



TAKING BIOTECH AS **FAR** AS IT CAN **GO**

CONFERENCE PROGRAM

HILTON GARDEN INN
FARGO, NORTH DAKOTA
NOVEMBER 6 & 7, 2018



WELCOME LETTER

On behalf of the entire staff of Aldevron, welcome to Fargo and to our inaugural Breakthrough Symposium.

The field of gene and cell therapy has seen unprecedented progress and success, with the first approvals of commercial products and many more in the pipeline. These achievements have been built on many years of challenging and innovative work by some of the best minds in science and medicine. As Aldevron contemplated how to celebrate our 20th anniversary and the opening of our first building on our new 10-acre campus, we concluded the best way would be to host an event that would honor the tremendous work done in the field and look forward with optimism to the breakthroughs ahead. It is our great pleasure to have you join us to learn from and network with leading researchers, scientists, educators, students, industry professionals and more.

We have gathered an outstanding group of speakers from all over the country who will share their most significant advances and most recent updates in gene and cell therapy. The biotech field is changing rapidly, and it is critical that we connect, participate, learn and exchange ideas towards solving some of our world's most challenging problems. We consider it a privilege to serve our clients who are leading a biotech revolution that is curing disease and helping to feed the world. Our mission has remained unchanged for 20 years: to improve lives through innovative partnerships and scientific excellence.

Although Fargo is a relatively small city in a small state, our pioneering spirit, work-ethic, and love of challenges has shaped a highly entrepreneurial mindset that has been a driving force of our company. Combine that with access to more than 10 universities within a 90-mile radius conducting their own breakthrough research and educating future scientists; the reasons to remain here are very clear – this is a great place to build and grow a company. We expect that for many this will be a first visit to Fargo and possibly to North Dakota. We hope you enjoy your time here!



Michael Chambers
CEO, Aldevron



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SHARE YOUR EXPERIENCE

#BTSymp2018



7:00 AM

Registration & Breakfast

8:10 AM



Opening Remarks & Overview

MICHAEL CHAMBERS

President and CEO, Aldevron

Bio

Michael Chambers has served as President and CEO of Aldevron since founding the company in 1998 as an undergraduate at North Dakota State University (NDSU) in Fargo. He and fellow student John Ballantyne started producing plasmid DNA in a small lab at NDSU for use by researchers in the biotech community.

Over the next 20 years, they expanded and grew the business in Fargo, North Dakota, to become one of the industry's leading – and most respected – contract manufacturing organizations. Aldevron products and services are key components of the world's most innovative gene and cell therapy commercial products.

This year, Michael was named one of the "100 Most Intriguing Entrepreneurs" by Goldman Sachs and honored at its "2018 Builders and Innovators Summit" in October.

Under his vision and leadership, Aldevron acquired Genovac Antibody Technology of Freiburg, Germany, in 2004. Five years later, the company expanded into protein production in Madison, Wisconsin. Recently, the company completed construction of a 70,000 square-foot building in Fargo, the largest plasmid DNA manufacturing facility in the world. Michael continues to lead the Aldevron team of more than 200 on the next phase of its growth trajectory.

Michael received Bachelor of Science degrees in biotechnology, chemistry and microbiology from North Dakota State University.

8:20 AM



Genetic Engineering: T-Cells from Bedside to Bench to Boardroom

LAURENCE J.N. COOPER, M.D., PH.D.

Chief Executive Officer, ZIOPHARM

Bio

Prior to becoming the Chief Executive Officer of ZIOPHARM in May 2015, Dr. Laurence Cooper was a tenured Professor (early/exceptional promotion) at The University of Texas MD Anderson Cancer Center (MDACC), with joint appointments in the Division of Pediatrics and Department of Immunology. He also served as Section Chief of Cell Therapy at the Children's Cancer Hospital at MDACC where, as a Visiting Scientist at MDACC, he will continue to lead scientific efforts to develop new treatment approaches which pair genetic engineering with immunotherapies.

287Dr. Cooper has coauthored dozens of peer-reviewed journal articles, abstracts, and book chapters. He has initiated multiple trials under INDs infusing T cells and NK cells. He is undertaking the first protocols using a new approach to gene therapy based upon the Sleeping Beauty transposon/transpoase system and has helped develop clinical-grade artificial antigen presenting cells for numerically expanding and activating lymphocytes. Dr. Cooper obtained his M.D. and Ph.D. degrees at Case Western Reserve University in Cleveland and then training in Pediatric Oncology and Bone Marrow Transplantation at the Fred Hutchinson Cancer Research Center in Seattle.

9:00 AM



Translating Genome Editing of Somatic Stem Cells to the Clinic: Using the Best Tools

MATTHEW PORTEUS, M.D., PH.D.

Associate Professor, Department of Pediatrics and Institute of Stem Cell Biology and Regenerative Medicine

Bio

Matthew Porteus M.D., Ph.D. is an Associate Professor in the Department of Pediatrics, the Institute of Stem Cell Biology and Regenerative Medicine, and the Child Health Research Institute at Stanford. He received his B.A. from Harvard University in "History and Science" and his M.D. and Ph.D. degrees at Stanford University. He completed his post-doctoral medical training in Pediatrics and Pediatric Hematology/Oncology at Boston Children's Hospital and the Dana Farber Cancer Institute, and his post-doctoral research training under the mentorship of Dr. David Baltimore.

His primary research focus is on developing genome editing as an approach to cure disease, particularly those of the blood but also of other organ systems as well. His research program has made important discoveries in advancing the field of genome editing including the first use of genome editing using engineered nucleases in human cells and optimizing the use of the CRISPR/Cas9 system in primary human stem cells.

He also works as an attending physician on the Pediatric Hematopoietic Stem Cell Transplant service at Lucile Packard Children's Hospital where he cares for children under going bone marrow transplantation for both malignant and non-malignant diseases. His goal is to combine his research and clinical interests to bring innovative curative therapies to patients. He served on the National Academy Study Committee of Human Genome Editing and as a History and Science major at Harvard he wrote his undergraduate thesis on the social interpretation of the recombinant DNA controversy in the early 1970s.

9:40 AM



Broadening the Reach of Gene Therapy by Discovery and Collaboration in a Competitive Space

LUK H. VANDENBERGHE, PH.D.

Associate Professor at Harvard Medical School; Associate Member of the Broad Institute of Harvard and MIT

Bio

Luk H. Vandenberghe, Ph.D., is an Assistant Professor at Harvard Medical School and an Associate Member of the Broad Institute of Harvard and MIT in Boston, MA. He directs the Grousbeck Gene Therapy Center at Massachusetts Eye and Ear Infirmary in Boston, USA, a part of the Ocular Genomics Institute, a bench to bedside research program to study, diagnose, and develop treatments for diseases of the eye. He received a degree in cellular and genetic engineering from the University of Leuven, Belgium. His previous work led to the discovery of novel AAV serotypes such as AAV9, novel insights into AAV structure-function, and vector immunobiology.

His laboratory at Harvard addresses mechanistic questions on AAV virology, develops technologies aiming to overcome hurdles to gene therapy clinical applications, and actively translates gene therapy programs in hearing and vision. His research focuses on delivery questions, specifically on the adeno-associated virus (AAV) for therapeutic gene delivery. Recent studies leverage structural and evolutionary information on AAV as a starting point for the design of synthetic viral vector systems, a first generation of which is referred to as AncAAVs which are now progressing to the clinic for a number of indications.

Dr. Vandenberghe previously co-founded GenSight Biologics and Akouos. He also is a founder, board member, and advisor to Odylia Therapeutics, a non-profit catalyzing translation for gene therapies within the challenging field of ultra-rare disorders. Dr. Vandenberghe has over 50 peer reviewed publications and more than a dozen licensed patents, mostly related to gene therapy methods, technologies, and applications.

10:20 AM

Networking & Exhibit Break

10:40 AM



Precise Gene Editing of Mutant Alleles Using CRISPR-Cas9 Ribonucleoprotein Complexes

KRISHANU SAHA, PH.D.

Assistant Professor, Department of Biomedical Engineering, University of Wisconsin-Madison

Bio

Krishanu Saha is an Assistant Professor in the Department of Biomedical Engineering at the University of Wisconsin-Madison. He is also a member of the Wisconsin Institute for Discovery, Carbone Cancer Center, and Stem Cell and Regenerative Medicine Center as well as the National Academies' Forum on Regenerative Medicine.

Prior to his arrival in Madison, Dr. Saha studied Chemical Engineering at Cornell University and the University of California in Berkeley. He was a Society in Science: Branco-Weiss fellow at the Whitehead Institute for Biomedical Research at MIT, and in the Science and Technology Studies program at Harvard University. Major thrusts of his lab involve gene editing and cell engineering of human cells found in the retina, central nervous system and blood.

11:20 AM



Discovery and Characterization of Novel SpCas9 and AsCas12a Mutants Isolated by Protein Engineering and Directed Evolution

CHRISTOPHER VAKULSKAS, PH.D.

