC and NE. Dispersing formulations in the fasted state simulated gastric fluid (FASSGF) and intestinal fluid (FASSIF) at 37°C resulted in no significant increase in particle size for SEDDS A and NE. However, particle size of SEDDS B increased when dispersed in fed state simulated intestinal fluid (FESSIF), while particle size of liposomes decreased when dispersed in both FASSIF and FESSIF.

We anticipate that results obtained from this study will help to uncover a lead CBD formulation to pursue as a preclinical candidate while providing the framework to develop novel pharmaceutical cannabinoid formulations suitable for human use.

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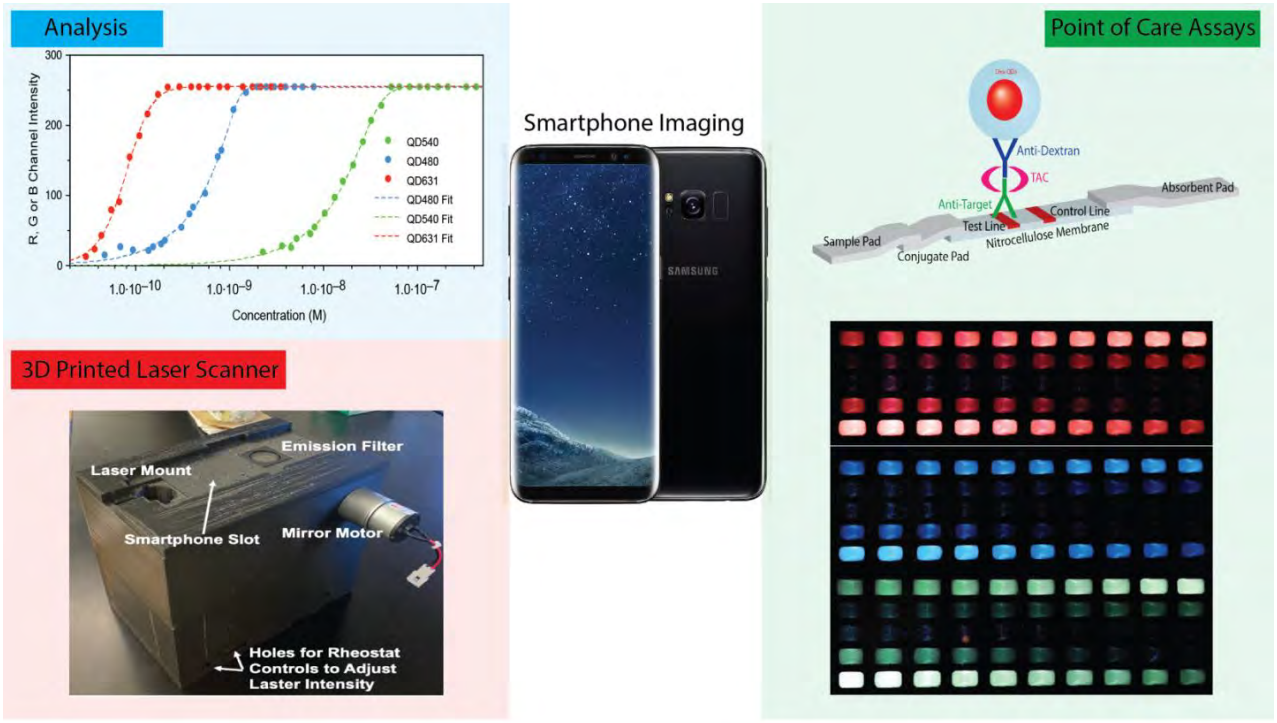
POSTER 4



A Laser Scanning Smartphone-Based Imaging Platform Toward Point-of-Care Diagnostic Testing

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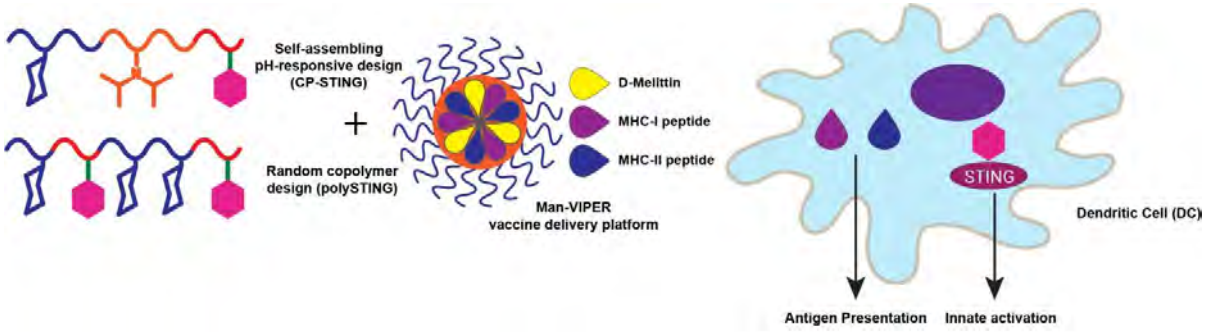
Smartphones are being widely adapted for point-of-care testing (POCT) due to their global ubiquity and on-board technologies. When smartphones are paired with advanced luminescent materials like quantum dots (QDs) and polymer dots (Pdots), they have sufficient sensitivity to become a viable alternative for many analyses with sophisticated laboratory instruments. To this end, we have developed and benchmarked a laser-scanning smartphone imaging platform (LS-SIP). Such a device is potentially ideal for readout of analytical technologies such as microwell plates, lateral flow immunoassays (LFIAs), and microfluidic or lab-on-a chip (LOCs) systems. As a flexible multi-purpose readout device, it helps avoid the possible problem of health care centers accumulating a myriad of different devices for POCT, each specific to a different assay.

The LS-SIP is composed of 3D-printed parts, simple optics, a DC motor, and a laser diode. A line-shaped laser beam is scanned over one of two sample areas that have been designed to accommodate both large and small assays or systems. During the scan, the smartphone acquires a movie that is then flattened into a complete image. The laser-scanning format enables imaging over a relatively large area with better sensitivity than an epi-illumination format. The analytical performance of the device was evaluated using QDs and Pdots, which have properties ideally suited to the device design and its limitations. Proof-of-concept assays were tested with LFIA microwell plates formats. The LS-SIP has is capable of multicolor imaging with picomolar limits of detection.

Overall, this research is a step toward improving diagnostic capability and accessibility—and thus equity and inclusion in health care—in resource-limited settings such as rural and remote communities in Canada and worldwide.

POSTER 5



STING Agonist Drugamers Enhance Peptide Vaccine Delivery Efficacy of An Endosomolytic Peptide Delivery Platform

Dinh Chuong (Ben) Nguyen[1,2], Kefan Song[1], Simbarashe Jokonya[1], Omeed Yazdani[1], Tran Luu[1], Suzie H. Pun[1,2], Patrick S. Stayton[1,2]

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Peptide neoantigen vaccines hold promise as a safe, low-cost and well-defined treatment modality for cancer[1] but are limited by low efficacy due to the inherent low immunogenicity of peptides[2]. We developed the Mannosylated Virus-Inspired Polymers for Endosomal Release (Man-VIPER) platform to overcome the limitation of poor cytosolic delivery of cytotoxic lymphocyte (CTL)-stimulating epitopes to dendritic cells (DCs), which partially contributes to low immune stimulation[3]. Man-VIPER improved tumor antigen delivery, CTL stimulation and tumor growth suppression in an antigen-expressing murine melanoma model, but ultimately mice still succumbed to progressing disease. The inclusion of innate pattern recognition receptor agonists, such as Stimulator of Interferon Genes (STING) agonists, can boost the efficacy of vaccine formulations[4]. Therefore, we hypothesized that STING agonist inclusion into Man-VIPER would improve its efficacy as a peptide neoantigen vaccine.

