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GENE & CELL THERAPY CONFERENCE

MARCH 21, 2024

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Avance Biosciences is a leading CRO/CTL providing GLP & GMP-compliant assay development, assay validation, and sample testing services to support biological drug development and manufacturing activities world-wide. We work with many biopharma clients, big and small, to support their outsourcing needs for preclinical, clinical and CMC testing activities. Avance is a recognized expert and market leader in supporting the bioanalytical needs for biodistribution toxicity and PK/PD studies under GLP. Additionally we support cell bank, drug substance and drug product testing for characterization and release under GMP. Our team has over 20 years of experience has in supporting preclinical IND enabling studies through clinical trial support.

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Our vision: Advancing the pharmaceutical sciences to drive prevention and cures.

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ORGANIZERS' WELCOME

Welcome to the 2024 Gene & Cell Therapy Conference.
Our organizers have gathered an excellent group of speakers for the first annual Gene & Cell Therapy Conference. The program is arranged to incorporate extensive audience participation and discussion. We encourage attendees to take full advantage of the opportunity to engage in discussion in order to receive the maximum benefit from the Gene & Cell Therapy Conference experience. Thank you for your participation.

ORGANIZING COMMITTEE

Nagendra Chemuturi, Eli Lilly
Jochem Gökemeijer, Bristol-Myers Squibb
Anshul Gupta, Editas Medicine
Vibha Jawa, Bristol-Myers Squibb
Steven Louie, Moderna
Dale Miles, Genentech
Hardik Mody, Genentech
Yan Ni, Passage Bio
Stephanie Pasas-Farmer, Ariadne Software

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2024 AGENDA

THURSDAY, MARCH 21

7:30 - 8:30 AM **Registration & Breakfast**

8:30 - 8:40 AM **Conference Opening**
Dr. Akintunde Bello, BMS

8:40 - 9:20 AM **PLENARY: *Past, Present, and Future of Gene Therapy***
Mark Milton, Retired (Novartis)

SESSION I: Current State-of-the-Art Gene Delivery
Moderators: Vibha Jawa, BMS & Steven Louie, Moderna

9:20 - 9:25 AM **Session Introduction**

9:25 - 9:50 AM ***Development of Viral Delivery for Precision Medicine***
R. Jude Samulski, UNC School of Medicine

9:50 - 10:15 AM ***ADME for In Vivo Genome Editing: Assessing Effectiveness of Delivery for Editing Cargo***
Everett Perkins, Eli Lilly

10:15 - 10:35 AM Break

10:35 - 11:00 AM ***The Role of DMPK in Non-Viral GT***
Nagendra Chemuturi, Eli Lilly

11:00 - 11:25 AM ***Assessment of ADME and DDI for LNP-mRNA Therapeutics***
Lei Ci, Moderna

SESSION II: Translational PK/PD and Clinical Pharmacology Aspects
Moderators: Hardik Mody, Genentech; Dale Miles, Genentech & Stephanie Pasas-Farmer, BioData Solutions

11:25 - 11:30 AM **Session Introduction**

11:30 - 11:55 AM ***Gene Therapy and Translational Sciences - How Are We Doing?***
Nicholas Buss, Eli Lilly

11:55 - 12:20 PM ***Preclinical Considerations for Development of GTx***
Glen Banks, BMS

12:20 - 1:35 PM Lunch

1:35 - 2:00 PM	***Construction of an Integrated Translational PBPK-QSP Modeling Platform to Investigate Tissue Distribution and Protein Expression Dynamics of LNP-mRNA Based Therapeutics*** Kenji Miyazawa, Moderna
2:00 - 2:25 PM	***Development of AAV-Based Retina Gene Therapy through the Eyes of the Clinical Pharmacologist*** Jennifer Ford, Janssen
2:25 - 2:35 PM	**VENDOR PRESENTATION** ***Troubleshooting in Flow Cytometry Method Development: Ions, Titers, and Bears (Oh My!)*** Brian Wile, Kcas Bio
2:35 - 2:55 PM	Break

SESSION III: Biomarkers and Clinical Translation Aspects of Gene Therapy

Moderators: Yan Ni, Passage Bio; Anshul Gupta, Editas Medicine & Stephanie Pasas-Farmer, BioData Solutions

2:55 - 3:00 PM	**Session Introduction**
3:00 - 3:25 PM	***Leveraging Clinical Diagnostic Assays for Rare Disease Drug Development*** Yan Ni, Passage Bio
3:25 - 3:50 PM	***Learnings from Duchenne Muscular Dystrophy: A Biomarker Perspective*** Jyoti Malhotra, Sarepta Therapeutics
3:50 - 4:15 PM	***Pre-Existing Antibody Assays for Patient Enrollment and Cdx - Title TBA*** Jennifer Jones, Spark Therapeutics
4:15 - 4:40 PM	***Can the Immunogenicity of AAV Vectors be Mitigated?*** Ronit Mazor, FDA
4:40 - 4:45 PM	Closing Remarks
4:45 - 5:45 PM	Reception