Senior Staff Scientist, Integrated DNA Technologies

Bio

Dr. Christopher Vakulskas, Ph.D. is a senior staff scientist in the molecular genetics research division of Integrated DNA Technologies. He has a Ph.D. in microbiology from the University of Iowa, where he studied genetic regulatory circuits in pathogenic bacterial species. He was an NIH Postdoctoral Fellow at the University of Florida where he studied RNA binding proteins and post-transcriptional gene regulation.

At IDT, Dr. Vakulskas has been involved in managing contract research services, leading process development for CRISPR protein purification and evolving novel CRISPR proteins with useful functions for commercialization. He conceived and executed a screen that led to the creation of a high-fidelity Cas9 mutant that was recently used to perform therapeutic gene editing in a recent issue of Nature Medicine.

12:00 PM



Development of a Novel Automation Platform and Bioinformatics for Engineering Cell Lines

TED TISCH

Chief Operating Officer, Synthego

Bio

Ted Tisch serves as COO of Synthego. He joined Synthego when it had 20 people and where he is now responsible for product development, operations and commercial functions, as the company develops full-stack engineering platforms delivering innovative genome engineering solutions.

Before joining Synthego, Ted spent 25 years in at Bio-Rad Laboratories developing leading reagent, instruments and software products in Research & Development, running Reagent Operations and serving six years as Vice President and General Manager at Bio-Rad Laboratories of the Protein Function Division (>\$200M revenue).

Ted holds a Master of Science in Organometallic Chemistry from the University of Nevada, Reno and a Bachelor of Science in Inorganic Chemistry from Fort Lewis College.

12:40 PM

Lunch & Networking

1:40 PM



Translation of Cell Based Gene Therapies for Cardio-Pulmonary Disorders

DAVID COURTMAN, PH.D.

Scientist and Director of Biotherapeutics, Ottawa Hospital Research Institute; Assistant Professor, University of Ottawa; Chief Scientific Officer, Northern Therapeutics

Bio

Dr. Courtman is trained in cellular and molecular pathology and biophysics with, extensive expertise in the development of novel therapies for cardiovascular disease. He has a long standing interest in the development of novel cardiovascular biomaterials and the regulation of biological responses within the unique spatially-oriented matricellular environment of blood vessels.

Dr. Courtman has participated in the development of a number of cellular-based gene therapies for the treatment of cardiopulmonary disorders taking them from initial concept, through appropriate animal disease models, and into first-in-human clinical trials. He is currently directing the manufacturing of these cell products for trials treating patients suffering from pulmonary arterial hypertension, myocardial infarction, and sepsis.

He is a Scientist and Director of Biotherapeutics at the Ottawa Hospital Research Institute, Assistant Professor at the University of Ottawa, and Chief Scientific Officer of Northern Therapeutics.

[Read an interview with Dr. Courtman as he discusses academic perspectives on cell therapy, trial design and building partnerships.](#)

2:20 PM



Microdystrophin Gene Therapy for Musculoskeletal Diseases

PALANI PALANIAPPAN, PH.D.

Head of Technical Operations, Sarepta Therapeutics

Bio

Palani Palaniappan, Ph.D., has more than 25 years of pharmaceutical experience and continues to contribute to commercial registration and clinical development of numerous candidates in small molecule, biologics, ADCs and cell therapy. He serves as the head of Technical Operations at Sarepta Therapeutics, and focuses on small molecule and antisense based RNA medicines. He leads Sarepta's gene therapy CMC and Manufacturing team and is responsible for all stages of development from research through commercial.

Prior to Sarepta, Palaniappan held increasing leadership positions and recently lead biologics and new modality development at Takeda Pharmaceuticals with responsibility for global development of drug candidates in variety of modalities including cell and gene therapy, microbiomes and antibody drug conjugates. Before Takeda, he spent a number of years each at Millennium Pharmaceuticals, Biogen, Gilead Sciences and Par Pharmaceuticals. He completed executive leadership education at Oxford University and Insead, post-doctoral work at the University of California Riverside and Virginia Commonwealth University Richmond. He received his Ph.D. from the Indian Institute of Technology Kanpur.

| SCHEDULE / TUESDAY, NOVEMBER 6

3:00 PM



Translational Gene and Cellular Engineering

MARK OSBORN, PH.D.

Assistant Professor, University of Minnesota

Bio

Dr. Mark Osborn is an Assistant Professor at the University of Minnesota in the Department of Pediatrics and the Division of Blood and Marrow Transplantation. He is a member of the Masonic Cancer Center, the Stem Cell Institute, The Institute for Engineering in Medicine, and the Center for Genome Engineering. His laboratory focuses on gene and cell therapy for inherited and acquired lethal pediatric disorders and applies the latest methodologies in gene therapy and genome engineering.

3:40 - 5:00 PM

Poster Session, Exhibit & Networking

4:00 - 6:30 PM

Aldevron Facility Tours & Reception



| Guided Tours of Our New Facility

Tuesday, November 6 | 4:30 PM - 6:30 PM

The new Aldevron building is the world's largest and most advanced plasmid DNA manufacturing facility. It has unparalleled GMP capacity and flexibility for clinical and commercial production. Transportation between the Hilton Garden Inn and Aldevron's new HQ is provided in a continual loop. Look for the bus outside the conference entrance.

7:00 PM

Breakfast & Networking

8:10 AM



Opening Remarks & Overview

MICHAEL CHAMBERS

President and CEO, Aldevron

8:20 AM



Challenges in Manufacturing AAV in a Phase I Environment: A Practical Approach to Meeting Demand and Managing Expectation in an Evolving Field

ANDREW MOREO

Operations Manager, GMP and Viral Vector Core Facilities, Nationwide Children's Hospital (NCH)

Bio

Andrew Moreo serves as Operations Manager for the GMP and Viral Vector Core Facilities at Nationwide Children's Hospital (NCH) in Columbus, Ohio.

Mr. Moreo has a B.S. in Biology from Purdue University and nearly two decades of experience in genetic and molecular research, with the last 11 years focused on AAV production. During his time at NCH, Andrew has led the development of an adherent AAV production platform that is robust, adaptable and high-yielding across a wide array of serotypes.

This platform has delivered over 1.5×10^{17} viral particles in support of 40+ Phase 1 INDs. He also led in the expansion of the GMP facility from a single product legacy space to a new multi-product facility capable of handling up to four independent products simultaneously.

In his role, Moreo continues to lend his expertise in the development of new vector production platforms as well as the transfer of existing technologies to clients wishing to pursue advanced phase production.

9:00 AM



Manufacturing Considerations in Cell Therapy Development

ANNE KANTARDJIEFF, PH.D.

Director of Plasmids and Small Molecules Manufacturing, bluebird bio

Bio

Anne Kantardjieff serves as Director of Plasmids and Small Molecules Manufacturing at bluebird bio. Prior to that, she was the Director of Early Stage Process Development at Alexion Pharmaceuticals, where her team was responsible for cell line development as well as upstream and downstream process development for Alexion's early clinical biologics portfolio.

Kantardjieff is the recipient of the 2018 ACS BIOT Van Lanen award, recognizing her service to the Biotechnology Division of the American Chemical Society division, where she also serves as secretary. She obtained her Ph.D. in Chemical Engineering from the University of Minnesota, and her B.Eng. in Chemical Engineering from McGill University in Montreal, Quebec, Canada.

9:40 AM



Standards Development in Gene and Cell Therapy

MARITZA C. MCINTYRE, PH.D.

President, Advanced Therapies Partners, LLC

Bio

Dr. McIntyre has 20 years of experience in the development, evaluation and regulation of biological and small molecule products within startup biotech firms, the Food and Drug Administration (FDA), and as a consultant. Dr. McIntyre was a product reviewer and ultimately Branch Chief in the Division of Cellular and Gene Therapies at FDA/CBER, where she was actively involved policy development and liaison activities to stakeholder groups. She has since worked in regulatory affairs and product development at Bavarian Nordic, REGENXBIO, Inc. and NanoCor Therapeutics. She served as Executive Vice President of Regulatory Affairs and Product Development at Bamboo Therapeutics where, as part of the senior management team, she participated in portfolio selection, product development and fundraising that resulted in an initial \$50 million finance round and ultimate the sale of the company to Pfizer.

As president of Advanced Therapies Partners LLC, Dr. McIntyre provides strategic regulatory and product development advice to biotech companies, academics, and venture capital firms. She has proven success in defining development strategies for products with complex regulatory challenges including special designations (orphan, RMAT, pediatric orphan drug designation), endpoint selection, accelerated approval, complete response letters and dispute resolution. She has also been involved in the preparation of some of the first BLA and MAA submissions for gene therapy products to FDA and EMA. She has multidisciplinary experience, including chemistry manufacturing and control (CMC), preclinical, and clinical with a wide range of product types, including novel gene and cell therapy products, vaccines, biological products and small molecules at varied stages of product development.

Through her participation in industry associations, including ASGCT and the Standards Coordinating Body she has continued to contribute to gene therapy regulatory policy development.

Dr. McIntyre received a Ph.D. in virology from the University of Chicago and graduated magna cum laude with an Honors B.S. in biology from Wayne State University.