Co-delivery of antigen and STING agonists is important to maximize specific immune activation[4]; among other reasons, it avoids abrogation of antigen uptake into prematurely adjuvant-stimulated DCs[5]. To that end, we developed two mannosylated polymeric STING agonist prodrugs ('drugamers'). The agonist is formulated into a cathepsin-labile prodrug monomer and co-polymerized with mannose. We synthesized two constructs – a linear random copolymer (polySTING) and a self-assembling pH-responsive amphiphilic diblock copolymer (CP-STING). The STING drugamers were co-formulated with Man-VIPER micelles to produce drugamer-adjuvanted Man-VIPER formulations. All drugamer + Man-VIPER formulations demonstrated superior induction of DC and CTL maturation, as well as CTL activity compared to Man-VIPER alone or with free STING agonist. In an antigen-expressing murine melanoma model, all drugamer + Man-VIPER formulations improved tumor suppression compared to Man-VIPER. Free STING agonist worsened tumor control, while co-micellized CP-STING significantly outperformed separately micellized CP-STING. These results highlight the importance of antigen-STING co-delivery, and demonstrate the utility of STING agonist drugamers as an adjuvant to Man-VIPER.

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POSTER 6

mRNA-LNP Transfection of Platelets is Compatible with Current and Alternative Storage Practices

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[4]NanoMedicines Research Group, University of British Columbia; Vancouver, V6T 1Z3, Canada.
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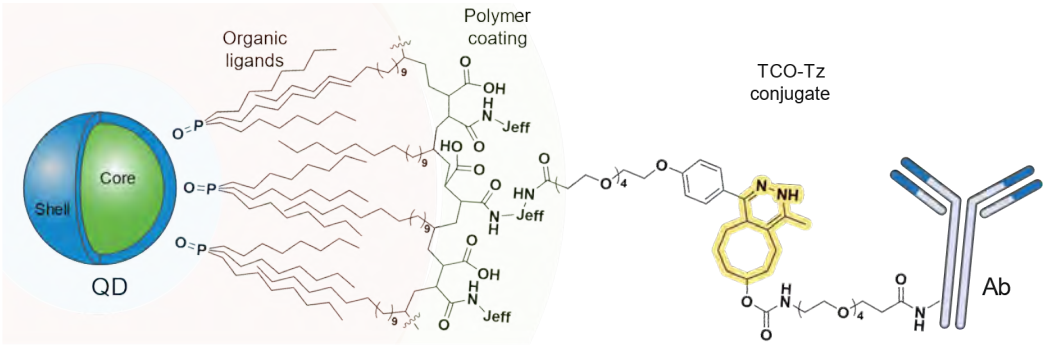
†These authors contributed equally to this work

Platelets are cells that detect and respond to sites of vascular damage where they play an important role in attenuating bleeding. However, in cases of major hemorrhage, transfused platelets can be ineffective because of limited coagulability. Therefore, there is a need for improved platelet products for use in clinics. A strategy to overcome limitations of current platelet products is to enhance the platelets' innate function using lipid nanoparticles (LNPs) to deliver exogenous cargo. Although anucleate, mature platelets contain all machinery required to translate proteins *de novo*, while maintaining other physiological functions. Additionally, advancements in the LNP technology have improved the delivery efficacy of exogenous cargo into target cells. Our group has shown that platelets can be engineered to express exogenous proteins when suspended in a crystalloid buffer. However, this buffer is an isotonic solution used mainly for tissue cultures and is not approved for use in clinical settings. Consequently, to establish genetically engineered platelets as a cell therapy platform, this technology would need to be developed with clinical compatibility in mind. Our group has identified an LNP formulation compatible with transfecting platelets in plasma and platelet additive solution (PAS), a clinically compatible solution. Additionally, we have found that alternative storage practices do not affect the transfectability of platelets, with platelets expressing exogenous cargo even after storage. Further development of mRNA-LNP modified platelets with clinical compatibility will introduce and expand the use of platelets as a platform cell therapy.

POSTER 7



Development of bright antibody conjugates of quantum dots (QDs) towards the analysis and characterization of extracellular vesicles (EVs) through single-molecule fluorescence (SMF) technology

Jasmine Bernal Escalante and Russ Algar

Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1 British Columbia, Canada; e-mail algar@chem.ubc.ca

Extracellular vesicles (EVs) are nanosized membranous structures actively secreted by cells that function as carriers of bioactive molecules and intercellular communication mediators. EVs are found in biological fluids and participate in multiple physiological and pathological processes [1], this makes them potential targets for liquid biopsy-based diagnostics and prognostics [2]. Due to their high heterogeneity and small size the development of effective methods to label and characterize EVs is an urgent need. The use of brighter fluorescent labels is one way to approach this problem. Here, we present preliminary results in the development of antibody conjugates of quantum dots (QDs)—high photoluminescent nanometer-sized semiconductors—for downstream EV analysis. Breast cancer (SKR-BR3) cells are selectively immune labeled as proof of concept. QDs were first polymer-coated and then conjugated with antibodies.

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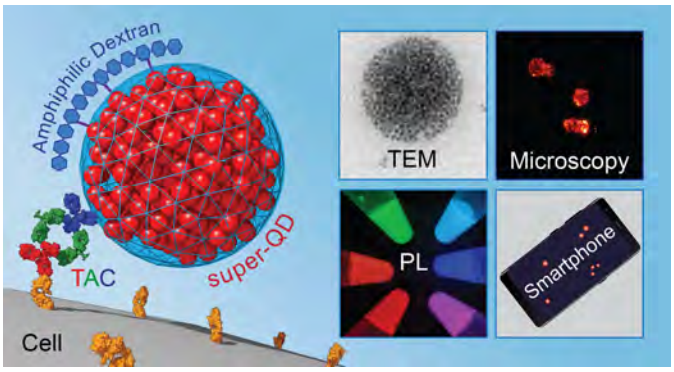
POSTER 8



Development of Dextran-Functionalized Super-nanoparticle Assemblies for Bioanalysis and Imaging

Kelly Rees[1], Ghinwa H. Darwish[1], W. Russ Algar[1]*

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Colloidal semiconductor quantum dots (QDs) are a popular material for applications in bioanalysis and imaging, for use alongside or as a replacement to fluorescent dyes [1]. Although individual QDs are bright, some applications benefit from even brighter materials. One approach to higher brightness is to form super-nanoparticle assemblies of many QDs. Here, we present the preparation, characterization, and utility of dextran-functionalized super-nanoparticle assemblies of QDs or "super-QDs" [2]. Amphiphilic dextran was synthesized and used to encapsulate many hydrophobic QDs via a simple emulsion-based method. The resulting super-QDs had hydrodynamic diameters of *ca.* 90–160 nm, were characterized at the ensemble and single-particle levels, had orders-of-magnitude superior brightness compared to individual QDs, and were non-blinking. Additionally, binary mixtures of red, green, and blue (RGB) colours of QD were used to prepare super-QDs, including colours difficult to obtain from individual QDs (*e.g.*, magenta). Tetrameric antibody complexes (TACs) enabled conjugation via self-assembly for selective cellular immunolabelling and imaging on both an epifluorescence microscope and a smartphone-based platform, with the super-QDs outperforming individual QDs in both cases due to their high brightness. Ongoing work is focusing on expanding the scope of amphiphilic dextran to super-nanoparticle assemblies of other nanomaterials (*e.g.*, gold or magnetic nanoparticles) and conjugated polymer nanoparticles. In all cases, the dextran provides good colloidal stability, low non-specific binding, and enables immunolabelling via TACs, suggesting that dextran-functionalized nanomaterials could have a wide range of applications in bioanalysis and imaging.