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ABSTRACTS

PLENARY

Gene Therapy: The Past, Present, and Future
Mark Milton, (Retired) Novartis

Although regarded as a novel therapies, gene therapies have been in development for over three decades. They have a checkered history with several false dawns which have been characterized by clinical challenges, including the demise of patients as a consequence of the administration of the gene therapy. This presentation will provide a summary of the history of gene therapies, review the current state of the art, and provide some insights as to how the development of gene therapies may evolve.

SESSION I

ADME for In Vivo Genome Editing: Assessing Effectiveness of Delivery for Editing Cargo
Everett Perkins, Eli Lilly

In vivo genome editing has become a reality, with multiple therapies currently in clinical trials. However, safe and effective delivery of gene editing cargo remains a challenge. The viral vectors and lipid nanoparticles commonly utilized to deliver the editing components to target tissues have limitations such as immunogenicity, limited capacity, poor efficiency, and suboptimal tissue tropism. Furthermore, these one-and-done therapies often lack of well-established translational models, which brings uncertainty to human dose projections and margin-of-safety assessments. The rapid advancements in next-generation delivery approaches will necessitate more precise assessment of efficiency, specificity, and cross-species comparability. This presentation will discuss these challenges and describe a mechanistic translational framework in the context of AAV-delivered gene editors.

SESSION II

Gene Therapy and Translational Sciences - How Are We Doing?
Nicholas Buss, Eli Lilly

It has been approximately 6 years since the approval of Luxturna followed by Zolgensma and more recently a further three AAV gene therapies were approved. Furthermore, there are many AAVs in clinical development for many different indications administered via ocular, otic, CNS or systemic routes increasing our preclinical and clinical experience with AAVs. But how are we doing from a translational perspective? This presentation will review key aspects of translational sciences in AAV gene therapy.

Construction of an Integrated Translational PBPK-QSP Modeling Platform to Investigate Tissue Distribution and Protein Expression Dynamics of LNP-mRNA Based Therapeutics
Kenji Miyazawa, Moderna

The application of physiologically based pharmacokinetic (PBPK) models is well-established in the research field of drug delivery. This study introduces the development of a translational platform PBPK-quantitative systems pharmacology (QSP) model to investigate the distribution of mRNA therapeutics encapsulated in lipid nanoparticles (LNPs). The model was calibrated with published literature where juvenile and adult rats were administered with LNPs encapsulating mRNA encoding uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1) protein at multiple single or repeated dose levels. Local and global sensitivity analysis were performed to explore the determinants of protein expression and pharmacodynamic (PD) response. Inclusion of LNP recycling, key to predicting secondary peaks in mRNA pharmacokinetics (PK) often observed experimentally, was discussed. Our findings highlight

that mRNA stability, translation, and uptake rate are crucial factors for modulation of protein exposure. Simulations suggest that fast LNP recycling and slow re-uptake can lead to a distinct secondary peak in plasma mRNA levels. Using the model, we generated virtual animal cohorts to explore optimal dosing and schedule, and further identified the potential strategy to enhance the treatment efficacy by manipulating the LNP-mRNA design properties. The potential of the PBPK-QSP model as a translational tool for mRNA therapeutics will also be addressed.

Development of AAV-Based Retina Gene Therapy through the Eyes of the Clinical Pharmacologist
Jennifer Ford, Janssen

Adeno-associated virus (AAV) vector-based gene therapy is an innovative modality being increasingly investigated to treat diseases by modifying or replacing defective genes or expressing therapeutic entities. With its unique anatomical and physiological characteristics, the eye constitutes a very attractive target for gene therapy. Specifically, the ocular space is easily accessible and is generally considered "immune-privileged" with a low risk of systemic side effects following local drug administration. As retina cells have limited cellular turnover, a one-time gene delivery has the potential to provide long-term transgene expression. Despite the initial success with Luxturna, the first approved retina gene therapy by FDA and EMA, there are still challenges to be overcome for successful clinical development of these products and scientific questions to be answered. The current presentation will discuss experience learned thus far for AAV-based retina gene therapy related to preclinical to clinical translation; first-in-human dose selection; relevant bioanalytical assays and strategies; clinical development considerations including trial design, biodistribution and vector shedding, immunogenicity, transgene expression, and pediatric populations; opportunities for model-informed drug development; and regulatory perspectives.

