INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

PSC-DERIVED CELL THERAPIES: CLINICAL ADVANCES, NEXTGEN TECHNOLOGIES, AND THE PATH TO SUCCESS

An ISSCR International Symposium

2–4 OCTOBER 2024
COPENHAGEN, DENMARK

IN PARTNERSHIP WITH

novo nordisk®

PROGRAM BOOK

WELCOME

Dear Attendees,

It is our pleasure to welcome you to the ISSCR Copenhagen International Symposium, "PSC-Derived Cell Therapies: Clinical Advances, NextGen Technologies, and the Path to Success." We developed this three-day program to explore how far we have come and the exciting future ahead for cell therapies. We will hear from pioneers at the forefront of the field and discuss together how lessons learned can inform and streamline the development of the next generation of PSC-derived cell therapies, from early preclinical work all the way through to clinical success.

PSC-derived cell therapies come with their own unique opportunities and challenges. This program was designed to gather our community together to not only share the latest data from ongoing clinical trials, but to openly discuss strategies for overcoming current challenges and bottlenecks, as well as share some of the most exciting new technologies and platforms that may shape the future of cell therapies.

On the first day we will hear from the first wave of therapies already in clinical trials for the treatment of AMD, retinitis pigmentosa, Parkinson's Disease, epilepsy, heart disease, as well as autoimmune diseases and cancer. Days two and three will delve into the critical steps in developing new cell therapies, including preclinical innovations, advances in manufacturing technologies, navigating the regulatory landscape, clinical trial considerations, strategies around immunosuppression and immune evasion, and exciting new technologies and platforms that are in the pipeline.

We invite you to participate in this symposium to its fullest. We have included four panel discussions throughout the program around key topics to provide a platform to share perspectives and strategies for success.

Ask questions. Join the conversation. Share your perspectives and expertise. Discuss your setbacks and successes. Only through collaborations with our global community that leverage our experiences can we effectively advance new therapies for patients.

We hope you enjoy the scientific program, reconnect with old friends and colleagues, and forge new and meaningful connections and partnerships that will accelerate the development of transformative new medicines.

Sincerely,

ISSCR Copenhagen Program Organizing Committee,

Melissa K. Carpenter, PhD, *Carpenter Consulting Corporation, USA (Co-Chair)*
Kapil Bharti, PhD, *NEI/NIH, USA (Co-Chair)*
Brendan Jones, MBBS, PhD*, Novo Nordisk, Denmark*
Charis Segeritz-Walko, PhD, *Novo Nordisk, Denmark*
Lorenz Studer, MD, *Memorial Sloan Kettering Cancer Center, USA*
Masayo Takahashi, MD, PhD, *Vision Care, Japan*

WELCOME

Dear Colleagues and Friends,

It is with great pleasure that I welcome you to the International Symposium of the International Society for Stem Cell Research (ISSCR) in Copenhagen. As the co-sponsor of this prestigious event, Novo Nordisk is thrilled to join forces with the ISSCR in hosting a gathering that promises to be a landmark in the field of pluripotent stem cell-derived cell therapies.

At Novo Nordisk, we are committed to advancing the frontiers of medical science through innovation and collaboration. This congress brings together world-renowned scientists, pioneering innovators, and clinical experts who are at the forefront of ESC and iPSC-derived cell therapies. Our shared goal is to accelerate the transition of these groundbreaking therapies from the lab to the clinic, changing the lives of people living with serious chronic diseases.

Thanks to events like the ISSCR International Symposium, where collaboration is fostered, we aim to share the knowledge, data, and capabilities needed to develop transformative cell therapies faster for those living with serious chronic diseases. This is the place where great minds bring us closer to the future we aspire to.

On behalf of Novo Nordisk, I extend my deepest gratitude to all participants for joining us in Copenhagen, the heart of our headquarters. Your dedication and contributions are vital to the advancement of this field, so thank you for being part of this journey.

Welcome to Copenhagen and enjoy this unforgettable and transformative experience.