10:20 AM

Networking & Exhibit Break

10:40 AM



Directed Evolution by Molecular Breeding

ROBERT WHALEN, D.SC.

Chief Scientific Officer, Altravax; Maxygen

Bio

Robert Whalen is Chief Scientific Officer at Altravax (since 2010) and more recently at Maxygen (since 2017), both in Sunnyvale, California. He oversees multiple programs applying molecular breeding to the creation of novel products in many areas of biotechnology from enzymology to therapeutics and vaccines.

Previously, Whalen was Director of Infectious Diseases at the original Maxygen (1999–2009), where he began to apply the molecular-breeding directed evolution approach to vaccine discovery. Prior to entering the biotechnology industry in 1999, he spent 27 years in France as a staff scientist and group leader at the Pasteur Institute in Paris. Much of his early work was centered on studies of muscle development. He was an early contributor to the development of DNA vaccines and has published a number of articles in that field.

In 1995, he created The DNA Vaccine Web site (now DNAvaccine.com) and served as a consultant to several biotechnology companies. From 1986–1996 he was a member of the Scientific Advisory Board of the French Muscular Dystrophy Group. He is the author of some 120 scientific papers, reviews, and book chapters in the fields of biochemistry, gene expression, developmental biology, molecular physiology, gene transfer, vaccinology, immunology, and directed evolution.

Robert Whalen obtained degrees from the Pennsylvania State University (B.S., 1970), Harvard University (A.M., 1974), and the French National Center for Scientific Research (D.Sc., 1991).

11:20 AM



Nonviral Synthetic Polymers Promote Cellular and In Vivo Delivery of Therapeutic Genes and Editing Systems

THERESA M. REINEKE, PH.D.

Distinguished McKnight University Professor, Department of Chemistry, University of Minnesota

Bio

Theresa M. Reineke is a Distinguished McKnight University Professor in the Department of Chemistry at the University of Minnesota. She also holds graduate faculty appointments in the Departments of Chemical Engineering/Materials Science and Pharmaceutics. Her educational background includes receiving a Bachelor of Science from the University of Wisconsin–Eau Claire, a Master of Science from Arizona State University, and a Ph.D. from The University of Michigan. She then received a National Institutes of Health Postdoctoral Fellowship to further her research background at the California Institute of Technology prior to beginning her independent faculty career.

Her research group is currently focused on enabling fundamental and applied technology advancements of polymers in the fields of gene and cell therapy, oral drug delivery, and sustainability for which she has published more than 110 peer-reviewed manuscripts and received several awards such as the American Society of Gene and Cell Therapy Outstanding Investigator Award, the National Institutes of Health Director's New Innovator Award, and the American Chemical Society Division of Polymer Chemistry Carl S. Marvel Creative Polymer Chemistry Award.

Reineke is also a founding Associate Editor of ACS MacroLetters and currently on the Editorial Advisory Boards of the ACS journals Biomacromolecules and Bioconjugate Chemistry.

12:00 PM



Plasmid DNA in Cell and Gene Therapy: From Bench to Bedside

JAMES BROWN, PH.D.

Vice President of Corporate Development, Aldevron

Bio

James Brown, Ph.D., joined Aldevron in 2015 and serves as Vice President of Corporate Development. His responsibilities include developing and implementing strategies for expanding Aldevron's DNA, mRNA, antibody, and protein products and production services. In this capacity Dr. Brown heads the product management team, which expands existing product features and develops new products, including pALD-X80, an off-the-shelf rAAV helper plasmid.

Prior to joining Aldevron, Dr. Brown was Vice President, Technical Operations, at REGENXBIO Inc., a gene therapy company. In this role he was responsible for contract manufacturing, vendor management, operations, quality assurance and biological reagent sales. Prior to REGENXBIO Dr. Brown served in roles of increasing responsibility in operations and quality assurance at MedImmune, Meso Scale Discovery and IGEN International, Inc. Dr. Brown holds a Ph.D. in chemistry from Stanford University and a B.S. in chemistry from Butler University.

12:40 PM

Lunch & Networking

2:00 PM



Welcome to Afternoon Sessions

MICHAEL CHAMBERS

President and CEO, Aldevron

2:10 PM



Coming of Age Together: Rare Disease Advocacy and Gene Therapy

MICHELLE BERG

Vice President, Patient Affairs & Community Engagement, Abeona Therapeutics

Bio

Michelle Berg joined Abeona in June of 2015, serving initially as Vice President, Patient Advocacy and transitioning in late 2017 to Vice President, Patient Affairs & Community Engagement to better reflect departmental focus. As lead for the Patient Affairs department, Michelle's role encompasses development and provision of education and information on rare disease, drug development, gene therapy, and advocating for change and awareness for the impact on communities served specifically through Abeona's pipeline programs. Berg is a contributing author and speaker on patient focused programming and rare disease advocacy.

Previously, she was the first hire with Aldevron, LLC, now a leading global contract manufacturing organization specializing in biological reagents for R&D through clinical trial use. During her time there, she held multiple positions with increasing responsibility, contributing to the growth and reach of the organization, focused on developing strong relationships, collaborations, and services. Additionally, she performed research on behalf of the Department of Plant Sciences, North Dakota State University. Her B.S. in Biotechnology was attained from North Dakota State University in 1997. She brings over 17 years of experience in the gene and cell therapy, vaccine, gene editing and molecular diagnostic fields.

2:40 PM



An Early Experimental Trial Participant at Penn Medicine for Dr. Carl June's CAR-T Therapy

ROBERT (BOB) W. LEVIS

A Patient's Perspective

Bio

After receiving a Bachelor of Science degree in metallurgical engineering from Grove City College and a Master of Science degree in materials science and physics from the University of Illinois, Bob joined Air Products & Chemicals, Inc., in 1975. He held various positions as an applications development engineer at the Allentown, Pennsylvania, corporate headquarters.

In 1979 he was assigned to Brazil as manager of Applied R&D, with Air Products Gases Industriais Ltd. The four-year experience in Brazil led to other overseas business assignments including five years each in Japan and Taiwan, where he managed Air Products' gases and equipment business through joint venture companies in these countries.

In June 1999, Bob was named Vice President & General Manager for Air Products Asia where he oversaw the company's gases and equipment business in Southeast Asia. This executive responsibility was expanded in 2002 as V.P. & General Manager for the company's largest division in Asia, based in Singapore for a total of eight years.

Bob is currently consulting through Asia & Brazil Connections, LLC with small and medium sized U.S. enterprises on Asia and Brazil market entry opportunities including ABEC, Inc (biopharmaceutical engineering and equipment manufacturer) where he was employed during 2011/2012 as Director, Asia Business Development.

Bob was diagnosed with Chronic Lymphocytic Leukemia (CLL) in 2002. He continued to "watch & wait" until 2008 when he moved back to the U.S. and underwent FCR (fludarabine, cytoxan, rituxan) treatment for his CLL. He maintained a complete remission for 3+ years when his CLL relapsed to the most aggressive 17p del form. Bob enrolled in a Pharmacyclics trial (ibrutinib vs. ofatumumab) at Penn Medicine in Philadelphia, and was randomized to the ofatumumab arm. The ofatumumab treatment did not stop the progression of his CLL and he was denied crossover access to ibrutinib.

Facing death while alive on hemoglobin and platelet transfusions, he enrolled as one of the early experimental trial participants at Penn Medicine for Dr. Carl June's CAR-T therapy. He received his genetically modified T-cells on March 12, 2013 and was in complete remission for another 3+ years. A second CAR-T therapy during 2017 at Penn Medicine reduced the CLL in his bone marrow to < 5.0 %. Now Bob maintains normal blood labs while on the oral kinase inhibitor, ibrutinib.

Bob and Carl June have become friends helping others to overcome their blood cancers. Bob remains an avid sportsman and competitor while consulting on patient advocacy initiatives. Google "Bob Levis Leukemia" for various media coverage of his CAR-T experience.

3:00 PM



Emily Whitehead's Journey to CAR-T

TOM WHITEHEAD

Co-Founder, Emily Whitehead Foundation

Bio

CTom Whitehead, a Penelec lineman and Emily's dad, is a co-founder of the Emily Whitehead Foundation. In January 2015, Tom and his wife Kari founded the Emily Whitehead Foundation in honor of their daughter, three-time leukemia survivor and first child in the world to receive CAR-T cell therapy, Emily Whitehead. Tom shares Emily's story to inspire others to take action in the fight to cure childhood cancer and focus their fundraising and awareness efforts on pediatric immunotherapy cancer research.