VENDOR PRESENTATION

Troubleshooting in Flow Cytometry Method Development: Ions, Titers, and Bears (Oh My!)
Brian Wile, Kcas Bio

We present a selection of three case studies in assay development to illustrate some of the potential challenges encountered and solutions employed when developing custom assays for cell and gene therapy trials. These case studies highlight the importance of buffer system selection (including the ionic strength of the solution), the importance of titrations on a relevant matrix, and some of the challenges inherent in RO assay validation, which can be a true bear.

The first case is a comparison study between a BD and eBioscience permeabilization buffer set for a small chemokine panel and present differences in fluorescent intensity of key chemokines depending on the buffer system used. The second case is a recent titration performed with stimulated PBMCs. First, optimal titers for antibodies to key cell subset markers were confirmed. These antibodies were then used as a 'backbone' for the remaining titrations, where both the stain index and % positivity were used to select the optimal titration on the cell types of interest. Finally, we discuss some of the inherent challenges associated with RO assay validation. We review some of the biology associated with interrogated receptors and the challenges encountered in RO analysis of clinical samples. We quantify these biological limitations through assay validation steps, use statistics from a recent project to demonstrate the challenges in determining appropriate acceptance criteria, and discuss options that we consider as a standard set.

SESSION III

Leveraging Clinical Diagnostic Assays to Enable Rare Disease Drug Development
Yan G. Ni, Passage Bio

Drug development for rare diseases presents numerous challenges, including a lack of methodology for evaluating the pharmacodynamic (PD) effect or efficacy of a drug candidate, which hinders the progression of clinical research. Diagnostic testing labs provide potent tools for patient identification and are frequently used to support clinical trial enrollment. Recently, more clinical diagnostic labs have begun offering these assays for PD and efficacy assessment, particularly for rare diseases clinical trial. It's important to recognize that, for a given assay, the context of use and quality attributes required for patient diagnosis and PD assessment vary significantly. This presentation will explore these differences and the analytical, operational, and regulatory considerations involved in using clinical diagnostic assays for rare disease drug development.

Pre-Existing Antibody Assays for Patient Enrollment and CDx
Jennifer Jones, Spark Therapeutics

Companion diagnostics are a specific type of in vitro diagnostic assay that provides information that is essential for the safe and effective use of a corresponding drug or biological product. For cell and gene therapies, companion diagnostics may be used to identify individuals who are less likely to respond to a specific therapy and thereby not candidates for enrollment in clinical trials or for treatment with a particular drug or biological product. Companion diagnostics are subject to oversight by regulatory agencies thus CDx developers must ensure compliance with regulatory requirements specific for assays used to select patients for treatment. Careful planning of assay development activities is required to ensure that the assay meets technical and regulatory requirements while being suitable for use by health care professionals and patients.

Can the Immunogenicity of AAV Vectors be Mitigated?
Ronit Mazor, FDA

Despite the high safety profile demonstrated in clinical trials, adeno-associated virus (AAV)-mediated gene therapy still faces considerable obstacles due to its immunogenicity. Here, we silenced the most immunodominant T cell epitope in AAV9 through rational design chimerism. The new chimeric vectors maintained their functions and potency, including yield, cellular specificity, in vitro and in vivo transduction efficacy, and biodistribution in mice, while not eliciting any cellular immune responses.

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SPEAKER BIOGRAPHIES

NICHOLAS BUSS, Eli Lilly
Nicholas Buss is currently leading Translational Research at Akouos in Lilly Genetic Medicine and has experience with AAVs across multiple routes of administration and indications.

LEI CI, PHD, Moderna
Dr. Lei Ci is an associate scientific director at Moderna Therapeutics. She has 15 years of experience in drug discovery and development. She received her PhD from Dr. Sugiyama Yuichi's lab at the University of Tokyo. After graduation, Lei worked at biotech companies leading the ADME and DMPK effort to support drug discovery. In her current role, Lei is leading DMPK and clinical pharmacology efforts for multiple high impact projects in Moderna's mRNA pipeline. She has actively participated in academic and regulatory consortia and professional societies. She recently published papers evaluating ADME, PK, and biodistribution of LNPs.

JENNIFER FORD, PHD, Jannsen
Dr. Jennifer Ford, PhD, is a Senior Scientist in the Department of Clinical Pharmacology and Pharmacometrics at Johnson & Johnson. She received her PhD in Nutritional Sciences with a focus in the application of mathematical modeling and compartmental analysis to study nutrient status and metabolism from the Pennsylvania State University. Dr. Ford later completed postdoctoral training at the University of North Carolina Eshelman School of Pharmacy using physiologically based pharmacokinetic modeling to study drug metabolism in children with obesity. In her current role as a Clinical Pharmacology Leader, Dr. Ford has supported clinical development of several retina gene therapy programs.