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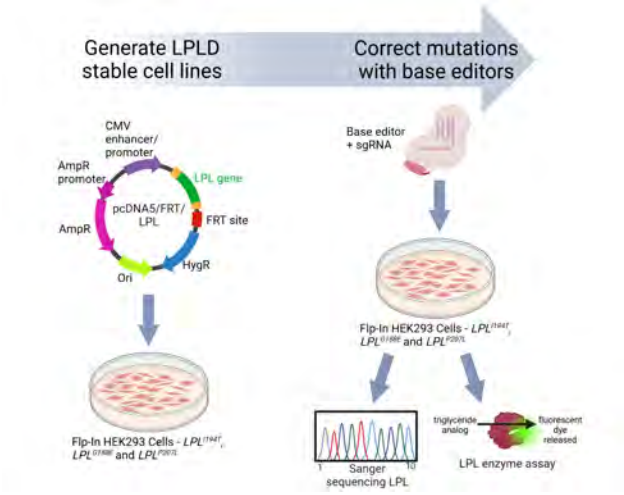
POSTER 9



Proof of Principle Base Editor Correction of Pathogenic Mutations in Lipoprotein Lipase Deficiency *In Vitro* Disease Model

Liam O'Keeffe[1], Tiffany Carlaw[1], Neel Mehta[2], Tessa Morin[1], Sarah Ng[1], Lin-Hua Zhang[1], Colin Ross[1]
Faculty of Pharmaceutical Sciences[1], Faculty of Medical Genetics[2]
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Lipoprotein lipase deficiency (LPLD) is a rare genetic disorder characterized by loss of LPL function leading to elevation of blood plasma triglycerides [1]. This hypertriglyceridemia has numerous downstream effects including recurrent acute pancreatitis and increased risk of diabetes mellitus. The most frequently occurring mutations for LPLD are three point mutation substitutions resulting in severe or complete loss of LPL activity: P207L, G188E, and I194T [2]. Current treatments are limited to low efficacy dietary plans restricting lipid intake. In recent years, CRISPR/Cas9 base editors have become viable tools capable of reliably inducing single nucleotide genomic edits [3,4]. In LPLD, base editors could restore endogenous gene expression and activity. P207L and G188E are amenable to ABEs (A-to-G conversions) and I194T is amenable to CBEs (C-to-T conversions). Here, we investigate the application of ABE and CBE base editors for the correction of LPLD mutations P207L, G188E, and I194T *in vitro*.

Stable cell lines were developed for the three most common LPLD mutations. Significant sequence correction was observed in all three cell lines. However, only one has demonstrated significant protein restoration. These foundational results justify future research expansion into *in vivo* mouse models for further assessment of LPL activity restoration and delivery optimization.

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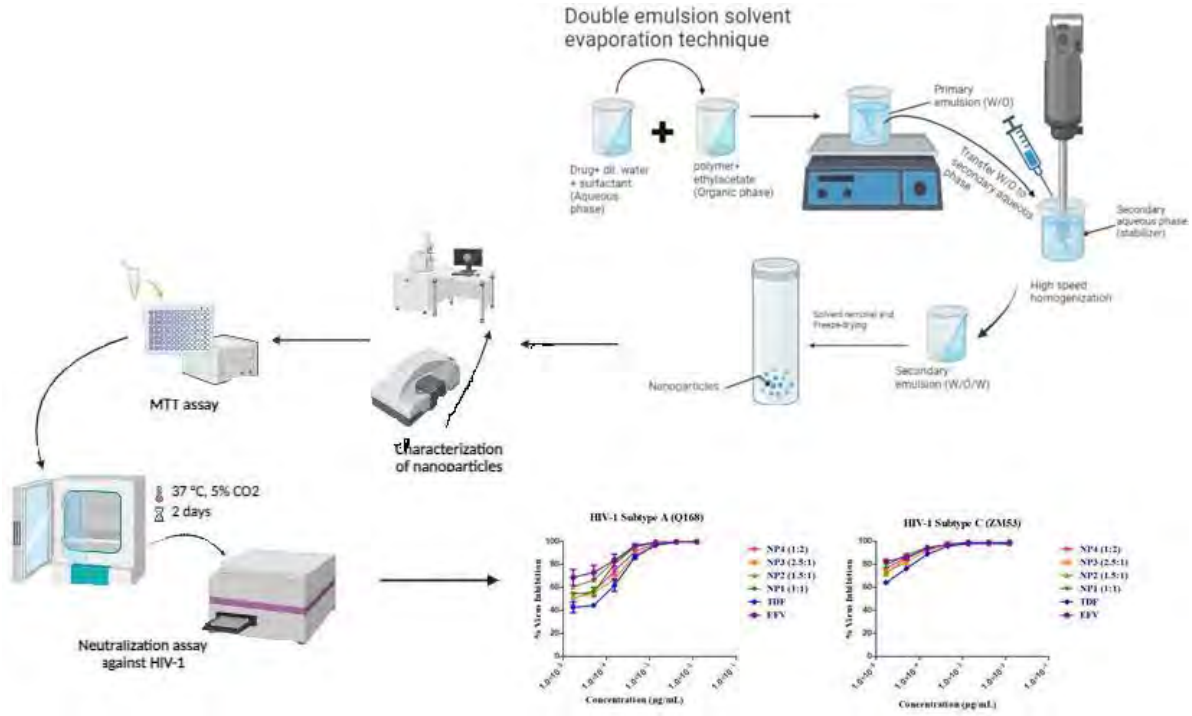
POSTER 10



Synthesis and characterization of poly(epsilon-caprolactone) nanoparticles loaded with tenofovir disoproxil fumarate: A novel anti-HIV treatment approach

Oluwafemi Obisesan[1*], Lesego Tshweu[2], Kabamba Alexandre[3] and Hazel Mufhandu[1*]

[1]Department of Microbiology, North-West University, Mafikeng, South Africa,[2]Biotherapeutics Delivery laboratory, Centre for Nanostructures and Advanced Materials, DSI-CSIR Nanotechnology Innovation centre, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa,[3]Council for Scientific and Industrial Research, Emerging Research Area Platform, Next Generation Health Cluster, Pretoria, South Africa; e-mail successfreak01@gmail.com

The remarkable ability of the human immunodeficiency virus (HIV) to evade the host's immune system and conventional antiretroviral therapy, has posed significant challenges in achieving complete eradication of the virus in people living with HIV (PLWHIV). However, nanotechnology has emerged as promising avenue for addressing some of the obstacles associated with the use of antiretroviral drugs by modifying drug molecules in nanoscale dimensions [1,2]. Hence, the present study explores the utilization of poly(epsilon- caprolactone) (PCL) as a carrier for encapsulating tenofovir disoproxil fumarate (TDF), offering an alternative treatment approach for HIV infection. TDF-loaded polymeric nanoparticles were successfully prepared and characterised. The characterisation of TDF-loaded polymeric nanoparticles at varied drug to polymer ratios showed that TDF was loaded in PCL with an encapsulation efficiency and drug loading capacity in the range of 23-46% and 4.8-19.9%, respectively. Of note, the neutralisation efficacy of TDF-loaded polymeric nanoparticles was more improved compared to free TDF. Encapsulation of TDF with PCL did not hinder the antiviral activity of TDF against HIV-1 infection but rather enhanced its potency.