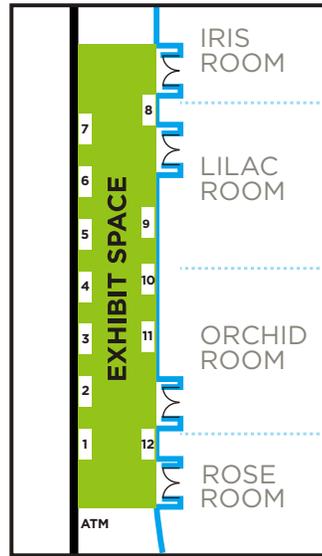
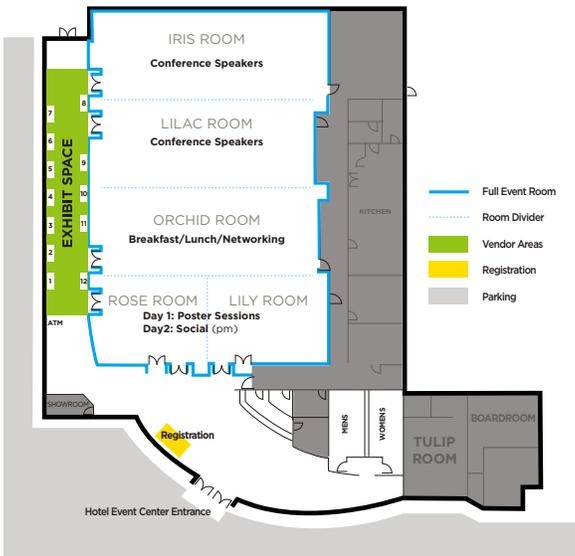
| SCHEDULE / WEDNESDAY, NOVEMBER 7 (CONT.)

4:00 PM

Closing Reception & Networking

EXHIBITORS

Exhibitor manual can be found at BreakthroughSymposium.com/Sponsors



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SECTION 1: GENE EDITING / THERAPY

01-01. Polymerization of Quinine for Applications in Gene Therapy

Craig Van Bruggen, David Punihaole, Andrew J. Schmitz, Samantha Linn, Jeffrey M. Ting, Yogesh K. Dhande, Zhe Tan, Mitra Ganewatta, Renee R. Frontiera, Theresa M. Reineke
University of Minnesota, Twin Cities, St. Paul, Minnesota

Abstract: Although the medical community is quickly embracing gene editing technologies, such as CRISPR/Cas9, for the development of new gene therapies, there are still significant drawbacks to current gene delivery methods which often focus on viral vectors. A promising alternative to viral vectors include polymer-based gene delivery. Cationic polymers can electrostatically bind with negatively-charged DNA to form nano-scaled complexes, called polyplexes, which shuttle DNA into cells via endocytosis in a process known as transfection.

Although many types of polymers have been made to increase internalization of DNA into human cells, efficient expression of the DNA is often limited by polyplex entrapment in degradative endosomes. Quinine, a naturally-sourced antimalarial with potential endosomolytic behavior, was polymerized with a variety of hydrophilic comonomers via free radical copolymerization in order to create a more effective polymer-based transfection reagent.

A small library of statistical copolymers containing quinine was screened for hits that can efficiently bind DNA, form polyplexes, and transfect several human cell lines in vitro. A copolymer of 2-hydroxyethyl acrylate (HEA) and quinine, poly(HEA-co-quinine), showed an exceptional ability to transfect both adherent and suspension human cell types with a GFP plasmid at levels comparable to commercial reagents, such as Lipofectamine or JetPEI, with limited cytotoxicity. Genome editing of HEK-293T cells in vitro with CRISPR/Cas9-encoding plasmids was enhanced when using poly(HEA-co-quinine) as a transfection reagent compared to commercial reagents.

In addition, preliminary work has shown that quinine's unique fluorescence and Raman activity, makes poly(HEA-co-quinine) well suited as a dual-threat therapy agent and diagnostic probe for understanding the polymer's DNA-binding behavior, trafficking within the cell, and the mechanisms by which it enhances transfection. Through this work, quinine's storied utility in medicine and synthetic chemistry is broadened as it becomes a potential new tool in the field of gene delivery.

01-02. Building Quality Assurance Measures into an Effective Evidence Based Precision Medicine Program

April Schultz, Pharm D Rph; Jordan Baye Pharm D, MA, BCPS; Natasha Petry Pharm D, BCACP; Russ Wilke MD PhD FACP; Jennifer Reiner PhD; Aissa Aifaoui Pharm D
Sanford Health, Sioux Falls, South Dakota

Abstract: Although the medical community is quickly embracing gene editing technologies, such as CRISPR/Cas9, for the development of new gene therapies, there are still significant drawbacks to current gene delivery methods which often focus on viral vectors. A promising alternative to viral vectors include polymer-based gene delivery. Cationic polymers can electrostatically bind with negatively-charged DNA to form nano-scaled complexes, called polyplexes, which shuttle DNA into cells via endocytosis in a process known as transfection.

SECTION 1: GENE EDITING / THERAPY

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01-03. Improving CRISPR-Cas9 Specificity with Chemical Modifications in Single-guide RNAs

Daniel Ryan¹, David Taussig¹, Israel Steinfeld¹, Smruti Phadnis¹, Benjamin Lunstad², Savita Shah¹, Madhurima Singh¹, Ryan McCaffrey², Magdalena Olesiak³, Bo Curry¹, Jeffrey Sampson¹, Douglas Dellinger², Laurakay Bruhn¹

¹Agilent Research Laboratories, Santa Clara, California; ²Agilent Research Laboratories, Boulder, Colorado; ³University of Colorado, Boulder, Colorado

Abstract: As CRISPR systems prove transformative for altering genomes with unprecedented ease, there remains keen interest in improving their specificity for perfectly matched targets to prevent unintended genomic alterations, especially in the context of therapeutic applications. Multiple approaches have been explored to further increase the specificity of CRISPR-Cas9 systems including truncation of the guide RNA and engineering or evolution of the Cas protein for higher specificity.

We developed a novel approach for enhancing specificity by employing site-specific chemical modifications in the 20-nucleotide DNA recognition sequence ("guide sequence") in single-guide RNAs (sgRNAs). Our deep sequencing results from transfected human cells show that a modification (2'-O-methyl-3'-phosphonoacetate, or "MP") incorporated at select sites in the ribose-phosphate backbone of sgRNAs can markedly reduce off-target cleavage activities, while maintaining high on-target performance as demonstrated in four clinically relevant genes.

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For instance, addition of a single MP modification to a guide RNA reported by several groups to be highly efficient for editing the sickle cell disease (SCD) allele dramatically reduced off-target activity at a site in chromosome 9, which typically undergoes very high rates of off-target cleavage, by an order of magnitude in three different cell types including CD34+ hematopoietic stem cells without compromising the on-target activity. Through extensive studies in which we systematically walked a single MP across the 20-nt guide sequences of multiple sgRNAs we have identified specific positions where MP and similar modifications of the backbone are particularly effective at improving specificity across a variety of guide sequences.

These results demonstrate that selective incorporation of modifications such as MP in the 20-nt guide sequence can allow users to tailor the specificity of sgRNAs for select targets and cellular environments, thus providing a versatile new tool for augmenting the performance of CRISPR systems for research, industrial and therapeutic applications.

01-04. Efficient *in vitro* Delivery of CRISPR/Cas9 Ribonucleoprotein Using Triblock Polymer Micelles

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Abstract: CRISPR Cas9 is a great revolution in genomics research and has rapidly developed into one of the most efficient gene editing tools for research and therapeutic applications. Due to the high cost and scale limitations of viral vectors, there exists an urgent need for developing affordable and efficient delivery vehicles to transport CRISPR Cas9 system into the cells and achieve gene editing *in vitro* and *in vivo*.

In this work, we present the synthesis and characterization of a polymeric micelle-based delivery system. This delivery systems has been designed with core-shell-corona architecture, which contains a hydrophobic core, a cationic midblock to bind with CRISPR Cas9 ribonucleoproteins (RNPs), and a hydrophilic corona to protect the complex from colloidal aggregation and enzymatic degradation. The successful binding of RNPs to micelles has been shown by agarose gel electrophoresis, and further supported by the complexes' negative to positive surface charge shift based on Zeta potential measurement.

The size of the nanoparticle delivery systems were characterized using DLS, and the complexes were visualized by Cryo-TEM imaging. Characterization via static light scattering has been employed to enable quantitation of RNP binding density on the surface of the micelles. The gene editing efficiency of the delivered RNPs with human embryonic kidney traffic light reporter (HEK293T TLR) cells was examined by flow cytometry and DNA sequencing. A higher gene editing efficiency (around 33% indels) compared to multiple highly efficient commercial delivery vehicles such as jetPEI and Lipofectamine 2000 was found with the polymer micelle-based delivery vehicles. This work reveals the promise of polymer micelle scaffolds as a new affordable method to encapsulate and deliver gene editing systems.

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01-05. Pre-clinical Work and Scale-up Processes Towards the Clinical Translation of Therapeutic Gene Correction of the Sickle Cell Disease Causing Variant in Somatic Hematopoietic Stem Cells

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Abstract: We developed a genome editing platform that utilizes the CRISPR/Cas9 nuclease system coupled with AAV6 to induce homologous recombination (HR) directed-correction of the sickle cell disease causing mutation. Here we present the preclinical research, demonstrating the safety and efficacy of genome editing of somatic hematopoietic stem and progenitor cells (HSPCs), and key scale-up processes enabling clinical translation.