JENNIFER JONES, Spark Therapeutics
Jennifer has more than 25 years of experience in IVD and CDx Development and is currently the Head of Companion Diagnostics at Spark Therapeutics.

JYOTI MALHOTRA, Sarepta Therapeutics
Jyoti Malhotra is currently the Executive Director at Sarepta in Translational Biology. She has drug discovery experience in conducting and directing research from early stage to clinical development in RNA, gene therapy and small molecules. She also has experience across multiple therapeutic areas such as neurodegenerative, muscular dystrophies, coagulation disorders as well as protein misfolding and rare genetic diseases. She has authored 40+ peer-reviewed publications and abstracts in drug discovery and academic settings. Jyoti has given numerous presentations in prestigious international and national conferences and symposia geared for the drug discovery industry. She has biomarker development experience for pre-clinical to clinical in neuromuscular (DMD) rare diseases and CNS therapeutic areas. She is a key team member in the accelerated approvals of Vyondys53, Amondys45 and Elevidys.

RONIT MAZOR, PHD, FDA
Dr. Ronit Mazor is a Principal investigator in the office of gene therapy (OGT), Office of Therapeutic Products (OTP) at the Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA). She leads a research group that focuses on cellular and humoral immune response to gene therapy vectors. Ronit performs CMC reviews for BLA and IND submissions of biological products focusing on viral vector gene therapies. Before joining the FDA, Ronit worked as a senior scientist in Medimmune/AstraZeneca in their Antibody discovery and protein engineering focusing on prediction and mitigation of immunogenicity of therapeutic proteins. Dr. Mazor

completed her graduate work in immunology from Tel Aviv University in Israel and her Post-doctoral training in the National Cancer Institute in Bethesda.

MARK N. MILTON, PHD, (Retired) Novartis
Mark Milton retired in March 2022, after almost 31 years in the pharmaceutical industry. Over the course of his career, he has managed a GLP-compliant bioanalytical laboratory, provided nonclinical and clinical support to LMW compounds, biologics, cell therapies, and gene therapies. He now provides gene therapy and biologics nonclinical and clinical consulting services through Lake Boon Pharmaceutical Consulting, LLC.

Mark received a B.Sc. in Biochemistry and Soil Science from UCNW, Bangor, a M.Sc. in Toxicology and Ph.D. in Biochemical Toxicology from the University of Surrey, England. Mark started his career at GD Searle in Skokie, Illinois before moving to Millennium Pharmaceuticals in Cambridge, Massachusetts. He then moved to Tempo Pharmaceuticals (Cerulean Pharmaceuticals) before joining Novartis in Jan 2009 where he held leadership positions in ocular PK/IG, the PK/PD/IG of Biologics, and Gene Therapies, as well as representing PKS Sciences on the Cell & Gene Therapy Steering Committee and Novartis's First in Human review board. Mark held a leadership position in the AAV Immunogenicity Task Force and the Immunogenicity Advisory Group. Mark supported many of Novartis's Gene Therapy projects, supported many of Novartis's anti-complement programs, wrote the nonclinical PK summary for the Kymriah BLA, was involved in the inlicensing of Luxturna, and the acquisitions of Avexis and Gyroscope, and provided nonclinical and clinical PK and immunogenicity support to Zolgensma post-market and for intrathecal administration of OAV101.

Mark is a past-chair of the BioSafe PKPD EWG, a member of the BioSafe LC, IQ Board of Directors, a member of the AAPS pre-existing antibody and Immunogenicity Risk Assessment Working Groups and was the BIO observer to the ICHS3A Q&A WG.

He has published over 30 peer-reviewed publications and book chapters and presented extensively on the development of NCEs, Biologics, and Gene Therapies. His interests include the PK/PD and immunogenicity of biotherapeutics, the contribution of PK (biodistribution) and IG to the development of gene therapies, ocular immunology, the selection of the clinical starting dose based upon the MABEL calculation, and alternative designs for FIH clinical trials of monoclonal antibodies in healthy volunteers.

KENJI MIYAZAWA, PHD, Moderna
Kenji Miyazawa is a dedicated scientist in the Clinical Pharmacology/Pharmacometrics/Quantitative Systems Pharmacology group at Moderna Therapeutics. Specializing in QSP modeling, Kenji brings particular expertise in PBPK modeling, with a focus on rare diseases and oncology. Prior to joining Moderna, Kenji completed a Ph.D. in Quantitative Biosciences and Engineering from the Colorado School of Mines. He is passionate about leveraging computational models to enhance drug development and personalized medicine, aiming to improve patient outcomes and advance treatment options in complex therapeutic areas.