Warm regards,

Joachim Fruebis
CVP of Cell Therapy
Novo Nordisk

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ABOUT THE ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

CONTACT US

The International Society for Stem Cell Research
630 Davis St, Suite 200
Evanston, IL 60201 USA
+1-224-592-5700
isscr.org

The International Society for Stem Cell Research (ISSCR) is a 501c(3) nonprofit organization with a mission to promote excellence in stem cell science and applications to human health. Our vision is a world where stem cell science is encouraged, ethics are prioritized, and discovery improves understanding and advances human health.

The ISSCR represents nearly 5,000 scientists, students, educators, ethicists, and business leaders from more than 80 countries. Each ISSCR member makes a personal commitment to uphold the ISSCR Guidelines for Stem Cell Research and Clinical Translation, an international benchmark for ethics, rigor, and transparency in all areas of practice.

Our Board of Directors and Committees represent leaders across research, academia, and industry who are committed to advancing the Society's mission.

Our work is made possible through generous support from our members and allied organizations towards strategic initiatives that support the mission:

- **Regulatory Affairs:** The ISSCR helps members navigate the regulatory landscape while assisting regulators by making scientifically informed recommendations for the development of stem cell therapies.
- **Policy:** The ISSCR advocates globally to support research funding, enforce ethical guidelines, and guard against unproven therapies.
- **Education:** The ISSCR provides resources and programs for the general public, educators, physicians, policy makers, and regulators. Aboutstemcells.org and ISSCR's patient handbook provide scientifically vetted resources for patients seeking unbiased and trusted information.
- **Standards** and **Guidelines:** The ISSCR sets international guidance for ethical and rigorous research, adopted by public and private organizations, regulatory bodies, funders, and publications. These references strengthen the pipeline of research and therapies, ultimately to benefit the patient.
- **International Conferences:** The ISSCR hosts a portfolio of international and digital meetings designed for knowledge sharing and collaboration to further the field. Discover upcoming programs, including the ISSCR 2025 Annual Meeting.
- **Publishing:** The ISSCR publishes *Stem Cell Reports*, an open access journal communicating basic discoveries in stem cell research alongside translational and clinical studies.

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CCRM Commercializing Living Therapies

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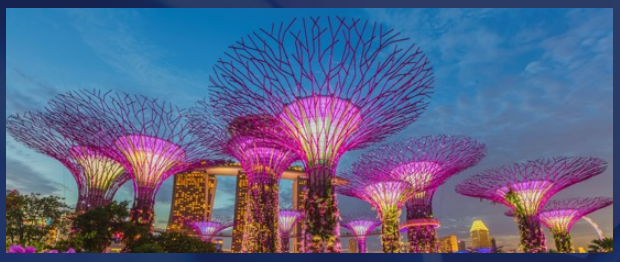
UPCOMING PROGRAMS



SINGAPORE INTERNATIONAL SYMPOSIUM
STEM CELLS IN TISSUE MAINTENANCE, REPAIR, AND AGING

11-13 DECEMBER 2024 | SINGAPORE
In partnership with Stem Cell Society Singapore (SCSS) & co-sponsored by STEMCELL Technologies

EDINBURGH SUMMIT
PLURIPOTENT STEM CELLS: SETTING STANDARDS, SHAPING THERAPIES*

12-13 FEBRUARY 2025 | EDINBURGH, UK
In partnership with Cell and Gene Therapy Catapult

ATHENS INTERNATIONAL SYMPOSIUM
NEURAL STEM CELLS: CAPTURING COMPLEXITY AND PLASTICITY FROM THE CELL TO THE ORGANISM

3-4 APRIL 2025 | ATHENS, GREECE
In partnership with Stem Cell Reports & co-sponsored by MaxWell Biosystems

ISSCR 2025 ANNUAL MEETING
THE GLOBAL STEM CELL EVENT

11-14 JUNE 2025 | HONG KONG
Co-Sponsored by The University of Hong Kong, The Chinese University of Hong Kong, & The Hong Kong University of Science and Technology

