ISSCR

American Society of Gene + Cell Therapy

IS MADISON

EMERGING THERAPIES

21-23 September 2022

at the Intersection of Genetic and Cellular Technologies

PROGRAM BOOK

Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR) and the American Society for Gene and Cell Therapy (ASGCT), welcome to the 2022 Madison International Symposium, "Emerging Therapies at the Intersection of Genetic and Cellular Technologies." This hybrid meeting is an extension of the digital webinar series our organizations delivered last year, where two leading stem cell and regenerative medicine societies have collaborated to deliver the latest findings in this rapidly advancing field.

The marriage of genomic and cellular advances holds tremendous potential for regenerative medicine. By leveraging and combining recent discoveries in stem cell biology and powerful new gene-editing technologies, researchers are developing potentially transformative new therapies.

Bookended by Keynote addresses from Donald Kohn and Leslie Thompson, this three-day scientific program is designed to highlight groundbreaking work driving new therapies for devastating diseases affecting a wide spectrum of tissue and organ systems ranging from the blood to the brain. Join us as we delve into the latest discoveries advancing potential novel therapeutics for Parkinson's and Huntington's Diseases, Epilepsy, Motor Neuron Disease, hemoglobinopathies, diabetes, heart disease, Muscular Dystrophy, Epidermolysis Bullosa, macular degeneration, and cancer. Expand your knowledge as we explore strategies to overcome complex challenges unique to manufacturing cell and gene therapies for the clinic.

We invite you to immerse yourself in the more intimate setting of this smaller, focused international symposium to build new connections and enrich ongoing collaborations that will help advance your research. Thank you for your ongoing support of the ISSCR and the ASGCT – we hope that you come away from this meeting inspired with new ideas and relationships that will drive advances as we strive together to reach the therapeutic potential of regenerative medicine that will improve human health.

Sincerely,

The Madison International Symposium Organizing Committee

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Shen Shen, PhD, *Vertex Pharmaceuticals, USA*

Assem Ziady, PhD, *Cincinnati Children's Hospital Medical Center, USA*

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health.

The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research – a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

Contact Us

The International Society for Stem Cell Research
5215 Old Orchard Road, Suite 270
Skokie, Illinois 60077, USA
+1-224-592-5700
www.isscr.org
@ISSCR

American Society of Gene + Cell Therapy

ABOUT ASGCT

The American Society of Gene & Cell Therapy is the primary professional membership organization for gene and cell therapy. The Society's members are scientists, physicians, patient advocates, and other professionals. Our members work in a wide range of settings including universities, hospitals, government agencies, foundations, and biotechnology and pharmaceutical companies.

The mission of ASGCT is to advance knowledge, awareness, and education leading to the discovery and clinical application of genetic and cellular therapies to alleviate human disease. ASGCT's strategic vision is to be a catalyst for bringing together scientists, physicians, patient advocates, and other stakeholders to transform the practice of medicine by incorporating the use of genetic and cellular therapies to control and cure human disease.

Contact Us

The American Society for Cell and Gene Therapy
20800 Swenson Drive, Suite 300
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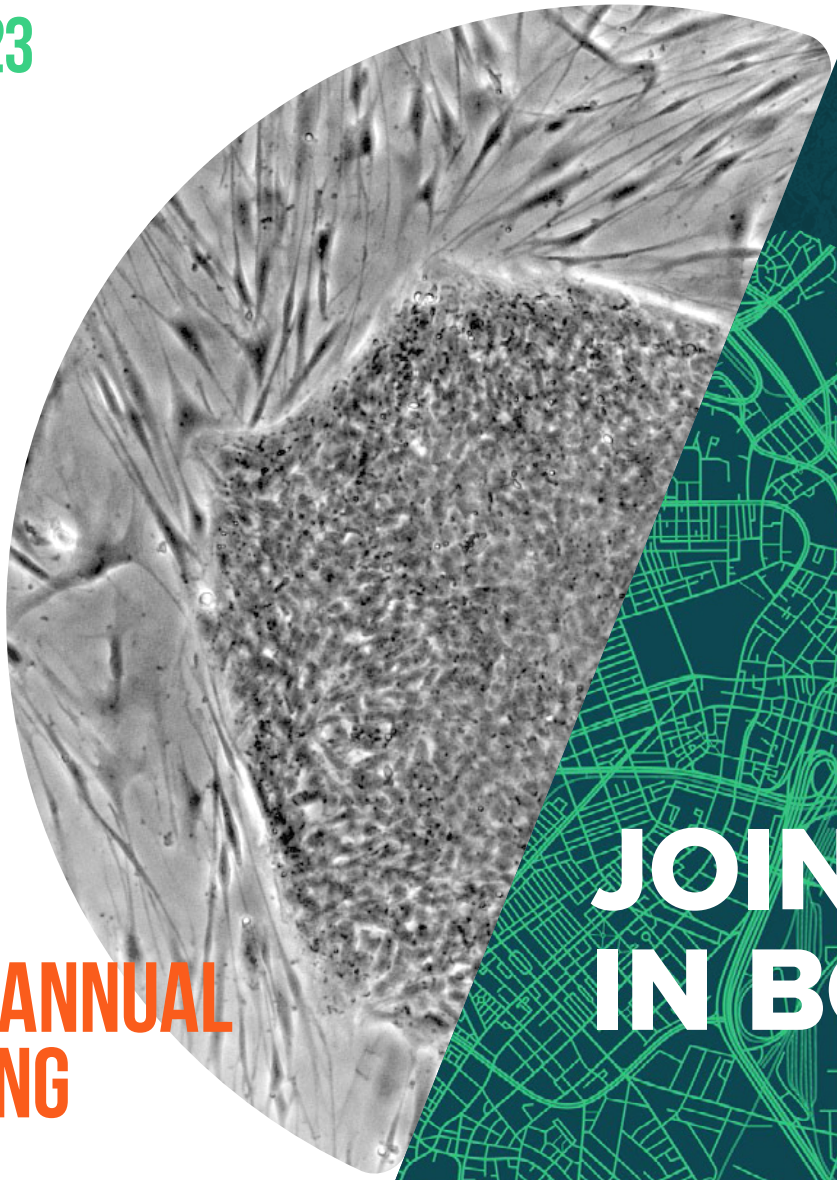

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ISSCR

Meeting Policies

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The ISSCR and ASGCT are committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination.

All participants are expected to treat others with respect and consideration, follow venue rules and alert staff or security, if onsite, of any dangerous situations or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics.

These policies comprise the Code of Conduct for ISSCR Meetings, which will be followed for this event, and apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the meeting and related events.

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ISSCR prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to ISSCR meetings staff at the Registration Desk or isscr@isscr.org.

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By registering for this meeting, you agree to the ISSCR's Recording Policy. It is strictly prohibited to record (photographic, screen capture, audio and/or video), copy, or download scientific results from the sessions, presentations and/or posters at the 2022 Madison International Symposium. Intent to communicate or disseminate results or discussion presented at the meeting in prohibited until the start of each individual presentation.

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Abstract content may not be announced, publicized, or distributed before the presentation date and time in any way including blogging and tweeting. ISSCR and ASGCT does permit promotion of general topics, speakers, or presentation times. This embargo policy applies to all formats of abstract publication.

ONSITE BADGE PICK UP

Pick-up your name badge in the registration area in the **Lakeside Commons Foyer, Level 1** area of the Monona Terrace Community and Convention Center during posted hours. Name badges are required for admission to all sessions, social events, and the Exhibit & Poster Hall. Badges may be picked up during the following times:

Wednesday, 21 September	7:30 AM – 6:00 PM
Thursday, 22 September	8:30 AM – 5:00 PM
Friday, 23 September	8:30 AM – 3:00 PM

COVID-19 VACCINATION VERIFICATION

Proof of vaccination is required to attend the in person program for the 2022 Madison International Symposium. **Attendee badges cannot be picked up until vaccination is verified.** Learn more about what is required for all attendees by visiting the Health & Safety page. Every attendee will be required to wear a mask, regardless of vaccination status, during all ISSCR events and gatherings both inside and outside the convention center.

VIRTUAL ASSISTANCE

Access the 2022 Madison International Symposium Virtual Platform and login with your ISSCR credentials. Click on the 'Ask ISSCR' chat icon located on the lower right side of the screen for any questions or email ISSCRdigital@isscr.org.

INTERNET ACCESS

The Monona Terrace Community and Convention Center offers free basic WiFi suitable for general email and internet browsing. To connect to the center's complimentary WiFi, select network name **Monona Guest**. Then, select **Free Service**. No password is needed.

RECORDINGS PROHIBITED

Still photography, screen capture, video and/or audio taping/recording of the sessions, presentations and/or posters at the 2022 Madison International Symposium is strictly prohibited. Intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation.

GENERAL INFORMATION

Nursing Mother's Room
The Monona Terrace Community and Convention Center has a designated private space available for nursing mothers. If you require access to the room, please visit the Information Desk near the main entrance to the center, located on Level 4. The desk attendant will direct nursing mothers to the room and provide the key to open the door and a privacy sign to place on the doorknob. Please note that there is no refrigerated storage available on the premises.

Smoking
Smoking is prohibited in the Monona Terrace Community and Convention Center.

Lost and Found
Please bring found items to the Registration area in the Lakeside Commons Foyer, Level 1 area of the Monona Terrace Community and Convention Center during posted hours. If you lost an item, stop by during registration hours for assistance.

Parking
Monona Terrace Community and Convention Center features a 600-space, automated parking structure available 24/7 and is accessible via West Wilson Street and the eastbound lanes of John Nolen Drive. Metered parking and additional short-term and all-day public parking is available throughout downtown Madison in various structures and lots. Prices and forms of payment will vary. Attendees are responsible for paying their own parking garage fees. Download a helpful parking map here.

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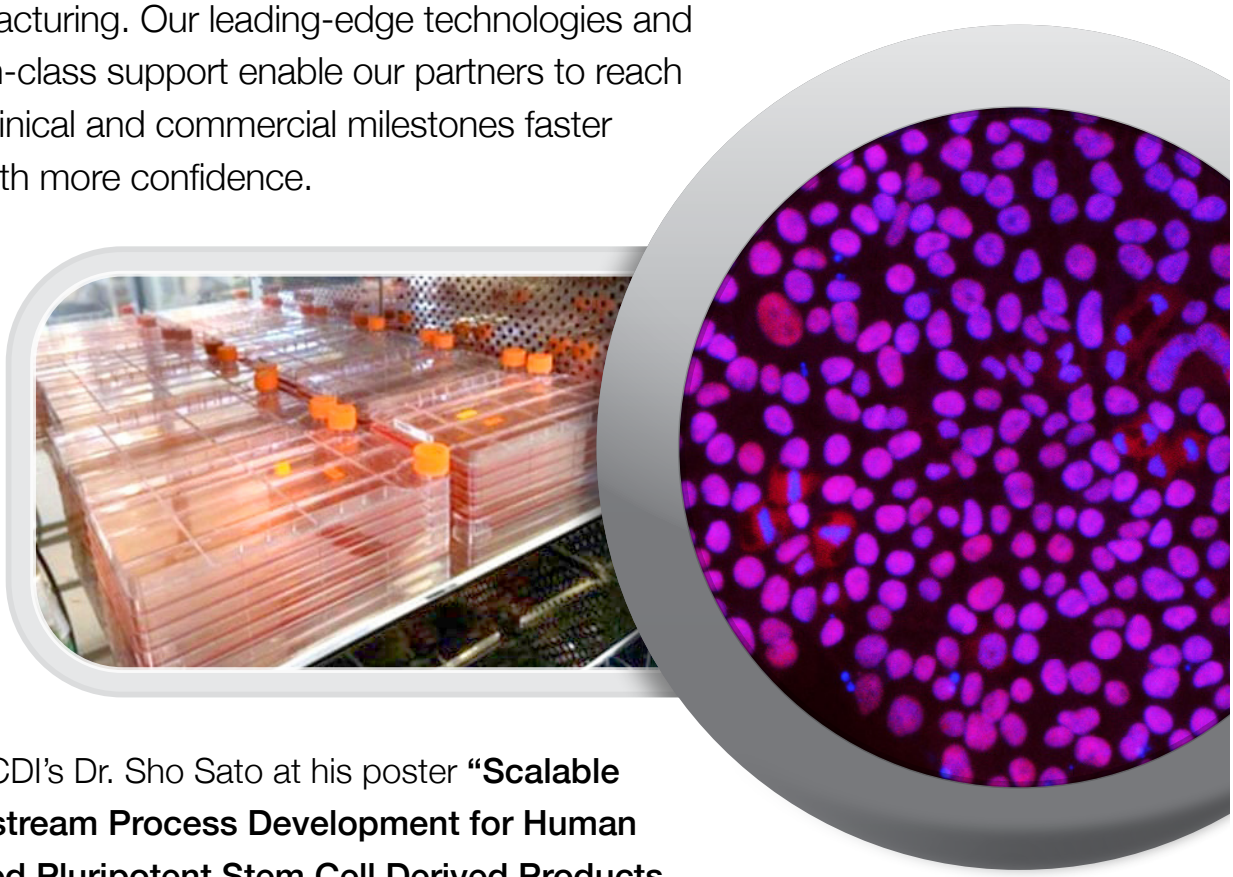

Join FCDI's Dr. Sho Sato at his poster **"Scalable Downstream Process Development for Human Induced Pluripotent Stem Cell Derived Products Manufacturing"** to learn how choices in culturing technology and operation scheme is impacted by volume reduction ratio, shear sensitivity, carryover limitations, and throughput requirements.

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Program Schedule

Wednesday, 21 September

2022 Madison International Symposium:
Emerging Therapies at the Intersection of Genetic and Cellular Technologies

Wednesday
21 September 2022

10:00 AM – 11:00 AM — OPENING PLENARY SESSION

Exhibit Hall A, Level 1

Chair: **Dan Marshak**, *Consulting & Advisory, USA*

10:00 AM – 10:05 AM

Welcome and Opening Remarks
Dan Marshak, *Consulting & Advisory, USA*

10:05 AM – 11:00 AM

Keynote Address
Donald Kohn, *University of California, Los Angeles, USA*
HEMATOPOIETIC STEM CELL GENE THERAPY FOR INHERITED BLOOD CELL DISEASES

11:00 AM – 11:45 AM — CENTRAL NERVOUS SYSTEM DISEASES I

Exhibit Hall A, Level 1

Chair: **Dan Marshak**, *Consulting & Advisory, USA*

11:00 AM – 11:30 AM

Catherine Priest, *Neurona Therapeutics, USA*
NRTX-1001: INHIBITORY NEURON CELL THERAPY FOR PHASE I/II CLINICAL INVESTIGATION IN PEOPLE WITH CHRONIC FOCAL EPILEPSY

11:30 AM – 11:45 AM

Yunfeng Ding, *University of Wisconsin-Madison, USA*
IPSC-DERIVED NEUROVASCULAR UNIT MODELING REVEALS THE ROLE OF APOE4 IN BLOOD-BRAIN BARRIER DYSFUNCTIONS

11:45 AM – 1:15 PM — LUNCH BREAK

Exhibit Hall B, Level 1

12:00 PM – 1:00 PM — INNOVATION SHOWCASES

Lecture Hall, Level 4

Presented by: **Synthego Corporation**

12:00 PM – 1:00 PM

HIGH THROUGHPUT CRISPR EDITING AND GENOMIC CHARACTERIZATION

**Lunches can be brought upstairs to enjoy during Innovation Showcases*

1:15 PM – 1:45 PM CENTRAL NERVOUS SYSTEM DISEASES II

Exhibit Hall A, Level 1

Chair: **Not available at time of printing**

1:15 PM – 1:45 PM

Malin Parmar, *Lund University, Sweden*
DEVELOPING A STEM CELL BASED THERAPY FOR PARKINSON'S DISEASE; THE INTERPLAY OF EXPERIMENTAL STUDIES AND TRANSLATIONAL EFFORTS

1:45 PM – 3:00 PM IN UTERO CELL AND GENE THERAPY

Exhibit Hall A, Level 1

Chair: **Not available at time of printing**

1:45 PM – 2:15 PM

Charlotte Sumner, *Johns Hopkins University, USA*
ADVANCING IN UTERO TREATMENT FOR THE GENETIC MOTOR NEURON DISEASE SMA
**Virtual Presentation*

2:15 PM – 2:30 PM

Caleb Dillingham, *University of Wisconsin-Madison, USA*
POSTTRANSCRIPTIONAL CONTROL OF PLURIPOTENCY BY HISTONE DEMETHYLASES

2:30 PM – 3:00 PM

Tippi Mackenzie, *University of California, San Francisco, Benioff Children's Hospital, USA*
PRENATAL THERAPIES FOR SINGLE GENE DISORDERS

3:00 PM – 3:30 PM REFRESHMENT BREAK

Exhibit Hall B, Level 1

Sponsored by: **Promega Corporation**

3:30 PM – 5:00 PM HEMATOPOIETIC DISEASES

Exhibit Hall A, Level 1

Chair: **John Tisdale**, *NIH/NHLBI, USA*

3:30 PM – 4:00 PM

Boro Dropulić, *Caring Cross, USA*
IMPROVING ACCESS OF CAR-T CELL AND OTHER GENE-MODIFIES CELLULAR THERAPIES BY MANUFACTURING THE FINAL PRODUCT AT THE PLACE-OF-CARE

4:00 PM – 4:15 PM

Saritha D'Souza, *University of Wisconsin-Madison, USA*
EVALUATING THE SAFETY AND IMMUNOGENICITY OF MHC HOMOZYGOUS IPSC-DERIVED CD34+ HEMATOPOIETIC PROGENITORS IN A NHP MODEL

4:15 PM – 4:30 PM

Kyle Cromer, *University of California, San Francisco, USA*
TAKING A CUE FROM CLINICAL GENETICS TO ENGINEER A COMPETITIVE ADVANTAGE INTO RED BLOOD CELLS

4:30 PM – 5:00 PM

Igor I. Slukvin, *University of Wisconsin-Madison, USA*
ADVANCING PLURIPOTENT STEM CELL TECHNOLOGIES FOR IMMUNOTHERAPIES

5:00 PM – 7:00 PM WELCOME RECEPTION AND POSTER SESSION I

Exhibit Hall B, Level 1

Sponsored by: **WiCell**

5:00 PM – 6:00 PM **ODD NUMBER POSTERS**

6:00 PM – 7:00 PM **EVEN NUMBER POSTERS**

Program Schedule

Thursday, 22 September

Thursday

22 September 2022

9:00 AM – 10:15 AM CELL & GENE THERAPY MANUFACTURING

Exhibit Hall A, Level 1

Sponsored by: **FUJIFILM Cellular Dynamics, Inc.**
Chair: **Gerard Bauer**, *University of California, Davis School of Medicine, USA*

9:00 AM – 9:30 AM
David Dismuke, *Forge Biologics, USA*
TITLE NOT AVAILABLE AT TIME OF PRINTING

9:30 AM – 9:45 AM
Namita Khajanchi, *University of Wisconsin-Madison, USA*
CHROMATIN MODULATION FOR EFFICIENT CRISPR-CAS9 GENE EDITING OF HUMAN PLURIPOTENT STEM CELLS

9:45 AM – 10:15 AM
Austin Thiel, *ElevateBio, USA*
TECHNICAL AND REGULATORY CONSIDERATIONS FOR THE MANUFACTURE OF GENE-EDITED iPSCs

10:15 AM – 10:45 AM REFRESHMENT BREAK

Exhibit Hall B, Level 1

10:45 AM – 11:45 AM CELLULAR TRANSPLANTS

Exhibit Hall A, Level 1

Chair: **Gerard Bauer**, *University of California, Davis School of Medicine, USA*

10:45 AM – 11:15 AM
Michele De Luca, *University of Modena and Reggio Emilia, Italy*
COMBINED CELL AND GENE THERAPY FOR EPIDERMOLYSIS BULLOSA
**Virtual Presentation*

11:15 AM – 11:45 AM
Sally Temple, *Neural Stem Cell Institute, USA*
ADULT RETINAL PIGMENT EPITHELIAL STEM CELL THERAPY FOR PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

11:45 AM – 1:15 PM LUNCH BREAK

Exhibit Hall B, Level 1

12:00 PM – 1:00 PM INNOVATION SHOWCASES

Madison Ballroom AD, Level 4

Presented by: **MaxWell Biosystems**

12:00 PM – 1:00 PM
NEXT-GENERATION IN-VITRO ASSAYS: CHARACTERIZING THE ACTIVITY OF HUMAN IPSC-DERIVED NEURONS IN 2D AND 3D CULTURES AT HIGH RESOLUTION
**Lunches may be brought upstairs to enjoy during Innovation Showcases*

12:00 PM – 1:00 PM SPECIAL SESSION

Madison Ballroom BC, Level 4

Developed in Partnership with: **the ISSCR & ASGCT Ethics Committees**

12:00 PM – 1:00 PM
ETHICS SESSION: IN SEARCH OF DEFENSIBLE LIMITS AT THE INTERSECTION OF GENETIC AND CELLULAR TECHNOLOGIES
**Lunches may be brought upstairs to enjoy during the Special Session*

1:15 PM – 2:30 PM IMMUNO-ONCOLOGY: CAR-T AND NK CELL THERAPIES

Exhibit Hall A, Level 1

Sponsored by: **STEMCELL Technologies**
Chair: **Not available at time of printing**

1:15 PM – 1:45 PM
Michel Sadelain, *Memorial Sloan Kettering Cancer Center, USA*
CAR T CELLS AND THE LIVING DRUG CONCEPT
**Virtual Presentation*

1:45 PM – 2:00 PM
Lauren Sarko, *University of Wisconsin-Madison, USA*
GENOME-EDITED, CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS TO TARGET NEURODEGENERATION AND SENESCENCE

2:00 PM – 2:30 PM
Bob Valamehr, *Fate Therapeutics, USA*
FT536: A FIRST-OF-KIND, OFF-THE-SHELF IPSC-DERIVED CAR-NK CELL PRODUCT CANDIDATE FOR SOLID TUMORS DESIGNED TO UNIQUELY TARGET MICA/B STRESS PROTEINS AND OVERCOME MECHANISMS OF EVASION

2:30 PM – 3:00 PM REFRESHMENT BREAK

Exhibit Hall B, Level 1

3:00 PM – 4:30 PM DIABETES & METABOLIC DISEASES

Exhibit Hall A, Level 1

Chair: **Austin Thiel**, *ElevateBio, USA*

3:00 PM – 3:15 PM
Bayley Waters, *University of Wisconsin-Madison, USA*
ISLET ARCHITECTURE IN ADULT MICE IS ACTIVELY MAINTAINED BY ROUNDABOUT RECEPTORS

3:15 PM – 3:30 PM
Devon Ehnes, *University of Washington, USA*
INVESTIGATING HYPER-STABLE COMPUTATIONALLY DESIGNED TIE2 PROTEINS TO MITIGATE DIABETIC VASCULOPATHY AND PROMOTE VASCULAR STABILITY

3:30 PM – 3:45 PM
Holger Russ, *University of Colorado, USA*
CD9 MARKS A HUMAN BETA CELL SUBPOPULATION WITH INCREASED IMMUNOGENICITY

3:45 PM – 4:00 PM
Sara Sackett, *University of Wisconsin-Madison, USA*
VALIDATING EXPRESSION OF BETA CELL MATURATION-ASSOCIATED GENES IN HUMAN PANCREAS DEVELOPMENT Y

4:00 PM – 4:30 PM
Doug Melton, *Harvard University and Vertex Pharmaceuticals, USA*
STEM CELL-DERIVED PANCREATIC ISLETS TO TREAT INSULIN-DEPENDENT DIABETES
**Virtual Presentation*

4:30 PM – 5:30 PM POSTER SESSION II AND NETWORKING

Exhibit Hall B, Level 1

Friday
23 September 2022

9:00 AM – 10:15 AM MUSCLE DISORDERS

Exhibit Hall A, Level 1

Chair: **Shen Shen**, *Vertex Pharmaceuticals, USA*

9:00 AM – 9:30 AM
Seng Cheng, *Pfizer, USA*
PROGRESS TOWARDS DEVELOPING GENE THERAPY FOR DUCHENNE MUSCULAR DYSTROPHY

9:30 AM – 9:45 AM
Amish Raval, *University of Wisconsin-Madison, USA*
HUMAN IPSC-DERIVED-COMMITTED CARDIAC PROGENITOR CELLS ENGRAFT AND IMPROVE CARDIAC CONTRACTILITY RESERVE IN A SWINE ISCHEMIC CARDIOMYOPATHY MODEL

9:45 AM – 10:15 AM
Christine Mummery, *Leiden University, The Netherlands*
HUMAN CARDIOVASCULAR DISEASE MODELS BASED ON HIPSC: FROM DISEASE MECHANISMS TO DRUG DISCOVERY

10:15 AM – 10:45 AM REFRESHMENT BREAK

Exhibit Hall B, Level 1

10:45 AM – 11:45 AM NEW TECHNOLOGIES AND FUTURE DIRECTIONS I

Exhibit Hall A, Level 1

Chair: **Assem Ziady**, *Cincinnati Children's Hospital Medical Center, USA*

10:45 AM – 11:15 AM
Shuibing Chen, *Weill Cornell Medical College, USA*
CHEMICAL APPROACHES TO GENERATE FUNCTIONAL HUMAN ISLETS FOR TRANSPLANTATION

11:15 AM – 11:30 AM
Andrew Khalil, *Harvard University, USA*
METABOLIC GLYCOENGINEERING AFFORDS HIGHLY EFFICIENT INTRACELLULAR DELIVERY OF CAS9 RIBONUCLEOPROTEIN IN HUMAN CELLS

11:30 AM – 11:45 AM
Xinzhi Zou, *Stanford University, USA*
A PROGRAMMABLE SYSTEM FOR REWIRING ABERRANT CANER SIGNALING TO THERAPEUTIC EFFECTOR RELEASE

11:45 AM – 1:15 PM LUNCH BREAK

Exhibit Hall B, Level 1

1:15 PM – 2:30 PM NEW TECHNOLOGIES AND FUTURE DIRECTIONS II

Exhibit Hall A, Level 1

Chair: **Shen Shen**, *Vertex Pharmaceuticals, USA*

1:15 PM – 1:45 PM
Rubina Parmar, *Intellia Therapeutics, USA*
ADVANCES IN THE THERAPEUTIC APPLICATION OF CRISPR/CAS9 FOR GENOME EDITING

1:45 PM – 2:00 PM
Ruosen Xie, *University of Wisconsin-Madison, USA*
PH-RESPONSIVE POLYMER NANOPARTICLES FOR EFFICIENT DELIVERY OF CAS9 RIBONUCLEOPROTEIN WITH OR WITHOUT DONOR DNA

2:00 PM – 2:30 PM
Viviana Gradinaru, *California Institute for Technology, USA*
GETTING ACROSS BARRIERS: GENE DELIVERY ACROSS THE BLOOD-BRAIN BARRIER FOR PRECISE MINIMALLY-INVASIVE STUDY AND REPAIR OF NERVOUS SYSTEM
**Virtual Presentation*

2:30 PM – 3:30 PM CLOSING KEYNOTE

Exhibit Hall A, Level 1

Chair: **Shen Shen**, *Vertex Pharmaceuticals, USA*

2:30 PM – 3:25 PM
CLOSING KEYNOTE
Leslie Thompson, *University of California Irvine, USA*
HUMAN NEURAL STEM CELL TRANSPLANTATION FOR HUNTINGTON'S DISEASE

3:25 PM – 3:30 PM
CLOSING REMARKS
Shen Shen, *Vertex Pharmaceuticals, USA*


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Table 16
Interactive Virtual Exhibit Available

FUJIFILM Cellular Dynamics, Inc. (FCDI), is a leading developer and manufacturer of human induced pluripotent stem cells (iPSCs) that are utilized to revolutionize drug discovery and cell therapies. Leveraging its expertise in iPSC technologies, FCDI is committed to enhance the beauty of modern cell biology, drive innovations in discovery, and advance medicine. Additionally, FCDI utilizes its iPSC platform to advance the progress of therapeutic candidates in the clinic and provides contract development and manufacturing (CDMO) services. FCDI's life science highly pure iCell® Products are available in industrial quantities required for drug and cell therapy development. For more information, visit: FujifilmCDI.com

MAXWELL BIOSYSTEMS

Albisriederstrasse 253
8047 Zurich, Switzerland
+41 44 244 24 24
www.mxwbio.com

Table 10
Interactive Virtual Exhibit Available

MaxWell Biosystems is a technology leader engineering advanced CMOS (complementary metaloxide-semiconductor)-based high-density microelectrode arrays (HD-MEAs) which are at the core of our easy-to-use systems. The technology allows integrating circuitry with thousands of electrodes per square millimeter on the same chip at high spatial resolution. MaxOne (single-well) and MaxTwo (multi-well), equip scientists to record electrical signals of neurons in in-vitro 2D and 3D models. MaxWell Biosystems' HD-MEA technology allows the capture of neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' HD-MEA platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

STEMCELL TECHNOLOGIES INC

1618 Station St
Vancouver, BC V6A1B6 Canada
+1 604-675-7575
www.stemcell.com

Table 7
Interactive Virtual Exhibit Available

At STEMCELL, science is our foundation. Driven by our mission to advance research globally, we offer over 2,500 tools and services supporting discoveries in stem cell research, regenerative medicine, immunotherapy and disease research. By providing access to innovative techniques like gene editing and organoid cultures, we're helping scientists accelerate the pace of discovery. Inspired by knowledge, innovation and quality, we are Scientists Helping Scientists.