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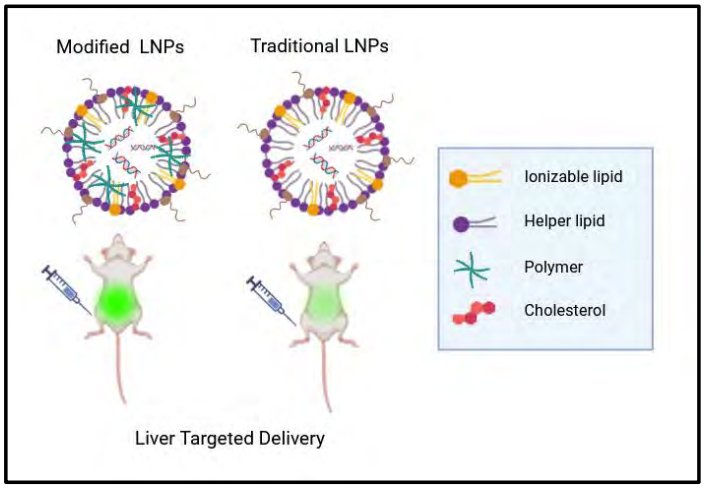
POSTER 11

Polymer Modified Lipid Nanoparticles (LNPs) for mRNA Delivery: Improved Endosomal Escape and Liver Targeting

Ramya Kannan [1#], Quan Le [1#], Vivekjot Brar [1], Sophie Roesger [1], Lukas Hohenwarter [1], Po-Han Chao [1], Angel Lee [2], Jing-Ping Liou [3], and Shyh-Dar Li [1]

[1]*Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada*
[2]*NanoStar Pharmaceuticals, Vancouver, BC, Canada*
[3] *School of Pharmacy, Taipei Medical University, Taipei, Taiwan; # Equal contributions*



Lipid nanoparticles (LNPs) are attractive vehicles for nucleic acid delivery. However, less than 2% of the nucleic acid payload was released in the cytosol by the LNP-mediated delivery.[1] To address this major barrier, we have synthesized a novel polymer that can destabilize lysosomes and be incorporated within LNPs. LNP formulations were optimized by incorporating varied amounts of the polymer, and the formulations were characterized by dynamic light scattering for particle size and polydispersity, electrophoresis for zeta potential, and RiboGreen assay for mRNA encapsulation efficiency. The optimal polymer-LNP and standard LNP displayed comparable physiochemical properties, while the polymer-LNP significantly enhanced luciferase gene expression in the liver and muscle in mice through IV and IM injections, respectively, compared to the standard LNP carrying a luciferase mRNA. This polymer could be incorporated into different LNP formulations composed of different ionizable cationic lipids and yielded comparable results.

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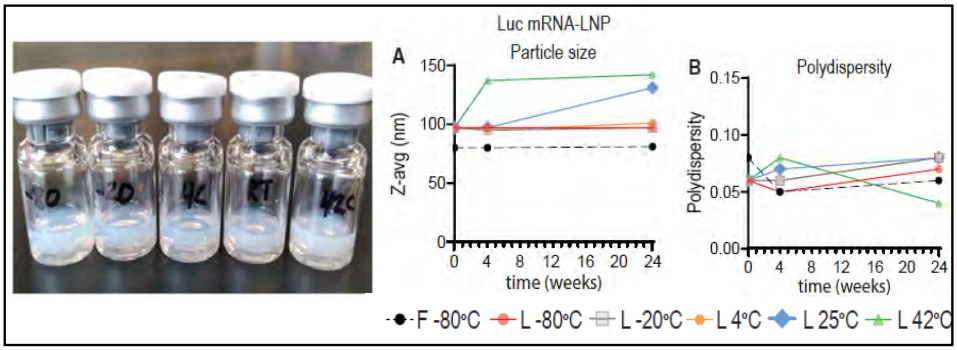
POSTER 12



Routine and Advanced Characterization of Non-Viral Vectors by Multiple Technologies (DLS + NTA + DSC + SAXS)

Jonathan Mehtala[1], Matthew Brown[1], Kiel Jones[1],Kuo-Chih Shih[1], Robert Cocciardi[2]
Malvern Panalytical, Westborough, USA [2] Malvern Panalytical, a Division of Spectris Canada Inc., St-Laurent, QC, email: *matthew.brown@malvern.com*

Lipid Nanoparticle (LNPs) and other non-viral vectors are critical components in the development of mRNA-based vaccines and gene therapies. Non-viral vectors have several key advantages over viral vectors, including ease of synthesis, high encapsulation efficiency, and minimal processing. A key challenge in the development of mRNA-LNP therapies is the development of robust methods that can measure critical quality attributes (CQA's) to ensure quality, safety, efficacy in addition to advanced structural analysis for long term stability and storage. Four technologies, Dynamic Light Scattering (DLS), NanoTracking Analysis (NTA), Differential Scan Calorimetry (DSC), and Small-Angle X-ray Scattering (SAXS) are key for the characterization of mRNA-LNPs including LNP properties of size, charge, particle concentration, thermal stability, lamellar structure, and comparability during lyophilization. LNP size is critical to determine sample quality, and correlates to many important attributes, such as stability, encapsulation efficiency, *in-vivo* immune response, cellular uptake, endosomal escape, and transfection efficiency.

DLS was used to measure LNP size over a range of synthetic conditions [1] with LNP size correlating to *in-vivo* immune response in mice and LNP lamellar structure. Lyophilization was also used to enhance the long-term stability of mRNA-LNP samples [2], LNP size and RNA integrity measurements of frozen and lyophilized mRNA-LNP samples were taken at different temperatures and time points. Both LNP size and RNA integrity degraded when stored at 25 and 42 degrees Celsius for at least 4 weeks. NTA was used as a complimentary analysis to the DLS measurements and provided deeper insights into the polydispersity and aggregate state of a LNP samples with regards to LNP aggregate state, PEG layer thickness, and absolute particle concentration. DSC was used to compare thermal stability of free mRNA in solution and encapsulated by LNPs [2]. The encapsulated RNA had a Tm between 65-80 C, whereas the free RNA had a broader thermal transition from 35-90 C. Finally, SAXS was used to characterize LNPs of different sizes (60, 90, 120, and 170 nm diameter) and found that both size and lamellar structure correlated to antibody titer immune response in mice [1]. Overall, we demonstrate the utility of these 4 characterization techniques for assessing the properties of LNPs.

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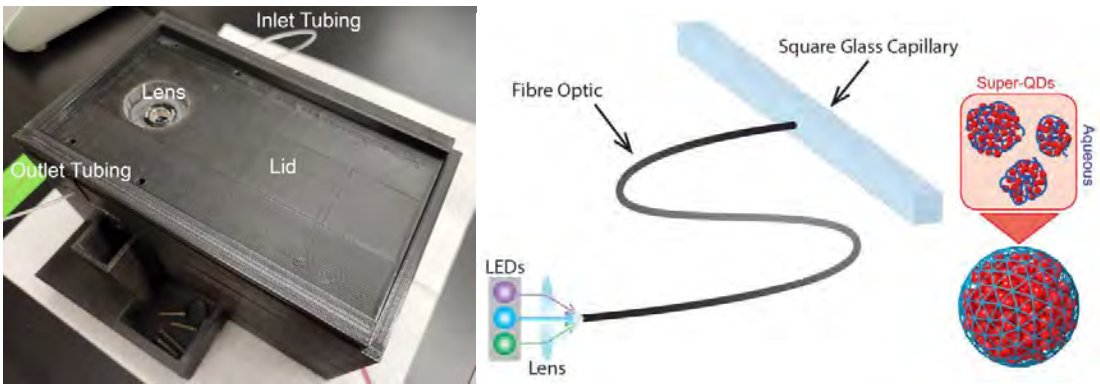
POSTER 13