SEATTLE INTERNATIONAL SYMPOSIUM
AI AND MACHINE LEARNING: THE FUTURE OF DIGITAL BIOLOGY*

9-10 OCTOBER 2025 | SEATTLE, USA

BOSTON INTERNATIONAL SYMPOSIUM
LOST IN TRANSLATION: BRIDGING THE GAPS FROM BEDSIDE TO BENCH*

11-12 DECEMBER 2025 | BOSTON, USA

Learn more at ISSCR.org/upcoming-programs

**Title subject to change*

MEETING INFORMATION

All times are listed in Central European Summer Time (CEST)

ONSITE BADGE PICK UP

Pick up your name badge at the designated area during the times listed below. Name badges are required for admission to all sessions, social events, meals/breaks, and the Exhibit & Poster area. Badges can be picked up during the following times:

Registration Desk Hours
The Black Diamond – Royal Danish Library
Søren Kierkegaards Pl. 1, DK 1221 Copenhagen K, Denmark

Tuesday, 1 October	*3:00 PM – 5:00 PM*
Wednesday, 2 October	*8:00 AM – 6:00 PM*
Thursday, 3 October	*8:00 AM – 5:00 PM*
Friday, 4 October	*8:00 AM – 4:00 PM*

ISSCR PROGRAM AGENDA

There will be no printed program book for the 2024 Copenhagen International Symposium. You can access the online version of the program agenda here: isscr.org/copenhagen-agenda.

Livestream will not be available for this event. However, registrants can access the audio and slide recordings on-demand after the event by logging into the Member Library with their ISSCR credentials. If you have trouble logging in, try resetting your password. If the problem persists, please contact isscrdigital@isscr.org. Please allow approximately two weeks after the event for the on-demand content to be published.

ABSTRACT REVIEWERS

Kapil Bharti, Christian Honoré, Sara Howden, Henning Kempf, Howard Kim, Jane S. Lebkowski, Tenneille E. Ludwig, Heather Main, Jonathan Niclis, Amanda Rickard, Charis Segeritz-Walko, Wolfram Zimmermann

SMOKING

Smoking or the use of e-cigarettes is prohibited inside The Black Diamond – Royal Danish Library.

LOST AND FOUND

Please bring found items to the ISSCR Registration Desk during posted hours. If you lose an item, visit the registration desk during posted hours for assistance.

PARKING

The Black Diamond – Royal Danish Library does not have dedicated parking facilities for guests. The closest public parking spaces are at:

- Slotsholmsgade
- Frederiksholms channel
- BLOX parking with 350 parking spaces, which has easy access from Vester Voldgade

DISABILITY PARKING

Motorists with a disability parking permit can park at Søren Kierkegaards Plads when visiting The Black Diamond – Royal Danish Library. The site has six disability parking spaces, but they cannot be reserved in advance.

POSTER INFORMATION

Each poster will be presented during a 45-minute session in the Queen's Hall Foyer of The Black Diamond – Royal Danish Library. **Poster presenters must adhere to the scheduled date and time of poster set-up, presentation, and take-down.** Presenters are responsible for removing their posters at the end of their presentation time. Any posters not removed at the end of the session will be discarded.

WEDNESDAY, 2 OCTOBER

Poster Session I
Set-up: 3:15 PM – 3:45 PM
Presentation: 4:45 PM – 5:30 PM
Take-down: 5:30 PM

Poster Session II
Set-up: 5:30 PM – 5:45 PM
Presentation: 5:45 PM – 6:30 PM
Take-down: 6:30 PM

THURSDAY, 3 OCTOBER

Poster Session III
Set-up: 2:45 PM – 3:15 PM
Presentation: 5:00 PM – 5:45 PM
Take-down: 5:45 PM

Every cell type we create can give rise to multiple next generation therapies

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2880 Bagsværd
Denmark
www.novonordisk.com

Novo Nordisk is a leading global healthcare company, founded in 1923 and headquartered in Denmark. Our purpose is to drive change to defeat diabetes and other serious chronic diseases such as obesity and rare blood and endocrine disorders. We do so by pioneering scientific breakthroughs, expanding access to our medicines, and working to prevent and ultimately cure disease. Novo Nordisk employs about 61,400 people in 80 countries and markets its products in around 170 countries.