Sponsor and Exhibitor Directory

SILVER SPONSORS

PROMEGA CORPORATION

2800 Woods Hollow Rd
Fitchburg, WI 53711 USA
techserv@promega.com
www.promega.com

Table 17
Interactive Virtual Exhibit Available

With a portfolio of >4,000 products covering the fields of genomics, protein analysis and expression, cellular analysis, drug discovery and genetic identity, Promega provides a variety of solutions to meet the fast-growing demands in cell biology and cell and gene therapy development. Our solutions include reporter bioassays, cell health assays, simple and fast Lumit™ immunoassays, advanced bioanalytical tools for mass spec analysis, automated and manual systems and reagents for DNA/RNA extraction from any sample type, cell line authentication support, and polymerases and master mixes for PCR and qPCR testing and much more.

SYNTHEGO CORPORATION

3696 Haven Avenue, Suite A
Redwood City, CA 94063 USA
www.synthego.com

Synthego is a genome engineering company that enables the acceleration of life science research and development in the pursuit of improved human health. The company leverages machine learning, automation, and gene editing to build platforms for science at scale. With its foundations in engineering disciplines, the company's full-stack platform vertically integrates proprietary hardware, software, bioinformatics, chemistries, and molecular biology to advance both basic research and therapeutic development programs. By providing both commercial and academic researchers and therapeutic developers with unprecedented access to cutting-edge genome engineering products and services, Synthego is at the forefront of innovation in engineered biology.

BRONZE SPONSORS

GENEZEN

9900 Westpoint Dr, Suite 128
Indianapolis, IN 46256 USA
info@genezen.com
www.genezen.com

Table 3
Genezen is a cell and gene therapy Contract Development and Manufacturing Organization (CDMO), focused on supporting the demands of the current and future gene and cell therapy manufacturing market worldwide—making viral vector production accessible to both early-stage, growth-oriented companies and established industry leaders. Genezen offers early-phase process development, GMP vector production, and analytical testing services, building on the company's expansive knowledge and experience in the industry and working with the nation's leading institutions.

MAXCYTE

22 Firstfield Rd, Ste 110
Gaithersburg, MD 20878-1795 USA
www.maxcyte.com

Table 13
Interactive Virtual Exhibit Available

MaxCyte® is a leading provider of cell-engineering platform technologies and helps bring next-generation cell-based therapies to life. Our Flow Electroporation® technology and next-generation ExPERT™ platform enables 20 of the top 25 biopharmaceutical companies to accelerate, streamline, and improve the drug development process from early research stages to commercialization.

Sponsor and Exhibitor Directory

BRONZE SPONSORS

MORGRIDGE INSTITUTE FOR RESEARCH

330 N Orchard St
Madison, WI 53715 USA
news@morgridge.org
www.morgridge.org

As an independent research organization, the Morgridge Institute for Research explores uncharted scientific territory to discover tomorrow's cures. In affiliation with the University of Wisconsin-Madison, we support researchers who take a fearless approach to advancing human health in emerging fields such as regenerative biology, metabolism, virology and biomedical imaging. Through public programming, we work to inspire scientific curiosity in everyday life. Learn more at: morgridge.org.

UNIVERSITY OF WISCONSIN-MADISON STEM CELL & REGENERATIVE MEDICINE CENTER

8457 WIMR II
1111 Highland Avenue
Madison, WI 53705 USA
stemcells.wisc.edu

Table 8
The UW–Madison Stem Cell and Regenerative Medicine Center (SCRMC) provides a central point of contact, information, and facilitation for all stem cell research activities on campus. The center's mission is to advance the science of stem cell biology and foster breakthroughs in regenerative medicine through faculty interactions, research support, and education.

EXHIBITORS

AXION BIOSYSTEMS, INC

1819 Peachtree Road NE, Suite 350
Atlanta, GA 30309 USA
info@axionbio.com
www.axionbio.com

Table 5
Axion BioSystems is a leading life sciences tools company focused on innovative live-cell assays used to study the function of cells in vitro for drug discovery, disease modeling, cancer immunotherapy, safety/toxicity, and more. The Maestro platform is the world's most advanced microelectrode array (MEA) and impedance system, allowing non-invasive evaluation of your cells in an easy-to-use assay. Whether monitoring the intricate, electrical activity of excitable cells (e.g. neurons and cardiomyocytes), or tracking the growth and death of cancer cells, Maestro allows you to investigate the functionality of your cells label-free in a multiwell plate. Axion's CytoSMART line of products are the next generation in kinetic live-cell imaging, combining compact and fast imaging hardware with powerful image analysis algorithms. Generate high-quality, robust data with the latest in automated time-lapse imaging.

BRAINXELL, INC

455 Science Drive, Suite 210
Madison, WI 53711 USA
www.brainxell.com

Table 11
BrainXell provides high-purity, iPSC-derived human neurons and glia for research and development with a focus on drug discovery. Utilizing proprietary technology, we generate high-purity, subtype-specific neurons that mature rapidly and are quickly and easily ready for a variety of assays. Multiple neuron subtypes relevant to a range of disorders are available. Additionally, each neuron subtype can be made in custom batches from 50 million to 10 billion neurons from unique iPSC or ESC lines. We are dedicated to delivering the highest quality products for off-the-shelf neurons and custom service projects.

EXHIBITORS

CAI

One Park Fletcher
2601 Fortune Circle East, Suite 301B
Indianapolis, IN 46241 USA
+1 317-271-6082
cai.admin@cagents.com
cagents.com

Table 21

CAI is passionate about helping companies get their product to market faster. Any misstep on the path to commercialization drains capital, impacts valuation, and delays time to revenue. Most importantly, delays can impact patient access to novel, life-saving therapies. Our industry experts work with you to characterize your product and process, and to apply the principles of quality by design, quality risk management and industry best practices to help you reach each milestone on the path to commercialization in the most efficient manner. Our integrated project delivery model enables you to accelerate process validation, tech transfer, manufacturing scale-up, vendor selection, quality system implementation and regulatory affairs from early clinical trials through scale-up to commercial manufacturing. CAI has over 25 years of experience and is an employee-owned company. We have a reputation for exceeding customer expectations. CAI is the partner you need to meet a higher standard.

CULTURETRAX (BY ACUMIUM LLC)

717 John Nolen Drive
Madison, WI 53713 USA
+1 608-310-9700
hello@culturetrax.com
www.culturetrax.com

Table 6

CultureTrax is a modern productivity tool designed specifically for cell culture scientists. It combines the structure and tracking capability of sophisticated Laboratory Information Management Systems with the ease of an Electronic Lab Notebook. Workflow data is captured semantically in searchable fields, offering unlimited opportunities for use. It offers significant data management power and lets you interrogate and share your valuable data in new and creative ways.

CultureTrax is an example of how Acumium is supplying valuable technology solutions to the Biotechnology industry by zeroing in on the biggest problems and finding areas where technology can add value.

DAI SCIENTIFIC EQUIPMENT INC.

25677 N Hillview Ct
Mundelein, IL 60060-9436 USA
+1 847-719-6222
customerservice@daiscientific.com
www.daiscientific.com

Table 4

Founded in 1967, D.A.I. Scientific Equipment has a rich history of working with market-leading manufacturers to provide products and services to analytical laboratories in the pharmaceutical, educational, biotechnology, and clinical industries. We offer a wide range of products to support a number of applications.

What sets us apart from other equipment suppliers is our commitment to understanding and supporting our products. Our highly qualified field staff is educated by our principal manufacturers in equipment applications and their proper use, serving as consulting specialists to an array of research professionals, architects, and contractors.

GETINGE

1 Geoffrey Way
Wayne, NJ 07470 USA
www.getinge.com

Table 15

With a firm belief that every person and community should have access to the best possible care, Getinge provides hospitals and life science institutions with products and solutions that aim to improve clinical results and optimize workflows. The offering includes products and solutions for intensive care, cardiovascular procedures, operating rooms, sterile reprocessing, life science, and Applikon bioreactor systems. Getinge employs over 10,000 people worldwide and the products are sold in more than 135 countries.

EXHIBITORS

HEIDOLPH NORTH AMERICA

1235 N Mittel Blvd Suite B
Wood Dale, IL 60191 USA
+1 224-265-9600
sales@heidolph.com
www.heidolphna.com

Table 2
Heidolph North America is a German laboratory manufacturer that sells and services premium solutions and installation services for cell culture cultivation, chemical synthesis, process development, work-up and evaporation chemistry applications. Our product portfolio includes best-in-class, high quality laboratory and research capital equipment designed to focus on cost reduction, safety, and ease of use operation. All Heidolph manufactured offerings include an industry leading 3-year full-service warranty and a 10 year lifespan.

Heidolph North America Portfolio Offerings include: 3D Cell Culturing Plates, Smart Incubators, Platform Shakers, Autoclaves/Sterilizers, High Quality Peristaltic Pumps for Biology or Chemistry Applications, Rotary Evaporators, Chillers, Vacuum Pumps, Magnetic Stirring Hotplates, Overhead Stirrers, Chemical Reactors, Control Software, Glove Boxes for Air Sensitive Applications.

JANGOCELL

2997 Yarmouth Greenway Dr
Fitchburg, WI 53711 USA
+1 833-465-2646
info@jangocell.com
www.jangocell.com

Table 14
JangoCell, LLC is a laboratory products and cell biology services provider specializing in stem cell technologies. We carry a wide selection of ADSCs, MSCs, PBMCs and T Cells based on species, age, sex, and tissue, as well as classic cell culture medias and consumables to support your ongoing research. JangoCell also offers a wide range of custom cell biology services to help propel your research forward, faster. We have an expert team of scientists with decades of experience eager to help and committed to providing technical expertise, dependable service, and high-quality results.

MILLIPORESIGMA

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To place an order or receive technical assistance in the U.S. and Canada, call toll-free +1 800-645-5476
For Customer Service, call +1 800-325-3010
For Technical Service, please visit:
SigmaAldrich.com/techservice

Table 20
Together, we impact life and health with science. We offer one of the broadest portfolios in the industry for scientists, best-in-class products for pharmaceutical development and manufacturing, and a fully integrated service organization to support CDMO and contract testing across traditional and novel modalities. Our vision is a world where our innovative products, services, and digital offerings help create solutions for people globally and a sustainable future for generations to come.

MISSION BIO

400 E Jamie Ct., Ste 100
South San Francisco, CA 94080 USA
+1 415-854-0058
marketing@missionbio.com
missionbio.com/applications/cell-and-gene-therapy/

Table 12
Mission Bio is a life sciences company that accelerates discoveries and cures for a wide range of diseases by equipping researchers with the tools they need to better measure, characterize, and predict our resistance and response to new therapies. Mission Bio's multi-omics approach improves time-to-market for new therapeutics, including innovative cell and gene therapies that provide new pathways to health. The company's Tapestri Platform is being utilized by customers at leading research centers, pharmaceutical, and diagnostics companies worldwide. A Tapestri assay recently entered GMP qualification and validation, indicating that single-cell analysis is on its way to routine use in clinical trials to support the next wave of life-saving CGT treatments.

EXHIBITORS

NAMOCELL

2485 Old Middlefield Way, Suite 30
Mountain View, CA 94043 USA
www.namocell.com

Table 19

Namocell is a leading provider of high-performance single cell sorting and dispensing systems to empower single cell research, therapeutics development and diagnostics. Namocell's Single Cell Dispensers are the fastest and easiest solution to identify and isolate single cells, nuclei, protoplasts, bacteria, yeast and fungi, and enable users to accomplish single cell sorting and dispensing in one step while being gentle to the cells to preserve cell viability and integrity. We serve researchers and scientists in a wide range of applications, including cell line development and engineering, CRISPR and iPSC cloning, single cell genomics, cell and gene therapy, antibody discovery, rare cell isolation, single cell proteomics, and synthetic biology. Learn more at www.namocell.com, and follow us on LinkedIn: https://www.linkedin.com/company/namocell/.

THERMO FISHER SCIENTIFIC

5225 Verona Rd
Madison, WI, 53711 USA
www.thermofisher.com

Table 18

Thermo Fisher Scientific supplies innovative solutions for the world's stem cell research. With applications that span basic research and commercial scale-up to disease modeling and downstream clinical research – we provide a broad range of products and services including high quality media, non-integrating reprogramming technologies, reagents and instruments for characterization and analysis, and cutting edge plastics.

WAISMAN BIOMANUFACTURING

1500 Highland Ave
Madison, WI 53705 USA
+1 608-262-9547
waisbio@gmail.com
gmpbio.org

Table 1

The primary mission of Waisman Biomanufacturing is to deliver efficient translation of scientific discoveries for early-stage clinical trials by developing manufacturing processes and quality control methods in conjunction with providing overall product development and regulatory support. Our goal is to accelerate the advancement of novel biological therapies and vaccines by offering the manufacturing and support services required for cGMP production of drugs used in human clinical trials. Waisman Biomanufacturing has a proven track record for producing high-quality clinical trial material for both large and small biotech companies, universities, and federal agencies such as the National Institutes of Health (NIH).

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BIOSYSTEMS

MaxTwo

Multi-Well High Density
Microelectrode Array
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MaxOne

Single-Well High Density
Microelectrode Array
(HD-MEA System)

MaxWell Biosystems is a technology leader providing instrumentation and solutions to boost scientific research and development in neurosciences, stem cell and tissue engineering, ophthalmology, and other fields involving electrogenic cells. The company engineered advanced **high-density microelectrode arrays (HD-MEAs)** as the core of easy-to-use platforms, **MaxOne (Single-Well)** and **MaxTwo (Multi-Well)**, that equip scientists to record electrical signals of neurons in in-vitro **2D and 3D models**. MaxWell Biosystems' HD-MEA technology allows to capture neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

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Speaker Abstracts

WEDNESDAY, 21 SEPTEMBER

10:00 AM – 11:00 AM
Opening Plenary Session

10:05 AM – 11:00 AM
KEYNOTE ADDRESS

HEMATOPOIETIC STEM CELL GENE THERAPY FOR INHERITED BLOOD CELL DISEASES

Kohn, Donald B.

Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA, USA

Treatment of inherited blood cell diseases using autologous transplantation of gene-modified stem cells (gene therapy) has been advancing over the past 3 decades. Adenosine deaminase Severe Combined Immune Deficiency (ADA SCID) was the first disorder approached by gene therapy. In studies done in collaboration with investigators at University College London/Great Ormond Street Hospital (UCL/GOSH), we treated 50 ADA SCID patients with the EFS-ADA lentiviral vector (LV) and busulfan reduced intensity conditioning (RIC)h busulfan. 48/50 (96%) achieved sustained engraftment of ADA gene-corrected stem cells with immune reconstitution. Similar high frequencies of immune reconstitution have been achieved in a trial using a LV and RIC for X-linked SCID (XSCID) in a trial performed at UCL/GOSH, Boston Children's Hospital and UCLA with all 11 patients achieving sustained immune reconstitution. We have also performed clinical trials of gene therapy for two disorders of neutrophil dysfunction, X-linked Chronic Granulomatous Disease (XCGD) and Leukocyte Adhesion Deficiency I (LAD I). Both trials used the same LV backbone with a chimeric myeloid enhancer/promoter driving expression of the relevant cDNA (CYBB and ITGB2, respectively) and cytoablative busulfan conditioning (target AUC 65-75 mg/L*hr.). While the 5 adult patients with XCGD achieved sustained engraftment of gene-corrected HSC with >10% oxidase (DHR)+ neutrophils and absence of subsequent opportunistic infections, all 4 pediatric patients suffered significant decline in gene-marked cells in peripheral blood cells and BM CD34, 3-6 months after gene therapy, stabilizing at ~0.5% DHR+ neutrophils. The basis for the decline in gene-marked cells in the pediatric patients is unknown. In contrast, all 9 of the LAD I patients, treated between 0.5-9 years of age, have shown stable persistence of gene marked blood cells and ~20-70% CD18-expressing neutrophils. Use of adenine base editing in HSC to correct a founder mutation in the CD3D gene that causes SCID in a Mennonite population is highly efficient and restores the ability of the corrected HSPC to produce mature T lymphocytes in vitro in an Artificial Thymic Organoid system. These studies demonstrate the potential to apply HSC gene therapy for the treatment of blood cell diseases.

Keywords: Gene Therapy, Hematopoietic Stem Cells, Primary Immune Deficiencies

11:00 AM – 11:45 AM
Central Nervous System Diseases I

11:00 AM – 11:30 AM

NRTX-1001: INHIBITORY NEURON CELL THERAPY FOR PHASE I/II CLINICAL INVESTIGATION IN PEOPLE WITH CHRONIC FOCAL EPILEPSY

Priest, Catherine[1], Banik, Gautam[2], Blum, David[3], Bulfone, Alessandro[4], Parekh, Mansi[1], Hampel, Philip[1], Kim, Hannah[1], Sevilla, Eric Steven[1], Adler, Andrew F.[1], Feld, Brianna[1], Fuentealba, Luis[1], Watson, Michael[2], Salter, Naomi[2], Lee, Seonok[2], Kriks, Sonja[5], Havlicek, Steven[5], Maury, Yves[5], Bershteyn, Marina[5], Cuzon Carlson, Verginia[6], Burchiel, Kim J.[7], Nicholas, Cory R.[8]

[1]Preclinical Development, Neurona Therapeutics Inc, South San Francisco, CA, USA, [2]Process Development and Manufacturing, Neurona Therapeutics, Inc, South San Francisco, CA, USA, [3]Clinical Development, Neurona Therapeutics, Inc, South San Francisco, CA, USA, [4]Operations, Neurona Therapeutics, Inc, South San Francisco, CA, USA, [5]Discovery Biology, Neurona Therapeutics, Inc, South San Francisco, CA, USA, [6]ONPRC, Oregon Health and Science University, Portland, OR, USA, [7]Department of Neurological Surgery, Oregon Health and Science University, Portland, OR, USA, [8]CEO, Neurona Therapeutics, Inc, South San Francisco, CA, USA

Drug-resistant seizures represent a significant unmet medical need for more than one-third of people diagnosed with epilepsy. Surgical resection or ablation of the seizure focus may be an option for some patients with focal-onset epilepsies; however, these procedures are destructive to surrounding tissues and are not indicated for all individuals. A cellular therapeutic delivering GABA to focal regions of seizure onset could restore balanced neural activity and suppress chronic seizures without destruction of tissue. NRTX-1001 is a human inhibitory neuron cell therapy

candidate that may provide such an option. NRTX-1001 is derived from clinical grade human pluripotent stem cells and comprises GABAergic, post-mitotic, migratory interneurons. The NRTX-1001 product is characterized and tested for safety, identity, strength, purity and potency, and cGMP clinical product lots have been manufactured. The translational development of NRTX-1001 in support of an investigational new drug application will be presented, including functional assessments in a rodent model of chronic, drug-resistant focal hippocampal seizures; extensive safety testing to understand proliferative capacity, biodistribution, toxicology and tumorigenicity of the cells after transplantation; and demonstration of MRI-guided delivery into the hippocampus of non-human primates. A phase I/II clinical trial (NCT05135091) to evaluate NRTX-1001 in people with drug-resistant temporal lobe epilepsy was cleared by the FDA and has begun enrollment. The clinical trial design includes an open-label dose escalation, followed by a randomized, controlled evaluation of NRTX-1001 safety and efficacy in people with chronic temporal lobe epilepsy.

Funding Source
Funded in part by the California Institute for Regenerative Medicine (DISC2-10525; TRAN1-11611; CLIN2-13355)
Keywords: cellular therapeutic, GABAergic interneuron, clinical trial

11:30 AM – 11:45 AM

IPSC-DERIVED NEUROVASCULAR UNIT MODELING REVEALS THE ROLE OF APOE4 IN BLOOD-BRAIN BARRIER DYSFUNCTIONS

Ding, Yunfeng, Palecek, Sean P., Shusta, Eric V.

Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA

APOE4 is the strongest genetic risk factor for late-onset Alzheimer's Disease (AD), and is known to affect multiple neuronal and glial cell types in the central nervous system (CNS). Recent clinical research has suggested that increased leakage at the blood-brain barrier (BBB) is commonly observed among AD patients. However, it remains unclear whether and how APOE4 could contribute to BBB dysfunction. To explore this, we differentiated isogenic induced pluripotent stem cell (iPSC) lines with different APOE genotypes into brain microvascular endothelial cell-like cells (BMECs) and brain pericyte-like cells (PCs). BMECs and PCs are the two major component cell types of the human neurovascular unit. We found that while isogenic BMECs with different APOE genotypes had similar trans-endothelial electrical resistance, tight junction integrity and efflux transporter activity, the presence of APOE4 significantly hindered the amyloid clearance capabilities of BMECs in a Transwell model, suggesting that APOE4 could contribute to reduced amyloid clearance at the BBB. Subsequently, isogenic PCs with different APOE isoforms were compared for their APOE secretion levels and amyloid uptake capabilities. We found that when compared to APOE3 and APOE2 PCs, APOE4 PCs demonstrated similar levels of APOE secretion, but had a significantly higher level of amyloid deposition, an observation in line with vascular pathologies in AD patients. Our results reveal that although APOE4 did not directly affect BMEC barrier properties in the iPSC-derived neurovascular unit model, it plays an important role in amyloid clearance and deposition at the BBB. Furthermore, our findings highlight that iPSC-derived neurovascular models can capture amyloid pathologies of AD at the BBB, enabling such in vitro models in future disease modeling and drug screening research.
oral lobe epilepsy.

Funding Source
NIH 5R01NS109486
Keywords: Alzheimer's Disease, Blood-Brain Barrier, Disease Modeling

12:00 PM - 1:00 PM
Sponsored Innovation Showcase

Lecture Hall, Level 4

12:00 PM –1:00 PM

HIGH THROUGHPUT CRISPR EDITING AND GENOMIC CHARACTERIZATION

Presented by Synthego Corporation

Nugent, Rebecca[1], Saha, Krishanu[2], Zheng, Tueting[1]

[1]*Synthego Corporation, CA, USA,* [2]*University of Wisconsin, WI, USA*

The power of CRISPR gene editing is reflected in its rapid development from a research tool to a therapeutic. A key problem for researchers is balancing throughput and speed for developing editing approaches with downstream clinical compatibility and ensuring cell safety profiles. In this presentation we highlight Synthego's synthetic guide RNA platform HALO and the linkage to our engineered cells platform ECLIPSE. We end by detailing analytical approaches to assessing genomics characterization and safety profiles.

1:15 PM – 1:45 PM
Central Nervous System Diseases II

1:15 PM – 1:45 PM
DEVELOPING A STEM CELL BASED THERAPY FOR PARKINSON'S DISEASE; THE INTERPLAY OF EXPERIMENTAL STUDIES AND TRANSLATIONAL EFFORTS

Parmer, Malin

New York Stem Cell Foundation, NY, USA and Lund University, Sweden

Cell based transplantation aimed at the replacement of lost dopamine (DA) neurons holds great potential for the treatment of Parkinson's disease (PD). Considerable progress has been made in generating fully functional and transplantable dopamine (DA) neurons from human pluripotent stem cells (hPSCs) and the field is rapidly developing. We have developed robust and efficient differentiation protocols resulting in the formation of authentic and functional DA neurons from human embryonic stem cells (hESCs). Here, I will summarize the translational trajectory and describe how experimental studies feed in along the way of bringing hESC-derived dopamine cell product to clinical trial for PD. I will also assess the potential trajectory of this line of translational and clinical research and address its current limitations and future possibilities.