CD34+ HSPCs were isolated from fresh plerixafor-mobilized apheresis products from healthy donors. Several HSPCs culture conditions were tested, together with a large-scale electroporation system. We used a high-fidelity Cas9 and a chemically modified sgRNA, delivered as ribonucleoprotein complexes, and developed a scalable AAV6 production process (gcSCD-AAV6, toxicology lot). We quantified the alleles modification frequency in liquid culture and the allelic distribution in edited HSPCs derived-colonies.

We were able to achieve up to 60% allele correction of the sickle cell mutation with this approach, thus showing proof of efficacy in the scale-up process. Data on colony forming unit and single cell genotyping will also be presented. Overall, we have proved manufacturing reproducibility and matched the required specifications for clinical-scale production (150 million cells showing >20% HR, >70% viability and >80% CD34+). In order to investigate safety, edited HSPCs (gcHBB-SCD cell product) and donor matched untreated cells were karyotyped and injected in the NSG animal model to perform a long-term tumorigenicity/toxicology study. We will present the ongoing results and the plan to file an IND to initiate a phase I/II clinical trial testing the strategy in patients.

01-06. Training Biotechnology Students through the Use of Modern Gene Editing in the Classroom...and Beyond

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Abstract: Recombinant DNA technology is possible because of four major discoveries: (1) plasmid cloning vectors, (2) identification/purification of restriction enzymes, (3) polymerase chain reaction (PCR) to amplify DNA, and (4) DNA sequencing. Over the past three decades, these discoveries have been adapted and merged with other discoveries to not only allow for sequencing of whole genomes on a chip, but also for the manipulation genes with relative ease, to the point where these technologies are no longer the limiting factors for disease diagnosis and therapeutics.

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The field of recombinant DNA technology is evolving at an amazingly rapid rate. Modern medicine and medical research has also evolved, such that these fields now often require the use of DNA identification and manipulation to identify disease-causing DNA perturbations, to prescribe the best therapeutics (personalized medicine), and/or to provide for more permanent treatment options through targeted gene therapy. Modern agriculture is also taking advantage of gene editing to generate crops resistant to pests and disease in order to increase yields and nutritional value.

At NDSU, the BIOC 474: Methods of Recombinant DNA Technology course has been using the CRISPR-Cas9 system to practice, from start-to-finish, how one does modern-day gene editing through a semester-long project. Students emerging from this class will have gained fundamental knowledge and experience for manipulating gene function in cells. This study outlines the gene-editing strategy used in the class and presents students' results from preliminary studies using CRISPR-Cas9 technology.

01-07. Aldevron's CRISPR-associated Nucleases for Gene Editing: Tools to Support Discovery and Therapeutic Programs

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Abstract: Gene editing using CRISPR-associated nucleases, including SpCas9, offers a novel approach to treat many diseases. SpCas9 now is being evaluated in multiple preclinical and clinical programs. While many vendors offer research-grade SpCas9, these nucleases are not applicable to clinical trials due to construct design and quality grade.

Aldevron offers SpCas9 and AsCpf1 nucleases optimized for development and clinical applications. In addition to wild-type SpCas9, Aldevron now offers SpyFi Cas9 which supports gene editing with significantly reduced off-target effects. Aldevron's gene editing proteins are supported by a robust, scalable manufacturing protocol and an extensive quality package.

These proteins are available in a range of quality grades from research grade to GMP, allowing for a seamless transition from research applications to the clinic.

01-08. Gene Editing Immune Cells with Ribonucleoproteins for Chimeric Antigen Receptor Therapy

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SECTION 1: GENE EDITING / THERAPY

Abstract: Chimeric antigen receptor (CAR) T cells are genetically engineered T cells that have been used to target cancer-associated antigens. The profound success of CAR T cells in treating hematological malignancies (e.g. leukemia by targeting CD19) has led academic centers and industry to scale up and manufacture these engineered cell therapies. While biomanufacturing processes to produce these therapies have been devised, critical quality attributes are still under development to define the most efficacious and safe cell product. In order to manufacture efficient and predictable CAR T cells, the functional effects of different sources of heterogeneity in CAR T cells needs to be taken into account.

To address this issue, CAR T cells were engineered using CRISPR-Cas9 as opposed to conventionally used viruses. Viruses can insert one or more copies of the transgene into various random locations in the genome, near different regulatory elements, all which can influence the final expression of the CAR gene. Using precise gene editing with CRISPR Cas9, we can gain tighter control of the over the CAR expression in the final CAR T cell product.

Primary T cells are isolated from whole blood from healthy volunteers using RosetteSep (Stem Cell Technologies) and then cultured in Immunocult. T cells are nucleofected using a Lonza Nucleofection kit to deliver Cas9 protein (Aldevron), sgRNA targeting a safe harbour locus, and the donor CAR plasmid. Fluorescence activated cell sorting is performed on the nucleofected T cell population to isolate CAR+ T cells. Similar transfection methods were also used to create CAR+ NK cells using the standard cell lines.

SECTION 2: NUCLEIC ACIDS

02-01. Functional Evaluation of Isoforms Present in pDNA Vaccines

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Abstract: Nucleic acid vaccines (DNA or RNA) are experiencing a significant resurgence in the treatment of infectious diseases, allergy and oncology. Plasmid DNA (pDNA) provides the elements required for propagation in a bacterial host (e.g. replication origin, selection marker) but more importantly, they contain all the elements required for directing target-gene expression in human patients. Our technology provides an additional element, the gene encoding Lysosomal Associated Membrane Protein (LAMP) to help traffic the target gene into the MHC-II pathway thus eliciting a strong immune response.

As with other parenteral drugs, pDNAs made for use in clinical studies must meet minimal requirements for safety, identity, purity and potency. One such attribute is Forms Analysis, which relates to the proportion of supercoiled (SC), Open Circular (OC), Linear (L) or other forms like concatamers that are routinely found in the pDNA product. While all these forms are identical in nucleic acid composition, it is generally accepted that preparations containing a higher proportion of SC pDNA will be taken up more readily by cells and ultimately be more efficacious.

In the present study, we prepared different isoforms of pDNA encoding either a GFP reporter or a LAMP fusion gene, confirmed the forms by Agarose Gel Electrophoresis (AGE) and HPLC and analyzed their ability to direct gene expression in vitro (transfection or electroporation) and in vivo (injection into mice). Our results demonstrate that the OC form of pDNA is competent at directing gene expression in vitro. Unexpectedly, a linear form with an intact LAMP fusion gene was also able to direct gene expression. Confirmation of these results in vivo, could be used to substantiate a reduced specification for SC pDNA which would directly impact product yield and manufacturing COGs.

02-02. mRNA Synthesis Reagents and Manufacturing: Research Through Clinical Development

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Abstract: Messenger RNA (mRNA) unravels new possibilities for the treatment and prevention of diseases. As a relatively new therapeutic modality, mRNA offers advantages: It can instruct the ribosome to make almost any protein, it functions without entering the cell nucleus, and it degrades without leaving behind unwanted or harmful genetic traces. The clinical use of mRNA was initially limited due to low efficacy and undesirable immunogenicity.

These issues have been addressed through optimization and modification of mRNA elements such as the 5' cap, nucleoside structures, poly(A) tail and 5'/3' UTRs. As a result, there is an exploding demand for clinical use is straining manufacturing capacity. Aldevron is meeting this latest challenge by expanding our capabilities to satisfy the rapidly growing need for high quality mRNA in research through clinical applications.

SECTION 2: NUCLEIC ACIDS

To reduce supply chain risk, minimize lead time and ensure the highest quality for our clients, Aldevron manufactures the linear plasmid DNA (pDNA) template and recombinant enzymes used for mRNA synthesis performed by in vitro transcription (IVT). The production of mRNA at Aldevron can begin with the electronic construct sequence or supercoiled pDNA provided by the client. During mRNA synthesis, Cap1 and poly(A) tail structures are added enzymatically or alternatively co-transcriptionally using a cap analog and template-encoded poly(A) tail sequence.

Modified bases can also be incorporated dependent on project requirements. Purification is accomplished by column chromatography. A robust process was established by rigorous development. mRNA production is available in a range of quality grades from research grade to GMP and the process is identical between the service grades, allowing for a seamless transition from research applications to the clinic.

02-03. Production of Plasmid DNA in Gas Permeable Low-density Polyethylene Bags

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Abstract: Personalized ex vivo gene therapy and immunotherapy has increased the demand for small to medium scale plasmid production. Historical and current methods to produce plasmids from *E. coli* grown at volumes of 250-ml to 10-L rely on classic shake flasks. The related glassware (or plasticware) is expensive especially for clinical-grade manufacturing. Also, the venting required for the use of shake flasks can allow cross contamination from other nearby plasmid growths and/or phage contamination from the environment.

With the use of a novel GFP reporter plasmid, SUPERGLO, this project demonstrates the feasibility of growing plasmid-containing *E. coli* in completely closed low-density polyethylene bags (LDPB). Milligram quantities of SUPERGLO were grown and subsequently purified from a K12 *E. coli* strain using commercially available LDPBs. GFP expression and plasmid yields were comparable to plasmids made in conventional shake flasks.