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UK
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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.

OFFICIAL MEETING JOURNAL

STEM CELL REPORTS

STEM CELL REPORTS

www.cell.com/stem-cell-reports/home

Stem Cell Reports is an open access forum communicating basic discoveries in stem cell research, in addition to translational and clinical studies. *Stem Cell Reports* focuses on manuscripts that report original research with conceptual or practical advances that are of broad interest to stem cell biologists and clinicians. *Stem Cell Reports* participates in Cell Press Multi-Journal Submission, allowing authors to simultaneously submit their papers for consideration by multiple journals at once.

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Dorothy Hodgkin Building
Babraham Research Campus Cambridge
Cambridge, England Cb22 0AX
UK
www.bit.bio

bit.bio is a synthetic biology company focused on human cells that is advancing medicine and enabling curative treatments. bit.bio's opti-ox™ precision cell programming technology enables conversion of induced pluripotent stem cells (iPSCs) into any desired human cell type in a single step, at industrial scale, while maintaining exceptional purity and consistency. bit.bio's cell therapy pipeline, based on txCells™, is focused on serious diseases that lack effective treatments. The lead candidate, bbHEP01 based on txHepatocytes, is in development as a treatment for patients suffering from acute liver failure and acute-on-chronic liver failure. The ioCells™ research cell product portfolio is opening up new possibilities for studying human biology and developing new medicines in research and drug discovery.

PEPTIGROWTH

9-10, Nihonbashi Horidomecho 1-chome
Nihonbashi Life Science Building 7
Chuo-ku, Tokyo, 1030012
Japan
peptigrowth.com/en

PeptiGrowth develops novel synthetic peptide growth factors. Conventional recombinant growth factors and cytokines used in regenerative medicine and cell therapy often face various quality challenges, such as lot-to-lot variation, potential contamination with biological impurities, low stability, and high cost. PeptiGrowth has been working on developing a series of synthetic peptides that address these challenges while maintaining equivalent or better functionality to conventional recombinant growth factors and cytokines available in the market. Our peptides are completely chemically synthesized and animal component-free, enabling completely chemically defined cell culture conditions.

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Berenkoog 41
1822 BH Alkmaar
Netherlands
www.amsbio.com

AMSBIO is a leading provider of premium products designed for the field of cell and gene therapy. Our extensive portfolio encompasses stem cells derived from diverse sources, animal-origin free culture media, reprogramming agents, and cryopreservation media that meet the highest GMP standards. We go beyond by offering a comprehensive range of stem cell characterization tools, differentiation reagents, distinctive assay platforms, and specialized packaging solutions for AAV and lentivirus. AMSBIO stands out with the industry's broadest selection of recombinant ECMs and AOF growth factors ensuring unparalleled productivity and seamless regulatory compliance. Introducing cutting-edge hydrogel matrix products tailored for PDX applications, organoid, spheroid, and pluripotent stem cell culture, such as Extragel—a like-for-like replacement for Matrigel™, Geltrex™, and Cultrex™ Basement Membrane Extract (BME), and MatriMix—a fully-defined alternative to existing options.

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P.O. Box 13901
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Research Triangle Park, NC 27709
USA
www.bwfund.org

The Burroughs Wellcome Fund (BWF) is an independent private foundation that serves and strengthens society by nurturing a diverse group of leaders in the biomedical sciences to improve human health through education and powering discovery in frontiers of greatest need. BWF makes grants primarily to degree-granting institutions or nonprofit research institutes on behalf of individual researchers. To complement these competitive award programs, BWF also makes grants to nonprofit organizations conducting activities intended to improve the general environment for science.