Keywords: Embryonic stem cells, dopamine neurons, Parkinson's Disease

1:45 PM – 3:00 PM
In Utero Cell and Gene Therapy

1:45 PM – 2:15 PM
ADVANCING IN UTERO TREATMENT FOR THE GENETIC MOTOR NEURON DISEASE SMA

Sumner, Charlotte

Neurology and Neuroscience, Johns Hopkins University, Baltimore, MD, USA

The autosomal recessive motor neuron disease spinal muscular atrophy (SMA) has traditionally been the leading inherited cause of infant and early childhood mortality. Over the last 6 years, three new gene targeting therapeutics have been approved for patients with SMA. Markedly improved therapeutic efficacy with early drug administration has led to population wide neonatal screening for SMA in the U.S. However, for those infants with the most common and severe forms of SMA, this is often insufficient to ensure optimal outcomes. We have demonstrated in human SMA tissues and SMA model mice that impairments of motor neuron development begin in utero and are followed by precipitous postnatal degeneration. In order to advance in utero treatment of SMA patients, we have been exploring fetal treatment of SMA mice either by treating pregnant dams with a permeant small molecule SMN2 splice modifier or by using a SMN2 targeted antisense oligonucleotide injected into the amniotic fluid. Both approaches can improve motor neuron development and maintenance compared to postnatal treatment alone in severe SMA mice.

Funding Source
NINDS R35 NS122306
Keywords: In utero therapeutics, Motor neuron disease, Genetic therapy

2:15 PM – 2:30 PM
POSTTRANSCRIPTIONAL CONTROL OF PLURIPOTENCY BY HISTONE DEMETHYLASES

Dillingham, Caleb M., Cormaty, Harshini, Morgan, Ellen, Thurston, Hailey, Sridharan, Rupa

Cellular and Regenerative Biology/Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA

Pluripotent stem cells such as embryonic stem cells (ESCs) can self-renew indefinitely and differentiate into any cell type given the right stimulus. The plasticity of ESCs is linked to a unique chromatin structure compared to somatic cells, which are less compacted and depleted for histone modifications associated with gene repression, such as H3K9 methylation. We have found that the H3K9me1/2 demethylases KDM3A and KDM3B have essential roles in the acquisition of pluripotency. Although the double knockout (DKO) of Kdm3a and Kdm3b is lethal in both mice and ESCs, there are very few changes in steady-state gene expression and the mechanism of gene regulation by these proteins remains unknown. Therefore, we performed unbiased immunoaffinity purification of KDM3A or KDM3B followed by mass spectrometry to identify associated proteins. Surprisingly, KDM3A/3B were associated with the RNA processing machinery rather than the expected heterochromatin proteins. We orthogonally validated the interaction of KDM3B with the splicing regulator PRMT5. To investigate if KDM3A/3B had a role in splicing we first generated KDM3B KO ESCs and knocked in a "degron" domain into KDM3A that allows for the rapid inducible degradation of the protein. We then performed whole transcriptome RNA sequencing after four hours of degradation and found misregulation of several canonical splicing pathways. Interestingly, the transcripts that

retained exons in the KDM3A/3B KO had functions in chromatin and transcription regulation as well as response to DNA damage. Transcripts that lost exons were enriched for kinase functions such as ATP binding. Thus, we have uncovered an unexpected function for histone demethylases in the splicing selection of transcripts. We propose that this data shifts our view of how these histone demethylases work in cancer, obesity, and other diseases and should be examined as potential targets for replacement therapies.

Funding Source
Histone Demethylase Control of Post Implantation Development 1RO1HD105151-01A1
Keywords: RNA Splicing, Histone Demethylase, Protein Interactions

2:30 PM – 3:00 PM

PRENATAL THERAPIES FOR SINGLE GENE DISORDERS

Mackenzie, Tippi

Broad Center for Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA

We are living in a golden age of medicine in which the availability of next generation sequencing and expanding tools in gene therapy/editing make it theoretically possible to repair almost any defect in the genetic code. Importantly, the ability to diagnose genetic disorders before birth and the presence of established surgical techniques enable these therapies to be delivered safely to the fetus. Prenatal therapies are generally used in the second or early third trimester for severe, life-threatening disorders for which there is a clear rationale for intervening before birth. Our group is currently conducting a phase 1 clinical trial of in utero hematopoietic stem cell transplantation for fetuses with alpha thalassemia major (NCT 02986698) as well as phase 1 clinical trial of in utero enzyme replacement therapy for fetuses with lysosomal storage diseases (NCT04532047). While there has been promising work for prenatal gene therapy in preclinical models, the path to a clinical prenatal gene therapy approach is complex. We and others are exploring the use of antisense oligonucleotides, gene replacement, and gene editing strategies (for somatic cells only) in single gene disorders that are severe enough to warrant therapy. It is essential to conduct multidisciplinary conversations that include patients, regulatory experts, and other stakeholders as we define a roadmap for safe and effective clinical applications.

Keywords: Prenatal Therapy, Gene Therapy, Genetic Disorders

3:30 PM – 5:00 PM
Hematopoietic Diseases

3:30 PM – 4:00 PM

IMPROVING ACCESS OF CAR-T CELL AND OTHER GENE-MODIFIED CELLULAR THERAPIES BY MANUFACTURING THE FINAL PRODUCT AT THE PLACE-OF-CARE

Dropulić, Boro

Caring Cross, Gaithersburg, MD, USA

The current price for a one-time commercial CAR-T cell therapy, not including hospital costs is at least $350,000 per dose. Such pricing is not sustainable in high-income countries, let alone in low- and middle-income countries, leaving many without access to these transformative therapies. A centralized model currently used for the manufacture and distribution of commercial CAR-T cells drives up their cost due to the logistical and infrastructure needs of this model. A more cost-effective way is to manufacture the final CAR-T cell product locally at the place-of-care. In a recent studies, the overall response rate of a CAR-T cell therapy for adult B-cell Lymphoma and Pediatric Leukemia using locally manufactured CAR-T cells produced outstanding clinical outcomes [Nat Med, 2020, https://www.nature.com/articles/s41591-020-1081-3; Nat Com, 2021, https://www.nature.com/articles/s41467-021-27312-6]. Caring Cross is collaborating with clinical centers around the world to develop and improve the access of CAR-T and other gene-modified cellular therapies that are manufactured locally at, or close to, the hospitals treating the patients needing these therapies. This place-of-care manufacturing significantly reduce the costs of manufacturing CAR-T and other gene-modified cellular products and promises to improve their affordability and access around the world.

Keywords: CAR-T cell, Place-of-Care Manufacturing, Affordability and access around the world

4:00 PM – 4:15 PM

EVALUATING THE SAFETY AND IMMUNOGENICITY OF MHC HOMOZYGOUS IPSC-DERIVED CD34+ HEMATOPOIETIC PROGENITORS IN A NHP MODEL

D'Souza, Saritha S.[1], Kumar, Akhilesh[1], Maufort, John[1], Weinfurter, Jason S.[2], Raymond, Mathew[1], Strelchenko, Nick S.[1], Perrin, Elizabeth S.[1], Coonen, Jennifer S.[1], Mejia, Andres S.[1], Simmons, Heather S.[1], Torbett, Bruce E.[1], Reynolds, Mathew S.[1], Thomson, James S.[1], Slukvin, Igor I.[1]

[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA, [2]School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA

Ex vivo expanded somatic myeloid progenitors' administration has been explored as a way to facilitate a more rapid myeloid recovery and improve overall survival following myeloablation. Advances in induced pluripotent stem cell (iPSC) technologies have created alternative platforms for supplying off-the-shelf immunologically compatible myeloid progenitors, including cellular products derived from major histocompatibility complex (MHC) homozygous "superdonors", potentially increasing the availability of MHC- matching cells and maximizing the utility for stem cell banking. However, it is unclear the teratogenic and tumorigenic potential of iPSC-derived progenitor cells and whether they will induce alloreactive antibodies upon transfer. Here, we evaluated the safety and efficacy of using CD34+CD45+ hematopoietic progenitors derived from MHC homozygous iPSCs (iHPs) for treating cytopenia following myeloablative HSC transplants in a Mauritian cynomolgus macaque (MCM) nonhuman primate (NHP) model. We demonstrated that infusing iHPs is well-tolerated and safe, observing no teratomas or tumors in the MCMs up to one year after HSC transplant and iHP infusion. Importantly, the iHPs also did not induce significant levels of alloantibodies in MHC-matched or -mismatched immunocompetent MCMs, even after increasing MHC expression on iHPs with IFNg. These results suggest feasibility of iHP use in the setting of myeloablation and that iHP products pose a low risk of inducing alloreactive antibodies.

Funding Source
This research was funded by National Institutes of Health (NIH) R01HL132891 grant and NIH grant P51 OD011106-54 to the Wisconsin National Primate Research Center.
Keywords: iPSC derived hematopoieitc progenitors, Non Human Primate model, alloimmunization

4:15 PM – 4:30 PM

TAKING A CUE FROM CLINICAL GENETICS TO ENGINEER A COMPETITIVE ADVANTAGE INTO RED BLOOD CELLS

Cromer, Kyle[1], Camarena, Joab[2], Hampton, Jessica P.[2], Luna, Sofia E.[2], Majeti, Kiran R.[2], Porteus, Matthew H.[2]

[1]Department of Surgery, University of California, San Francisco, CA, USA, [2]Pediatrics, Stanford University, Stanford, CA, USA

Due to disease prevalence and amenability of hematopoietic stem cells (HSCs) to ex vivo culture and transplantation, multiple genome editing trials are underway to treat the hemoglobinopathies. However, the greatest barrier to a functional cure is achieving high engraftment levels of corrected HSCs in the bone marrow (BM), which is currently only possible using devastating myeloablation regimens to clear space in the HSC niche prior to transplant. While genome-edited HSCs produce cells of all lineages (T cells, B cells, macrophages, etc.), the only cell type of clinical relevance to the hemoglobinopathies is the RBC. Therefore, technology to bias edited HSCs toward the erythroid lineage would allow low corrected HSC chimerism in the BM to yield high levels of corrected RBC chimerism in the bloodstream. Taking a cue from clinical genetics, we know that truncations in the erythropoietin receptor (EPOR) can drive non-pathogenic hyper-production of RBCs. Using CRISPR-mediated genome editing we demonstrated that truncation of the endogenous EPOR as well as expression of a truncated EPOR (tEPOR) cDNA is able to dramatically bias HSCs toward the erythroid lineage in vitro. Next, we coupled tEPOR expression with a genome editing strategy to correct beta-thalassemia, which significantly enriched for corrected RBCs following editing in patient-derived HSCs. We then transplanted human HSCs edited with this correction+enrichment vector into NSG mice and showed that edited cells are capable of long-term engraftment and lineage reconstitution. We also demonstrated that engrafted cells retained the ability to dramatically enrich for corrective editing events over the course of RBC differentiation. Therefore, we believe our work demonstrates a strategy by which correction of the hemoglobinopathies could be achieved even with low engraftment of edited HSCs. This would therefore allow us to reduce or eliminate the need for high-morbidity myeloablation regimens and simultaneously amplify the efficacy of current therapies in the clinic. In doing so, we believe our results have the potential to improve safety and accessibility to effective treatments for the large number of patients suffering from the hemoglobinopathies worldwide.

Keywords: Genome editing, Cell engineering, Hematopoietic stem cells

4:30 PM – 5:00 PM

ADVANCING PLURIPOTENT STEM CELL TECHNOLOGIES FOR IMMUNOTHERAPIES

Slukvin, Igor I.[1], Majumder, Aditi[2], Zhang, Jue[3], Smith, Portia[2], Jung, Ho Sun[2], Uenishi, Gene[2], Park, Mi Ae[2], Kumar, Akhilesh[2], D'Souza, Saritha[2], Suknuntha, Kran[2], Mesquitta, Walatta-Tseyon[2], Brok-Volchanskaya, Vera[2], Forsberg, Matthew[4], Capitini, Christian[4], Thomson, James[3]

[1]Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI, USA, [2]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA, [3]Regenerative Biology, Morgridge Institute for Research, Madison, WI, USA, [4]Pediatrics, University of Wisconsin, Madison, WI, USA

Cellular therapies combined with genetic engineering technologies have emerged as increasingly powerful tools for immunotherapies of cancers and viral infections. The use of induced pluripotent stem cells (iPSCs) as an unlimited source of blood progenitors and terminally differentiated cells can further expand applicability of cellular immunotherapies by offering "off-the-shelf" therapeutic products to fit specific clinical needs for a broad group of patients. During development, hemogenic endothelium (HE) is the main source of blood cells in the embryo. To improve blood manufacturing from human iPSCs for immunotherapies, it is essential to define the molecular determinants that enhance HE specification and promote development of the desired lineage of immune cells from HE. We revealed that activation of arterial program in HE through overexpression of ETS1, SOX17 or modulation MAPR/ERK signaling at mesodermal stage of development dramatically increases the formation of DLL4+CXCR4+ arterial-type HE highly enriched in frequency of T cell precursors. In contrast, abrogation of arterial programming following deletion of artery-specific enhancer within intron 3 of DLL4, impairs T cell production from iPSCs. NK cell specification from HE can be enhanced by overexpression of SOX18 or treatment of CD34+CD43+ progenitors with UM171. We also demonstrated that HE endothelium can be induced directly from iPSCs by overexpression ETV2 modified mRNA (mmRNA). Using this system, we developed a defined and rapid protocols for production of neutrophils (iN) and macrophages (iM). Following knockout SIRPA or integration of GD2-CAR into AAVS1 locus of iPSCs we generated myeloid cells with potent anti-tumor activities. SIRPA-KO iM and iN demonstrated significantly increased phagocytic and cytotoxic capabilities of multiple CD47+ cancer cell lines. GD2-CAR iNs and iM selectively killed GD2-expressing melanoma and neuroblastoma cell lines in vitro and showed superior anti-tumor activities in vivo in NSG mice with melanoma or neuroblastoma xenograft. Overall, our studies provided innovative strategies to aid in generation of lymphoid and myeloid cells from iPSCs for immunotherapies through modulation of HE specification and diversification.

Keywords: iPSC, Immunotherapy, CAR-neutrophils

THURSDAY, 22 SEPTEMBER

9:00 AM – 10:15 AM
Cell & Gene Therapy Manufacturing

Sponsored by FUJIFILM Cellular Dynamics Inc.

9:00 AM – 9:30 AM

TITLE NOT AVAILABLE AT TIME OF PRINTING.

Dismuke, David

Forge Biologics, USA

Abstract not available at time of printing.

9:30 AM – 9:45 AM

CHROMATIN MODULATION FOR EFFICIENT CRISPR-CAS9 GENE EDITING OF HUMAN PLURIPOTENT STEM CELLS

Khajanchi, Namita[1], Molugu, Kaivalya[2], Lazzarotta, Cicera R.[3], Das, Amritava[4], Tsai, Shengdar Q.[3], Saha, Krishanu[5]

[1]Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [2]Biophysics, University of Wisconsin, Madison, WI, USA, [3]Hematology, St. Jude Children's Research Hospital, Memphis, TN, USA, [4]Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA, [5]Biomedical Engineering, Wisconsin Institute for Discovery, Madison, WI, USA

Genome-edited human induced pluripotent stem cells (iPSCs) have broad applications in disease modeling, drug discovery, and regenerative medicine. Despite the significant development of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system, the gene editing process can be inefficient and can take several weeks to months to generate edited iPSC clones. One of the major challenges is that chromatin compaction limits the Cas9 protein access to the target DNA. To overcome these challenges, we developed a strategy to improve the speed and efficiency of the iPSC gene editing process via the application of a histone modifier, Trichostatin A (TSA; Class I and II histone deacetylase inhibitor or HDAC inhibitor). We observed that TSA-treated iPSCs had increased chromatin accessibility (via single nuclei ATAC-seq) and gene-editing efficiency in these cells increased ~3.5 fold at several closed and open chromatin loci without any increases in off-target modifications. The edited iPSCs retained full pluripotency and genomic integrity. We also

further developed an in situ nuclear imaging-based pipeline to quantify the TSA-induced change in global chromatin changes to enable rapid visual identification of iPSCs that are more amenable to gene editing. These methods to generate edited iPSCs rapidly and efficiently could advance the biomanufacturing of therapeutically-relevant, gene-edited iPSCs.

Funding Source
National Science Foundation (CBET-1350178), National Institutes of Health (U01 EY032333), UW-Madison's Stem Cell & Regenerative Medicine Center, Wisconsin Alumni Research Foundation, Wisconsin Institute for Discovery
Keywords: gene editing, induced pluripotent stem cells (iPSCs), chromatin modulation

9:45 AM – 10:15 AM
TECHNICAL AND REGULATORY CONSIDERATIONS FOR THE MANUFACTURE OF GENE-EDITED IPSCS

Thiel, Austin

ElevateBio, Cambridge, MA, USA

The ability to generate cell therapies from pluripotent stem cells is changing the way we think about diseases and how to treat them. Regenerative medicines derived from induced pluripotent stem cells (iPSCs) have the potential to treat a large number of diseases, many of which currently lack efficacious therapies, by identifying the deficient or non-functional cell type involved in the disease and generating the corresponding healthy cell type from pluripotent stem cells. The production of high quality iPSCs that are suitable for clinical and commercial manufacturing of differentiated cell therapies is a critical step in product development. In addition, gene editing of iPSCs to enhance the function or durability of PSC-derived therapies is becoming increasingly common. Technical and regulatory aspects must be considered when developing processes for iPSC reprogramming and editing. Having access to integrated solutions could be the key to catalyzing these two scientific disciplines to revolutionize regenerative, cell, and gene therapies for patients around the world.

Keywords: iPSC, editing, regulatory

10:45 AM – 11:45 AM
Cellular Transplants

10:45 AM – 11:15 AM
COMBINED CELL AND GENE THERAPY FOR EPIDERMOLYSIS BULLOSA

De Luca, Michele

Centre for Regenerative Medicine S. Ferrari, University of Modena and Reggio Emilia, Modena, Italy

LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB) was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. We report long-term follow-up of life-saving regeneration of the entire epidermis on a JEB child suffering from a devastating form of JEB. The regenerated transgenic epidermis remained stable throughout the entire 6-year follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes. In studying the different behavior of JEB and COL7A1-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway and consequent alteration of the expression of FOXM1. This pathway is not altered in RDEB, explaining the different behavior of transgenic RDEB cells vs transgenic JEB cells. This notion imposes the development of a different gene correction strategy to successfully tackle RDEB. We also discuss preliminary data aimed at gene editing of dominantly inherited genodermatoses, as EB Simplex.

Keywords: Gene therapy, Epidermolysis bullosa, Epidermal stem cells

11:15 AM – 11:45 AM
ADULT RETINAL PIGMENT EPITHELIAL STEM CELL THERAPY FOR PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

Temple, Sally

Neural Stem Cell Institute, Rensselaer, NY, USA

The retinal pigment epithelium (RPE) is a central nervous system tissue specialized to support the neural retina and is essential for vision. Dysfunction of the RPE can lead to blinding conditions such as age-related macular degeneration (AMD), a highly prevalent, neurodegenerative disease. Several RPE cell-replacement strategies are being pursued, including using embryonic or induced pluripotent stem cells and, in our work, adult RPE stem cells. We previously

showed that the adult human retina contains a rare population of cells capable of being activated to a stem cell state after exposure to mitogens in culture. Starting with adult cadaver eyes donated to eye banks, the RPE layer is extracted, and retinal pigment epithelial stem cells (RPESCs) are activated to proliferate, expanding in culture to produce over a billion cells per single donor. Preclinical testing demonstrated promising safety and efficacy following subretinal transplantation of a progenitor stage cell derived from the adult RPESC in animal models. These findings supported an investigational new drug application to the FDA for a cell replacement therapy for patients suffering from age-related macular degeneration (AMD) and launch of a Phase I/IIa clinical trial. Translating basic stem cell research towards the clinic presents challenges, including how to manufacture and define these complex cell products more efficiently and reliably. Characterization of the adult RPESC product demonstrates significant differences from pluripotent stem cell-derived RPE cells, which are already showing promise in clinical trial. This comparison between cell products opens future opportunities to study the impact of cell characteristics on transplant outcome for patients suffering from AMD, helping to define critical quality attributes that reliably define effective cell replacement.

Funding Source
Funding support from the National Eye Institute, NIH Regenerative Medicine Innovation Project (RMIP), and Luxa Biotech
Keywords: retina, age-related macular degeneration, cell therapy

12:00 PM - 1:00 PM
Sponsored Innovation Showcase

Madison Ballroom AD, Level 4

12:00 PM –1:00 PM

NEXT-GENERATION IN-VITRO ASSAYS: CHARACTERIZING THE ACTIVITY OF HUMAN IPSC-DERIVED NEURONS IN 2D AND 3D CULTURES AT HIGH RESOLUTION*

Presented by MaxWell Biosystems

Obien, Marie[1], Hornauer, Philipp[2], Sundberg, Maria[3], McSweeney, Danny[4]

[1]MaxWell Biosystems, Switzerland, [2]Bio Engineering Laboratory, ETH Zurich, Switzerland, [3]Sahin Lab, Boston Children's Hospital, MA, USA, [4]Park Lab, University of Massachusetts, Amherst, MA, USA

Both 2D and 3D brain models derived from human induced pluripotent stems cells (hiPSCs) are emerging as promising tools for investigating brain development and disease progression, as well as to test drug toxicity and efficacy in-vitro. In order to adopt hiPSC-derived 2D and 3D neuronal networks for rapid and cost-effective phenotype characterization and drug screening, it is necessary to assess their cell type composition, gene expression patterns, and physiological function. In this innovation showcase, our invited speakers will showcase studies where MaxWell Biosystems' advanced highdensity microelectrode arrays (HD-MEAs) as the core of easy-to-use platforms, MaxOne (single-well) and MaxTwo(multi-well), allowed to capture neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks and facilitated the characterization of the neuronal activity of hiPSC-derived neurons. During this session speakers will introduce how brain development disorders are modeled in 2D and 3D in-vitro. Overall, the presentations will provide an overview on how HD-MEA technology can efficiently advance research in 2D and 3D hiPSC-derived brain models and accelerate drug discovery for neurodegenerative diseases.

**This Innovation Showcase will be available in-person and on the virtual meeting platform.*

12:00 PM - 1:00 PM
Special Session

Madison Ballroom BC, Level 4

12:00 PM –1:00 PM

ETHICS SESSION: IN SEARCH OF DEFENSIBLE LIMITS AT THE INTERSECTION OF GENETIC AND CELLULAR TECHNOLOGIES

Developed in Partnership with ISSCR & ASGCT Ethics Committee

Saha, Krishanu[1], Hurlbut, Benjamin[2], Jasanoff, Sheila[3]

[1]University of Wisconsin-Madison, WI, USA, [2]Arizona State University, AZ, USA, [3]Harvard University, MA, USA

With emerging biotechnologies, we have seen the emergence of a global patchwork of laws, policies, recommendations, proposals, conventions, and declarations that define and delimit the field of permissible research, for instance, in embryo culture and genome editing. Numerous expert bodies, including the ISSCR and ASGCT, have taken an active role in specifying limits for responsible research but have tended not to explicitly define their own remit and responsibilities. This session aims to clarify more directly the concepts of limits that are reflected in these developments and the stakes associated with setting them. We will discuss 1) the nature and use of limits to address ethics concerns, 2) the authority and remit of professional groups to do this, and finally, 3) the ideal processes and their associated strengths and limitations.

1:15 PM – 2:30 PM
Immuno-Oncology: CAR-T and NK Cell Therapies

Sponsored by STEMCELL Technologies Inc.

1:15 PM – 1:45 PM
CAR T CELLS AND THE LIVING DRUG CONCEPT

Sadelain, Michel

Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract not available at the time of printing.