YAN NI, PHD, Passage Bio
Dr. Yan Ni is an Executive Director of Biomarkers and Precision Medicine at Passage Bio. The Biomarkers and Precision Medicine team is responsible for the overall biomarker strategic planning and execution for all clinical and preclinical programs in the Passage Bio drug development portfolio. Before Passage Bio, Yan was an Associate Director at the Precision Medicine Group of Regeneron Pharmaceuticals and was responsible for clinical biomarker planning and implementation for multiple disease areas from First-in-human to phase III. Yan also worked in the BioAnalytical Sciences Department at Bristol-Myers Squibb for five years, where her team provided biomarker

assay development, validation, and outsourcing support for the clinical pipeline. Her industry career started at Merck Research Laboratories at Rahway, New Jersey.

Yan actively volunteers for the American Association of Pharmaceutical Scientists (AAPS) and is a founding member of the AAPS Biomarkers and Precision Medicine Community and the Gene and Cell Therapeutic Product Communities. She is an inventor of several patents and is recognized for her expertise in clinical biomarker development and translational science. Yan receives her PhD in Neuroscience from University of California, Irvine, and holds a Master of Science degree from the Institute of Basic Medical Sciences in Beijing.

EVERETT PERKINS, JR., PHD, Eli Lilly
Everett (Rhett) Perkins is currently Executive Director in ADME at Eli Lilly and Company. Dr. Perkins received a B.S. in Biology from Millsaps College and a PhD in Pharmacology and Environmental Toxicology from the University of Mississippi. He has worked in the pharmaceutical industry for 25 years, with areas of focus in gene therapy, oligonucleotides, carboxylesterases, prodrugs, and mechanistic pharmacokinetic modeling. Dr. Perkins has guided the discovery, development, and regulatory interactions for numerous drug candidates, and has over 30 research publications in the fields of drug metabolism and environmental toxicology.

JUDE SAMULSKI, PHD, UNC School of Medicine
Dr. Samulski received his Ph.D. in medical microbiology and immunology from the University of Florida. His graduate work (1978-82) demonstrated the first use of AAV2 as a viral vector which culminated in the first U.S. FDA approved gene therapy drug for Leber Congenital Amaurosis (LCA).

After completing post-doctoral training at Princeton, Dr. Samulski was hired at University of Pittsburgh where his lab demonstrated the first use of AAV for gene delivery to the Central Nervous System (CNS) which eventually led to the initial treatment of AAV for Canavan's disease. In 1993, he was recruited to the University of North Carolina (UNC) to initiate, build and direct the UNC Gene Therapy Center. In this setting, development of self-complementary AAV vectors (currently used in approved SMA gene therapy drug), Chimeric AAV capsids (first example of synthetic vector used in humans - DMD trial), and critical research that enabled large scale production was carried out. Research in the Samulski Lab, headed by Dr. Xiao Xiao, Ph.D., demonstrated the first AAV-mediated long-term gene transduction in muscle (J. Virology, 1996). This finding precipitated efforts to develop 1st generation AAV vectors to treat Duchenne muscular dystrophy that is now in Phase 3 clinical trials.

In total, Dr. Samulski has worked with AAV for 40 years, and for 25 years, he was director of the University of North Carolina Gene Therapy Center. He was the scientific founder of Bamboo Therapeutics, Inc. and served as the Chief Scientific Officer and Executive Chairman of the company until its acquisition by Pfizer in 2016. Upon its acquisition, Dr. Samulski joined Pfizer, as VP Gene Therapy, to ensure the successful transition of Bamboo's Duchenne muscular dystrophy therapeutic. Dr. Samulski is a former member of the Recombinant DNA Advisory Committee (RAC), a committee tasked with assisting the FDA with gene therapy clinical trial approvals in the U.S. He also frequently serves as a gene therapy consultant to the FDA. In 2008, Dr. Samulski was recognized by the American Society of Cell and Gene Therapy with their inaugural Outstanding Achievement Award for his work and later served as President of the Society. He was also invited by Pope Francis to the Vatican in recognition for his work in the treatment of Canavan's disease.

Dr. Samulski has advanced therapeutics into human clinical trials for hemophilia, Duchenne muscular dystrophy, giant axonal neuropathy, Pompe disease and congestive heart failure, to name a few. He has published more than 350 articles and holds more than 300 patents related to AAV technology. Currently, Co-founder, Chairman and

Chief Scientific Officer of Columbus Children's Foundation that has focused on developing AAV gene therapies for "ultra-rare" disorders as well as Co-Founder and Chief Scientific Officer of Asklepios Biopharmaceuticals, a clinical stage gene therapy company that develops AAV gene therapies for genetic and complex disorders.