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750 Main Ave
Cambridge, MA 02139
USA
www.cellinobio.com

Cellino is on a mission to make stem cell-derived cell therapies accessible for patients. Stem cell-derived regenerative medicines are poised to cure some of the toughest diseases within this decade, including Parkinson's, diabetes, and heart disease. However, current therapeutic production processes are not scalable due to extensive manual handling, high variability, and expensive facility overhead. Cellino's vision is to make personalized regenerative medicines viable at large scale for the first time.

Cellino's technology combines label-free imaging, artificial intelligence-based image analysis, and high-precision laser-based cell removal to automate cell reprogramming, expansion, and differentiation in a closed cassette format, enabling thousands of clinical-grade cell samples to be processed in parallel in a single facility.

EVOTEC

Essener Bogen 7
Hamburg 22419
Germany
www.evotec.com

Evotec is a life science company with a unique business model focused on delivering highly effective new therapeutics to patients. The Company leverages its multimodality platform for proprietary projects and within a network of partners including Pharma, Biotech, academics, and other healthcare stakeholders. With more than 5,000 highly qualified people at 17 sites, Evotec aims to create the world-leading co-owned pipeline for innovative therapeutics.

Evotec's iPSC infrastructure represents one of the largest and most sophisticated platforms in the industry. It has been developed over the last years with the goal to (1) industrialize iPSC-based drug screening in terms of throughput, reproducibility and robustness to reach the highest industrial standards, and to (2) deliver off-the-shelf iPSC-based cell therapy products to patients.

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Forskaren, Hagaplan 4
Stockholm County, 113 66
Sweden
www.hoyaconsulting.se

At HOYA Consulting, we specialize in meeting the quality and commercial needs essential for bringing hPSC-derived Cell Therapy to market. Our Nordic-based team is engaged and driven by curiosity, leveraging deep scientific expertise and industry knowledge to keep our clients at the forefront of this rapidly evolving sector. We pride ourselves on being approachable and open to new ideas, fostering respectful and collaborative relationships not only with clients but also with other industry stakeholders. As competent experts in our field, we advance the translation of innovative science into patient therapies with confidence and determination.

MEDICON VALLEY ALLIANCE

Arne Jacobsens Allé 15, 2
DK-2300 Copenhagen S
Denmark
www.mva.org

Medicon Valley Alliance (MVA) is the non-profit membership organization for the Danish-Swedish life science cluster Medicon Valley. Our 300+ members represent the region's triple helix and include universities, hospitals, life science businesses, regional governments, selected municipalities and service providers. We create value for our members by co-hosting, launching and driving meetings, networks, seminars, conferences and projects that strengthen the collaboration, networking and knowledge-sharing in the regions' life science community, create critical mass and help realizing the full potential of Medicon Valley. Our events serve as a meeting and marketplace for Nordic life science and we also assist foreign companies, which would like to explore the business and partnership potential in the region.

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Sylviusweg 62
2333 BE Leiden
Leiden Bio Science Park
Netherlands
necstgen.com

NecstGen is a non-profit CDMO and centre of excellence for Cell and Gene Therapy, located in a purpose-built GMP facility on the largest bio-cluster in the Netherlands, Leiden Bio Science Park. Here, NecstGen provides critical contract development, manufacturing and rental services to academic and industrial therapy developers to deliver a new generation of therapies to patients.

STEMCELL TECHNOLOGIES, INC.

1618 Station St
Vancouver, BC V6A1B6
Canada
www.stemcell.com

At STEMCELL Technologies, 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 are helping scientists accelerate the pace of discovery. Inspired by knowledge, innovation, and quality, we are Scientists Helping Scientists.

CONTRIBUTING SPONSOR

CCRM

661 University Avenue
Suite 1002
Toronto, ON M5G 1M1
Canada
www.ccrm.ca

CCRM is a Canadian public-private partnership established with seed funding by the Government of Canada, the Province of Ontario, and leading academic and industry partners. It supports the development of regenerative medicine-based technologies, and cell and gene therapies. CCRM has a 40,000-square-foot space dedicated to advanced cell manufacturing that includes a GMP facility for producing cells and viral vectors. CCRM accelerates the translation of scientific discovery into new companies and marketable products for patients with specialized teams, dedicated funding and unique infrastructure. In 2022, CCRM established OmniaBio Inc., a pre-clinical to commercial-scale CDMO. CCRM is hosted by the University of Toronto.