Prototype Smartphone-Based Device for Flow Cytometry

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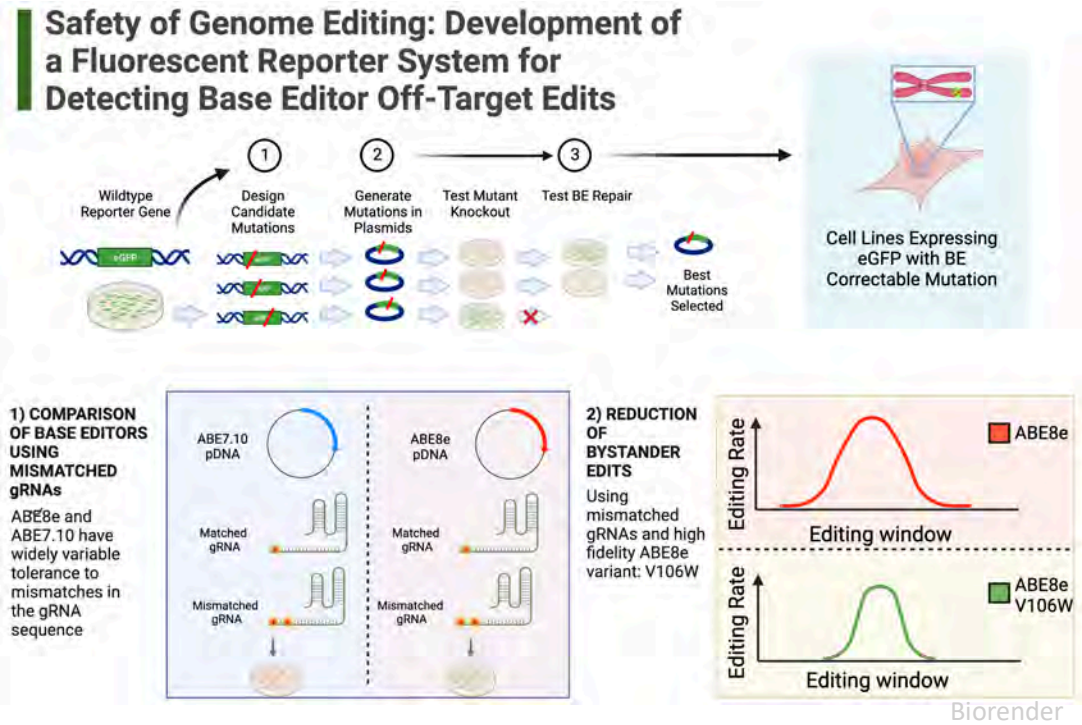
Many areas of research and healthcare require the ability to detect, enumerate, and type different cells. Flow cytometry is one of the most widely used methods for solving this problem, but the instruments are very large and expensive, and are typically limited to specialized laboratories in wealthy and populous centres. This lack of access to a gold-standard method for cellular analysis is a major barrier to equity in healthcare. One possible method for reducing the cost of flow cytometry is the use of a smartphone-based device. With their global proliferation and built-in optoelectronic components, smartphones have excellent potential for use as detectors and platform devices to increase the accessibility of cellular and other laboratory analyses.

Our group has recently developed a prototype smartphone-based device for flow cytometry. This device uses the smartphone camera to track immunofluorescently-labeled cells as they pass through a microfluidic flow cell. Here, we describe development of the next generation of this device with multimodal imaging capability. Features of the device include a violet laser and/or violet LED for the excitation of multi-colour fluorescence from super-QD immunoconjugates, a blue LED for the excitation of a live-cell permeant fluorescent nuclear stain, a green LED for the imaging of red-coloured cells, improved magnification to allow for sizing of cells and their nuclei, and forward light scattering. Potential applications of this device include the identification of cancerous cells from urine or blood samples and the complete blood cell count, which is the most common type of diagnostic test carried out in the world. The technology developed through this research will help improve accessibility, equity and inclusion for advanced diagnostic health care in rural, remote, and other resource-limited communities. The previous device was able to accurately count two different cell types in a mixture using high-brightness supra-nanoparticle assemblies of colloidal semiconductor quantum dots. Cell autofluorescence has been observed with the current device, demonstrating the ability for achieving our potential applications.

POSTER 14



Safety of Genome Editing: Development of a Fluorescent Model System to Investigate Reducing Off-Target Genome Edits by Base Editors

Tessa Morin[1], Tiffany Carlaw[2], Siyue Yu[1], Liam O'Keeffe[1], Linhua Zhang[1] and Colin Ross[1,2]
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[2]Department of Medical Genetics, University of British Columbia, Vancouver, Canada; email tessiemn@student.ubc.ca

Gene editing is a potential curative treatment for genetic diseases. However, current gene editing technologies are limited in their use and safety. Newer gene editors have more specificity, such as CRISPR/Cas9-derived base editors, which enzymatically convert a target base to another[1]. However, base editors are also capable of creating unwanted mutations, known as off-target edits, which can potentially lead to tumourgenesis[2]. The difficulty of detecting these base changes has resulted in a poor understanding of factors involved in off-target edits.

In our study, we investigated the on-target and off-target editing efficiencies of the new base editor ABE8e, which is known for its promiscuous editing activity[1]. To reduce off-target edits, the safety mutation V106W was added to the deaminase[1]. **We hypothesize that using ABE8e^{V106W} with off-target mismatched sgRNAs will have reduced genome editing compared to ABE8e.** We successfully developed a human cell fluorescent model system using a mutated *Green Fluorescent Protein* (*GFP*) reporter gene with a premature stop codon. After successful base editing to correct the mutation, the cells are analyzed using flow cytometry to quantify the editing efficiency. To simulate short guide RNA (sgRNA)-dependent off-target editing, we designed multiple mismatched sgRNAs with intentional mismatches to the target *GFP* sequence. These mismatched sgRNAs mimic the true *GFP* sgRNA matching incorrectly to sequences similar to *GFP* in the genome. Our research demonstrated a higher specificity with the ABE8e^{V106W} in combination with a mismatched sgRNA, with two-fold reduced bystander editing ($p<0.0001$) compared to ABE8e.

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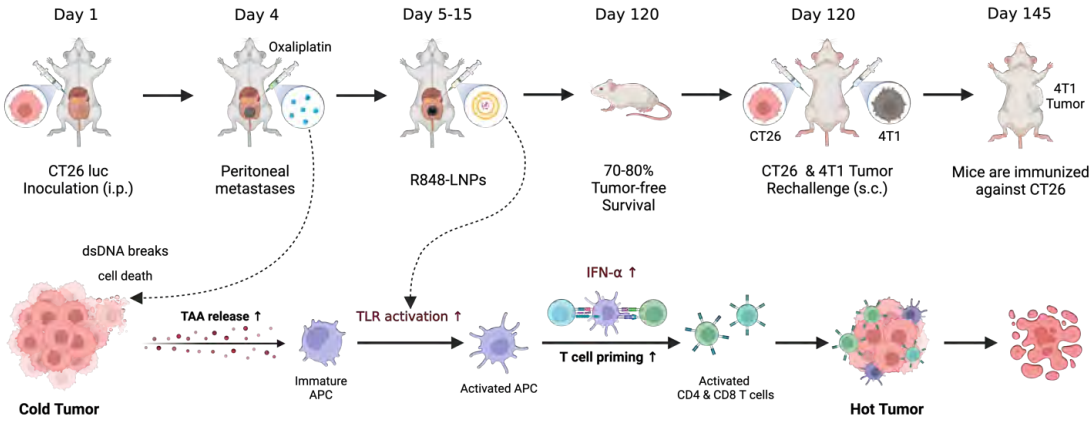
POSTER 15