BRIAN WILE, Kcas Bio

Brian is currently the General Manager at KCAS Bio-Philadelphia, where he works with more than 50 scientists and support staff to deliver GLP, GCLP, and exploratory flow cytometry services to clients in the drug development space. The laboratory has experience with a variety of indications and treatment modalities, but are especially experienced in oncology and autoimmune indications and cell therapy applications, where the team at KCAS Bio has helped cell therapy researchers develop PK, PD, and immunophenotyping assays for 3 of the top 5 commercially available CAR-T therapies and is actively partnering with dozens of innovators in the space.

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POSTER ABSTRACTS

Assessing Human Immune Response to Empty/Full AAV Capsids in an In Vitro Assay

Haruka Nishiumi*#, Kirk Haltaufderhyde¥, Tamako Garcia¥, Ellie Deju Calixto¥, Mitchell McAllister¥, Beth McGonigal¥, Christine Boyle¥, Anne S. De Groot¥, Susumu Uchiyama*

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Graduate School of Engineering
Department of Biotechnology
Laboratory of Macromolecular Biotechnology (Uchiyama Lab.)

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The use of adeno-associated virus (AAV) vectors in gene therapies and gene delivery protocols presents a promising avenue for treating gene-deficiency diseases and for the introduction of novel genes in CAR-T systems, respectively. However, the presence of empty capsids in the final product complicates the use of AAV gene delivery due to potential immunogenicity issues. This study investigates the relationship between the final dose of empty capsids in the gene delivery product and the immune responses observed in recipients of AAV therapy. We hypothesized that higher doses of empty capsids, in relation to full capsids containing transgenes, correlates with increased immune reactions in the host. To test this hypothesis, we conducted an ex vivo immunogenicity assay using AAV empty and full capsids at varying ratios. Human peripheral blood mononuclear cells obtained from blood center leukopack filters were cultured with different ratios of empty to full capsids in an adapted In Vitro Immunogenicity Protocol (IVIP). Our results demonstrate a significant increase in immune responses when empty capsids are added to full capsids, with ratios greater than 1:1 eliciting two to three-fold higher in vitro immunogenicity reactions. These findings suggest that the prevalence of empty capsids directly influences the immunogenicity of AAV therapy, highlighting the importance of optimizing capsid compositions for enhanced therapeutic outcomes.

Using a Semi-Mechanistic Model to Predict Efficacious Dosing Regimens and Optimal Drug Design Properties for a LNP-delivered mRNA UGT1A1 Replacement Therapy in Crigler-Najjar Syndrome Type 1 Patients

Jamie Nosbisch, Saheli Sarkar, David Bassen, Carter Johnson, Diana Marcantonio, Joshua Apgar, David Flowers

Applied BioMath, LLC, 561 Virginia Road, Suite 220, Concord, MA 01742

PURPOSE:
Crigler-Najjar syndrome type 1 (CN1) is an autosomal recessive disease caused by a marked reduction in glucuronosyltransferase family 1 member A1 (UGT1A1) enzymatic activity which results in toxic accumulation of bilirubin in the blood. Treatment options are currently limited for CN1 patients. While liver transplants can be used to restore UGT1A1 activity, an alternative approach considers using gene therapy to restore UGT1A1 activity by delivery of mRNA encoding UGT1A1 protein via lipid nanoparticle (LNP). Utilizing a semi-mechanistic model, we explored the feasibility of dose amounts and frequencies that could sustain reductions in bilirubin levels over time and investigated the therapeutic design variables that most strongly influence dose projections to help prioritize optimization efforts.

METHODS:
A semi-mechanistic model was developed to describe an mRNA therapy delivered to the liver via a LNP. Processes described by the model include LNP delivery to the liver, endosomal escape of the mRNA, translation of the target protein UGT1A1, and UGT1A1 catalysis of bilirubin to bilirubin monoglucuronide. Model parameters were set to values for lipid-delivered mRNA therapeutics taken from the literature. The delivered mRNA intracellular half-life and UGT1A1 enzyme half-life were fixed to typical values. To illustrate the usage of the model for a feasibility assessment, no parameters were derived from clinical or preclinical studies.

RESULTS:
Model simulations predict that prohibitively large doses are required to maintain the bilirubin reduction target of 25% of baseline over time. The model predicts that doses larger than 0.07 mg/kg achieve sustained reduction in bilirubin concentration at Q1W dosing frequency; however, increasing the dosing interval requires increasing doses, and at Q3W doses need to be prohibitively high to maintain desired bilirubin levels throughout the dosing period. This is because the mRNA is cleared at an exponential rate inside the cells, creating a nonlinear relationship between dosing frequency and sustainability of response.