This is made possible by the inherent gas permeability of the LDPB. This new method offers advantages to clinical-grade plasmid manufacturing, and has application to routine research-grade plasmid production. LDPBs are readily available, clean, inexpensive, and they take up a fraction of the space required for shake flasks. Importantly, LDPBs offer complete segregation of individual plasmids, even if they are growing in the same incubator. Further work is underway to assess the robustness of the described methods and bag sterilization methods. Industry-wide adoption of this method could save millions of dollars per year, and reduce the waste and energy associated with the use of conventional shake flasks – thereby helping to enable personalized medicine.

SECTION 3: BIOPROCESSING AND ANALYTICS

03-01. An Industrial Purification Platform for AAV

BIA Separations

Abstract: This poster presents fully scalable non-affinity purification strategy that has been proven to be effective for all AAV serotype tested to date. Cell lysate is directly subjected to column purification after removal of cell debris without requiring a concentration step using tangential flow filtration. The process consists of three chromatographic steps.

Hydrophobic interaction chromatography on a CIMmultus™ OH monolith is used for initial virus capture and purification. Precipitating salts are used at 1.0–2.0 M to achieve virus binding. Most of the small molecule contaminants and proteins are eliminated in the flow-through. AAV co-elutes with a highly reduced population of contaminating proteins. DNA-protein complexes are very strongly retained and require NaOH for removal. Intermediate polishing is performed with a CIMmultus™ SO3 cation exchange monolith.

The AAV fraction from the capture step is titrated to a pH value of 3.5–5.0 and diluted to binding conditions. Sugars and surfactants are added to suppress non-specific interactions with tubing and containers, and the product is eluted in a salt gradient. Final polishing is conducted on a CIMmultus™ QA anion exchange monolith which separates empty capsids from full capsids. This is achieved in a salt gradient at alkaline pH.

03-02. Sample Displacement Chromatography of Plasmid DNA Isoforms

BIA Separations

Abstract: Preparative scale chromatographic separation of open-circular (oc) from supercoiled (sc) plasmid DNA (pDNA) isoforms has been already established on CIM® C4 with high ligand density (C4 HLD) monolithic columns with sample loading in 3.0 M ammonium sulphate (AS).

The process requires high molarity of AS, increasing the overall cost of the process. Sample displacement chromatography (SDC) can be used as an alternative to decrease the AS concentration required during loading onto hydrophobic chromatographic supports.

This study compares three chromatographic monoliths with different hydrophobic ligands on the surface (C4 HLD, pyridine and histamine) for the purification of different pDNA vectors in SD mode. Optimal AS loading concentration range was determined by analytical separation of two pDNA isoforms in descending AS concentration gradient.

03-03. Purification and Analytics of pDNA Downstream Bioprocessing Using Hydrophobic Chromatographic Monoliths in Sample Displacement Mode

BIA Separations

SECTION 3: BIOPROCESSING AND ANALYTICS

Abstract: Plasmid DNA (pDNA) used in vaccination and gene therapy has to be highly pure and homogenous, which point out necessity to develop efficient, reproducible and scalable downstream process. Convective Interaction Media (CIM) monolithic chromatographic supports being designed for purification of large molecules and nanoparticles seem to be a matrix of choice for pDNA purification.

In present work we describe a pDNA purification process designed with two chromatographic steps on CIM monolithic columns: capture step based on anion-exchange (AEX) chromatography (CIMmultus™ DEAE) and polishing step based on hydrophobic interaction chromatography (HIC) chemistry in sample displacement (SDP) mode on high ligand density butyl-modified (CIMmultus™ C4 HLD) monolithic support.

Classical polishing step in HIC mode requires high concentration of ammonium sulfate (AS) during loading step and elution is then achieved by descending AS gradient. On the other hand, SDP utilizes different relative binding affinities of components in a sample mixture and separates pDNA isoforms under overloading conditions, where sc pDNA isoform acts as a displacer of oc or linear pDNA isoform.

Combination of both chromatographic steps using optimized CaCl₂ precipitation enabled production of pure pDNA, satisfying all regulatory requirements. Process was found to be reproducible and exhibits high productivity. In addition, at-line monitoring of pDNA purification process is shown, using CIMac pDNA analytical columns using PATfix™ HPLC system.

03-04. A Rapid Alternative to Culture Based Mycoplasma Detection

ThermoFisher Scientific

Abstract: Per regulatory requirements, cell-culture based therapies must be free of mycoplasma. Manufacturers have traditionally outsourced testing to labs that specialize in the 28-day culture-based test method. For manufacturers of gene and cell therapy products, as well as other low-dose and short shelf-life therapeutics, it is not feasible to wait 28 days for test results. Thus, the need for rapid mycoplasma test results has also increased.

Real-time PCR based assays provide a viable alternative to the culture-based method and provide results in hours while meeting the required sensitivity. Following validation, regulatory filing and review, users across multiple therapeutic modalities have received regulatory acceptance to use of a rapid real-time PCR assay for lot release testing.

Poster abstracts are available at BreakthroughSymposium.com/Posters

SECTION 4: BASIC RESEARCH

O4-01. Progression of Prostate Cancer in Bone Mimicking 3D Tissue Engineered Scaffolds

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Abstract: Translational models that mimic skeletal metastasis of prostate cancer are very critical to investigate the extreme stage of the disease. Herein, we report a polymer-nanoclay based in vitro tumor model which recapitulates extreme stage of prostate cancer (PCa) skeletal metastasis.

A unique cell culture system termed as 'sequential culture' has been applied to create a more bone-mimetic niche for colonization of prostate cancer cells. Sequentially cultured PCa cells with MSCs formed self-organized multicellular tumoroids with distinct tight cellular junctions and hypoxic core regions. We demonstrated that PCa cells undergo mesenchymal to epithelial transition (MET) in the tissue engineered bone microenvironment mimicking the late stage of PCa metastasis.

We studied the effect of metastasized PCa cells on bone mineralization and extracellular matrix formation. We used the in vitro tumor model to investigate the alteration in mechanical properties of PCa cells during MET and during the progression of the disease at the metastatic site.

We found that PCa cells become softer and mechanical plasticity increases with the disease progression at the metastatic site. We investigated the interconnection between cytoskeletal changes and alteration in mechanical properties of PCa cells and found that F-actin predominantly regulates the softening of PCa cells at the metastatic site.

Therefore, our sequential culture-based tumor model can be applied to recapitulate more consistent osteotropic cancer cell behavior in understanding tumor biology. This model also can be implemented for drug screening to target early colonization stage of prostate cancer cells in the bone microenvironment.

O4-02. Nanoclay Based 3D *in vitro* Breast Cancer Bone Metastases Model to Study Later Stage of Cancer Pathogenesis in Bone

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Abstract: TBreast cancer (BrCa) is the second leading cause of cancer death in women, after lung cancer. Metastatic breast cancer preferentially spreads to bone and colonizes within the bone marrow to cause bone metastases. To date, the mechanisms of breast cancer-related metastasis to bone are poorly understood. This may be partly due to the overreliance on in vivo xenografts. Recent studies have shed light on the fact that these xenografts did not have not a human bone microenvironment which increasingly considered to be the critical component of metastases.

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To address this drawback, we have developed a nanoclay based 3D in vitro model of breast cancer bone metastasis using human mesenchymal stem cells (MSCs) and human breast cancer cells (BrCa) which mimics later stage of breast cancer pathogenesis in the metastatic site. Initially, MSCs were seeded on the scaffolds to obtain 'new' bone with similar stoichiometry, mechanics and biological function as that of human bone.

Further, human breast cancer cells (MDA-MB-231 and MCF-7) were seeded on this newly formed bone to mimic metastasis conditions. The sequential culture of MSCs and BrCa cells showed the occurrence of mesenchymal to the epithelial transition of cancer metastasis in this model as evidenced by gene expression analysis and immunocytochemistry, thus providing a platform to study biochemical cues that underlie disease progression.

The changes to migratory capabilities and invasiveness in MDA-MB-231 as compared to tumor growth with MCF-7 was directly observed on the 3D model. Together, a novel bone-mimetic 3D in vitro breast cancer model has been developed that could be used to shed light on the mechanism that governs the later stage of cancer pathogenesis in bone.

O4-03. Effect of Kisspeptin on the Intracellular Calcium Regulation in Asthmatic Human Airway Smooth Muscle Cells

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Rationale: The pathophysiology of asthma involves inflammation and alterations in the structure and function of airway smooth muscle (ASM). Notably, prepubertal boys are more likely to have asthma, while following puberty; women show decreased asthmatic prevalence during postmenopausal years. Accordingly, in the context of understanding sex differences in asthma, it seems important to identify pathways closely relevant to sex steroids. Kisspeptins (KISS1) originally identified as a key protein that regulate GnRH, bind to their receptor (KISS1R) to regulate sexual differentiation and puberty onset. Considering this, we hypothesize a major role of kisspeptin and KISS1R in sex difference in asthma. Here, we test our hypothesis using primary ASM cells as an model.

Methods: Primary human ASM cells were cultured in DMEM-F12 and serum deprived for 72 hours. Standard procedures followed for western blot analysis and rtPCR techniques. Immunofluorescence analysis was done using Biotek LFX Imager. 4 uM Fura-2 loaded ASM cells exposed to KISS1 agonist metastin were imaged to assess for intracellular calcium ([Ca²⁺]_i) responses using Olympus confocal microscope.