Liposomal Resiquimod for the Treatment of Peritoneal Metastasis of Colorectal Cancer

Vanessa Chan[1], Po-Han Chao[1], Shyh-Dar Li[1]

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University of British Columbia, Vancouver, BC, Canada, vgchan@student.ubc.ca

Colorectal cancer is projected to become the fourth most diagnosed cancer in Canada for 2023. In its late stages, this disease typically metastasizes to the peritoneal cavity. The current therapy is aggressive, and the rate of recurrence is 41% at 1 year post-treatment, 73% at 3 years, and 76% at 5 years[1]. We have developed two cationic liposomal formulations (LNP1 and LNP2) for targeted delivery of TLR7/8 agonist Resiquimod (R848) to the peritoneal cavity. Both formulations were found to locally increase levels of IFN-α in the peritoneal fluid while maintaining low levels in the plasma. Combination therapies of Oxaliplatin (OXA) and LNP1-R848 or OXA and LNP2-R848 were tested at varying dosages and dose frequencies in 120-day survival experiments. Cured mice were then rechallenged with the original tumour line to assess the development of immune memory. Immune uptake and depletion experiments were also performed to parse out the mechanism of action of LNP1-R848 and LNP2-R848. At our optimized dose and dosage frequency, we achieved a survival rate of 80% from LNP1-R848 and 70% from LNP2-R848. The LNP formulations increased infiltration of anti-tumour immune cells, facilitating the tumour-free survival. All mice treated with LNP-R848 rejected the original tumour upon rechallenge. Both formulations were found to be taken up by dendritic cells with LNP2-R848 showing favoured uptake by macrophages as well. The immune depletion experiment showed that $CD8^+$ T cells played the strongest role in treatment success. Overall, we demonstrated the potential for LNP1-R848 and LNP2-R848 in the treatment of peritoneal metastasis of colorectal cancer.

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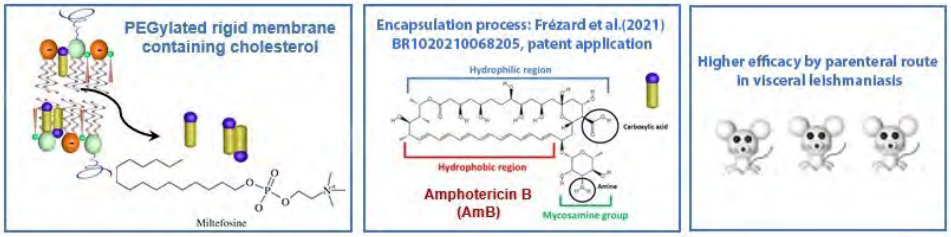
POSTER 16



INNOVATIVE LIPOSOMAL NANOFORMULATION CO-INCORPORATING AMPHOTERICIN B AND MILTEFOSINE FOR THE THERAPY OF VISCERAL LEISHMANIASIS

Virgínia M.R. Vallejos[1*], *Guilherme S. Ramos*[1*], *Helane L.O. De Morais*[1], *Gabriel S.M. Borges*[2], *Marta M.G. Aguiar*[2], *Christian Fernandes*[2], *Ricardo T. Fujiwara*[3], *Lucas A.M. Ferreira*[2], *Frédéric Frézard*[1].

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Federal University of Minas Gerais (UFMG), Belo Horizonte (MG), Brazil; e-mail: mendesvc@yahoo.com.br

Liposomal amphotericin B (AmB) or AmBisome® and oral miltefosine (Milt) are two major therapeutic options for visceral leishmaniasis (VL). Nevertheless, the clinical efficacy of these drugs is limited in severe cases of HIV/VL co-infection and cutaneous leishmaniasis (CL). Previously, our group introduced a novel method for encapsulation of AmB into pre-formed liposomes and demonstrated that PEGylated liposomal AmB is more effective than AmBisome® by both parenteral and oral routes in the treatment of murine CL [1]. Considering the great potential of drug combination for further improving leishmaniasis chemotherapy, the aim of this work was to develop a novel liposomal nanoformulation co-incorporating AmB and Milt and evaluate its therapeutic efficacy in a hamster model of VL. AmB was incorporated into pre-formed PEGylated liposomes containing Milt. The mixed PEGylated formulation showed vesicles with average diameter of 135 (±14) nm and low polydispersity index (PI<0.3), with AmB encapsulation efficiency of 90%. Circular dichroism characterization evidenced a low AmB aggregation state in comparison to AmBisome®. The mixed PEGylated formulation was compared to PEGylated formulations containing solely AmB and Milt and to AmBisome® in *Leishmania infantum*-infected hamsters for its effects on the parasite load in liver and spleen after 10 doses of treatment, given every day by IP route (5 mg/kg of AmB and 3.9 mg/kg of Milt). Animals, either untreated or treated IP with empty PEGylated liposomes, were used as negative controls. Treatments with the mixed AmB/Milt liposomal formulation, the liposomal formulation of AmB and AmBisome®. promoted significant parasite load reduction in the liver in comparison untreated control. Strikingly the mixed formulation showed the highest level of parasite reduction (94.3%) in comparison to Ambisome® (88.3%) and PEGylated liposomal AmB (84,2%). Significant reduction was also observed in the spleen, at a similar level, for all the AmB/Milt liposomal formulations. This study supports the increased efficacy of the mixed PEGylated formulation and its potential for the treatment of severe cases of VL and for situations where there has been therapeutic failure with the use of Milt or AmB alone (Work supported by the Brazilian agencies FAPEMIG, CNPq and FINEP).

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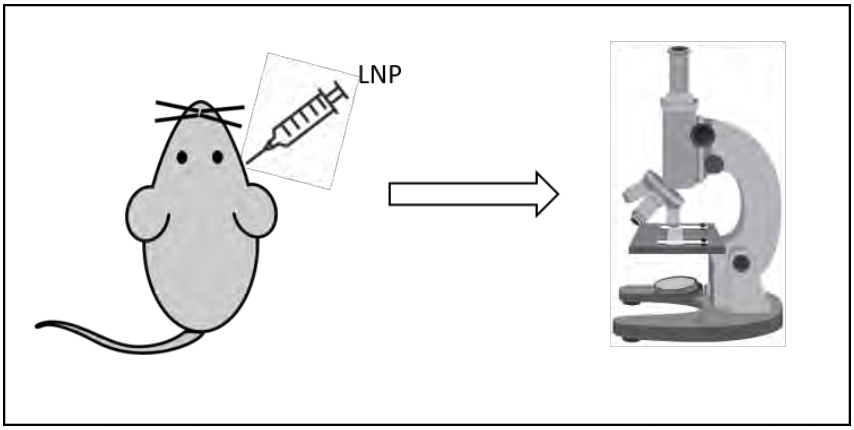
POSTER 17



Lipid Nanoparticle-Mediated Nucleic Acid Delivery to Mouse Brain via Local Administration

Wenchen Zhao[1], Kieu Lam[1], James Heyes[1]