To achieve longer dose intervals with lower dose amounts, it may be possible to engineer the properties of the lipid or the mRNA for improved efficacy. Three drug design parameters were scanned over a realistic range of values to assess the possibility of attaining the feasibility criteria for bilirubin reduction at 0.5 mg/kg dose Q3W. While the efficiency of mRNA escape from endosomes is known to be a major bottleneck in intracellular drug delivery, the model predicts that increasing endosomal escape efficiency may provide some benefit but not enough to attain the feasibility criteria at this dose amount and interval. Similarly, more efficient translation, which can be achieved through codon-optimization of the mRNA, does not result in enough benefit to reach feasibility. The stability of the mRNA, on the other hand, which is represented in the model by the intracellular mRNA half life, is a key design variable that impacts the feasibility for UGT1A1

replacement and its pharmacology. With an mRNA half life of 30 hours, the drug achieves 25% reduction at all times at 0.5 mg/kg. Increasing the half life to 40 hours reduces the required dose five-fold to 0.1 mg/kg.

CONCLUSIONS:

In summary, a semi-mechanistic model was developed and parameterized to predict efficacious dose and dosing intervals and to help prioritize optimization of liver-targeted mRNA delivery of an enzyme designed to treat CN1. Using this model, we determined that it is feasible for liver UGT1A1 to restrict bilirubin to non-toxic levels at frequent (Q1W-Q2W) dosing intervals, but dose amounts would be prohibitively high at less frequent intervals (Q3W). With further model analysis, we identified UGT1A1 mRNA stability as the primary design variable that may be modified to enable a longer dosing interval at reasonable dose amounts. This case study highlights how mechanistic modeling can be used for making early feasibility assessments for novel gene therapy treatments and can help inform the design properties for these therapeutics.

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AAV8 Shedding Assay to Support Gene Therapy Clinical Trials

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Gene therapy has already demonstrated its potential of bringing life-changing therapies to patients with devastating diseases and a growing number of clinical trials have been approved in recent years. One of the requirements in clinical development is to monitor vector shedding to control the potential environmental risk associated with the therapy. The assay read-out is qualitative, providing the sponsor and investigator with the relevant information about vector-shedding clearance after therapy. A sensitive and reliable method is needed in several different matrices, typically including blood, plasma, faeces, semen, urine and saliva. Viral shedding assays involve the extraction of viral DNA from patient secretions and excreta, the presence of which is then measured by selective and sensitive molecular methods (qPCR or ddPCR).

Here we present the development of a shedding method for the detection of a AAV8 vector in several matrices, including whole blood, plasma, faeces, semen, urine and saliva. The method development is split into 3 distinct phases: primer/probe design, PCR optimization and extraction. In first part of method development, we designed 5 sets or primers/probes. In the second phase we optimised the best qPCR conditions by comparing the five primers/probe sets and four master mixes to identify the combination, showing the best amplification efficiency and linearity and the lowest limit of quantification (LLOQ), limit of detection (LOD), and background noise. In the third phase, we compared seven commercially available DNA extraction kits and evaluated them based on the viral DNA recovery efficiency and, most importantly, the sensitivity, measured in genome copies per µl (or mg) of starting material.

While we chose a single combination of primer/probe set and master mix, we observed that different DNA extraction kits have to be used depending on the matrix of interest in order to achieve the best sensitivity which ranged from 0.6 to 7.4 GC/µl (mg) obtained from 50-200 µl (mg) of starting material.

In this presentation we share the unique requirements and challenges in developing vector shedding assays, based on the results of a streamlined AAV8 shedding assay method development. We are sharing an extensive comparison of most commercially available extraction kits. We conclude with our recommendation for validation parameters, acceptance criteria and results reporting to support clinical and preclinical gene therapy studies.

Optimizing Cell Viability in Cell Therapy: A Comparative Study of Whole Blood Sample Stabilization Techniques

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This abstract details a pivotal investigation into optimizing sample preservation techniques for cell therapy applications, focusing on the critical aspect of maintaining leukocyte viability and functionality in whole blood samples. The study meticulously evaluates the performance of three preservation strategies—10% DMSO, TransFix, and Cyto-Chex—within the context of cell therapy, where the integrity of immunophenotypic markers is paramount. Leveraging samples from five donors, the research contrasts fresh, non-stabilized samples against those treated with stabilizers, employing stringent criteria to ensure cellular integrity. The findings reveal the limitations of commercial stabilizers TransFix and Cyto-Chex in meeting their stability claims. Conversely, an in-house 10% DMSO solution, when combined with -80°C storage, emerges as the most effective method, preserving cell types across multiple freeze-thaw cycles. This breakthrough underscores the potential of the 10% DMSO technique for enhancing cell therapy outcomes by enabling reliable sample transport, long-term storage, and high-throughput analysis. The study not only challenges existing stabilization methodologies but also sets a new standard for flow cytometry sample preparation in cell therapy research and development.