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Results: Our novel findings indicate the ubiquitous expression of KISS1 and KISS1R in human ASM cells. Interestingly, there is a significant difference in the expression levels of KISS1 and KISS1R between males and females with females showing lower expression of both KISS1 and KISS1R at a basal level. Furthermore, our studies show decreased expression of KISS1 and increased expression of KISS1R in asthmatic cells compared to non-asthmatic ASM cells. Our functional studies showed that metastatin was effective in reducing $[Ca^{2+}]_i$ responses in non-asthmatic and asthmatic ASM cells. This effect was found to be significantly more in asthmatic ASM cells.

Conclusion: Overall, we demonstrate an altered expression and function of kisspeptin and KISS1R in both males and females in normal and asthmatic conditions suggesting a novel role of kisspeptin signaling in asthma.

O4-04. Divergent Estrogen Receptor Signaling on Intracellular Calcium Regulation in Human Airway Smooth Muscle Cells

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Purpose:

Asthma incidence has been shown to be higher in pre-menopausal women and aging men, strongly indicating that estrogen may be involved in both sexes. Recent studies demonstrated that airway smooth muscle (ASM) from both male and female expresses estrogen receptors (ER) – ER α and ER β and these receptor expressions are upregulated during asthma or inflammation. We hypothesize that these receptors have differential roles in intracellular calcium $[Ca^{2+}]_i$ regulation in ASM cells.

Methods:

Human ASM cells were enzymatically dissociated from tissue obtained incidental to lung surgery. Cells were plated onto 8-well glass plates and grown to 70% confluence, exposed to pro-inflammatory cytokines TNF α (20ng/ml) and/or IL-13(50ng/ml) in the presence or absence of 1nM 17 β -estradiol (E2), 10nM PPT (ER α agonist) or WAY (ER β agonist) for 24h. These were then loaded with 4 μ M Fura-2, AM in HBSS for 1h and subsequently washed. The $[Ca^{2+}]_i$ level was measured from the intensity change observed following exposure to 10 μ M histamine, using Olympus confocal microscope.

Results:

WAY was effective in reducing $[Ca^{2+}]_i$ levels in ASM cells compared to control. The cytokines significantly increased $[Ca^{2+}]_i$ compared to vehicle. In combination with cytokines, WAY significantly reduced $[Ca^{2+}]_i$ in ASM cells, while PPT did not elicit any notable changes compared to cytokines alone. We further examined mechanism of estrogen signaling via several membrane channels and pumps. It was found that ER β signals primarily through L-type calcium channels and Sarcoplasmic Reticulum Calcium Reuptake pump (SERCA).

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O4-05. Virulence Assessment of *Listeria Monocytogenes* in the Insect Larvae Model *Galleria Mellonella* and Impact on Clinical Outcomes

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Abstract: *Listeria monocytogenes* (Lm) is frequently found as a contaminant in food and animal feed and has been associated with different clinical manifestations in humans and farmed ruminants such as central nervous system (CNS) infections, bacteremia (BAC) and maternal-neonatal (MN) infections. Among strains Lm variances in virulence has been reported. Data on clinical isolates from ruminants in Europe, have provided evidence of hypervirulent clones causing rhombencephalitis in lineage I and have described sequence type (ST) 1 as predominant, suggesting its increased neurotropism. However, there is also evidence of hypervirulent clones in lineage II indicating that this feature is not restricted to strains from lineage I.

Our goal was to compare the virulence potential among strains of Lm belonging to different CCs and isolated from patients with different clinical outcomes using *G. mellonella* as a biological model.

This study was conducted using 23 strains from clinical cases of listeriosis in ruminants and humans. We selected representative isolates from different CCs based on three clinical outcomes (CNS, MN and BAC) to inoculate the *G. mellonella*. Larvae injected with different concentrations of Lm were incubated and monitored over seven days for time needed to kill 50% of larvae (LD50) and to determine the survival rate of *G. mellonella*.

We found that CC14 (II) strains associated with MN infections showed lower LD50 (higher virulence) than Lm strains from CC1 and CC7 recovered from CNS infections and BAC, at all inoculum concentrations. The difference, however, was dose and strain- dependent.

These findings suggest that there are differences in virulence potential that may be associated to specific clinical outcomes, and indicate that *G. mellonella* is an accessible animal model to test Lm pathogenesis and virulence. It also contributes to the identification of CCs with a higher ability to cause disease, thus facilitate surveillance and management of listeriosis.

Acknowledgements: Veterinary Diagnostic Lab (NDSU), RCA Small Grant Program (NDSU), Colciencias Scholarship (Colombia).

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04-06. Can TA-65 Influence Telomere Dynamics During Development in House Sparrows (*Passer Domesticus*)?

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Abstract: Research over the past decade has demonstrated that age is the main risk factor for many major diseases that persist in modern society such as cancer, cardiovascular disease, and neurodegeneration. Thus, understanding the underlying mechanisms of aging is an important avenue of research in biomedicine.

One mechanism that may be involved in cell death and aging are telomeres. Telomeres, protective sequences of DNA on the ends of eukaryotic chromosomes, naturally shorten during cell division and in response to stress. Once telomeres reach a critically short length this leads to cellular senescence and death. Recent evidence has demonstrated early life telomere length can be predictive of lifespan.

Therefore, to fully understand the relationship between telomere length and organismal aging it is imperative to be able to manipulate telomere dynamics (length and loss rate). One way that telomeres can be manipulated is through the enzyme telomerase, which adds nucleotides onto the ends of telomeres, restoring their length. Mutations in telomerase activity, which is characterized by down-regulated telomerase and greater telomere shortening, have been linked to several diseases.

An extract from the Chinese root, *Astragalus membranaceus*, called TA-65 has been shown to lengthen telomeres by increasing telomerase activity. To investigate the causal role of telomeres in early life, we experimentally manipulated TA-65 in growing free-living house sparrows (*Passer domesticus*) and examined the effect on telomere length and loss. Nestlings were randomly assigned to either an experimental or control treatment group and given a daily oral dose of TA-65 or sterile water, respectively. Blood samples were collected from nestlings on day 2 and 10 post-hatch to measure telomere length and loss.

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We predicted that experimental nestlings would have less telomere loss and longer overall telomere length than controls. Manipulation of telomere dynamics through TA-65 could yield insight into organismal aging and disease prevention strategies.

04-07. GSTP1 Knockdown and Inhibition Impairs Pancreatic Ductal Adenocarcinoma (PDAC) Growth

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Abstract: Glutathione S-transferase pi 1 (GSTP1) is a key cytosolic detoxification enzyme that metabolizes xenobiotic compounds and byproducts of cellular metabolism. GSTP1 is overexpressed in many tumors, particularly ovarian, non-small cell lung, breast, colon, and pancreas. Moreover, GSTP1 is overexpressed in drug-resistant cancer cell lines. The reasons for increased expression ratios compared to normal tissues or wild-type cell lines are not well understood. We investigated the role of GSTP1 in the pathogenicity of pancreatic ductal adenocarcinoma (PDAC). We generated two GSTP1 knockdown lines in metabolically diverse PDAC cells. GSTP1 knockdown impaired the in vitro growth and proliferation of PDAC cells.

Additionally, GSTP1 knockdown cells exhibited elevated reactive oxygen species (ROS) levels and a prolonged G0/G1 phase of the cell cycle. Pharmacological inhibition of GSTP1 recapitulated the results obtained by the genetic inactivation of the same. Additionally, orthotopic implantation of GSTP1 knockdown cells in the pancreata of the athymic nude mice resulted in reduced tumor weight and volume compared to the control.

The growth trajectory of the tumors was monitored and the tumor volume was calculated every ten days using a Vevo-3100 ultrasound imaging system. Histological analysis of the tumors generated from GSTP1 knockdown PDAC cells revealed reduced expression of Ki67 while activated (cleaved) caspase3 was found to be elevated in these tumors compared to the control. GSTP1 knockdown PDAC cells exhibit a significant change in the expression of p53-responsive genes and oxidative stress-responsive genes involved in cell death and DNA repair pathways.

We report activation of JNK and upregulation of HMOX-1 and SOD1 in GSTP1 knockdown PDAC cells. We propose that overexpression of GSTP1 provides selective advantages to the PDAC cells by preventing the accumulation of ROS and suppressing the expression of apoptotic genes. Collectively, our data suggest that GSTP1 plays a salient role in the growth and proliferation of PDAC cells and is a novel therapeutic target for PDAC.

04-08. Combined Effect of Sleep Deprivation and Exogenous Melatonin on Zebrafish IL-8 and TNF-Alpha Expression Levels Post-injury

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SECTION 4: BASIC RESEARCH

Abstract: The immune system and the circadian rhythm are evolutionarily conserved and intertwined processes. Sleep loss has been linked to altered immune function, and melatonin has been shown to exhibit strong regulatory effects on the immune system. Previous studies have shown that the secretion of the pro-inflammatory cytokines IL-8 and TNF- α is increased in response to exogenous melatonin.