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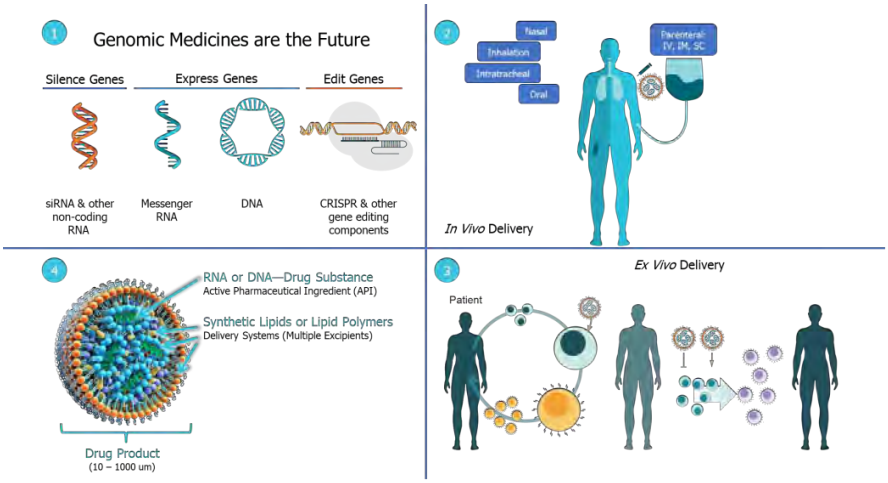
Lipid nanoparticles (LNPs) have proven a successful platform for the delivery of nucleic acid (NA)-based therapeutics and vaccines. It is well established that LNP is liver-centric, meaning after intravenous administration of nucleic acid encapsulated in LNP, the payload accumulates rapidly in liver. Therefore, there is increasing demands of leveraging LNP technology to target extrahepatic tissues such as spleen, bone marrow, brain, etc. Brain, in particular, is challenging to target because the presence of blood-brain barrier (BBB) prevents most of the peripheral molecules from entrance. Although there have been studies utilizing BBB penetrant formulations, the level of expression in brain is still relatively low. Thus, little is known about how encapsulating mRNA in LNP affects payload activity and distribution in brain. A study examining the impact of LNP delivery on mRNA cellular uptake and potency in different brain cell types would be invaluable in advancing brain-targeting LNP technology.

To bypass the BBB we delivered LNP encapsulating Cre recombinase mRNA directly to Ai9 transgenic mouse brain via either intracerebroventricular (ICV) injection to ventricle or intraparenchymal injection to entorhinal cortex or hippocampus. The expression of Cre mRNA excises (floxed) stop codon that precedes tdTomato gene, leading to its expression. We examined tdTomato expression via light sheet microscopy at 24h, day 7, 14, 21, and 28d post injection and observed strong signals at all timepoints for all administration route with signal peaking at day 14. In addition, we characterized localization of tdTomato in brain by immunohistochemistry (IHC) using anti-tdTomato antibody. IHC analysis showed that tdTomato is primarily expressed in neurons with limited expression in other brain cell types such as astrocyte and microglia. Overall, we demonstrate the potential of delivering nucleic acid by LNP to brain.

POSTER 18



Well Characterized Lipid Nanoparticle Library Accelerates Development of Next Generation Genomic Medicines

Zhengyu Chen[1] (Presenting Author), Nikita Jain[1] (Corresponding Author), Sedigheh (Cece) Nazaripour[1], Suraj Abraham[1], Leanna Yee[1], Sams Sadat[1], Mehar Gayatri Namala[1], Helena Son[1], Tony Wu[1], Sijo Chemmannur[1], Kobe Ticker[1], Gertrude Henneh[1], Malathi Anantha[1], Ruchi Sharma[1], Srinivas Abbina[1], Seetalakshmi Thambatti[1], Vinay Mayya[1], Emily Soon[1], Jay Paquette[1], Anitha Thomas[1]

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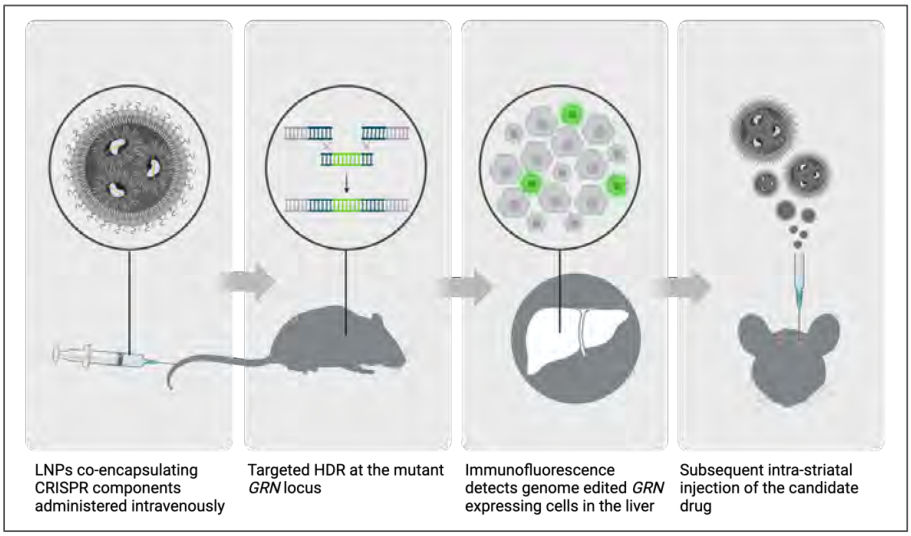
Introduction:
Ionizable amino lipids are a major constituent of the lipid nanoparticles (LNP) for delivering nucleic acid therapeutics (e.g., DLin-MC3-DMA in ONPATTRO®, ALC-0315 in Comirnaty®, SM-102 in Spikevax®). Scarcity of lipids that are suitable for cell therapy, vaccination, and gene therapies continue to be a problem in advancing many potential diagnostic/therapeutic/vaccine candidates to the clinic. Herein, we describe the development of novel ionizable lipids to be used as functional excipients for designing vehicles for nucleic acid therapeutics/vaccines *in vivo* or *ex vivo* use in cell therapy applications.

Methods:
We studied the transfection efficiency (TE) of LNP-based mRNA formulations of novel ionizable lipid candidates in primary human T cells and established a workflow for engineering of primary immune T cells towards non-viral CAR T therapy. Lipids were also tested in rodents for vaccine applications using self-amplifying RNA (saRNA) encoding various antigens. We have then evaluated safety and efficacy of various ionizable lipid candidates and their biodistribution using various LNP compositions. Further, using ionizable lipids from the library, we have shown gene editing of various targets in rodents.

Results:
Proprietary ionizable lipids and novel LNP compositions showed very high TE over MC3- LNPs in primary human T cells. Following IV administration of human erythropoietin (EPO) mRNA encoded LNPs, human EPO was generated resulting in an increase in hematocrit. Proprietary lipids comprising LNP vaccine candidates using self-amplifying RNA encoded for SARS-CoV-2 spike protein showed IgG levels specific to spike protein. The IgG levels were comparable to to that of SM-102 and ALC-0315 lipids used in the current mRNA vaccines. Preliminary results show case gene knock out of transthyretin and PCSK9 protein. Finally, We were able to show that physico-chemical profiles of ionizable lipids are going to be different for cell therapy, protein replacement, gene editing and vaccine applications.

Conclusions:
PNI proprietary lipid and or LNP compositional library can be used to mitigate the challenges in genomics medicine development by plug and play nature of the technology. We believe that these studies will pave the path to the advancement in nucleic acid based therapeutics and vaccines, or for cell & gene therapy agents for early diagnosis and detection of cancer, and for targeted genomic medicines.

POSTER 19



Gene Correction in Humanized Mutant Progranulin Mice for the Treatment of Progranulin-Associated Frontotemporal Dementia

Pardis Kazemian[1], Alissandra de Moura Gomes[1], Benjamin Life[1], and Blair R. Leavitt[1,2]

[1]Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada; [2]Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada; email: bleavitt@cmmt.ubc.ca

Loss of function mutations in the progranulin (*GRN*) gene are a prevalent etiological factor in familial frontotemporal dementia (FTD)[1]. There are currently no approved therapies for FTD stemming from GRN deficiency. An ideal therapeutic strategy would seek to restore *GRN* expression by correcting the deleterious mutations in affected patients.