Single-Cell Lentiviral Vector Integration Sites and Clonal Tracking Assay for Cell and Gene Therapy

Matt Cato, MissionBio

Lentiviral vector (LV) has been widely adopted as an efficient vehicle to deliver transgenes into cells due to its long-term efficacy. However, the semi-random integration of LV has raised safety concerns due to its potential to trigger tumorigenesis during CAR-T therapy. To mitigate this issue, characterization of vector integration on clonal expansion after gene therapy has become a crucial practice to monitor the activity of viral vectors on in vivo selection of patient clones. Here, we developed a single-cell resolution lentiviral vector integration site assay to survey the co-occurrence of specific integration sites and somatic genomic variants. Based on a set of LV transduced cell lines with known integration sites validated by orthogonal data, a targeted panel was designed to cover both the 5′ and 3′ ends of each integration site with predefined integration orientations. Samples include negative control and LV transduced cell lines with known vector copy numbers ranging from one to four copies. Using chemistry optimized for LTR junctions, we demonstrate the capability to quantitatively detect individual cells harboring specific vector integration sites and longitudinal tracking of cell clones with different vector copy numbers. Furthermore, besides amplicons targeting integration sites, in the same reaction, another set of internal vector amplicons (10-plex) and human genome amplicons (99-plex) are included to estimate vector copy number (VCN) and characterize somatic variants in the cell population.

Taken together, a high throughput single-cell multi-omics platform enabled us to simultaneously identify somatic variants along with vector integration events in individual cells, providing both potential functional mutation identification and clonal tracking capabilities. The development of single-cell lentiviral vector integration sites and clonal tracking assay provides a unique opportunity to better study longitudinally CAR-T cell clonal dynamics and lead to a more effective therapeutic agent.

Decoding the Mosaicism of Genome Editing with Single-cell Multi-omics Analysis

George Dorfman, MissionBio

Genome editing has emerged as a revolutionary force within the life sciences, wielding transformative potential in applications such as cell and gene therapy development, disease modeling, and functional genomics. Despite the promise of precision of advanced genome editors, editing outcomes remain largely unpredictable. Different cells subjected to the same editing regimen can yield distinct combinations of edits, varying not only across multiple on-target sites but also between on-target and off-target locations. From the perspective of the fundamental biological unit—a single cell— the zygosity disparity (mono-allelic, bi-allelic), heterogeneity in variants (homozygous, heterozygous, compound heterozygous), and their functional impact all contribute to the layer of complexity in the mosaicism of editing outcomes. Current genome editing analyses primarily rely on bulk methods, which, though valuable, provide only an average editing efficiency (at the allelic level) of a population. The nuanced cell-to-cell variation of edits remains elusive within these traditional approaches. Here, we present compelling evidence that the Tapestri Genome Editing Solution offers a breakthrough in the analysis of knockout (KO) and base editing (BE) experiments. We demonstrate the technology's unique single-cell multi-omics capability to furnish intricate details regarding zygosity and the co-occurrence of on- and off-target edits, thereby affording researchers the granularity needed for precise experimental outcomes.

Quantification of Single Cell Vector Copy Number in CAR-T Cell Products Utilizing a Novel Microfluidic Technology

Nori Ueno, MissionBio

Chimeric antigen receptor T-Cell (CAR-T) immuno-therapies have been transformative solutions to treat cancer patients. As most CAR-T therapies rely on the introduction of CAR into host cells using lentiviral vectors followed by re-introducing the modified T-cell back into patients, the quality of CAR-T is extensively regulated. Key safety and efficacy attributes such as transduction efficiency and transgene copy number, or viral vector copy number (VCN), needs to be accurately measured. However, conventional methods for measuring gene transfer lack the resolution and representation to truly reflect sample composition and either report a population average (bulk) or involve laborious and time-consuming clonal outgrowth. Mission Bio has developed an end-to-end solution from panel design to data analysis for single-cell targeted DNA sequencing to interrogate transgenes. Here, using the Tapestri platform we demonstrate that single-cell DNA sequencing identifies transduced versus non-transduced cells with exceptional accuracy and precision, as well as measures the single-cell level vector copy number (VCN) for populations of thousands of cells with single nucleotide resolution while reducing sample processing time from weeks to days. In addition, utilizing our oligo conjugated antibody system, we demonstrate single-cell multi-omics characterization of protein expression (functional) and transgene (genomic) information of CAR products.

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