TNF- α has been shown to increase in humans in response to sleep deprivation. In this study, zebrafish embryos were used to examine the effects of sleep deprivation and melatonin on IL-8 and TNF- α expression following an injury-induced immune response. Samples were collected either 3 h or 6 h after injury for qPCR analysis of IL-8 and TNF- α expression levels. The 3 h timepoint showed a 1.7- and 4.9-fold increase in IL-8 and TNF- α expression, respectively, in larvae that were both sleep deprived and exposed to melatonin, as compared to larvae that weren't exposed to either of those conditions.

The 6 h timepoint showed no significant differences between any of the groups. Results found here support the findings of previous studies and provide insight into the interaction between sleep loss, melatonin, and their combined effects on the immune system.

04-09. Elucidating the Complimentary Action of Transcription Factors in Brain Area Patterning

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Abstract: The brain starts out as a collection of neuronal precursor that give rise to neurons with a with non-specific axonal connections. As the brain matures, some of those connections are removed as the neurons specialize. Brain area patterning describes the process by which a group of neurons specialize into a specific area. As for the sensory areas, each is optimized to receive and process specific sensory input.

Recent research has been done to determine some of the important transcription factors involved in regulating this process. Two in particular have been shown to have a significant effect on determining the size of the primary Visual area (V1) in mammals, Emx1 and Emx2. Deletion of one of those transcription factors in mice causes similar phenotypes, a shrinking and posterior shift of the V1.

However, we do not understand how they work well enough to propose a clear mechanism. Is their action complimentary or cooperative? This study attempts to answer this question by using different multiple-allele deletion combinations and a double-knockout of the Emx1 and Emx2 genes and comparing the changes in area size using in situ hybridization and immunohistochemistry

04-10. JNK/ERK Signaling Mediates Piperlongumine-Induced PDAC Cell Death

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SECTION 4: BASIC RESEARCH

Abstract: Piperlongumine (PL), a glutathione S-transferase pi 1 (GSTP1) inhibitor, reduces glutathione levels and elevates reactive oxygen species to induce cancer-selective cell death. However, the signaling mechanisms that lead to cancer cell death have not been clearly identified, especially for pancreatic ductal adenocarcinoma (PDAC).

Given that c-Jun NH2-terminal kinase (JNK) is activated upon oxidative stress and GSTP1 directly interacts with JNK, we hypothesized PL activates JNK signaling to enhance PDAC cell death. Furthermore, we have previously reported that PL inhibits PDAC cell proliferation in vitro and in vivo by enhancing ROS and DNA damage. Given that ROS-induced ERK activation leads to decreased cell proliferation, we hypothesized PL activates ERK signaling to suppress PDAC cell proliferation.

Our results show PL induces PDAC cell death in a concentration-dependent manner. PL (10⁻⁶ M) treatment of PDAC cells resulted in inhibition of glutathione activity, dissociation of JNK from GSTP1, robust JNK and ERK activation, and induction of cleaved caspase-3 and cleaved PARP (apoptosis markers). Downstream effectors of JNK, c-Jun and ATF-2, were also activated by PL treatment in PDAC cells.

The JNK inhibitor, SP600125, significantly blocked PL-mediated PDAC cell death and partially inhibited the phosphorylation of c-Jun and ATF-2. The ERK inhibitor, U0126, significantly blocked PL-induced cell proliferation. PL resulted in >20-fold induction of heme oxygenase-1 (HMOX-1) gene expression, which was partially blocked by pretreatment with the JNK inhibitor or ERK inhibitor. We propose JNK and ERK signaling are involved in PL-mediated oxidative stress-induced growth inhibition and cell death in PDAC cells.

04-11. Identification of EMX2 Targets Using Based Proteomics Label-Free Mass Spectrometry: Wild Type and Conditional EMX2 Knockout Mice Comparison

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Abstract: Transcription factors (TFs) are proteins that positively or negatively regulate gene expression. This enables genes to be expressed in the correct cells at the appropriate level in each cell of an organism. Studies have shown the significance of various TFs in the development of the neocortex. One key TF is a homeodomain protein, Emx2, which is involved in determining the size and position of the primary visual area (V1) along a concentration-dependent gradient.

Conditional deletion of Emx2 in mice has been associated with a posterior shift of anterior cortical areas and reduction in V1 size. Previous studies have investigated changes in transcription following Emx2 deletion. However most of these studies were done at late embryonic time points after Emx2 has been down-regulated and no longer plays a significant role in neocortical development. Very little is known about the impact Emx2 deletion will have on the protein expression.

In this study, we will be looking at changes in protein expression by comparing a conditional knock-out (cKO) mouse to a wild type control at embryonic day 13.5, when the production of neurons regulated by Emx2 is at its greatest. Protein abundance and

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04-12. Invariant BECN1 C-X-X-C MOTIFS are Essential for Starvation-Induced Autophagy

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Abstract: Autophagy is an essential catabolic cellular homeostasis process conserved in all eukaryotes. BECN1, a protein essential for autophagosome nucleation, contains two invariant C-x-x-C motifs (18CxxC21 and 137CxxC140 in human BECN1) that bookend a large BECN1 intrinsically disordered region (IDR). We use circular dichroism spectroscopy to elucidate differences in secondary structural content of the wild-type and CysTetrad mutant BECN1 IDR.

Additionally, we are using small angle X-ray scattering analyses to further understand the impact of the CysTetrad mutation on the structure of the BECN1 IDR. We also show that helicity of the BECN1 IDR increases upon addition of 2,2,2-trifluoroethanol (TFE), suggesting that it may undergo binding-associated helical transitions. Further, we use ICP-MS to demonstrate that BECN1 binds Zn²⁺ in a 1:1 molar ratio under both, reducing and non-reducing environments.

Lastly, we show that both C-x-x-C motifs are required, while the IDR is also important, for starvation-triggered up-regulation of autophagy.

Support or Funding Information

NIH grants RO3 NS090939 and R15 GM122035 (S.C.S.) National Science Foundation grant MCB-1413525 (S.C.S.)

04-13. Effects of Differential Estrogen Receptor Activation in a Murine Model of Asthma

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Rationale: Evidences suggest airway hyperresponsiveness (AHR) to be a characteristic feature of asthma. Sex differences play a crucial role in the etiology of asthma and epidemiological studies show incidence and severity of asthma is greater in women, suggesting a critical role of female sex steroid hormones (estrogen). Earlier studies reported the role of sex differences in airway remodeling, but estrogen receptor (ER) specific signaling during inflammation or asthma is still a question. Our recent studies indicate increased expression of ER β in asthmatic airway smooth muscle (ASM) and its activation altered airway remodeling. In this study, we have investigated the differential role of ER activation in modulating lung mechanics using male, female and ovariectomized (OVX) C57BL/6J mice.

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Methods: Animals were challenged with a mixed allergen (MA) followed by implantation of sustained release pellets of placebo, ER α -agonist (PPT) and/or ER β -agonist (WAY) and airway mechanics, histology, laser capture micro-dissection (LCM) and western blotting were performed.

Results: MA exposure showed abnormalities in lung function and have significantly increased airway resistance, elastance, tissue-damping and decreased compliance. Females showed significant increase in lung abnormalities than males and OVX mice. PPT treatment showed limited changes, whereas WAY treatment significantly reversed MA induced changes. ASM mRNA isolated using LCM showed increased fibronectin, collagen-1 and vimentin with MA exposure, which was not altered with PPT whereas significantly downregulated with WAY treatment.

Conclusion: This novel study indicates activation of ER β signaling downregulates AHR and airway remodeling and may stand out as a potential target to treat asthma.

O4-14. Downstream Purification Solutions for Viral Vectors: Enabling Platform Approaches to Advance Cell and Gene Therapies

ThermoFisher Scientific

Abstract: The areas of gene and cell therapy have been of growing interest due to their potential of correcting the genetic cause of the disease rather than chronically treating symptoms. Viral vectors have shown to be most successful in delivering therapeutic genetic material into target cells and the adeno associated virus (AAV) sub-classes have emerged as the vector of choice for many therapies.

The lack of large scale manufacturing platform technologies for viral vector purification is one of the major challenges the field faces. In order to increase productivity and meet market needs, efficient commercial manufacturing capabilities need to get established. By reducing the number of purification steps and maximizing productivity, affinity chromatography already offers a significant improvement to the downstream process of biomolecules.

This technology offers scalability and process consistency, thereby providing a platform solution to the industry. Herein we outline the benefits of implementing affinity chromatography as platform in the downstream purification of viral vectors, using the CaptureSelect™ technology as the basis of generating high-binding affinity ligands. The methods described will reveal the benefits of affinity chromatography related to specificity, capacity, process yields as well as process scalability for the purification of AAV viral vectors. Specifically, the properties of POROS™ CaptureSelect AAV8, AAV9, and AAVX affinity resins will be discussed as tools to capture and purify a broad range of AAV serotypes. The utilization of these affinity resins will be a significant improvement to the downstream processing, by reducing the purification steps and maximizing productivity, offering scalability and processing consistency.

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