1:45 PM – 2:00 PM
GENOME-EDITED, CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS TO TARGET NEURODEGENERATION AND SENESCENCE

Sarko, Lauren E.[1], Traynor, Roshini[2], Givand, David[2], Forsberg, Matthew[3], Shepley, Claire[2], Capitini, Christian[3], Saha, Krishanu[4]

[1]Cellular and Molecular Pathology, University of Wisconsin, Madison, WI, USA, [2]Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA, [3]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA, [4]Department of Biomedical Engineering, Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA

Cellular senescence is a biological process that reduces proliferation and helps to prevent propagation of damaged cells. Controlling senescence within the body can be critical, as senescent cells can also play an important role in the pathophysiology of various disorders, including Alzheimer's disease (AD). Abnormally high numbers of senescent cells have been found in diseased tissues, and removal of these high senescent cell populations in animal studies by lysing senescent cells (i.e., "senolytic" approaches) can prevent neurodegeneration from occurring. Targeting the brain with therapeutic agents, however, has been a key challenge in treating these disorders as the blood-brain barrier (BBB) has evolved to restrict the transport of large molecules. Our research focuses on the use of T cells as a "Trojan horse" to deliver therapeutic molecules as they naturally surveil the brain. We exploit new abilities to program the specificity of T cell activation using synthetic receptors, called chimeric antigen receptors (CARs). Recent work indicates that these engineered T cells can be utilized to target senolytic agents for senescence-associated diseases, like liver fibrosis, by targeting the cell surface protein urokinase plasminogen activator receptor (uPAR). We have developed a new platform to genetically edit T cells to target and eliminate senescent cells while co-expressing additional factors. We leverage CRISPR-Cas9 genome editing and CAR T cell (CART) technology that redirects T cell specificity and effector functions to attack the desired target. We have successfully generated genome-edited uPAR-CART cells to recognize and specifically kill uPAR-positive senescent fibroblasts in vitro. Currently we are adapting this strategy to a murine model of AD by using human T cells with a mouse targeting uPAR to evaluate efficacy of this senolytic strategy in vivo. We anticipate that genome-edited senolytic CART cells could provide biological insights into targeting and enhancing the brain's immune defense against neurodegenerative disease.
Keywords: CAR-T, Neurodegeneration, Senescence

2:00 PM – 2:30 PM
FT536: A FIRST-OF-KIND, OFF-THE-SHELF IPSC-DERIVED CAR-NK CELL PRODUCT CANDIDATE FOR SOLID TUMORS DESIGNED TO UNIQUELY TARGET MICA/B STRESS PROTEINS AND OVERCOME MECHANISMS OF EVASION

Valamehr, Bob

Research and Development, Fate Therapeutics, San Diego, CA, USA

The advent of chimeric antigen receptor (CAR)-T cell therapies has revolutionized the treatment of hematological malignancies; however, broader therapeutic success has been challenged by observed toxicities, including on-target, off-tumor engagement of non-cancerous cells, limited tumor antigen expression and availability, and the ineffectiveness of single-antigen targeted CAR T cells to eradicate heterogeneous tumors. In addition, the inherent variability that arises from the use of patient- and donor-sourced T cells and the engineering of these T-cell populations as part of each manufacturing campaign results in significant drug product inconsistencies, which can impact safety, efficacy, and therapeutic reach. We have developed FT536, a first-of-kind, induced pluripotent stem cell (iPSC)-derived NK (iNK) cell product candidate that expresses a novel CAR and ubiquitously targets cancer cells through canonical stress ligand recognition. FT536 recognizes the conserved α3 domain of the pan-tumor associated MICA and MICB (MICA/B) stress proteins, a novel targeting strategy that mitigates a key tumor immune evasion mechanism. In addition to the CAR, FT536 is derived from a clonal master iPSC line that incorporates multiple genetic edits to enhance NK cell effector function, persistence, and multi-antigen targeting. Preclinical assessment of the product candidate's unique CAR modality demonstrated potent antigen-specific cytolytic activity against an array of solid and hematological tumor cell lines. FT536 is also armed with a high-affinity, non-cleavable CD16 (hnCD16) Fc receptor, which provides the potential to target additional tumor antigens in combination with tumor-targeting

antibodies. To this end, FT536 demonstrated significant tumor growth inhibition in multiple solid and liquid in vivo xenograft models as a monotherapy and in combination with therapeutic antibodies. An Investigational New Drug (IND) application for FT536 was allowed by the U.S. Food and Drug Administration (FDA) in December 2021, and a first-in-human clinical study of FT536 as monotherapy and in combination with tumor-targeting monoclonal antibody therapy for the treatment of multiple solid tumor indications is expected to commence in 2022.
Keywords: Off-the-shelf CAR NK Cell Therapy, Immuno-Oncology, Novel Cancer Antigen, Induced Pluripotent Stem Cells

3:00 PM – 4:30 PM
Diabetes & Metabolic Diseases

3:00 PM – 3:15 PM

ISLET ARCHITECTURE IN ADULT MICE IS ACTIVELY MAINTAINED BY ROUNDABOUT RECEPTORS

Waters, Bayley J., Blum, Barak

Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA

Islets of Langerhans are clusters of endocrine cell types within the pancreas responsible for regulating blood glucose in vertebrates. The spatial arrangement of cell types within these clusters is not random, and increasing evidence suggests that this islet architecture serves a functional purpose. Notably, insulin-producing beta cells in the islet exhibit enhanced hormone secretion when arranged adjacent to other beta cells, whereby they can synchronize insulin release. We recently reported that the axon guidance receptor family Roundabout (Robo) is required in the beta cell to establish islet architecture during development. Deleting Robo1 and Robo2 in the beta cells of mice during early postnatal development results in a total abolishment of classic murine islet architecture and a marked loss of beta cell-beta cell contacts. Interestingly, these mice also show reduced synchroneity of insulin secretion among their beta cells, as well as defects in glucoregulation, providing evidence that loss of islet architecture may affect islet function. However, whether islet architecture is “set” during development, or requires continuous maintenance throughout life, has not been determined. We hypothesized that islet architecture is maintained throughout life, and that Robo continues to regulate islet architecture subsequent to its role in establishing architecture in development. To assess this hypothesis, we used an inducible tissue-specific mouse model to delete Robo2 in the beta cells (Robo2 beta-KO) of adult mice. We labeled and visualized the different cell types in the islet using immunofluorescent staining and confocal imaging. Here we show that deleting Robo2 in adult beta cells results in significant loss of murine islet architecture, indicating that Robo2 may play a critical role in actively maintaining islet architecture throughout life. With continued focus on creating or preserving functional islets for transplant into diabetic patients, understanding the factors required to maintain cell type arrangement and thus optimize islet function becomes increasingly important for developing future islet replacement therapies.
Keywords: islet architecture; Roundabout receptors; beta cell

3:15 PM – 3:30 PM

INVESTIGATING HYPER-STABLE COMPUTATIONALLY DESIGNED TIE2 PROTEINS TO MITIGATE DIABETIC VASCULOPATHY AND PROMOTE VASCULAR STABILITY

Ehnes, Devon D.[1], Zhao, Yan-Ting[2], Phal, Ashish[3], Saini, Shally[4], Ueda, George[5], Somasundaram, Logeshwaran[6], Hoffman, Cameron[6], Ben-Sasson, Ariel[5], Sahib, Aya[6], Orozco, Alister[7], Mathieu, Julie[8], Sellers, Drew L.[3], Baker, David[6], Ruohola-Baker, Hannele[6]

[1]Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA, [2]Oral Health Sciences, Institute for Stem Cells and Regenerative Medicine, Seattle, WA, USA, [3]Bioengineering, University of Washington, Seattle, WA, USA, [4]Biochemistry, Institute for Stem Cells and Regenerative Medicine, Seattle, WA, USA, [5]Biochemistry, Institute for Protein Design, Seattle, WA, USA, [6]Biochemistry, University of Washington, Seattle, WA, USA, [7]Biochemistry and Molecular Biology, Reed College, Portland, OR, USA, [8]Comparative Medicine, University of Washington, Seattle, WA, USA

Diabetics are at increased risk of vascular diseases resulting from glucose-driven misregulation of vascular permeability via loss of adherens junctions. Currently, medical intervention relies on metabolic regulation, with no way to mitigate vasculopathy once it begins. The angiopoietin-Tie2 pathway regulates vascular stability, remodeling, and permeability. Recent studies show that inhibition of the Tie2 signaling axis results in increased atherosclerosis, and that hyperglycemia increases expression of inhibitory Ang2, suggesting that strong activation of this axis could help mitigate diabetic vascular instability. We used computationally designed protein scaffolds conjugated with the Ang1 F-domain at a range of valencies and geometries to evaluate Tie2 signaling output and tight junction formation. We found scaffolds presenting 6, 8, 12, 30, or 60 F-domains have Ang1 like

activity, upregulating pAKT, and inducing migration and tube formation, with one scaffold, H8, acting as a superagonist. Cells treated with H8 recruit junction factors Integrin, VE-Cadherin, ZO1, Occludin, and Claudin-5, and show accelerated ZO1 reassembly after chemical disruption. Interestingly, we observed two functionally distinct Tie2 clusters, one that recruits integrin for pAKT/FOXO regulation, and another that regulates junctional permeability. To assess the potency of our scaffolds in promoting endothelial identity and maturity, we evaluated H8 in iPSC-endothelial (iENDO) differentiation. We found these scaffolds were able to drive Tie2 receptor superclustering during endothelial maturation, resulting in accelerated and enhanced endothelial maturity. Importantly, we observed that Tie2 receptor clusters recruited TJP1 to the membrane to enhance tight junction formation at a very early stage of differentiation (d8) and 15-fold higher than controls. Finally, sc-Seq of d14 iENDOs showed that treated cells were overrepresented in clusters with mature transcriptional signatures and exhibited enriched expression of ZO1 compared to controls, showing a sustained effect of H8 scaffolds. Our data suggest that H8 could be used to attenuate diabetic vasculopathy by ameliorating vascular permeability, representing a first-of-its-kind therapeutic for one of the most prevalent diabetic comorbidities.

Keywords: diabetic vasculopathy, tight junctions, vascular stability

3:30 PM – 3:45 PM

CD9 MARKS A HUMAN BETA CELL SUBPOPULATION WITH INCREASED IMMUNOGENICITY

Russ, Holger A.[1], Anderson, Amanda[1], Barra, Jessie[1], Beard, Scott K.[2], Benninger, Richard[1], Dwulet, JaeAnn[1], Laundry, Lauri[1], Nakayama, Maki[1], Shilleh, Ali H.[1], Tse, Hubert[4]

[1]Barbara Davis Center For Diabetes, University of Colorado, Aurora, CO, USA, [2]University of Colorado Health Sciences Center, Denver, CO, USA, [3]Microbiology, University of Alabama, Birmingham, USA

The destruction of insulin producing pancreatic β-cells by an autoimmune attack is a hallmark of Type 1 diabetes (T1D). Despite tremendous research efforts focusing on elucidating the underlying mechanisms resulting in human T1D, the field currently lacks detailed understanding on how and why the disease develops in individuals. While T1D has been classically viewed as a disease of the immune system, increasing evidence suggest a critical role for the pancreatic beta cells in attracting their own immune destruction. Indeed, subpopulations of pancreatic beta cells that can withstand or critically contribute to autoimmunity have been identified using mouse models prompting the intriguing concept that beta cell heterogeneity is central in T1D development. However, if a human beta cell subpopulation with differential immunogenicity exist has not been comprehensively investigated as of yet. Using two complementary human beta cell models, cadaveric islets and stem cell derived beta cells (sBC), we identified a small beta subpopulation marked by CD9 that exhibits key markers of senescence, a senescence associated secretory phenotype, reduced function and an enrichment in immune response genes. HLA matched human beta cell and autoreactive T cell co-cultures showed increased T cell stimulation by CD9/senescent beta cells in these novel, functional assay systems. Our findings highlight the first demonstration of differential immunogenicity of human beta cell subpopulations. Furthermore, transplantation of cadaveric islets or sBC results in a dramatic increase in CD9/senescent beta cells providing important insights for current cell therapy efforts. In sum, our study offers important novel knowledge on beta cell heterogeneity and immunogenicity and carries critical implications for our current understanding of T1D development and cell replacement approaches.

Keywords: Autoimmune diabetes, immunogenicity, senescence

3:45 PM – 4:00 PM

VALIDATING EXPRESSION OF BETA CELL MATURATION-ASSOCIATED GENES IN HUMAN PANCREAS DEVELOPMENT

Sackett, Sara D., Tremmel, Daniel M., Gupta, Sakar, Mikat, Anna M., Mitchell, Samantha A., Odorico, Jon S.

Surgery, University of Wisconsin, Madison, WI, USA

Stem cell-derived islets (SC-islets) hold promise as a beta cell replacement therapy to treat diabetes. After transplantation, SC-islets attain improved maturation and functional profiles compared to in vitro culture. The acquisition of maturation is important in these cells in order to properly secrete insulin to control blood glucose levels; under-secretion of insulin will not be sufficient to reverse diabetes, while over-secretion of insulin could cause deadly hypoglycemia. One method for measuring maturation in SC-islets is based on function, but functional assays are time consuming, expensive, and require high numbers of living cells. The identification of markers for human beta cell functional maturation would therefore be informative for improving SC-islet differentiation, could facilitate the sorting of more mature beta cells from the pool of differentiated cells, and will stimulate a better understanding of normal human islet development. While several candidate factors to mark beta cell maturation have been identified, much of the data supporting these

markers come from animal models or SC-islets; it is unknown how well these expression profiles mirror primary human islet development. One such marker is Urocortin-3 (Ucn3), which is expressed only postnatally in mice when islets become functional. We explored Ucn3 expression at the gene and protein level in human fetal pancreas (HFP), adult human islets (AHI), and SC-islets and found that Ucn3 is expressed in human fetal islets well before the acquisition of functional maturation, and show that SC-islets that express Ucn3 are no more functionally mature than those that have no Ucn3 expression. We then utilized our tissue bank and SC-islet resources to test an array of other candidate maturation markers, and identify CHBG, G6PC2, FAM159B, Glut1, IAPP, MAFA, NTPDase3, SIX2, and SIX3 as markers with expression patterns that correlate developmentally with the onset of functional maturation in human beta cells.

Funding Source
NIH 1F31DK125021-01, JDRF 3-SRA-2017-364-SB
Keywords: Maturation marker; Beta cells; Stem cell-derived islets

4:00 PM – 4:30 PM

STEM CELL-DERIVED PANCREATIC ISLETS TO TREAT INSULIN-DEPENDENT DIABETES

Melton, Douglas A.

Harvard University, Cambridge, MA, US

One of the goals of regenerative medicine is to renew or replace cells and tissues that cause disease. A prime example of using stem cells to this end is the project aimed at making pancreatic beta cells, the insulin producing cells, for transplantation into people who have insulin-dependent diabetes. It has now been demonstrated that pluripotent stem cells can be induced by in vitro differentiation to produce functional human beta cells as well as other endocrine islet cell types. These stem cell-derived islets, sc-islets, have been shown to be functional and therapeutically effective in a human clinical trial. Research aimed at gaining more complete control of the cell type composition of sc-islets will be discussed as will experiments aimed at avoiding immune rejection that follows sc-islet transplantation.

Funding Source
Harvard Stem Cell Institute, HHMI
Keywords: diabetes, manufactured islets, genetic screens

FRIDAY, 23 SEPTEMBER

9:00 AM – 10:15 AM
Muscle Disorders

9:00 AM – 9:30 AM

PROGRESS TOWARDS DEVELOPING GENE THERAPY FOR DUCHENNE MUSCULAR DYSTROPHY

Cheng, Seng H.

Rare Disease Research Unit, Pfizer, Cambridge, MA, USA

Duchenne muscular dystrophy (DMD) is a devastating and life threatening X-linked disease caused by mutations in the gene encoding dystrophin that is needed for proper muscle membrane stability and function. Patients present with muscle degeneration that progressively worsens with age such that they require wheel-chair assistance when they are in their early teens, and succumbing to their disease by the time they are, on average, 28 yrs old. To address this, a recombinant adeno-associated virus serotype-9 vector (fordadistrogene movaparvovec) containing a truncated dystrophin gene (mini-dystrophin) was engineered, which aims to restore functional protein to cardiac and skeletal muscle. Following preclinical testing for safety and potential efficacy in animal models, a phase 1b, multicenter, single-arm, open-label trial was conducted in 19 ambulatory boys (n=3 low-dose; n=16 high-dose) with a genetic diagnosis of DMD and receiving a stable, daily glucocorticoid regimen. Median age at gene therapy infusion was 8.8 yrs (range 6.2–13.0 yrs); median baseline North Star Ambulatory Assessment (NSAA) total score was 27 (range 17–32). Functional end-points measured included change from baseline in the NSAA and other measures of motor and respiratory function. Three treatment-related serious adverse events occurred (dehydration, acute kidney injury, thrombocytopenia) as previously reported; all resolved within 15 days. For partici-pants in the high-dose group, mean mini-dystrophin levels were 22% and 40% of normal at 2 and 12 months, respec-tively (by liquid chromatography-mass spectrometry). Dystrophin-positive fibers were 39% and 62% at 2 and 12 months, respectively (by immunofluorescence). A consistent trend towards improved function was seen at 1-year following treatment with fordadistrogene movaparvovec compared with the decline observed in the external control cohort (placebo trial participants of similar age, weight, baseline function, stable steroid use). These preliminary results indicate that fordadistrogene movaparvovec has an accept-able safety profile in this population, provides for substantial expression of mini-dystrophin that increases (on average) between 2 and 12 months, and has the potential to benefit ambulatory DMD patients across a range of functions.

Keywords: Duchenne muscular dystrophy, AAV-mediated gene therapy, North Star Ambulatory Assessment

9:30 AM – 9:45 AM

HUMAN IPSC-DERIVED-COMMITTED CARDIAC PROGENITOR CELLS ENGRAFT AND IMPROVE CARDIAC CONTRACTILITY RESERVE IN A SWINE ISCHEMIC CARDIOMYOPATHY MODEL

Raval, Amish.[1], Roy, Sushmita[1], Zhou, Tianhua[1], Schmuck, Eric G.[1], Saito, Yukihiro[2], Conklin, James T.[1], Hacker, Timothy A.[3], Hsieh, Patrick[4], Koonce, Chad[5], Kamp, Timothy J.[1]

[1]Medicine, University of Wisconsin, Madison, WI, USA, [2]Cardiovascular Medicine, Okayama University Hospital, Okayama, Japan, [3]Cardiovascular Research Center, University of Wisconsin, Madison, WI, USA, [4]Institute in Biomedical Sciences, Academia Sinica, Tapei, Taiwan, [5]Cardiovascular Program, Fujifilm Cellular Dynamics International, Inc., Madison, WI, USA

Ischemic cardiomyopathy remains a major cause of morbidity and mortality. Cell therapy to remuscularize the failing heart holds promise, but the optimal cell product and therapeutic approach are unknown. Human iPSC-derived committed cardiac progenitor cells (CCPs) robustly differentiate into cardiomyocytes and endothelial cells in vitro. We hypothesized that catheter-based transendocardial injection (TEI) of CCPs in conjunction with a novel injectable cardiac fibroblast derived extracellular matrix (cECM) cell retention agent results in cardiac tissue grafts and improves functional outcomes in an immunosuppressed porcine ischemic heart failure (HF) model. Coronary artery balloon-occlusion myocardial infarction was induced in Yucatan mini-swine. After 1 month, 300M CCPs with and without 50 mg cECM particles were delivered in 14 intramyocardial injections into the infarct and peri-infarct region using a steerable injection catheter via an electroanatomic roadmap in comparisons to vehicle control. Human and cell-type-specific immunolabeling was used to define the area, composition and distribution of human grafts. Cardiac MRI and invasive pressure-volume assessment with dobutamine stimulation were used to evaluate viability and functional parameters. Human cardiac grafts composed predominantly of cardiomyocytes and some endothelial cells were detectable in 27 of 28 (96%) hearts up to 2 months following injections. At 1 month, MRI-measured left ventricular ejection fraction trended to improvement in pigs treated with CCPs alone (8.6 +/-8.2%) and with CCPs+cECM (3.8+/-10.2%), but not with Control (0+/-10.7%). The changes in EF were not statistically significant, but pre-load independent end systolic pressure volume relationship (ESPVR) following dobutamine challenge, which is a measure of left ventricular contractility reserve, significantly improved for both CCP alone (7.6 +/-1.1) and CCPs+cECM (5.4 +/-0.9) compared to baseline (p=0.004 and p=0.002 respectively) but not in the control group (4.0 +/-0.7) (p=0.26). In conclusion, in an immunosuppressed swine ischemic HF model, TEI of CCPs + cECM yields persistent human cardiac tissue grafts and improves left ventricular contractility reserve. These findings encourage further investigation of TEI of CCPs as a clinical therapy for HF.

Funding Source
National Institutes of Health Regenerative Medicine Innovation Project, Fujifilm Cellular Dynamics International, Inc., Cellular Logistics, Inc., UW-Madison School of Medicine and Public Health
Keywords: heart failure, cardiac progenitor, iPS cell

9:45 AM – 10:15 AM

HUMAN CARDIOVASCULAR DISEASE MODELS BASED ON HIPSC: FROM DISEASE MECHANISMS TO DRUG DISCOVERY

Mummery, Christine L., Bellin, Milena, Davis, Richard, Orlova, Valeria V.

Department Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Our lab creates models for cardiovascular disease based on pluripotent stem cells (hPSCs). We use these for understanding disease mechanisms and cardiotoxic effects of drugs. We can predict the toxic effects of test drugs with almost 80% accuracy in (immature) cardiomyocyte monolayer cultures When we require mature cells, we combine hPSC-cardiomyocytes with cardiac fibroblasts and endothelial cells in “microtissues”. The cardiomyocytes develop electrical, metabolic and functional features allowing us to model postnatal onset diseases or dissect which cell types in the heart are actually responsible for the disease phenotype. We showed for example fibroblasts in the heart can contribute to abnormal heart contraction in patients with arrhythmogenic cardiomyopathy. These complex cell systems are paving the way towards better understanding of disease mechanisms and drug discovery.

Funding Source
Netherlands Organ-on-Chip Initiative: an NWO Gravitation project (024.003.001); reNEW: Novo Nordisk Foundation Center for Stem Cell Medicine
Keywords: cardiovascular disease models, Human pluripotent stem cells, cardiac organoids and microtissues

10:45 AM – 11:45 AM
New Technologies and Future Directions I

10:45 AM - 11:15 AM
CHEMICAL APPROACHES TO GENERATE FUNCTIONAL HUMAN ISLETS FOR TRANSPLANTATION

Chen, Shuibing

Surgery, Weill Cornell Medical College, New York, NY, USA

Diabetes affects 462 million people worldwide and consumes one out of eight health care cost dollars from the citizens of the United States. The biology of the Islets of Langerhans, which contain mainly endocrine cells and endothelial cells, is central to the development of diabetes, and therefore plays a key role in development of therapeutic strategies. An efficient strategy to build functional human islets is important for both transplantation therapy and disease modeling of diabetes. We have performed several chemical screens to identify the small molecules promoting the human pancreatic beta cell generation, proliferation, and survival. These small molecules not only facilitate understanding the molecular mechanism controlling beta cells, but also help the generation of functional islets for transplantation.

Keywords: Chemical screen, human pluripotent stem cells, human islets

11:15 AM – 11:30 AM
METABOLIC GLYCOENGINEERING AFFORDS HIGHLY EFFICIENT INTRACELLULAR DELIVERY OF CAS9 RIBONUCLEOPROTEIN IN HUMAN CELLS

Khalil, Andrew[1], Mladenova, Tsvetilina[2], Young, Alicia[3], Chandler, Whitney[3], Markoulaki, Styliani[3], Wang, Hua[4], Jaenisch, Rudolf[5], Mooney, David[6]

[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA, USA, [2]Regenerative Medicine and Technology, Utrecht University, Utrecht, Netherlands, [3]Genetically Engineered Mouse Core, Whitehead Institute for Biomedical Research, Cambridge, MA, USA, [4]Department of Materials Science and Engineering, University of Illinois Urbana-Champaign, IL, USA, [5]Whitehead Institute for Biomedical Research, Cambridge, MA, USA, [6]John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

Efficient intracellular delivery of biomolecules, such as nucleic acids and proteins, to eukaryotic cells is essential to numerous biotechnology and medical applications. While many existing technologies for intracellular delivery of these molecules exist, e.g., as viral vectors, lipid nanoparticles, and electroporation, current approaches frequently present challenges with balancing delivery efficacy, toxicity, and safety. As such, the continued development of new intracellular delivery technologies, particularly for difficult-to-transfect/infect human cells, is of significant interest. Here, we describe a novel intracellular biomolecule delivery method with low toxicity, high efficiency and throughput, and then demonstrate its utility by delivering Cas9 ribonucleoproteins. Specifically, we developed an intracellular protein delivery method utilizing metabolic glycoengineering techniques, whereby sugar molecules used ubiquitously in cellular metabolism are modified to include highly specific azide click-chemistry functional groups. As these sugars are taken up by cells when added to the culture medium, glycosylation events throughout the cells decorate endogenous proteins with these click-functionalized sugars, some of which are trafficked to label the cell membrane with clickable azide groups. This approach resulted in uniform “sugar-labeling” of both stem- and primary-derived mammalian cells with low toxicity. After sugar-labeling, subsequent addition of dibenzocyclooctyne (DBCO)-modified Cas9 ribonucleoproteins (RNPs) complexed with single-guide RNA (sgRNA) to the culture medium afforded a highly-specific and low toxicity delivery mechanism, as RNP complexes clicked to the cell membrane were rapidly internalized. Using this strategy afforded Cas9-mediated editing efficiency of up to 50.5 and 75.0% in human pluripotent stem cells and mouse embryos, respectively. Furthermore, reimplanted mouse embryos edited using this technique resulted in 30.0% of live pups carrying INDELs at the targeted locus. Together, this metabolic glycoengineering strategy represents a novel and highly efficient method of intracellular protein delivery strategy with particular applications in Cas9-mediated genetic engineering of primary cells.

Funding Source
National Institute of Biomedical Imaging and Bioengineering, HOPEl Wellcome Leap
Keywords: Genetic engineering, Gene and drug delivery, CRISPR/Cas9

11:30 AM – 11:45 AM
A PROGRAMMABLE SYSTEM FOR REWIRING ABERRANT CANER SIGNALING TO THERAPEUTIC EFFECTOR RELEASE

Zou, Xinzhi, Lin, Michael

Bioengineering, Stanford University, Stanford, CA, USA

Oncolytic viruses are currently approved only for intratumoral injection to limit toxicity from infection of normal tissues. However, this does not allow treatment of disseminated disease while tumors are small or deeply located. A long-desired solution is a virus with sufficient specificity for tumor cells to be administered systemically for delivery throughout the body. To restrict viral replication to tumor cells, we recently developed a synthetic signaling

pathway that responds to hyperactive ErbB (EGFR and HER2) by releasing a viral replication protein, a system we call rewiring aberrant signaling to effector release (RASER). Using RASER, we engineered recombinant Adenovirus (Ad) and Vesicular Stomatitis Virus (VSV) whose replication is gated by ErbB hyperactivity. We showed that the resulting Ad-ErbB-RASER and VSV-ErbB-RASER selectively kill cancer cells with hyperactive ErbB signaling. VSV-ErbB-RASER is non-lethal to mice at 100 times the lethal dose for wild-type VSV, despite retaining wild-type viral replication proteins, demonstrating that RASER successfully limits replication in normal tissues. We have also successfully applied RASER to create viruses specific for cancer cells with hyperactive c-Met. Thus RASER may be a general method for rationally engineering viruses that replicate selectively in cancer cells with exquisite specificity, potentially serving as an effective systemic treatment for disseminated disease.
Keywords: Oncolytic virus, cancer therapeutics, Protein engineering

1:15 PM – 2:30 PM
New Technologies and Future Directions II

1:15 PM – 1:45 PM
ADVANCES IN THE THERAPEUTIC APPLICATION OF CRISPR/CAS9 FOR GENOME EDITING

Parmar, Rubina G.