CRISPR/Cas9-mediated gene editing is a novel approach to targeting monogenic hereditary disorders[2] such as *GRN*-dependent FTD. To develop a CRISPR-mediated gene therapy drug, we employ a 'humanized' mutant mouse model of FTD developed recently in our laboratory. Our existing mouse model that expresses a copy of the human *GRN* gene in the absence of murine Grn[3] has been further bioengineered to carry a common pathogenic *GRN* variant that results in the absence of GRN production. We deliver CRISPR/Cas9 as the ribonucleoprotein (RNP) complex co-administered with guide-RNAs and DNA repair templates via a novel lipid nanoparticle (LNP) formulation developed by our industry partners at Incisive Genetics. This approach induces homology-directed repair (HDR) at the mutant *GRN* locus. Leveraging the liver's inherent affinity for LNPs[4,5], we evaluate drug delivery and subsequent on-target genome editing efficiency *in vivo* by intravenous administration of the drug, followed by the assessment of HDR-mediated correction in hepatic cells. In a preliminary investigation involving an array of guide-RNAs and DNA repair templates, we conducted immunohistochemistry staining using a primary antibody against GRN to assess treated versus untreated animals' liver sections. These results demonstrate successful *GRN* expression restoration in select liver cells. Ongoing studies encompass quantification and dosage-dependent studies to enhance *GRN* correction in hepatic tissue. Subsequently, the optimized formulation resulting from these experiments will be employed in intra-striatal injections of the drug, facilitating the evaluation of *GRN* correction within brain cells.

This project serves as a proof-of-concept study for the efficacy of LNP-CRISPR gene correction within the brain, specifically for the treatment of hereditary neurological disorders. Furthermore, it signifies the advancement of this targeted genome editing approach for FTD patients.

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POSTER 20

Genetically Engineered Transfusable Platelets using mRNA-Lipid Nanoparticles

Colton Strong,[1,2,3]† Jerry Leung,[1,2,3,4]† Katherine E. Badior,[5]† Madelaine Robertson,[1,2,3,4] Xiaowu Wu,[6] Michael A. Meledeo,[6] Emma Kang,[3,7] Manoj Paul,[5] Yusuke Sato,[8] Hideyoshi Harashima,[8] Andrew P. Cap,[6] Dana V. Devine,[2,3,7,9] Eric Jan,[2] Pieter R. Cullis,[2,4] Christian J. Kastrup[1,2,3,5,10*]

[1]Michael Smith Laboratories, University of British Columbia; Vancouver, V6T 1Z4, Canada. [2]Department of Biochemistry and Molecular Biology, University of British Columbia; Vancouver, V6T 1Z3, Canada. [3]Centre for Blood Research, University of British Columbia; Vancouver, V6T 1Z3, Canada. [4]NanoMedicines Research Group, University of British Columbia; Vancouver, V6T 1Z3, Canada. [5]Blood Research Institute, Versiti Wisconsin; Milwaukee, 53226, USA. [6]Blood and Shock Resuscitation Program, United States Army Institute of Surgical Research; JBSA-FT Sam Houston, Houston, 78234, USA. [7]Department of Pathology and Laboratory Medicine, University of British Columbia; Vancouver, V6T 2B5, Canada. [8]Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University, Hokkaido, 060-0812, Japan. [9]Centre for Innovation, Canadian Blood Services; Vancouver, V6T 1Z3, Canada. [10]Departments of Surgery, Biochemistry, Biomedical Engineering, and Pharmacology and Toxicology, Medical College of Wisconsin; Milwaukee, 53226, USA. *Corresponding author. Email: ckastrup@versiti.org †These authors contributed equally to this work

Platelet transfusions are an integral treatment for managing bleeding and thrombocytopenia. Engineering platelets to express therapeutic proteins offers a potential solution to improve platelet products and expand platelets as cell therapy; however, there is no appropriate method for genetically modifying platelets collected from blood donors. Although anucleate, mature platelets synthesize protein de novo during circulation and storage, making them amenable to mRNA gene therapy. Advancements in lipid nanoparticle technology has enabled leading COVID-19 vaccines and is an efficient method to deliver nucleic acids into target cells. Here, we describe a transfection technique using platelet-optimized lipid nanoparticles containing mRNA (mRNA-LNPs) to enable exogenous protein expression in platelets ex vivo. Within the library of mRNA-LNPs tested, exogenous protein expression did not require, nor correlate with, platelet activation. LNP engineered platelets retained hemostatic function and agonist responsiveness in vitro and controlled bleeding comparably to unmodified platelets after transfusion into coagulopathic rats. We are now using this technology to express of proof-of-concept protein targets including antifibrinolytic peptides to deliberately alter platelet function. Further development of this technology will lead to the development of more effective platelet therapies.

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NOTES

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Note about Allergens :

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- Peanuts: Food is peanut free and cooked in peanut free oil
- Items that contain dairy: Butter Chicken & Paneer Tikka
- Channa Masala is vegan

Session 4:			Chair: Sabrina Leslie
1:15 PM	**Colin Ross**	**Nanomedicines for Gene Therapy: Optimization of Lipid Nanoparticles for Therapeutic Genome Editing**	**Pharmaceutical Sciences, UBC**
1:45 PM	**Melissa Radford**	**Dual Functionalization of Iron Oxide Nanoparticles**	**Simon Fraser University**
2:00 PM	**Lennart Bohrmann**	**Aptamer Conjugated Nanoparticles for Cancer Targeting: In Vitro and In Vivo Evaluation**	**University Hospital Basel, Switzerland**
2:15 PM		**BREAK**	
Session 5:			Chair: Marcel Bally
2:45 PM	**Abishek Wadhwa**	**Comprehensive mRNA-LNP Characterization using Single-Cell Techniques Reveals Tumour Microenvironment Reprogramming for Cancer Immunotherapy**	**Microbiology and Immunology, UBC**
2:57 PM	**Adrian Grzedowski**	**DNA Nano-Cubes as Highly Tunable Scaffold for Biosensors Engineering**	**Chemistry, UBC**
3:09 PM	**Nick Dragojlovic**	**Innovation Headroom for a More Accurate PD-L1 Companion Diagnostic in Early-Stage Non-Small Cell Lung Cancer**	**Pharmaceutical Sciences, UBC**
3:21 PM	**XuXin Sun**	**A Novel Liposomal Irinotecan Formulation Is a Potent Immunogenic Cell Death Inducer - Should It Be Developed for Use in Patients with Colorectal Cancer?**	**BC Cancer Research / Faculty of Medicine, UBC**
3:33 PM	**Virginia Vallejos**	**Development of Pegylated Liposomal Amphotericin B for Enhanced Treatment of Cutaneous Leishmaniasis Through Parenteral and Oral Administration**	**Federal University of Minas Gerais (UFMG), Brazil**
Session 6:			Chair: Colin Ross
3:45 PM	**Ethan Mickelson**	**Engineering Polymers for Resuscitation of Trauma Patients**	**University of Washington, Seattle, USA**
4:00 PM	**Joel Finbloom**	**Directing the Biointerface of Polyelectrolyte Nanocomplexes to Treat Bacterial Biofilm Infections**	**Pharmaceutical Sciences, UBC**
4:15 PM	**Hannah Safford**	**Ionizable Lipid Nanoparticles for In Vivo mRNA Delivery to the Placenta**	**University of Pennsylvania, Philadelphia, PA, USA**
4:45 PM		**RECEPTION and POSTER SESSION – with Beer and Pretzels**	
5:50 PM	Abishek Wadhwa	**Poster Prizes**	
6:00 PM	Urs Hafeli	**End of Vancouver Nanomedicine Day 2023**	