Chemistry and Delivery Sciences, Intellia Therapeutics, Acton, MA, USA

At Intellia, we are building a full-spectrum genome editing company. We are deploying the industry's broadest and deepest toolbox, including novel editing and delivery solutions, to harness the immense power of CRISPR-based technologies for in vivo and ex vivo therapeutic applications, each with the potential to revolutionize the future of medicine. For in vivo programs we use our proprietary lipid nanoparticle (LNP) platform to deliver to the liver a two-part genome editing system: a guide RNA specific to the targeted gene and messenger RNA that encodes the SpCas9 enzyme, which carries out the precision editing. With this technology we can deactivate a defective gene producing a toxic protein or insert a gene for gain of function. For ex vivo programs we have a novel allogeneic technology designed to overcome rejection by host T and NK cells without the need for host immune suppression. In this presentation, we will share the advances in the therapeutic application of CRISPR/Cas9 for genome editing.
Keywords: CRISPR/Cas9, Delivery, Editing

1:45 PM – 2:00 PM
PH-RESPONSIVE POLYMER NANOPARTICLES FOR EFFICIENT DELIVERY OF CAS9 RIBONUCLEOPROTEIN WITH OR WITHOUT DONOR DNA

Xie, Ruosen[1], Xie, Ruosen1, Wang, Xiuxiu[1], Wang, Yuyuan[1], Ye, Mingzhou[2], Zhao, Yi[2], Yandell, Brian S.[3], Gong, Shaoqin[1]

[1]Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI, USA, [2]Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [3]Department of Statistics, University of Wisconsin, Madison, WI, USA

CRISPR-Cas9 may offer new therapeutics for genetic diseases through gene disruption via nonhomologous end joining (NHEJ) or gene correction via homology-directed repair (HDR). However, the clinical transition of CRISPR technology is limited by the lack of safe and efficient delivery systems. Although non-viral vectors have been developed for Cas9 ribonucleoprotein (RNP) delivery in recent years, to date, few non-viral nanoplatforms have been developed for co-delivery of Cas9 RNP and donor DNA templates. Herein, pH-responsive polymer nanoparticles capable of safely and efficiently delivering Cas9 ribonucleoprotein alone (termed NHEJ-NP, diameter = 29.4 nm), or together with donor DNA (termed HDR-NP, diameter = 33.3 nm) were fabricated and evaluated in vitro and in vivo. Intravenously, intratracheally, and intramuscularly injected NHEJ-NP induced efficient gene editing in mouse liver, lung, and skeletal muscle, respectively, in transgenic Ai14 mice. Furthermore, intramuscularly injected HDR-NP also led to muscle strength recovery in a Duchenne muscular dystrophy mouse model (i.e., mdx mice). mPEG-PC7A was synthesized by ATRP and characterized by NMR. The size and morphology of NHEJ-NP and HDR-NP were studied by DLS and TEM. In vitro studies were performed using HEK-293T cells and WA09 hESCs. The in vivo gene editing efficiencies were evaluated in Ai14 transgenic mice or mdx mice and analyzed by immunofluorescence staining, trichrome staining, Sanger sequencing, and four-limb hanging time assay. The biocompatibility was investigated by H&E staining, RT-qPCR, and hematological analyses. NHEJ-NP and HDR-NP possess many desirable properties including high payload loading content, small and uniform sizes, high editing efficiency, good biocompatibility, low immunogenicity, and ease of production, storage, and transport, thereby making them desirable for various genome editing applications with clinical potentials.
Funding Source
The National Institutes of Health (4-UH3-NS111688)
Keywords: Non-viral vector, In vivo delivery, Gene therapy

2:00 PM – 2:30 PM

GETTING ACROSS BARRIERS: GENE DELIVERY ACROSS THE BLOOD-BRAIN BARRIER FOR PRECISE AND MINIMALLY-INVASIVE STUDY AND REPAIR OF NERVOUS SYSTEMS

Gradinaru, Viviana

California Institute for Technology, CA, USA

Protein engineering and data science have helped overcome challenges in optogenetics and gene delivery, with microbial opsins tolerated by mammalian cells and viral capsids that cross the blood–brain barrier. These tools are applied to neurodevelopmental and neurodegenerative disorders, for e.g. to understand circuits underlying locomotion and sleep for Parkinson's disease. By understanding how engineered capsids work and leveraging them as vehicles for targeted gene delivery via the vasculature, we are now closer to precise noninvasive study and repair of nervous systems.

2:30 PM – 3:30 PM
Closing Keynote

2:30 PM – 3:25 PM

HUMAN NEURAL STEM CELL TRANSPLANTATION FOR HUNTINGTON'S DISEASE

Thompson, Leslie M.[1], Reidling, Jack[2], Holley, Sandra[3], Cepeda, Carlos[3], Fury, Brian4, Monuki, Edwin[5], Meshul, Charles[6], Bauer, Gerhard[4], Levine, Michael[3]

[1]Psychiatry and Human Behavior/Neurobiology and Behavior, University of California, Irvine, CA, USA, [2]UCI MIND, University of California, Irvine, CA, USA, [3]Semel Institute, University of California, Los Angeles, CA, USA, [4]Institute for Regenerative Cures, University of California, Davis, CA, USA, [5]Pathology, University of California, Irvine, CA, USA, [6]Behavioral Neuroscience and Pathology, Oregon Health and Science University, Portland, OR, USA

Huntington's disease (HD) most overtly impacts the striatum, with progressive loss of medium spiny neurons and atrophy of the cortex. At a molecular level, the disease is accompanied by a progressive loss of neuronal proteins, including the neurotrophic factor BDNF that supports the survival of striatal neurons. Further, aberrant accumulation of aggregated huntingtin (HTT) protein species corresponds to disease pathogenesis. Stem cell-based approaches are promising as a treatment option for HD with potential to modulate pathology in complex tissues such as the brain. We have developed and extensively evaluated a GMP-compliant human embryonic stem cell (ESC)-derived neural stem cell (NSC) product, ESI-017 hNSCs, for transplantation into the striatum of HD patients, with the intent of slowing or preventing the progression of the disease. The transplanted ESI-017 hNSCs have been tested in multiple mouse models of HD. In both R6/2 and zQ175 mice, cells engraft and differentiate to neuronal populations, express BDNF and reduce mutant HTT accumulation. These molecular and histological improvements correlate with improvement in behavioral in HD mice. Transplantation of human NSCs is challenged by the need for long-term functional integration. Following long-term transplantation in zQ175 mice, host tissue appears to form synaptic contacts with transplanted cells as evidenced by EM, suggesting they may provide new circuitry to reduce the aberrant cortical excitability that occurs in human HD. hNSCs appear to receive synaptic inputs, innervate host neurons, and improve membrane and synaptic properties. Single nuc RNAseq on transplanted tissues following 6 months transplantation indicates three major populations of cells. Analysis of the host cells suggests significant rescue of the mouse transcriptional dysregulation. Overall, the findings support hNSC transplantation for further evaluation and clinical development for HD. IND enabling safety studies in preclinical models of HD are currently ongoing.

Funding Source
CIRM
Keywords: neural stem cells, Huntington's disease, cell therapy

Poster Abstracts

DAY 1: WEDNESDAY, 21 SEPTEMBER

5:00 PM – 6:00 PM
Poster Session I – ODD

6:00 – 7:00 PM
Poster Session I – EVEN

Cell and Gene Therapy Manufacturing

W-101

CTR9 DRIVES OSTEOCHONDRAL LINEAGE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM/STROMAL CELLS (MSCS) VIA EPIGENETIC REGULATION OF BMP-2 SIGNALING

Chan, Ngai Ting[1], Lee, Ming-Song[2], Galipeau, Jacques T.[3], Li, Wan-Ju[2], Xu, Wei[4]

[1]Department of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI, USA, [2]Department of Orthopedics and Rehabilitation, University of Wisconsin, Madison, WI, USA, [3]Department of Medicine, University of Wisconsin, Madison, WI, USA, [4]Department of Oncology, University of Wisconsin, Madison, WI, USA

Cell-fate determination of human mesenchymal stem/stromal cells (hMSCs) is precisely regulated by lineage-specific transcription factors and epigenetic enzymes. We found that CTR9, a key scaffold subunit of Polymerase Associated Factor Complex (PAFc), selectively regulates hMSC differentiation to osteoblasts and chondrocytes, but not to adipocytes. An in vivo ectopic osteogenesis assay confirmed the essentiality of CTR9 in hMSC-derived bone formation. CTR9 counteracts the activity of EZH2, the epigenetic enzyme that deposits H3K27me3. Accordingly, CTR9 knockdown MSCs gain H3K27me3, and the osteogenic differentiation defects can be partially rescued by treatment with EZH2 inhibitors. Transcriptome analyses identified Bone Morphology Protein-2 (BMP-2) as a downstream effector of CTR9. BMP-2 secretion, membrane anchorage as well as the BMP-SMAD pathway were impaired in CTR9 knockdown MSCs, and the effects were rescued by BMP-2 supplementation. This study uncovers an epigenetic mechanism engaging the CTR9-H3K27me3-BMP-2 axis to regulate osteochondral lineage differentiation of hMSCs.

Funding Source
National Institutes of Health grant R01 CA213293 (W.X.)
National Institutes of Health grant R01 CA268183 (W.X.)
National Institutes of Health grant R01 CA236356 (W.X.)
National Institutes of Health grant R01 AR064803 (W.J.L)
Keywords: Osteogenesis; lineage differentiation; transcription

W-102

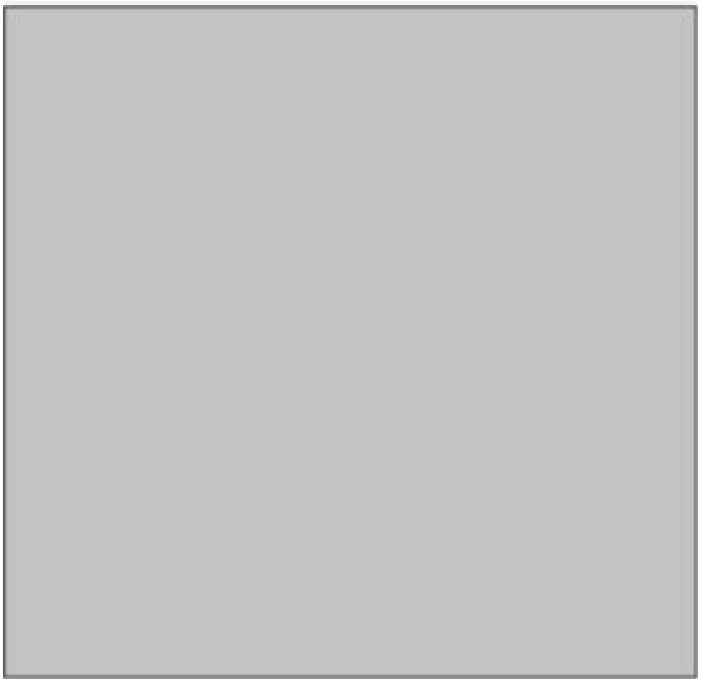
SCALABLE LENTIVIRAL PURIFICATION PROCESS WITH IN-LINE STERILE FILTRATION

Cherukuri, Pratima[1], Chakraborty, Saikat[2], Greenwald, Andrew[1], Trinh, Hung[3], Wu, Andrew[2]
[1]Process Development, Genezen, Indianapolis, IN, USA, [2]Analytical Development, Genezen, Indianapolis, IN, USA, [3]Downstream Process Development, Genezen, Indianapolis, IN, USA

Lentiviral vectors (LVV) are commonly used as safe and efficient tool for the delivery of transgenes into dividing and nondividing cells, enabling long-term gene expression. Production methods must be capable of making large quantities of high-quality functional LVV in an efficient and cost-effective way. The current cGMP LVV production processes lack the use of sterile filters due to the low stability of lentiviral vectors. The result of which is the consumption of precious final product on microbiological testing. As an attempt to solve this issue, Genezen evaluated the performance of two different commercially available sterile filters and two different buffers compositions, that can be incorporated into the process of LVV production. Our aim was to establish a scalable, fast, and robust downstream processing with a sterile filter to maximize final recovery of lentiviral vectors. Our traditional purification process consisting of three-unit operations (clarification, AEX and TFF) was evaluated with sterile filters before final fill and the entire downstream purification strategy was devised with single-use and easily scalable technologies. To summarize the results, the sterile filtration step was successfully performed with LVV recoveries of up to 23%. This data exhibits the potential benefits of including sterile filters to the LVV purification process for a more efficient LVV manufacturing process.

Keywords: Lentiviral Vector Manufacturing; GMP Viral Vector Manufacturing; Viral Vector Downstream Purification

W-103 POSTER WITHDRAWN



W-104

CONTROLLING MICROBIAL RISKS TO STEM CELLS WITHOUT ANTIMICROBIALS: USING THE PHYSICAL ATTRIBUTES OF THE CLOSED MANUFACTURING ENVIRONMENT

Henn, Alicia, Darou, Shannon, Henn, Stassa P., Yerden, Randy

Scientific, BioSpherix Medical, Parish, NY, USA

Traditional cleanrooms expose stem cells to not only variable room air cell handling conditions, but all the microbial risks that come from sharing the environment with humans. Extensive disinfection with harsh chemicals, as well as antibiotics and antimycotics, are widely used to mitigate these microbial risks. These measures add risk to stem cell cultures. Next generation isolators (NGI) are closed cell manufacturing systems that reduce risks for stem cells by providing a physical barrier between cultures and human operators. NGI also provide absolute control over the physical cell culture environment. Oxygen, carbon dioxide, temperature and relative humidity (RH) are controlled at all times. We have previously published on using low RH to reduce microbial risks when the cell processing chamber floor was contaminated. In this study, we extended those findings to add microbes to other surfaces of the cell processing chamber and processing items. Our null hypothesis was that microbes on non-floor surfaces would be able to contaminate mock cell cultures, even in low RH. We used coupons inoculated with 5 problem microbes (A. brasiliensis, B. subtilis, C. albicans, S. aureus, and P. aeruginosa) and placed them on the walls and ceilings of the cell processing chamber as well as the floor. We also contaminated exterior surfaces of cell culture processing items. We performed 9 media runs over 10 weeks with highly permissive microbial broth, adding a new set of microbes each run (thaw, medium change, and harvest operations). Almost 3 billion microbes accumulated in the system over the study. There was no contamination of test media except for positive control samples that were deliberately inoculated in vials. We disproved our hypothesis in that microbes on non-floor surfaces, even at very high numbers, were unable to contaminate mock cell cultures in the low RH environment of the NGI. This low-risk environment for stem cells can reduce the need for antibiotics/antimycotics as well as the use of harsh disinfectants, lowering the total risk to stem cell manufacturing operations.

Keywords: manufacturing; bioburden; stem cells

W-105

AN END-TO-END AUTOMATED CELL THERAPY MANUFACTURING PLATFORM ENABLING SCALABLE ALLOGENEIC CAR-T PRODUCTION

Li, Jingling[1], Cappabianca, Dan[2], Deans, Robert[1], Kasparek, Kassandra[1], Maures, Travis[1], Mendez, Pedro[1], Razinkov, Ivan[1], Saha, Krishanu[2], Thu, Myo[1], Thu, Ricky[1], Tran, Cac[1], Zheng, Yueting[1]

[1]Research and Development, Synthego Corporation, Redwood City, CA, USA, [2]Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA

Adoptive cell therapies are efficacious in fighting various cancers; however, high cost and low throughput manufacturing systems remain major obstacles. Synthego is developing an end-to-end, automated cell therapy manufacturing platform, CARE. It enables high-throughput and parallelized bioprocessing by separating basic unit operations. CARE's efficiency and reduction in COGS helps bring engineered cell and gene therapies to all patients. Here, we demonstrate the CARE system using an allogeneic CAR-T workflow. First we assessed cell isolation. We show isolation of >80% of primary T cells from PBMCs with >95% purity. Next we show Synthego's cell culture consumable can robustly expand primary T cells similarly to the benchmark G-Rex system. To demonstrate CARE's genomic editing capability, we simultaneously knocked out T-cell receptor α chain (TRAC), beta-2 microglobulin (B2M), programmed cell death protein 1 (PD-1), and knocked in anti-GD2 chimeric antigen receptor (CAR) to the TRAC locus using existing electroporation platform. Flow cytometry confirmed greater than 85% of edited cells are triple-negative. Further analytics showed translocation events in edited cells were less than 2% determined by digital droplet PCR. Our data suggests the CARE platform can perform cell isolation, gene modification, and cell expansion to produce a complex edited T cell product. It is a high-throughput and scalable manufacturing platform adaptable for a broad range of cell and gene therapies.

Keywords: Cell Therapy Manufacturing; CAR-T; CRISPR/Cas9 Gene Editing

W-106

VSC100: A HUMAN IPSC-DERIVED VASCULOPROGENITOR CELL THERAPY FOR ALLOGENEIC TREATMENT OF MICROVASCULAR DISEASES

Mann, Dave[1], Cliff, Carter[2], Farmer, James[3], George, Matthew[4], Jetzer, Ashley[5], Stauber, Sara[6], Yoder, Merv[2]

[1]Vascugen, Inc., Madison, WI, USA, [2]Senior Management, Vascugen, Inc., Madison, WI, USA, [3]Quality Control, Vascugen, Inc., Madison, WI, USA, [4]Research and Development, Vascugen, Inc., Madison, WI, USA, [5]Quality Assurance, Vascugen, Inc., Madison, WI, USA, [6]Operations, Vascugen, Inc., Madison, WI, USA

VSC100, an induced pluripotent stem cell (iPSC)-derived vasculoprogenitor cell (iPSC-VPC) therapy, is being developed for the treatment of severe microvascular disease conditions characterized by abnormal perfusion. VSC100 was developed to have similar functionality to primary endothelial colony forming cells (ECFCs), including high-proliferative potential, formation of lumenized vessels, and rescue of perfusion and tissue necrosis in preclinical models of ischemia. VSC100 displays certain properties of immune privileged cells, including expression of several known anti-inflammatory surface antigens and lacking expression of HLA Class II antigens. The first planned clinical evaluation of VSC100 is in subjects suffering from Critical Limb Threatening Ischemia (CLTI). CLTI results from insufficient blood flow to maintain the viability of skin and muscle tissue, typically manifesting in the lower legs and feet. Despite advanced surgical and endovascular revascularization treatment options, 30% of the patients with CLTI will require amputation, with 1-year mortality rates ranging from 15%-40%. For the “poor-option” patients, defined here as CLTI patients unsuitable for revascularization by one or more vascular surgical interventions, major amputation remains the only treatment option for providing symptomatic relief. Vascugen is in the process of executing process-qualification manufacturing of VSC100 in our onsite cGMP facility in support of planned GLP studies to enable the submission of an investigational new drug (IND) application for clinical exploration in CLTI.

Funding Source
Vascugen is a privately held company.
Keywords: Vascular Cell Therapy; iPSC; Allogeneic

Central Nervous System Diseases

W-107

CO-ACTIVATION OF CANONICAL WNT AND NOTCH SIGNALING IN HUMAN PSC-DERIVED ENDOTHELIAL PROGENITORS INDUCES BLOOD-BRAIN BARRIER PROPERTIES

Boutom, Sarah M.[1], Gastfriend, Benjamin D.[2], Shusta, Eric V.[2], Palecek, Sean P.[2]

[1]Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [2]Chemical & Biological Engineering, University of Wisconsin, Madison, WI, USA

Induction of blood-brain barrier (BBB) properties in central nervous system (CNS) endothelial cells during human development is incompletely understood and our knowledge of this process is derived mainly from animal models. In vitro BBB models derived from human pluripotent stem cells (hPSCs) can be used to study human BBB development and cerebrovascular disease. Human-derived in vitro models can be used to probe the relative importance of specific signaling pathways, including Wnt/β-catenin and Notch, on induction of BBB properties in naïve endothelium. These signaling pathways are typically attributed to ligands derived from neural progenitors and pericytes in the developing CNS. We differentiated endothelial progenitor cells (EPCs) expressing both CD31 and CD34 from hPSCs and treated the hPSC-EPCs with CHIR99021 to activate the Wnt/β-catenin signaling cascade and Notch1-intracellular domain (N1-ICD) lentiviral overexpression to simulate signaling through the Notch1 receptor. We find that co-activation of Wnt/β-catenin and Notch1 signaling resulted in simultaneous upregulation of Glut-1, a BBB-enriched glucose transporter, and decreased expression of both PLVAP and caveolin-1, two vesicular transcytosis-associated proteins. These findings suggest that the combination of these two signaling inputs yields induction of certain important barrier properties, including expression of a nutrient transporter and global reduction of transcytosis-associated protein expression. Future studies will investigate if this signaling combination is sufficient for induction of other major BBB properties in hPSC-derived endothelial cells. The identification of pathways with potential importance for developmental specification of BBB properties in humans may also advance efforts to model the human BBB in vitro.

Funding Source
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Keywords: Blood-brain barrier; Endothelial; Vascular

W-108

IMPROVED METHODS FOR LARGE HDR KNOCK-INS USING ALT-R(TM) HDR DONOR BLOCKS AND ALT-R HDR ENHANCER V2

Turk, Rolf, Woodley, Jessica, Thommandru, Bernice, Schubert, Mollie, Murugan, Karthik, Kurgan, Gavin, McNeill, Matthew, Jacobi, Ashley, Rettig, Garrett

Molecular Genetics, Integrated DNA Technologies, Coralville, IA, USA

CRISPR-based homology-directed repair (HDR) is an invaluable tool to facilitate specific mutations in a genomic region of interest. While many methods have been reported for improving HDR efficiency, achieving precise changes via HDR remains a challenge particularly for large knock-ins. HDR repair outcomes are most efficient with single-stranded DNA (ssDNA) templates when small insertions, deletions, or SNP changes are desired edits. For these applications, synthetic oligonucleotides (ssODN) have been studied and optimized with modifications for enhanced efficacy in HDR. Larger insertions can be generated via HDR using enzymatically generated ssDNA or double-stranded DNA (dsDNA) donor templates. Here, we present work demonstrating that improved HDR efficiency for large insertions can be obtained when dsDNA donor templates include novel end-modifications. These modifications improve the frequency of HDR and reduce homology-independent (blunt) insertion events that can occur at both on- and off-target CRISPR edits relative to unmodified dsDNA. The use of Alt-R modified dsDNA improved the ratio of HDR:Blunt repair events 4.3-fold on average relative to unmodified dsDNA templates for short inserts and reduced blunt insertion of large templates 4.6-fold at a mock off-target site. We demonstrate further improvement to HDR rates when using Alt-R HDR Enhancer V2, a small molecule that increases the rate of HDR in varied cell types including iPSCs and primary human T-cells. Together the use of Alt-R modified repair templates and the Alt-R HDR Enhancer V2 improved HDR rates up to 5- to 10-fold across knock-in experiments. Finally, we present investigations into design considerations for large dsDNA HDR templates including homology arm length and the placement of blocking mutations to disrupt Cas9 re-cleavage.

Keywords: Genome Editing; CRISPR; HDR

W-109

ACUTE SHEAR STRESS AND PIEZO1 ACTIVATION REDUCE EFFLUX TRANSPORT ACTIVITY IN BLOOD-BRAIN BARRIER IN VITRO MODELS

Foreman, Koji[1], Katt, Moriah[2], Daneman, Richard[3], Palecek, Sean[1], Shusta, Eric[1]

[1]Chemical and Biological Engineering Department, University of Wisconsin, Madison, WI, USA, [2]Neurosurgery, West Virginia University, Morgantown, WV, USA, [3]Pharmacology, University of California, San Diego, CA, USA

The human brain vasculature uniquely regulates the influx of various nutrients into the brain parenchyma while restricting the brain penetration of toxins and blood constituents. Blood-brain barrier (BBB) properties include efflux transporter activity which combines with passive barrier properties to limit the brain uptake of pharmaceuticals, creating a road-block for neurotherapeutic development. Understanding the regulation of efflux transport is key to improving the neurotherapeutic development pipeline. Previous research has demonstrated that regional neuronal activation in a mouse model results in a local increase in permeability to a blood-borne dye and substrate for the efflux transporter P-glycoprotein (P-gp). We hypothesized that this increase in permeability may be partly driven by a reduction of P-gp activity by endothelial cells in response to the transient increase in blood flow that accompanies neuronal activation. To test this hypothesis, we utilized human pluripotent stem cell (hPSC) and primary rat brain microvascular endothelial cell (BMEC) models of the BBB. We observed that 3 hours of 16 dyne/cm2 shear stress was sufficient to reduce P-gp efflux transport of the Rhodamine123 fluorescent substrate in rat primary BMECs. In addition, to identify a potential molecular mechanism of the shear-induced phenotype, we investigated the impact of pharmacological activation of Piezo1, a recently discovered mechanosensitive ion channel thought to be involved in vascular pathfinding and development, at the BBB. Application of the selective Piezo1 agonist, Yoda1, in our hPSC BMEC-like cell and primary rat BMEC models led to a reduction in P-gp activity after 3 hours of treatment, but no changes in transcription. Utilizing an hPSC line with a genetically encoded calcium indicator (GCaMP), we confirmed that application of the Piezo1 agonist led to an influx of calcium, a known modulator of P-gp activity, in the hPSC BBB model. Overall, this suggests that shear stress and Piezo1 may result in calcium mediated inhibition of efflux transport at the BBB, a potential mechanism for energy conservation within the brain vasculature during neuronal activation.

Funding Source
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Keywords: blood-brain barrier; stem cell modeling; shear stress

W-110

EFFECT OF REELIN ON HPSC-DERIVED NEURAL CREST STEM CELLS AND ENDOTHELIAL CELLS

Holt, Hope, Palecek, Sean, Shusta, Eric

Department of Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA

Secreted by Cajal-Retzius cells, reelin is an extracellular matrix glycoprotein which regulates positioning processes in the developing brain through cell-cell interactions. The reeler phenotype, brought about by in the absence of functional reelin, is described in mice as an impairment in motor coordination with a reeling gait. On the cellular level, loss of functional reelin results in compromised blood-brain barrier integrity as well as abnormal cell positioning. The reelin signaling pathway is thought to be involved in neurodegeneration, specifically reelin binds to ApoE receptors and the lack of reelin is associated with the phosphorylation of Tau, while Dab1, a propagator of reeling signaling, binds to amyloid-beta precursor protein family members. Additionally, reelin expression is increased with altered glycosylation in neurodegenerative disorders including frontotemporal dementia, Parkinson's disease, and Alzheimer's disease. In several psychiatric disorders such as bipolar disorder and schizophrenia, reelin expression levels are decreased. In this study, the effects of reelin will be shown on both hPSC-derived neural crest stem cells and hPSC-derived endothelial cells to determine the downstream components of the reelin pathway. These effects are investigated in each cell type alone as well as in a co-culture environment to allow for additional interactions across the cell types. HEK293T cells are transfected with pCrl, a plasmid to produce reelin or MOCK transfected. Spent media is collected every 48 h, concentrated 15x-100x, and placed on neural crest stem cells and/or endothelial cells for 0.5-6 h. Downstream components in the reelin pathway are then determined.

Keywords: Reelin; Blood-brain barrier; Endothelial cells

Diabetes & Metabolic Diseases

W-112

OVERCOMING IMMUNOLOGICAL BARRIERS FOR OFF-THE-SHELF IPSC-DERIVED ISLETS

Hu, Xiaomeng[1], White, Kathy[1], Olroyd, Ari[1], DeJesus, Rowena[2], Dominguez, Antonia[2], Dowdle, William[2], Chu, Elaine[1], Friera, Annabelle[1], Young, Chi[1], McGill, Trevor[3], Gagnon, Allison[4], Rukstalis, Michael[4], Millman, Jeffrey[4], Schrepfer, Sonja[1]

[1]Hypoimmune Platform, Sana Biotechnology, San Francisco, CA, USA, [2]TechScience, Sana Biotechnology, South San Francisco, CA, USA, [3]In Vivo Pharmacology, Sana Biotechnology, South San Francisco, CA, USA, [4]Therapeutic Cell Differentiation, Sana Biotechnology, South San Francisco, CA, USA

Improved differentiation protocols allow large-scale generation of induced pluripotent stem cell (iPSC)-derived islets. In order to make allogeneic "off-the-shelf" islets clinically useful and avoid rejection absent immunosuppression (IS), they need to evade host immune responses, a property we refer to as being "hypoimmune". Rhesus macaque (NHP) iPSCs were engineered to knock-out function of MHC class I and II and overexpress CD47 (HIP iPSCs). HIP iPSCs and unedited iPSCs (wt iPSCs) were transduced to express luciferase and transplanted IM into four allogeneic NHPs per group. There was a strong IFN-γ ELISpot T cell response 1 week after wt iPSC transplantation, and increased production of wt-iPSC specific IgM antibodies (Ab) and IgG Abs. At all time points tested (up to 12 weeks), PBMCs killed wt iPSCs via direct and Ab-mediated cellular cytotoxicity. Furthermore, serum from NHPs killed wt iPSCs via complement-dependent cytotoxicity. In contrast, NHPs that received HIP iPSCs showed no measurable immune response against HIP iPSCs at all time points. After 6 weeks, NHPs initially receiving wt iPSCs were injected with HIP iPSCs. Although the NHPs maintained their strong immune response against wt iPSCs, they did not mount any response against HIP iPSCs. NHPs receiving HIP iPSCs first developed a strong cellular and Ab-response against the subsequently injected wt iPSCs but continued to have no reactivity against HIP iPSCs. Bioluminescence imaging in vivo revealed rejection of all wt iPSC grafts in both groups within 2-3 weeks after transplantation, while all HIP iPSC grafts survived the study period of 16 weeks. As a step to extending these studies to human iPSCs, we created human HIP iPSCs. We were able to differentiate these human HIP iPSCs into functional HIP islet cells that evaded host immune responses. This work suggests that HIP modifications do not impact islet cell differentiation or function. These data support the development of allogeneic HIP islets for the treatment of patients with T1DM.

Keywords: hypoimmune; iPSC-derived islets; immunology

W-113

THREE-DIMENSIONAL SCAFFOLD WITH ASSOCIATED HEMOXCELL FOR SUPPORTING IPCS IN DIABETIC NUDE MICE SUBCUTANEOUS TISSUE AS PROMISING PRECLINICAL TRIAL

Khater, Sherry[1], Elessawy, Mohga[2]

[1]Pathology Lab Department, Urology and Nephrology Center, Mansoura, Egypt, [2]Research, Urology and Nephrology Center, Mansoura, Egypt

The worldwide prevalence of Diabetes mellitus evoked the need for better treatment options. Tissue engineering showed a great promising modality. For this, we investigated the potential role of a three-dimensional, scalable scaffold to support IPCs survival and function in the streptozotocin-induced, diabetic nude mice subcutaneous tissue, and to address the limits of our previously tested encapsulation systems. Isolation and expansion of human mesenchymal stem cells were subsequently differentiated into IPCs according to our published trichostatin-A protocol. Forty mice, diabetes was induced in 30, and 10 served as normal controls. For each diabetic mouse, 3x106 IPCs were cultured with the cytoform-400 scaffold in xenofree media with the addition of HEMOXCell just before transplantation. HEMOXCell subcutaneous injections for 7 days were done to further improve niche conditions. Follow-up for 3 months was done. The glucose tolerance curves exhibited a normal pattern demonstrating that the cells were glucose-sensitive and insulin-responsive. Their fasting blood sugar levels were reduced in 5 of them and reached near normal values in 15 of them. The sera of all transplanted mice contained human insulin and C-peptide with a negligible amount of mouse insulin. Removal of the transplanted scaffolds was followed by a prompt return of diabetes. Intracytoplasmic insulin granules were seen by immunofluorescence in cells from the harvested scaffolds. Furthermore, all pancreatic endocrine genes were expressed. This study demonstrated that the scalable scaffold with modified niche conditions can provide adequate support, an important issue when stem cells are considered for the treatment of type 1 diabetes mellitus.

Keywords: Diabetes; scaffold; niche

W-114

CHARACTERIZING THE ROLE OF THE RNA-BINDING PROTEIN CPEB1 IN BETA CELLS

Nimkulrat, Sutichot D., Blum, Barak, Jayaraaman, Hariharan

Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA

Functionally mature pancreatic β cells tightly secrete insulin to maintain glucose homeostasis. In type 1 diabetes (T1D), β cells are attacked by the immune system, while in type 2 diabetes (T2D), β cells malfunction due to metabolic toxicity induced by obesity and peripheral insulin resistance. Using RNA sequencing of β cells from early development to adulthood, as well from mouse models of both T1D and T2D, we recently reported that the different types of stresses in T1D and T2D induce different transcriptional trajectories of β cell response to the different stress types. These dedifferentiated β cells may re-express some progenitor marker genes, but they do not resemble true β cell progenitors. This suggests that different regulatory proteins may regulate β cell response to stressors in each type of diabetes. To find novel diabetes type-specific regulators, we inferred potential gene regulatory networks and regulators in our RNA sequencing datasets by using the MERLIN algorithm. Our analyses have identified the RNA-binding protein, Cpeb1, as a potential new regulator linked to β cell failure in diabetes. Thus, the MERLIN-predicted gene regulatory network of Cpeb1 and its associated genes are down-regulated in development and diabetes, with the strongest down regulation seen in type 1 diabetes, and their expressions are up-regulated in mature β cells. While Cpeb1 is known to regulate extensive range of biological processes and its involvement in regulating genes involved insulin signaling and apoptosis in the liver have been reported, Cpeb1 function in β cells is unknown. We have knocked down Cpeb1 in INS-1 cells which results in statistically increase of cell apoptosis in response to cytokines treatment compared to controls. We further found that Cpeb1 is not involved in cell proliferation or cell death under normal condition and S961-induced stress in whole-body Cpeb1 knockout mice. We are currently testing the hypothesis that Cpeb1 is translationally important in regulating β cell death in vivo induced by cytotoxicity and oxidative stress in the context on T1D.

Keywords: β cells; translational regulation; cell death

W-115

HUMAN STEM CELL-BASED APPROACHES TO STUDY GENETIC RISK FOR CORONARY ARTERY DISEASE

Salido, Elsa[1], De Medeiros Vieira, Carolina[1], Yang, Huan[2], Lo Sardo, Valentina[1]

[1]Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA, [2]Department of Surgery, University of Wisconsin, Madison, WI, USA

Coronary Artery Disease (CAD) is the most common type of heart disease, and the primary cause of death worldwide. Several genetic risk factors have been identified through Genome Wide Association Studies (GWAS), but their function is largely unclear. Among these, the most impactful is the 9p21.3 CAD risk locus, a 60 kb gene desert region of the human genome, harboring several Single Nucleotide Polymorphisms (SNPs) in high linkage disequilibrium in most ethnicities. Previously, we identified a role for the risk haplotype at 9p21.3 in influencing vascular smooth muscle cells (VSMCs) homeostasis and function. iPSC-derived VSMCs from individuals carrying the risk haplotype at 9p21.3 showed a dedifferentiation phenotype and functional alterations consistent with the role VSMCs have previously been shown to play in early CAD. Specifically, the 9p21.3 CAD Risk haplotype induces downregulation of VSMC markers, increases proliferation rate, and causes loss of adhesion and contraction properties in VSMCs. Deletion of the risk haplotype restores the non-risk phenotype, suggesting a gain of function effect of the risk variants in this locus. Here, we present a single cell transcriptomic analysis of iPSC-derived VSMCs with different genotypes at 9p21.3 to dissect the role of this locus in causing cell state changes in the vascular wall. Analysis of the risk-driven cell state transition and the identification of disease-prone cell subpopulations suggest early pathological alterations acting in the muscle layer of the arteries. This study provides insights into the early stage of CAD pathogenesis driven by the 9p21 risk locus and offers a method to explore the functional impact of human genomic risk regions by using large scale genome editing approaches and single cell transcriptomic.

Funding Source

Funding from the University of Wisconsin Genetics PhD Training Grant and the SciMed GRS Fellowship

Keywords: 9p21; iPSC-derived Vascular Smooth Muscle Cells; Coronary Artery Disease

W-116

DOWN SYNDROME AND DIABETES: CHARACTERIZING A UNIQUE FORM OF AUTO-IMMUNE DIABETES USING ISOGENIC TRISOMY 21 IPSC-DERVIED BETA-CELLS

Sethna, Cyrus R.[1], Blum, Barak[2]

[1]Cell and Molecular Biology, University of Wisconsin, Madison, WI, USA, [2]Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA

Individuals with Down Syndrome (DS), caused by trisomy of human chromosome 21, are susceptible to a unique form of early-onset, often neonatal, auto-immune diabetes, not associated with the canonical HLA variants typically linked to type-1 diabetics. It is hypothesized that this unique manifestation of diabetes can, in part, be attributed to aberrant β-cell development, function, and/or immunogenicity in Down Syndrome patients. Currently this is supported by post-mortem analyses of pancreatic islets of Down Syndrome Patients which have demonstrated mitochondrial fragmentation, decreased insulin secretion with increased pro-insulin secretion, and increased islet amyloid polypeptide plaques. Furthermore, multiple genes on chromosome 21 have been linked to diabetes. However, given the lack of reliable animal models and scarcity of quality patient samples, studying the etiology of DS- related diabetes remains challenging. To this end, we have recently established isogenic trisomy 21 induced pluripotent stem cells (iPSCs) to be used as an in vitro disease model. Through differentiating these cells into stem-cell derived β-cells (SC-β cells), we plan to generate a novel isogenic model to study the complex abnormalities that govern this disease and test the hypothesis that SC-β cells with trisomy 21 have abnormal differentiation, maturation, function, response to stress, or immunogenicity.

Funding Source

Jerome Lejeune Foundation, Grant #GRT-2022A-2123

Keywords: Trisomy 21; Diabetes; Disease Modelling

Immuno-Oncology: CAR-T and NK Cell Therapies

W-118

BIOLUMINESCENT ASSAY PLATFORMS FOR THE DISCOVERY AND DEVELOPMENT OF ENGINEERED T CELL THERAPIES FOR CANCER

Bach, Maggie L.[1], Cong, Mei[2], Fan, Frank[2], Hartnett, Jim[2], Lazar, Dan[2], Wang, Jun[2], Binkowski, Brock[2], Gilden, Julia[2], Grailer, Jamison[2], Slater, Michael[2], Stecha, Pete[2]

[1]Strategic Portfolio Management, Promega Corporation, Fitchburg, WI, USA, [2]Promega Research and Development, Promega Corporation, Fitchburg, WI, USA

T cell therapies are a promising approach for the treatment of cancer. For this, autologous or allogeneic T cells are engineered with specific T Cell Receptors (TCR) or Chimeric Antigen Receptors (CAR) that redirect effector responses against tumor cells and then infused into patients. Development of these therapies relies on the ability to rigorously evaluate candidate TCRs and CARs, characterize the effector functions of cell therapy products, and reproducibly measure the potency of cells for development and lot release. Therefore, we have developed novel bioluminescent bioassays to address these bottlenecks. To facilitate the screening and characterization of transgenic TCRs, we developed two TCRαβ-null reporter T cell lines, which are CD4+ or CD8+. Reintroduction of peptide-specific TCR α and β chains into TCRαβ-null reporter T cell lines results in peptide-dependent TCR activation and luciferase reporter expression. The select expression of CD4 or CD8 in the TCRαβ-null reporter T cell lines can enable the development of transgenic TCRs for both MHCI- and MHCII-restricted tumor antigen targets. To quantitatively measure the functions of engineered T cells, we have used split NanoBiT luciferase technology to develop two homogenous assays: a HiBiT target cell killing assay and cytokine immunoassays. Incubation of CAR-T with target cells stably expressing HiBiT results in lysis of the target cells and release of HiBiT proteins. These HiBiT proteins then bind to LgBiT in the detection reagent and form functional NanoBiT Luciferase to generate luminescence. We have generated knockouts of common tumor antigens in HiBiT target cell lines to facilitate studies of antigen-specificity. Furthermore, cytokine production (e.g IL-2 and IFN-gamma from the engineered T cells can be quantitatively measured in the homogenous NanoBiT Immunoassays. These luminescent assays are homogenous, fast, highly sensitive, and have robust assay windows. They represent a new set of tools for the discovery and development of T cell-based immunotherapies.

Keywords: CAR-T; TCR; potency assay

W-119

ENGINEER CAR-NEUTROPHILS FROM HUMAN PLURIPOTENT STEM CELLS FOR TARGETED CANCER IMMUNOTHERAPY

Bao, Xiaoping

Chemical Engineering, Purdue University, West Lafayett, USA

Neutrophils, the most abundant white blood cells in circulation, are closely related to cancer development and progression. Healthy primary neutrophils present potent cytotoxicity against various cancer cell lines through direct contact and via generation of reactive oxygen species. However, due to their short half-life and resistance to genetic modification, neutrophils have not yet been engineered with chimeric antigen receptors (CARs) to enhance their antitumor cytotoxicity for targeted immunotherapy. Here, we genetically engineered human pluripotent stem cells with synthetic CARs and differentiated them into functional neutrophils by implementing a chemically-defined platform. The resulting CAR-neutrophils presented superior and specific cytotoxicity against tumor cells both in vitro and in vivo. Collectively, we established a robust platform for massive production of CAR-neutrophils, paving the way to myeloid cell-based therapeutic strategies that would boost current cancer treatment approaches.

Keywords: CAR-NEUTROPHILS; CANCER IMMUNOTHERAPY; Glioblastoma

W-120

CRISPR ANTI-GD2 CAR-T CELLS FOR TREATMENT OF CANINE SARCOMA PATIENTS

Denne, Nina L.[1], Capitini, Christian[2], Cho, Monica[2], Saha, Krishanu[3], Vail, David[4]

[1]Cell and Molecular Biology, University of Wisconsin, Madison, WI, USA, [2]Pediatrics, University of Wisconsin, Madison, WI, USA, [3]Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [4]Veterinary Medicine, University of Wisconsin, Madison, WI, USA

Chimeric Antigen Receptor T (CAR-T) cell therapies have had limited success in treating solid tumors, and likely need to be further engineered to overcome the tumor microenvironment and increase persistence. We have developed a novel virus-free strategy in primary T cells that uses CRISPR-Cas9 to precisely insert a CAR targeting the antigen disialogangli-oside (GD2) into the TRAC gene encoding for the existing T cell receptor (TCR). GD2 is overexpressed in many solid tumor types in humans and dogs, and has limited expression in healthy tissues, making it an ideal candidate for solid tumor immunotherapy. Transcriptional profiling and testing in immunocompromised NSG mice reported in our recent preprint indicates that these virus-free CRISPR (VFC) CAR T

cells have a favorable memory-like phenotype and show fewer exhaustion markers than CAR T cells manufactured using viral vectors. However, questions regarding the safety and long-term efficacy of this treatment in fully immunocompetent hosts remain unanswered. We propose to manufacture and introduce VFC canine anti-GD2 CAR T cells into a companion canine cancer patient population to develop a potential treatment for GD2+ solid tumors. We have successfully shown knockdown of the canine TCR and are currently optimizing editing strategies for manufacturing canine VFC CAR T cells. The knowledge gained through these studies are likely to inform translational immunotherapy strategies for both canine and human patients.
Keywords: CAR-T; CRISPR-Cas9; Translational

W-121

GENERATION OF GD2-CAR NEUTROPHILS FROM HPSCS FOR TARGETED CANCER IMMUNOTHERAPY OF SOLID TUMORS

Majumder, Aditi[1], Jung, Ho Sun[1], Zhang, Jue[2], Suknuntha, Kran[1], Smith, Portia[1], Thompson, James A.[2], Slukvin, Igor[1]

[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA, [2]Morgridge Institute for Research, Madison, WI, USA

Chimeric antigen receptor (CAR) T cell and NK cell therapies already been successful in the eradication of lymphoid malignancies. CAR-lymphocytes are incapable to enter into the solid tumors and they also lose their activity within immunosuppressive tumor environment. Thus, opportunities exist for new immunotherapies for specific targeting of solid tumors using CAR-weaponized neutrophils which are capable of cytotoxicity and migration into solid tumors. However, generation of CAR neutrophils from peripheral blood represent a significant challenge due to their very short life-span. Human pluripotent stem cells (hPSCs) are a logical alternative for large-scale production of CAR neutrophils due to their renewability and uniform quality. In our study, we generated hPSCs with GD2 CARs integrated into AAVS1 locus and differentiated into neutrophils using serum- and xeno-free differentiation system based on modified ETV2 mRNA. Disialoganglioside GD2 antigen is highly expressed in neuroblastoma, glioma and melanoma cancer, and therefore a viable target for immunotherapy. Neutrophils generated from GD2 CAR iPSCs, as compared to wild type (WT), demonstrated superior cytotoxicity in vitro against GD2+ WM266-4 melanoma and CHLA20 neuroblastoma, while no differences of cytotoxicity were observed against GD2-negative SKOV3 ovarian and SK-BR3 breast cancer cells, indicating the specificity of anti-tumor therapeutic effect of CAR neutrophils. To assess in vivo potential of GD2 CAR neutrophils, NCG and NSG mice were inoculated intraperitoneally (IP) with 3x105 Luc2-eGFP+ WM266-4 melanoma cells and engraftment was assessed by IVIS bioluminescent imaging. On day 4 post WM266-4 injection, mice were either treated with 107 WT or GD2 CAR neutrophils via IP injection every 7 days. Upon assessment over 30 days, GD2 CAR neutrophil-treated mice showed reduced tumor burden compared to WT neutrophil-treated or untreated mice. Collectively, our studies demonstrate a feasibility of using hPSC-derived CAR-neutrophils for immunotherapies against solid tumor cancers.
Keywords: GD2-CAR; HPSCS; CANCER IMMUNOTHERAPY

W-122

PRECLINICAL OPTIMIZATION OF GENE EDITING FOR ADOPTIVE T CELL THERAPY WITH HIGH FIDELITY CRISPR/CAS9 RIBONUCLEOPROTEINS

Sellman, Max[1], Papademetriou, Jason[2], Cory, Evan[3], Yoder, David[3], Barco, Patricia[2], Brady, James[2]

[1]Protein Business Unit, Aldevron, Fargo, ND, USA, [2]Internal Research and Development, MaxCyte, Gaithersburg, MD, USA, [3]Internal Research and Development, Aldevron, Madison, WI, USA

Adoptive cell therapy with T lymphocytes harnesses the immune system for cancer treatment. T lymphocytes engineered with transgenic T cell receptors (TCR) specific for tumor antigens are in clinical development for various cancers. Knockout of endogenous TCR gene may improve expression of TCR transgene, and knockout of PD-1 may reduce T cell exhaustion induced by tumor expressed PD ligands. Gene editing with CRISPR-Cas9 via electroporation (EP) offers a rapid, clinically scalable approach for multiplexed knockout. High editing efficiency may reduce the timeline and cost of cell therapy manufacturing, and increase the proportion of edited cells. Additionally, editing with Cas9 ribonucleoprotein (RNP) may reduce off-target edits relative to Cas9 nucleic acid substrates, particularly with high fidelity Cas9. We transfected high fidelity Cas9 RNP (SpyFi™ Cas9 Nuclease, Aldevron®) into activated human T cells to knockout TCR using MaxCyte's scalable, GMP-compliant electroporation technology. High editing efficiency was observed by flow cytometry using RNPs complexed with SpyFi or wild type enzyme (WT Cas9, Aldevron). Editing efficiency was dependent on RNP concentration and electroporation energy. Following electroporation, viability improvement and resumption of proliferation were observed within 5 days. Co-electroporation of RNPs directed to TCR alpha chain, TCR beta chain, and PD-1 resulted in dual knockout of TCR and PD-1, with comparable editing efficiency for WT or SpyFi RNPs. The frequency of off-target editing was compared at multiple genomic loci by next generation sequencing. Taken together, the results support editing of T lymphocytes lacking endogenous TCR and PD-1 with high fidelity RNPs.
Keywords: CRISPR; TCR; Off-target

Muscle Disorders

W-123

MULTI-OMIC CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOLLOWING LONG-TERM IN VITRO MATURATION

Feeney, Austin K.[1], Simmons, Aaron D.[2], Bayne, Elizabeth F.[3], Ge, Ying[4], Palecek, Sean[2]

[1]Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [2]Department of Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA, [3]Department of Chemistry, University of Wisconsin, Madison, WI, USA, [4]Department of Cellular and Regenerative Biology, University of Wisconsin, Madison, WI, USA

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) represent a human cell type to advance disease modeling, drug discovery and toxicity, as well as provide a potential cellular therapy. However, a critical pitfall in cardiomyocyte differentiation is that the final cell product more closely resembles fetal cardiomyocytes than mature adult cardiomyocytes in terms of ultrastructure, metabolic utilization, electromechanical properties, and molecular markers. Efforts to improve hPSC-CM maturation in vitro have focused on introducing metabolic cues, electromechanical stimuli, 3D culture, co-culture with non-cardiomyocytes, and long-term in vitro maturation with varying levels of success. Because long-term in vitro maturation is straightforward to implement across a variety of lab settings, we characterized hPSC-CMs matured in extended culture at various time points spanning 1-6 months through metabolomics, proteomics, and transcriptomics. We quantified 948 metabolites via untargeted metabolomics and 3556 proteins via bottom-up proteomics across the maturation time course. Globally, there were 305 metabolites and 1158 proteins that significantly changed (one-way ANOVA, $p< 0.05$) during long-term culture. When comparing day 30 to day 190 samples, a pathway analysis of metabolites indicated a significant increase (FDR adjusted $p< 0.05$) in oxidation of very long chain and branched chain fatty acids as well as other metabolic pathways. An integrated joint pathway analysis of the metabolomic and proteomic datasets indicated a significant increase (FDR adjusted $p< 0.05$) in glycerophospholipid metabolism. Following the completion of our bulk RNA sequencing, we anticipate integrative multi-omics analyses will help to uncover novel insights about hPSC-CM maturation in long-term culture. These analyses will highlight important metabolic pathways and novel markers of hPSC-CM maturation states. Furthermore, we anticipate these data will provide benchmarks for hPSC-CM maturation and will help to identify candidate pathways to manipulate with the aim of accelerating hPSC-CM maturation in vitro.

Funding Source
The financial support of this project was provided by NIH grant NIH R01HL148059.

Keywords: cardiomyocyte; multi-omics; maturation

W-124

A HUMAN IPSC MODEL FOR STUDYING THE IMPACT OF MUSCULAR DYSTROPHY ON THE HEART

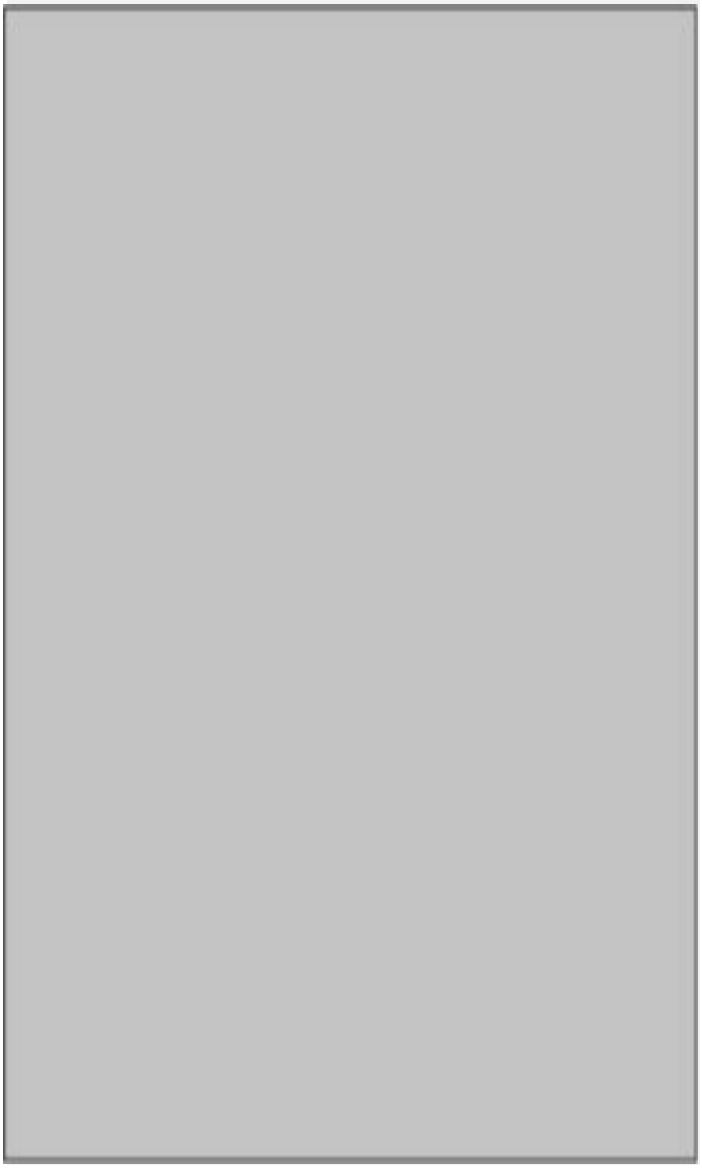
Rieger, Cara, Himmerich, Sarah A., Daigh, Christine A., Meyer, Nathan C., Vaidyanathan, Ravi, McMahon, Christopher W.

Research and Development, FUJIFILM Cellular Dynamics, Madison, WI, USA

Muscular Dystrophy is a devastating disease that affects approximately 1 in 6,000 males in the U.S. As treatments targeting skeletal muscle become available, patient quality of life and survival is dependent on understanding the impact of the disease on the cardiovascular system. Induced pluripotent stem cells (iPSC) are a powerful technology for disease modeling because large numbers of cells representing multiple lineages can be produced. Combining iPSC- derived cardiovascular cell types with a muscular dystrophy genotype (LMNA L35P) into 3D cardiac tri-cellular microtissues provides a new tool for studying the impact of muscular dystrophy on the heart. Blood was sourced from a muscular dystrophy patient carrying a defect in the lamin A/C gene (LMNA L35P) and reprogrammed to pluripotent iPSC. An isogenic corrected (LMNAcorr) control line was generated by gene editing and genotypes were confirmed. The LMNA L35P, corrected, and an apparently healthy normal (AHN) iPSC lines were differentiated to high purity cardiomyocytes, quiescent cardiac fibroblasts, and endothelial cells. All three cell types from the affected donor exhibited disorganized nuclear lamina structure, as reported in LMNA L35P skeletal myoblasts. Cardiomyocyte electrical activity and contraction of the three lines were characterized in monolayers. At high density, LMNA L35P cardiomyocytes showed irregular spontaneous electrical activity and contraction by impedance. Calcium transients in 3D microtissues were used to evaluate the contribution of the three cardiac cell types on cardiomyocyte function. When LMNA L35P cardiomyocytes were combined with ANH supporting cells in cardiac 3D microtissues in hypoxia, they exhibited a slower beat rate and decreased amplitude. In addition, unlike cardiac microtissues with normal healthy genotype and LMNAcorr, LMNA L35P isogenic cardiac microtissues did not display an inotropic response to isoproterenol. Interestingly, substituting LMNA L35P endothelial cells into microtissues containing AHN cardiomyocytes and AHN cardiac fibroblasts led to a decrease in cardiac calcium transient amplitude, suggesting a potential role for endothelial cells. These data demonstrate the power of iPSC models to study muscular dystrophy in the heart and provide new tools to develop treatments for this disease.

Keywords: Pluripotent stem cells; Muscular Dystrophy; Cardiac

W-125 POSTER WITHDRAWN



New Technologies and Future Directions

W-126

CONTROLLED STUDY MEASURING THE IMPACT OF A CELL CULTURE SPECIFIC SOFTWARE PLATFORM ON REPRODUCING A STEM CELL PROTOCOL

Conard, Kevin[1], Tubon, Thomas[2]

[1]CultureTrax, Acumium LLC, Madison, WI, USA, [2]Department of Workforce Development, BioMade, St. Paul, MN, USA

Many stem cell scientists experience challenges learning and reproducing protocols they have not previously run. Much of this difficulty arises from incomplete documentation of highly complex protocols and associated material recipes. There is also the problem of 'protocol drift'; a set of minor changes made to culture protocols that, for whatever reason, are not propagated across the entire organization. This leads to variability of results even though the 'same' protocol is being followed in the same lab. Interviews with representatives of both industry and academia were used to define the total effort required to train a new cell culture scientist to competency, which ranged from 6-12 months. The training costs include culture materials, BSC cabinet resources, and the mentorship of an experienced staff member. Another common observation was that staff members, regardless of the amount of training, did not all perform at the same level of competency. This NIH-funded controlled lab study tested the impact of CultureTrax software on scientists' ability to replicate a protocol they had not previously run, demonstrating dramatically improved success rates. Also tested was the ability of the experimental group to function at a consistent level of competency as compared to the control group when the protocol was known to be identical across the experimental group.

Funding Source

This research was supported by the National Institutes of General Medical Science of the National Institutes of Health under an STTR grant, award number R41GM12548.

Keywords: Process development; reproducible cell culture; stem cell training

W-127

PROMOTING DOPAMINE NEURON GENESIS BY HUMAN EMBRYONIC STEM CELLS (WA09) USING A NOVEL TRANSCRIPTIONAL REGULATOR

Delano-Taylor, Merritt K., Chowdary, Puneet, Okros, Max, West, Mary, Sweeney, Oliva

Biomedical Science, Grand Valley State University, Allendale, MI, USA

Nato3 (Ferdl3), is a basic helix-loop helix protein that is expressed in dopamine neuron progenitors in the floor plate of the midbrain during early embryonic development. Our previous data using a chick embryo model system show that Nato3 overexpression drives pro-dopaminergic (DA) genes in vivo in the midbrain. Others have shown in mouse knockout models that deletion of Nato3 decreases the number of mature DA neurons by half. Our unpublished data show that ectopic expression of a phosphorylation mimicking mutant form of Nato3 (PM-Nato3) induces expression of a significant breadth of pro-dopaminergic genes in multiple regions of the developing CNS (including regions outside the midbrain). These genes include Shh, Foxa2, Lmx1b, as well as mRNA expression of the mature DA neuron maker tyrosine-hydroxylase (TH). To determine if the potency of PM-Nato3 could promote the speed of appearance or total number of dopamine neurons in the WA09 human embryonic stem cell line we generated stable cell line containing a cassette with TET-ON inducible expression of PM-Nato3 and Neurogenin 2 (NEUROG2). Additionally we tested the ability of PM-Nato3 alone to induce pro-dopaminergic gene expression in WA09 cells in the presence and absence of standard dopaminergic differentiation medium. Our preliminary data indicate that PM-Nato3 can promote speed of expression and magnitude of TH expression in WA09 cells in all conditions. Further, the ability of PM-Nato3 to promote early expression of other dopamine neuron related genes is influenced by the media conditions. These data suggest that PM-Nato3 could serve as a tool to promote the formation of dopamine neurons by hESC.

Funding Source

ADVANCE Grant Proof-of-Concept Fund (Michigan Economic Development Fund); Tech Transfer Network postdoctoral fellowship support (Michigan Economic Development Fund); Campbell Foundation Research Grant; NIH R21-NS105436-01

Keywords: Neurogenesis; Dopamine; in vitro

W-128

ELUCIDATING EXPRESSION OF A CORRECTIVE ENZYME FOR MPS I THROUGH ADMINISTRATION OF A MODIFIED ADENOVIRAL VECTOR

Hurt, Sarah[1], Le, Steven Q.[1], Mendonça, Samir[2], Sorensen, Alexander[1], Curiel, David T.[2], Dickson, Patricia[1]

[1]Pediatrics, Washington University in St. Louis, MO, USA, [2]Radiation Oncology, Washington University in St. Louis, MO, USA

Mucopolysaccharidoses type I (MPS I) is a devastating lysosomal storage disorder that affects approximately 1 in 100,000 newborns. MPS I is characterized by mutations in the α-L-Iduronidase (IDUA) gene that lead to an accumulation of glycosaminoglycans in lysosomes. While treatment is available, it is non-curative, monetarily costly, and time-consuming. To this end, alternative therapies for MPS I have been highly sought after and one such approach is gene therapy. We are exploring the use of an adenoviral vector for the treatment of MPS I. Pursuing a gene therapy strategy with adenoviral vectors will allow us to target previously unreachable cell types such as the pulmonary endothelium through modification of the vector. To understand the outcomes of utilizing an adenoviral-based gene therapy for MPS I, we are administering an adenovirus encoded with the open reading frame for IDUA to the MPS I murine model and evaluating the consequential enzyme outcomes. We have also modified the vector to preferentially target pulmonary endothelial cells by modifying the adenoviral fiber to include a myeloid binding peptide that favorably transduces these cells. We hypothesize that a non-liver cellular source can subserve the role of cellular production of IDUA and traverse liver targeting, and, through future employment of gene editing technology, long-term correction can be achieved. These findings will better determine the feasibility of utilizing modified adenoviral vectors as a gene therapy vessel for treatment method for MPS I and other inherited, monogenic disorders.

Funding Source

This work was funded by an NSF Graduate Research Fellowship DGE-1745038

Keywords: Adenoviral; Mucopolysaccharidosis type I; Gene Therapy Vectors

W-130

DEVELOPMENT OF PURITY ANALYSIS METHODS FOR SYNTHETIC PRIME EDITING GUIDE RNAS FOR USE IN PRIME EDITING RESEARCH APPLICATIONS

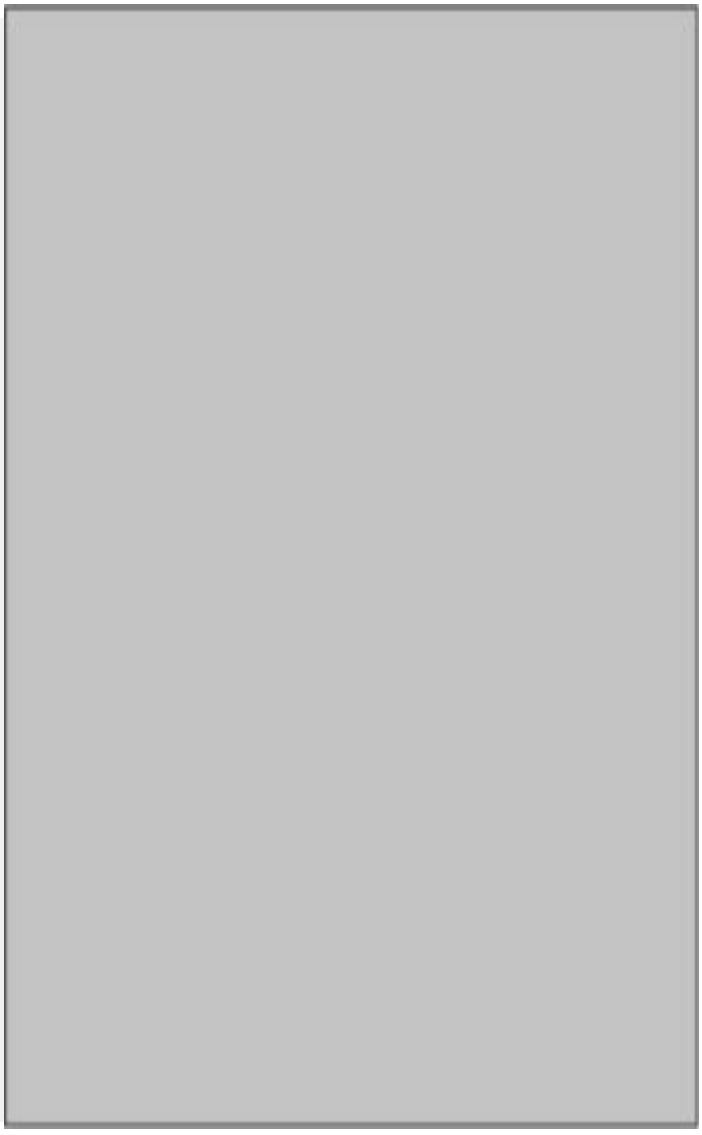
Jacobi, Ashley[1], Sturgeon, Morgan[1], Li, Tingting[2]

[1]Molecular Genetics, Integrated DNA Technologies, Coralville, IA, USA, [2]Applications Development, SCIEX, Brea, CA, USA

Prime Editing (PE) is a gene editing strategy that can perform targeted, small insertions, deletions, and substitutions without introducing double-stranded breaks in the genome. Prime editing utilizes a novel dCas9-reverse transcriptase (RT) fusion protein and a guide RNA (pegRNA) that targets the complex to the correct location in the genome. The pegRNA also enables RT priming and serves as a site-specific DNA repair template. In some applications, synthetic RNA oligonucleotides are favored because they can be characterized as discrete gRNA molecules for use in genome editing. However, the length requirement of pegRNAs (120–200 nt) as well as potential secondary structures due to complementary or partial complementary sequences present analytical quality control challenges. These secondary structures can lead to retention times for RNA oligos that do not resolve in a size-dependent manner on capillary electrophoresis. Therefore, there's a need for the development of purity analysis methods to overcome the formation of energetically favorable secondary structures of these chemically synthesized RNA molecules. The use of common denaturants in analytical analysis cannot entirely disrupt the hydrogen bonds of these molecules nor maintain the denatured state, resulting in inaccurate determination of the purity of the chemically synthesized pegRNA. Here, we describe a method that uses capillary gel electrophoresis laser-induced fluorescence method (CGE-LIF), which can offer high resolution and provide purity quantification of pegRNAs. We also study the effect of different purity levels on prime editing in functional studies using K562 cells.

Keywords: Prime Editing; Gene Editing; CRISPR

W-132 POSTER WITHDRAWN



W-133

REAL-TIME AND CONTINUOUS OXYGEN SENSING FOR CHARACTERIZATION OF STEM CELL CULTURE ENVIRONMENTS

Phang, Katelynn[1], Bryan, Richard[2], Inman, Walker[3]

[1]Research and Development, Lucid Scientific, Inc., Atlanta, GA, USA, [2]Software, Lucid Scientific, Inc., Atlanta, GA, USA, [3]Research, Lucid Scientific, Inc., Atlanta, GA, USA

Numerous factors have been shown to affect stem cell growth and maintenance of stemness, but little is known about the effects of oxygen concentration in media on stem cell proliferation and differentiation. It has long been assumed that typical ambient air oxygen concentration is sufficient for stem cell growth, but recent discoveries have highlighted the importance of in media and environmental oxygen concentration levels reflecting in vivo conditions as closely as possible. These advances in stem cell culture and

differentiation are leading to changes in the setup of in vitro stem cell culture experiments that require better control and characterization of the oxygen environment in the media around the cells. This environment is a function of both the atmospheric oxygen concentration as well the changes in oxygen concentration caused by cellular consumption. To address the aforementioned need, we have developed a real-time, oxygen sensing platform for measuring both oxygen concentration and oxygen consumption rates (OCR) of many cell types (including iPSCs) in standard multiwell plates. Here, we describe the operating principles and usage of the system as well as present oxygen data measured from cell culture experiments showing cell growth over time. These experiments show the stark differences in oxygen consumption and oxygen concentration between different seeding densities of C2C12 cells through the culture period. For low seeding densities (500, 750, 1000, 1250, and 1500 cells/well), OCR stabilizes at 50-60 fmols/mm2/s after 65-90 hours of culturing and the cells are observed to be partially differentiated. Initial OCR differences between the low densities are read to be 1 fmols/mm2/s per 250 cell difference. OCR rises to and then plateaus at 120 -150 fmols/mm2/s around 30 hours after a media change as the cells become fully differentiated. For higher densities (5k, 10k, 20k, and 40k cells/well), OCR also stabilizes at the same level (50-60 fmols/mm2), but this stabilization occurs much faster (around 30 hours for 40k cells/well). These results demonstrate the value of the use of real-time oxygen sensing for better characterization and understanding of cell cultures, including stem cell cultures, and differentiation over time.

Keywords: oxygen; real-time; characterization

W-134

STREAMLINED DETECTION OF CRISPR EDITING USING THE RHAMPSEQ CRISPR ANALYSIS SYSTEM

Rettig, Garrett, Jacobi, Ashley, Kurgan, Gavin, McNeill, Matthew, Sturgeon, Morgan

Research and Development, Integrated DNA Technologies, IA, Coralville, USA

Next generation sequencing (NGS) is the gold-standard method for characterizing the efficiency and specificity of genome editing with CRISPR systems. As the field progresses, CRISPR is being used as a screening tool and for therapeutics development. As a result, robust and accurate high-throughput genome editing analysis methods are needed. We have developed the rhAmpSeq CRISPR system, which includes straight-forward primer design, library preparation, and editing analysis for single target interrogation associated with CRISPR screening projects. Additionally, the rhAmpSeq CRISPR system facilitates highly multiplexed, targeted NGS library preparation for simultaneous characterization of on- and off-target editing, used for lead target characterization. Here, we highlight a streamlined workflow using rhAmpSeq CRISPR for high-throughput genotyping of 95 edited genes involved in DNA repair and compare the editing analysis to other available pipelines. Additionally, we highlight the workflow for quantifying off-target editing levels of the lead gRNA targets. To ensure broad accessibility, we developed a web interface hosting our NGS analysis software solution, CRISPAltRations, which uses cloud-based resources to enable high-throughput batch analyses. Our results highlight the ability for the rhAmpSeq system to support high-throughput CRISPR screening analysis and quantification of off-target editing at 1000's of genomic loci (predicted or empirically defined to be of risk) in one multiplexed PCR. Last, we present the sensitivity and specificity afforded by coupling these tools with an in-depth evaluation of 20 genomic targets containing indel frequencies ranging from 0.05-3.5%. When applying the specific requirements our investigation suggests sensitivity and specificity in the evaluation of CRISPR editing can approach 95% confidence.

Keywords: CRISPR; Genome Editing; NGS Analysis

Visual System

W-135

POTENTIAL THERAPIES FOR KIR7.1 CHANNELOPATHY

Kabra, Meha[1], Shahi, Pawan K.[1], Spillane, Allison[2], Wang, Yuyuan[3], Sinha, Divya[1], Newby, Gregory A.[5], Edwards, Kimberly[4], Theisen, Cole, Saha, Krishanu[6], Gamm, David M.[7], Liu, David R.[5], Macdonald, Susan[8], Hall, Sarah M.[8], Sabados, Jeff[8], Gong, Shaoqin Sarah[9], Pattnaik, Bikash R.[10]

[1]Department of Pediatrics, McPherson Eye Research Institute, University of Wisconsin, Madison, WI, USA, [2]Department of Pediatrics, University of Wisconsin, Madison, WI, USA, [3]Department of Biomedical Engineering, Department of Materials Science and Engineering, University of Wisconsin, Madison, WI, USA, [4]McPherson Eye Research Institute, Waisman Center, University of Wisconsin, Madison, WI, USA, [5]Merkin Institute of Transformative Technologies in Healthcare, Broad Institute of Harvard and MIT, Department of Chemistry and Chemical Biology, Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA, [6]Department of Biomedical Engineering, McPherson Eye Research Institute, University of Wisconsin, Madison, WI, USA, [7]Department of Ophthalmology and Visual Sciences, McPherson Eye Research Institute, Waisman Center, University of Wisconsin, Madison, WI, USA, [8]Hubble Therapeutics, Boston, MA, USA,

[9]*Department of Biomedical Engineering, Department of Materials Science and Engineering, Department of Chemistry, University of Wisconsin, Madison, WI, USA,* [10]*Department of Pediatrics, Department of Ophthalmology and Visual Sciences, McPherson Eye Research Institute, University of Wisconsin, Madison, WI, USA*

Leber Congenital Amaurosis 16 (LCA16) is a monogenic inherited ocular channelopathy caused by several biallelic point mutations in KCNJ13, which affect the retinal pigmented epithelial (RPE) layer of the retina. W53X (c.158G>A; tGg>tAg) mutation in KCNJ13 results in a truncated Kir7.1 protein. In the current study we aim to use two therapeutic approaches to restore the Kir7.1 channel function in W53X-LCA16-patient derived iPSC RPE and compared the outcomes for potency, efficiency, and safety (1). clinical grade AAV5-Kir7.1 HUB101 gene-therapy: Expression profile of AAV5-Kir7.1 transduced iPSC RPE cells indicated dose-dependent expression of Kir7.1 transcript. Protein expression confirmed the successful translation of exogenous Kir7.1 and trafficking to the membrane. Electrophysiology assay showed the rescue of Kir7.1 protein function in transduced cells, comparable to isogenic iPSC RPE cells. (2). Adenosine-CRISPR-base editor (ABE), delivered by silica nanoparticles (SNP): The screening of ABEs (mRNA and protein as RNP) for W53X (TaG>TgG) correction in HEK293 stable cells showed that the mRNA approach (50% efficiency) is better than RNP (25%). Nanoparticle-mediated delivery of ABE-mRNA in fibroblasts (47%) and post-mitotic hiPS-RPE (20%) established efficient therapeutic base editing that could now be undertaken for in vivo BE experiment. On target Indel mutagenesis (< 3%) and deep sequencing of potential off-target sites (< 1%) reassured the safety of ABEs. Electrophysiology showed the rescue of channel function in the edited iPSC RPE. We conclude that, endogenous gene correction via ABE delivered using SNPs has the upper hand (long-term, permanent change free of immune response unlike AAVs) over exogenous supplementation of Kir7.1 via AAVs, but it requires mutation-specific design to correct disease phenotype. Also, Channelopathies like LCA16 require bi-allelic gene correction in the diseased cell to produce a multimeric functional channel. AAV-gene augmentation is a universal approach and beneficial for hard to edit mutations because of sequence complexity. Together, these findings demonstrate that both the therapies have significant outcomes and can be developed as potential therapies for the treatment of pediatric blindness.

Funding Source

SCRMC Postdoc fellowship, R24EY032434, R01EY024995, MERI

Keywords: Gene-therapy; CRISPR-Base-Editing; Childhood blindness

W-136

IN VITRO MODELING AND RESCUE OF CILIOPATHY ASSOCIATED WITH MUTATIONS IN IQCB1 (NEPHROCYSTIN 5) USING PATIENT-DERIVED CELLS

Kruczek, Kamil[1], Qu, Zepeng[2], Welby, Emily[3], Shimada, Hiroko[4], Hiriyanna, Suja[5], English, Milton A.[2], Zein, Wadih M.[6], Brooks, Brian P.[6], Swaroop, Anand[2]

[1]*National Eye Institute, National Institutes of Health, Bethesda, MD, USA,* [2]*Neurobiology, Neurodegeneration & Repair Laboratory, National Eye Institute, National Institutes of Health, Bethesda, MD, USA,* [3]*Department of Cell Biology, Medical College of Wisconsin, Milwaukee, WI, USA,* [4]*Department of Physiology, Keio University, Tokyo, Japan,* [5]*Ocular Gene Therapy Core, National Eye Institute, National Institutes of Health, Bethesda, MD, USA,* [6]*Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, USA*

Mutations in IQCB1/NPHP5 gene encoding the ciliary protein Nephrocystin 5 cause early-onset blinding disease Leber congenital amaurosis (LCA), together with kidney dysfunction in Senior-Løken Syndrome. For in vitro disease modeling, we obtained dermal fibroblasts from NPHP5-LCA patients, which were reprogrammed into induced pluripotent stem cells (iPSCs) and differentiated into retinal pigment epithelium (RPE) and retinal organoids. Patient fibroblasts and RPE demonstrated aberrantly elongated ciliary axonemes. Organoids revealed impaired development of outer segment structures, which are modified primary cilia, and mislocalization of visual pigments to photoreceptor cell soma. All patient-derived cells showed reduced levels of CEP290 protein, a critical cilia transition zone component interacting with NPHP5, providing a plausible mechanism for aberrant ciliary gating and cargo transport. Disease phenotype in NPHP5-LCA retinal organoids could be rescued by AAV-mediated NPHP5 gene augmentation therapy. Our studies thus establish a human disease model and a path for treatment of NPHP5-LCA.

Funding Source

This research was supported by the Intramural Research Programs of the National Eye Institute (ZIAEY000450 and ZIAEY000546 to A.S.)

Keywords: Ciliopathy; Disease modeling; Retina

DAY 2: THURSDAY, 22 SEPTEMBER

4:30 PM – 5:30 PM
Poster Session II

Cell and Gene Therapy Manufacturing

T-101

HMGB1 BOX A GENE THERAPY FOR PREVENTION OF CELLULAR SENESCENCE IN AGING ORGANOID MODEL OF THE LACRIMAL GLAND

Rodboon, Teerapat[1], Bhummaphan, Narumol[2], Ferreira, Joao[3], Matangkasombut, Oranart[4], Mutirangura, Apiwat[2]

[1]Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand, [2]Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, [3]Avatar Biotechnologies for Oral Health and Healthy Longevity Research Unit, Chulalongkorn University, Bangkok, Thailand, [4]Microbiology and Research Unit on Oral Microbiology and Immunology, Chulalongkorn University, Bangkok, Thailand

Lack of tear secretion caused by age-related lacrimal gland (LG) impairment is a major cause of dry eye disease (DED). It is characterized by degeneration of secretory epithelia, and there is no effective treatment that can restore homeostasis. Also, there is neither a tearing nor an aging model of the LG that hold a cellular complexity reproducible enough for mechanistic investigations or drug screening applications. Therefore, the development of an aging LG model is essential to discover novel DED therapies. An in vitro three-dimensional (3D) magnetic bio-assembled organoid model induced by chemical mutagenesis is proposed as a tool for studying cellular senescence via spatial proteogenomics and to validate anew gene therapy to prevent age-related DED. Primary cells from porcine LG were used for fabricating a functional LG organoid with a magnetic 3D bioprinting (M3DB) platform. The M3DB-derived LG organoids displayed functional compartments of acinar, ductal, and myoepithelial like in native tissue. The organoids can be stimulated by carbachol, an acetylcholine agonist, by displaying calcium immobilization after 2h. To develop an aging model, endogenous DNA damage was induced to the organoids by treatment with 10 μM etoposide, a telomerase inhibitor. Then, aging hallmarks were assessed including mitochondrial-driven ATP activity, accumulation of senescence-associated oxidative stress and β-galactosidase, and amylase functional activity. The etoposide-induced LG organoids displayed all aging markers above. Hence, this model was utilized as a LG aging model mimicking DED to validate a gene therapy strategy with HMGB1 Box A. The HMGB1 Box A, a key aging therapeutic gene that was proven by our collaboration on stabilizing the DNA durability, was transfected into the organoid before aging induction. Gene therapy retained cell viability and functionality in our aging model by displaying high numbers of viable cells and eliciting amylase activity. Meanwhile, the organoids without gene therapy showed a complete loss of 3D integrity and cell viability. These findings support the roles of HMGB1 Box A on genomic stability and aging prevention in LG cellular senescence and aging. Moreover, this aging LG organoid model could pave the way for in vitro applications aiming for DED mechanistic studies and drug discovery.

Funding Source
T.R. is supported by a Postdoctoral Fellowship, Ratchadapisek Somphot Fund, Chulalongkorn University.
Keywords: Dry eye disease; Cellular Senescence; Gene therapy

T-102

SCALABLE DOWNSTREAM PROCESS DEVELOPMENT FOR HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED PRODUCTS MANUFACTURING

Sato, Sho[1], Kurakazu, Tomoaki[1], Kagawa, Hideaki[1], Santiago-Rodriguez, Yina[1], Fuhrken, Peter[2]

[1]Process Engineering, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA, [2]Operation, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA

Many in vitro culture systems for the differentiation of induced pluripotent stem cells (iPSC) are an extension of the basic laboratory cell culture methods used to pioneer the differentiation of these cells. These T-flask or spinner flask-based cultures and associated open centrifugation operations for downstream processing can be sufficient for initial market penetration – in the case of a research tool product – or pre-clinical and early clinical trials – for cellular therapies. As demand for these products increase, it is necessary to scale up or scale out these operations. Moreover, for clinical application, it is advantageous to use a single-use, closed and controlled system for cell processing. While much attention is often paid to the upstream cell culture operations in cell therapy manufacturing, it is equally important to utilize robust downstream operations including cell washing, concentration, formulation, and cryopreservation. Toward this end, we have evaluated multiple scale-up and closed system technologies for cell washing and concentration for iPSC and iPSC-derived cell

products in our manufacturing including conventional centrifugation and counter-flow centrifugation. We found that the appropriate downstream technology and its operation scheme varies depending on the volume reduction ratio, shear sensitivity, carryover limitations, and throughput requirements.
Keywords: Scaleup; Cell wash and concentration; Downstream processing

T-103

IDENTIFYING DIFFERENCES BETWEEN HUMAN PRIMARY VALVULAR INTERSTITIAL CELLS AND CARDIAC VENTRICULAR FIBROBLASTS

Shabnam, Fathima

Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA

Cardiac pathologies involve various cells of the heart and understanding the differences and functionalities of these subtypes is crucial for developing specific therapies. Quiescent valvular interstitial cells (qVICs) are a dynamic population of cells that are responsible for maintaining the physiological structure and function of valves. These cells arise mostly from endocardial cell progenitors whereas myocardial fibroblasts, such as left ventricular cardiac fibroblasts (LVCFBs), are mostly epicardial cell derived. Here, we explore the molecular and functional differences between qVICs and LVCFBs. We identify genetic markers from bulk transcriptomic analyses, differences in in vitro mineralization potential under metastatic and dystrophic conditions, and differences in extracellular matrix composition using mass spectrometry analysis.
Keywords: qVIC; LVCFB; hPSC

T-104

IDENTIFICATION OF PUTATIVE EARLY PREDICTORS AND MODULATORS OF CARDIOMYOCYTE DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

Simmons, Aaron[1], Bayne, Elizabeth[2], Ge, Ying[3], Palecek, Sean1, Zhu, Yanlong[3]

[1]Department of Chemical and Biological Engineering, University of Wisconsin, Madison, WI, USA, [2]Chemistry, University of Wisconsin, Madison, WI, USA, [3]Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA

Numerous protocols exist for differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes (CMs). Although these methods have improved in efficiency over the past decade, they remain highly variable in their resultant purities, not only among different source hPSC lines but also between batches in the same cell line. This substantial heterogeneity of hPSC-CM product outcomes points to poorly-understood, highly sensitive, and uncontrolled variables present within the overall process and results in poor overall process robustness. Herein, we have undertaken a multi-omic discovery approach to identify early dynamic differences in cell attributes between high- and low-purity hPSC-CM differentiations to provide systems-level insights into underlying mechanisms which drive these populations to divergent endpoints. Specifically, we are combining metabolomic, proteomic, lipidomic, and transcriptomic analyses collected throughout the differentiation process for high- and low-purity (as assessed by terminal %cTnT+ via flow cytometry) differentiation batches. To date we have identified novel putative candidate early quality attributes for process monitoring and cellular pathways which may be able to be modulated to augment process robustness in a scaled manufacturing context. Integration of data through the “omics cascade” is providing key insights into divergent temporal dynamics and signaling regulation which single-omic methods alone would not be able to achieve. Further confirmatory studies in additional cell lines, and initial investigations into enhancing process robustness (i.e. consistency of high-purity CM differentiations) via target pathway modulation are underway. In addition to gaining fundamental insights into the underlying biology of the differentiation process, our findings are being applied to 1) identify putative critical quality attributes for use in on- or at-line analytics for continuous process monitoring, 2) enhance process robustness through the development of protocols aimed at depressing off-target pathways and enhancing on-target ones, and 3) establish potential feedforward/feedback control schemes based on real-time analytics to respond to in-process intermediate quality attributes through rational adjustment of process parameters.
Funding Source
NIH 5 T32 GM008349 NSF EEC-1648035 NIH R01EB007534 NIH R01HL148059
Keywords: pluripotent stem cells; cardiomyocytes; process monitoring

Central Nervous System Diseases

T-105

THE ESSENTIALOME OF IMPRINTING GENES IN EARLY EMBRYONIC DEVELOPMENT REVEALS THERAPEUTIC TARGETS OF IMRPINTING DISORDERS

Kinreich, Shay, Benvenisty, Nissim

Genetics, Hebrew University of Jerusalem, Israel

Imprinted genes are expressed in uniparental fashion and their transcription is controlled by differentially methylated regions. Genomic imprinting regulates early human development and is involved in multiple disorders such as Angelman-syndrome, Beckwith-Wiedemann-Syndrome, Prader-Willi-syndrome, and Russel-Silver-syndrome. Previous works from our lab discovered that human embryonic stem cells (ESCs) that contain either only paternal epigenome (androgenetic ESCs) or only maternal epigenome (parthenogenetic ESCs) have unbalanced contribution to the different organs, and the maternal epigenome is essential for the proper development of the brain. We have recently established a genome-wide screening in human pluripotent stem cells, defining the essentialome of undifferentiated cells and their differentiated progenies. To uncover the role of imprinting in early human development we set out to determine the essentialome of imprinted genes. Our analysis demonstrated that none of the maternal imprinted genes are essential for the maintenance of pluripotency or for the differentiation of the germ layers, but there are several imprinted genes that are essential for neuron development. Using CRISPR-Cas9 technology, we created mutant ESCs for three of these imprinted genes, which are also involved in Angelman syndrome and Beckwith-Wiedemann syndrome. Neuronal differentiation of single and double mutants showed lower capacity of neurogenesis and suggested the involvement of cytokines in their regulation. These results clarify the effect of maternal genes in neuronal development and in neural imprinting disorders and should assist us in identifying novel therapeutic agents.

Keywords: Human pluripotent stem cells; Imprinting disorders; Epigenetics

T-106

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL ORGANOIDS INCORPORATING MICROGLIA FOR INTERROGATION OF NEURAL INFLAMMATION

Lebakken, Connie S., Favreau, Peter M., Greuel, Kaylie F., Kulas, Joshua M., Parham, Kailyn, Richards, William, Zimmermann, Joshua M.

Research and Development, Stem Pharm, Inc., Madison, WI, USA

Neuroinflammation is a complex response to brain injury involving activation of the innate immune response, release of inflammatory mediators, and the generation of reactive species resulting in downstream effects including vascular compromise, oxidative stress, and neurotoxicity. Neuroinflammation is a critical component in the etiology and progression of many diseases including neurodegenerative diseases, stroke, trauma, seizures, neuropsychiatric disorders, and brain cancers. There is a critical need to develop advanced neural microphysiological systems (MPS) that can model neuroinflammation and bridge the gap between simplistic cell models and clinical data. Stem Pharm has leveraged its proprietary synthetic hydrogel platform to enable the formation of complex, reproducible, induced pluripotent stem cell (iPSC)-derived neural organoids containing microglia and vascular cells that are well-suited to study neural inflammation. The neural organoids are formed in 96-well plates from iPSC-derived neural precursor cells, microglia, endothelial cells, and mesenchymal stem cells and are ready for screening 21 days after initial plating. Single cell transcriptional analysis demonstrates that the organoids are cell-type diverse, containing multiple neuronal subtypes, astrocytes, microglia, and endothelial cells. Bulk and single cell RNA-seq analysis demonstrates high intraclass correlation and low coefficients of variation between biological replicates. Incorporated microglia are distributed throughout the organoids, display ramified morphology resembling in vivo morphology, and demonstrate a gene expression signature that strongly correlates with in vivo microglia expression. Modulation of microglia within the organoids to pro- and anti-inflammatory phenotypes was validated through stimulation with lipopolysaccharides, interferon gamma, TGFβ & IL-10, or IL-4 & IL-13. These data demonstrate the promising application of Stem Pharm's advanced neural organoids for facilitating translation between pre-clinical and clinical discovery and development in the active area of neuroinflammation.

Funding Source

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Keywords: Neuroinflammation; microglia; organoids

T-107

OLIGODENDROCYTE PROGENITOR CELLS AS A THERAPY FOR DEMYELINATING DISORDERS

Lepack, Ashley, Wolfarth, Andrew, Luther, Raven, Stitt, Nick, LoSchiavo, Deven, Cutia, Thomas, Starikov, Lev, Petko, Alyssa, Efthymiou, Anastasia, Tam, Edmund, Wang, Jing, Hsu, Juliana, Irion, Stefan, Hudzik, Tom, Patsch, Christoph, Paladini, Carlos

Neurology, BlueRock Therapeutics, New York, NY, USA

Loss of myelination within the central nervous system (CNS) leads to the gradual loss of neurological function and subsequent symptoms including cognitive and motor deficits. Current treatments for demyelinating disorders include management of symptoms and supportive help. However, these treatments only alleviate specific symptoms, and to date there are no treatments to help prevent or rescue the long-term neurological outcomes. Oligodendrocyte progenitor cells (OPCs) are critical for maintaining CNS homeostasis and producing myelination. Given that OPCs are highly mobile and readily differentiate into mature axon-wrapping myelin, they present as a unique cellular medicine in treating demyelinating conditions in which endogenous OPCs are dysfunctional. We have recently developed a novel bioprocess to derive OPCs from human induced pluripotent stem cells (OG01) and have demonstrated that OG01 cells in vitro are molecularly and functionally comparable to endogenous OPCs. For our current studies, we assessed the efficacy of OG01 in the Shiverer mouse, a hypomyelinated mouse model. Briefly, we transplanted OG01 cells into the brainstem and cerebellum (regions with large volumes of white matter, and importance for motor coordination) of Shiverer mice. We found that OG01 rescued ataxic gait compared to vehicle controls, a key phenotype observed in clinical populations. Furthermore, rescue in ataxic gait correlated with functional remyelination of the cerebellum as measured by the presence myelin basic protein (MBP) expression that is absent in the Shiverer mouse. Additionally, we measured conduction velocity across major axon bundles, as a readout for functional myelination. Taken together, these data demonstrate that OG01 is sufficient to rescue molecular, functional, and subsequent behavioral phenotypes in a hypomyelinated mouse, suggesting that OG01 has the potential to be a treatment option for demyelinating disorders where existing therapeutics are limited.

Keywords: Oligodendrocyte; Myelination; Ataxic gait

T-108

HIGH-THROUGHPUT LABEL-FREE IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF AXONS IN NEURONAL NETWORKS

Li, Zhuoliang[1], Jäckel, David[1], Clément, Blandine[2], Thammavongsa, Anaïs[1], Obien, Marie Engelene[1]

[1]MaxWell Biosystems, Zürich, Switzerland, [2]Institute for Biomedical Engineering, ETH Zürich, Switzerland

Axons enable neuronal communication by propagating electrical signals within neuronal networks. Its dysfunction plays a central role in deliberating pathologies such as Parkinson's Disease and Amyotrophic Lateral Sclerosis. Therefore, access to axonal physiology is crucial for studying information processing within neuronal networks and accelerating drug development for neurological disorders. High-density microelectrode array (HD-MEA) technology enables chronic label-free in-vitro extracellular recordings of axonal action potentials in neurons. MaxOne (single-) and MaxTwo (multi-well) HD-MEA Systems (MaxWell Biosystems, Switzerland) simultaneously capture fast propagating action potentials along multiple axons. Here, we present the MaxLab Live AxonTracking Assay, a software which automatically detects and functionally characterizes axonal signals across hundreds of neurons within a network. We reliably identified and measured from axonal arbors in primary neuronal cultures as well as human iPSC-derived glutamatergic and motor neurons over multiple weeks. We tracked the signal propagation to deduce the conduction velocity, axonal length, and number of axonal branches. We found that the neuronal and branch propagation velocity significantly increased between DIV 13 and 20, corresponding to the maturation of the neuronal network. In conclusion, MaxLab Live AxonTracking Assay combined with HD-MEA technology enables reliable access to electrophysiological recordings of axons, providing a novel functional phenotype for neurological disease modelling and drug screening studies.

Funding Source

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Keywords: Microelectrode Array; iPSC derived neurons; Primary neurons

T-109

DIFFERENTIATING MESENCHYMAL STEM CELLS FROM IPSCS FOR ANALYSIS OF TYPE 2 DIABETES AND RELATED TRAIT GWAS LOCI

Ventresca, Christa[1], Varshney, Arushi[2], Orchard, Peter[2], Monteiro da Rocha, Andre[3], Laakso, Markku[4], Tuomilehto, Jaakko[5], Lakka, Timo[4], Mohlke, Karen[6], Boehnke, Michael[7], Scott, Lara[7], Koistinen, Heikki[5], Collins, Francis[8], Herron, Todd[3], Parker, Stephen[2]

[1]Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA, [2]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA, [3]Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI, USA, [4]Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland, [5]Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland, [6]Department of Genetics, University of North Carolina, Chapel Hill, NC, USA, [7]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA, [8]NHGRI, National Institutes of Health, Bethesda, MD, USA

Background Some type 2 diabetes (T2D) and related trait genome wide association study (GWAS) signals likely impact T2D risk via skeletal muscle, a primary insulin response tissue. We previously generated in vivo mesenchymal stem cell (MSC) data through single nucleus (sn-)multiomics (RNA+ATAC) profiling of 286 skeletal muscle biopsies and found that a subset of GWAS signals colocalize with cell-type specific e/caQTL active in skeletal muscle MSCs. One method to investigate these signals is to differentiate MSCs from induced pluripotent stem cell (iPSC) lines, allowing us greater flexibility to explore these pathways. From the same individuals with muscle sn-multiomics, we derived 50 iPSC lines from fibroblasts. To develop a greater understanding of T2D and related trait mechanisms, we can use techniques on the iPSCs differentiated to MSCs to nominate target genes and regulatory elements at each GWAS signal. These approaches include sn-multiomics and CUT&Tag, a sensitive method to analyze histone marks. We have already optimized both techniques on multiple cell types. Hypothesis We hypothesize that MSCs can be differentiated from iPSC lines for the purpose of investigating the impact of MSC-specific molecular mechanisms on T2D risk and related traits. Results Here, we demonstrate that MSCs can be derived from iPSC lines for deep molecular phenotyping. These iPSC-derived MSCs display MSC morphology, expression of MSC marker genes, and loss of expression of pluripotency and hematopoietic markers. Over the course of the differentiation, Oct3/4, a pluripotency marker, drops from 73.4% of cells expressing this down to 0.42%. Meanwhile, CD73, a MSC marker, increases from 7.25% of cells expressing up to 60.2%, based on flow cytometry analysis. We are currently performing a time course analysis with this experimental system with ten independent iPSC lines all multiplexed to explore the trajectory from iPSC progenitors to fully-differentiated MSCs, and compare in vitro sn-multiome signatures to those observed in matched in vivo MSCs. Future Directions We will use a cohort of 50 iPSC-derived MSC samples to investigate stimulatory state specific genetic regulatory effects (e/caQTL) within T2D pathways and will validate our results by performing CRISPRi knockdown and CRISPRa activation on a subset of nominated loci.

Keywords: type 2 diabetes; multiomics; mesenchymal stem cells

Immuno-Oncology: CAR-T and NK Cell Therapies

T-111

TARGETED TRANSGENE INSERTION IN NATURAL KILLER CELLS USING CRISPR-CAS9

Shankar, Keerthana[1], Zingler-Hoslet, Isabelle[1], Capitini, Christian[2], Saha, Krishanu[1]

[1]Biomedical Engineering, University of Wisconsin, Madison, WI, USA, [2]Pediatrics, University of Wisconsin, Madison, WI, USA

Natural killer (NK) cells are innate cytotoxic lymphocytes capable of killing virally infected cells and malignant tumors. Unlike T cells, NK cells are HLA-agnostic and cause little to no Graft vs. Host Disease in allogenic transfusions, making them excellent candidates for off-the-shelf therapies. However, current techniques to insert transgenes into NK cells primarily employ viral vectors. Viral methods can give rise to complications such as insertional mutagenesis, which can lead to gene silencing or oncogene activation. Here we have developed a non-viral editing strategy in primary NK cells. With this method, the transgene is encoded in a linear double stranded DNA (dsDNA) template and produced by polymerase chain reaction (PCR). The template includes homology to the intended target for insertion that is defined by nuclease mediated double strand break formation by a Cas9-single guide RNA (gRNA) complex. We show efficient (>80%) CRISPR knock-out of the inhibitory NKG2A receptor encoded by the KLRC1 gene within primary NK cells. Optimization of electroporation timing showed that delivery of 3 ug of donor DNA on day 4 of ex vivo expansion results in gene knock-in rates of up to 24% for transgenes as large as 2.5 kb. Lastly, we report that the use of K562-mbIL15-41BBL feeder cells improves expansion of CRISPR edited cells by approximately twenty-fold. This data provides a proof-of-principle for on-target integration of a long transgene within primary NK cells via CRISPR-Cas9 genome editing, which could be further optimized to enable the non-viral manufacture of genetically-programmed NK immunotherapies.

Keywords: CRISPR; Cell Manufacturing; Immunotherapy

T-112

SIRPA-KNOCKOUT MACROPHAGES FROM HUMAN PLURIPOTENT STEM CELLS HAVE SUPERIOR ANTI-TUMORIGENIC CAPACITY AGAINST CD47+ CANCERS

Smith, Portia[1], Majumder, Aditi[1], Emmen, Isla[1], Ayuso, Jose M.[2], Beebe, David[2], Slukvin, Igor[1]

[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA, [2]Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA

Solid tumor cancers are notoriously difficult to treat with current immunotherapies due to the immunosuppressive tumor microenvironment (TME). The TME actively excludes cytotoxic T cells while heavily recruiting tumor associated macrophages (TAMs) for means of growth and survival. The symbiotic relationship between TAM and tumor can be much attributed to cancer-expression of the "don't eat me" receptor, CD47, which is recognized by SIRPa on macrophages and blocks phagocytosis. To overcome this obstacle, we hypothesized macrophages without SIRPa protein will have enhanced anti-tumorigenicity. Macrophages have unique access to the hostile TME, long life-spans, and a natural capacity for phagocytosis and cytotoxicity; making them an attractive candidate for immunotherapy. Human induced pluripotent stem cells (iPSCs) serve as a renewable and genetically pliable source to generate iPSC-Macrophages (iMacs) for the treatment of solid tumors. We generated SIRPa-knockout (KO) iPSCs and developed a chemically defined xeno-free protocol for efficient generation of macrophages by 2D hemogenic endothelium formation followed by differentiation into monocytic cells and M0 macrophages using M-CSF, IL-3, and IL-6. Using these methods, we were able to generate between 1-3 x 10^8 macrophages from one million iPSCs. Wild type (WT) and SIRPa-KO iMacs displayed a similar phenotype and successfully polarize into M1 and M2 subtype when activated by LPS+IFN-gamma or IL-4, respectively. During in vitro cancer challenges, SIRPa-KO iMacs displayed superior phagocytosis and cytotoxicity of multiple CD47+ cancer cell lines with addition of cancer-specific monoclonal antibodies (mAb) compared to WT. We observed little to no phagocytosis of CD47+ cancer cells by SIRPa-KO or WT iMacs without mAb, substantiating the clinical safety of SIRPa-KO iMacs interacting with healthy CD47+ cells. Moreover, despite the presence of mAb, tumor growth was exacerbated when co-cultured with WT iMacs; contrary to the suppression observed with SIRPa-KO iMacs. In this novel system, we conclude that SIRPa-KO iPSCs produce functional macrophages that confer superior anti-tumor potential through enhanced phagocytosis, cytotoxicity, and tumor-growth-suppression against CD47+ cancers and may be clinically translated into an immunotherapy.

Keywords: Macrophages; Immunotherapy; Solid tumors

New Technologies and Future Directions

T-113

TUNABLE ENCAPSULATION OF STEM CELLS AND THEIR DERIVATIVES FOR SUSTAINED, LOCAL CELL-DELIVERY

Stover, Harald[1], Fuehrmann, Tobias[2], Cochrane, Nathan[2], Ellis, Carl[3], Ros, Samantha[4], Kornberg, Tatiana[5]

[1]Allarta Life Science Inc, Dundas, ON, Canada, [2]Regenerative Medicine, Allarta Life Science, Hamilton, ON, Canada, [3]Capsule, Allarta Life Science, Hamilton, ON, Canada, [4]Polymer, Allarta Life Science, Hamilton, ON, Canada, [5]Preclinical, Allarta Life Science, Hamilton, ON, Canada

Cell replacement therapies hold the promise of functional cures for a wide range of indications, including endocrine and inflammatory disorders. Although each disease follows a unique pathophysiology, there are common challenges, including immune rejection or clearance of transplanted cells, foreign body response to implanted materials, poor metabolic connection of the graft, and cell failure. Allarta has developed a scalable, immune-privileged platform based on synthetic hydrogels that can support either (I) cell containment for chronic disorders or (II) a sustained release over days to weeks of therapeutics for acute applications. Combining the encapsulation technology with human stem cells with safety switches, could further enable robust, safe cell therapies by 1. Protecting grafted cells from immune rejection while still allowing metabolic exchange. 2. Physically containing rapidly expanding cells, and 3. Providing added safety controls, by leveraging safe cell technology to ablate proliferating transplanted cells. Allarta's technology enables pluripotent and mesenchymal stem cells to survive as single cells or cell aggregates within alginate capsules for multiple weeks in vitro, depending on culture conditions. Stem cell proliferation could be controlled by varying extracellular matrix molecules concentrations within the capsules. While standard alginate capsules released proliferative cells within days to weeks, the non-degradable hydrogels limited cellular escape. These encapsulation systems allowed the normal function and Ganciclovir-triggered ablation of proliferative cells through the FailSafe™ system (cells obtained under a REA from PanCELLa, https://pancella.com/). As well, good persistence and minimal fibrosis was shown for empty and cell-containing capsules in C57BL/6 mouse models, with good cell survival, containment and function, including human donor islets. The current data describes an encapsulation technology platform that offers a range of hydrogels that can contain strongly proliferative cells or facilitate sustained release of therapeutics including cells for shorter term applications. Combining immune-privileged capsules with a safe-cell line offers unique opportunities to deliver functional and safe cell-based therapies.

Keywords: Encapsulation; Cell Therapy; Sustained Cell Delivery

T-115

IN VIVO TARGETED DELIVERY OF NUCLEIC ACIDS AND CRISPR GENOME EDITORS BY GSH-RESPONSIVE SILICA NANOPARTICLES

Wang, Yuyuan[1], Gong, Shaoqin[2], Pattnaik, Bikash R.[3], Shahi, Pawan K.[4], Wang, Xiuxiu[5], Xie, Ruosen[5], Zhao, Yi[5]

[1]University of Wisconsin, Madison, WI, USA, [2]Department of Ophthalmology and Visual Sciences, Department of Biomedical Engineering and Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA, [3]Department of Ophthalmology and Visual Sciences, Department of Pediatrics and McPherson Eye Research Institute, University of Wisconsin, Madison, WI, USA, [4]Department of Pediatrics and McPherson Eye Research Institute, University of Wisconsin, Madison, WI, USA, [5]Department of Biomedical Engineering, and Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA

One major hurdle to the clinical translation of gene therapy is the lack of safe and efficient delivery approaches for nucleic acids and genome editors. Herein we report a stimulus-responsive silica nanoparticle (SNP) capable of efficient and safe delivery of a broad range of biologics including nucleic acids and CRISPR genome editors. SNPs were prepared by a water-in-oil microemulsion method. The size and morphology of the SNPs were studied by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Targeting ligands (e.g., all-trans-retinoic acid (ATRA) or N-acetylgalactosamine (GalNAc)) can be conveniently conjugated onto the surface of the SNPs. The in vivo delivery efficiencies were evaluated in a Ai14 transgenic mouse model via subretinal and intravenous injections. The SNPs synthesized via the water-in-oil microemulsion method had a well-controlled nanoparticle size (~50 nm). A disulfide crosslinker was integrated into the silica network, endowing the SNP with glutathione (GSH)-responsive cargo release capability when internalized by target cells. An imidazole-containing component was incorporated into the SNP to enhance the endosomal escape capability. The SNP can deliver various cargos, including nucleic acids (e.g., DNA and mRNA) and CRISPR gene editors (e.g., Cas9/sgRNA ribonucleoprotein (RNP), and RNP together with a donor DNA) with excellent efficiency and biocompatibility. The SNP surface can be PEGylated and functionalized with different targeting ligands. In vivo studies showed that subretinally injected SNP conjugated with ATRA and intravenously injected SNP conjugated with GalNAc can effectively deliver mRNA and RNP to murine retinal pigment epithelium (RPE) cells and liver cells (Figure 1 C), respectively, leading to efficient gene editing. The delivery efficiency can be further enhanced by optimizing the SNP formulation. Using advanced targeting strategies, SNPs can be used to achieve gene editing in other types of organs/cells (e.g., the central nervous system). The multifunctional SNP is an efficient, biocompatible, and versatile nanoplatform for targeted delivery of a broad range of biomacromolecule cargos both in vitro and in vivo.

Funding Source
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Keywords: In vivo gene editing; non-viral vector; Silica nanoparticle

T-116

SINGLE-CELL MULTI-OMICS ASSAY TO ACCELERATE CELL AND GENE THERAPY DEVELOPMENT

Wang, Yue[1], Li, Alex[2], Parikh, Saurabh[2], Wang, Shu[3], Yang, Yilong[3], Mendoza, Daniel[4], Patel, Khushali[4]

[1]Commercial, Mission Bio, San Francisco, CA, USA, [2]Research and Development, Mission Bio, San Francisco, CA, USA, [3]Bioinformatics, Mission Bio, San Francisco, CA, USA, [4]Pharma Assay Development, Mission Bio, San Francisco, CA, USA

Cell and gene therapies are transformative solutions for a host of inherited and acquired diseases for which existing interventions are ineffective. Many such therapies rely on the introduction of transgenes into host cells using viral or non-viral vectors or directly gene editing of host genome. The accurate measurement of gene transfer and gene editing outcomes and efficiencies is critical to the development of therapeutic agents and is a key attribute for assessing their safety and efficacy. Conventional bulk measurement either reports a population average that miss the cell-to-cell variation or involve laborious colony picking and time-consuming clonal outgrowth. Mission Bio has developed an end-to-end solution from panel design to data analysis for high-throughput single-cell targeted multi-omics sequencing. Here, using the Tapestri platform we demonstrate that single-cell DNA sequencing identifies transduced versus non-transduced cells with exceptional accuracy and precision for populations of up to 10,000 cells while reducing sample processing time from weeks to days.

Keywords: Single-cell DNAseq; Gene Editing; Gene Transfer

T-117

PRINTING FUNCTIONAL HUMAN NEURAL TISSUES

Yan, Yuanwei, Li, Xueyan, Gao, Yu, Mathivanan, Sakthikumar, Kong, Linghai, Tao, Yunlong, Dong, Yi, Li, Xiang, Bhattacharyya, Anita, Zhao, Xinyu, Zhang, Su-Chun

Waisman Center, University of Wisconsin, Madison, WI, USA

Probing how the human neural networks operate is hindered by the lack of reliable human neural tissues amenable for dynamic functional assessment of neural circuits. We developed a 3D bioprinting platform to assemble tissues with defined neural cell types in a desired dimension. The printed neuronal progenitors differentiate to neurons and form functional neural circuits in and between tissue layers within weeks, evidenced by spontaneous synaptic currents and synaptic response to neuronal excitation. Printed astrocyte progenitors develop into mature astrocytes with elaborated processes and form functional neuron-astrocyte networks by calcium flux and glutamate uptake in response to neuronal excitation. These designed human neural tissues will likely be useful for understanding the wiring of human neural networks, modeling pathological processes, and serving as platforms for drug testing.

Keywords: 3D bioprinting; human pluripotent stem cells; neural circuits

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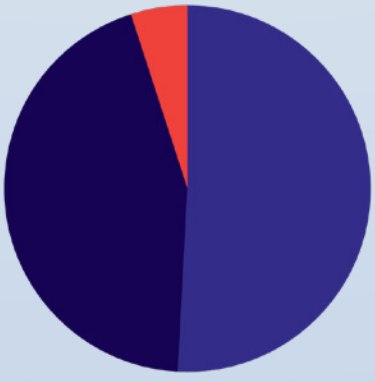

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