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AXIAL CELL SYSTEMS

C/O SU Holding
Villa Bellona
Universitetsvägen 8
106 91 Stockholm
Sweden
axialcellsystems.com

Axial Cell Systems is revolutionizing synthetic biology and disease modeling through our innovative neuromesodermal progenitor technology. Our proprietary method generates specific cell types of precise body position location, from any cell line, enabling accurate recreation of multiple cell types, including muscles, nervous system, and cartilage, that closely mimic natural functions. Our groundbreaking approach will improve disease modelling, facilitate drug screening and accelerate therapy development. By bridging the gap between in vitro models and human physiology, we offer pioneering opportunities for pharmaceutical companies, biotech firms, and academic researchers to advance their work in drug discovery, mechanisms research and restorative medicine.

BIOLAMINA

Löfströms alle 5
Stockholm 172 66
Sweden
www.biolamina.com

We offer an expansive portfolio of defined human recombinant laminin matrices, Biolaminin®, for a variety of applications, such as expansion of human pluripotent stem cells and differentiation and maintenance of different specialized cell types. The biologically relevant cell-matrix interaction leads to improved cell functionality, robust culture systems and safe cells for therapy. BioLamina's laminin technology has been scientifically validated in many high impact journals.

CORE BIOGENESIS

850 Bd Sébastien Brant BioParc
3 67400 Illkirch-Graffenstaden
France
corebiogenesis.com

Core Biogenesis provides next-generation recombinant proteins for stem cell research. Rooted by the principles of the bioeconomy, the company delivers its exclusive Ultra-Scalable Biomanufacturing as a Service (UBaaS) platform for the benefit of business leaders, scientists, and ultimately patients in the field of regenerative medicine. Our portfolio has a wide list of growth factors and cytokines for pluripotent, mesenchymal, and immune cells. More importantly, Core Biogenesis supply solutions enable for stock security and long-term batch reservation. Established from scientific rigor, our fast-growing team is fostered by diversity and inclusion in science, business, and technology, and we are fully devoted to help those making the world a better place through the discovery of novel treatments for life devastating diseases.

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MAXWELL BIOSYSTEMS

Albisriederstrasse 253
8047 Zurich
Switzerland
www.mxwbio.com

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.

MEDCHEMEXPRESS

Bergkällavägen 37C
19279, Sollentuna
Sweden
www.medchemexpress.com

MedChemExpress (MCE) offers a wide range of high-quality research grade bioactive molecules including chemical compounds, natural products, recombinant proteins, and peptides for stem cell research. We also provide custom synthesis service, protein production service, and various drug screening services. MCE strives to a competent and trustworthy partner for your research and scientific projects.

NANOCELLECT BIOMEDICAL

9525 Towne Centre Dr. Suite 150
San Diego, CA 92121
USA
nanocellect.com

NanoCellect is committed to empowering every scientist to make discoveries one cell at a time, by ensuring high cell viability required to advance cell-based research. We develop and deliver microfluidic based solutions that are affordable, compact, and easy-to-use. Our expanding portfolio of instruments and consumables enable biomedical scientists to analyze and sort cells required for drug discovery, single cell-omics, cloning, antibody discovery, and basic research.

PARSE BIOSCIENCE

700 Dexter Ave N Ste 600
Seattle, WA 98109
USA
www.parsebiosciences.com

Parse Biosciences is a global life sciences company whose mission is to accelerate progress in human health and scientific research. Empowering researchers to perform single cell sequencing with unprecedented scale and ease, our pioneering approach is enabling groundbreaking discoveries in cancer treatment, tissue repair, stem cell therapy, kidney and liver disease, brain development, and the immune system. Founded based on a transformative technology invented at the University of Washington, Parse is used by over 2,000 labs across the world. Our growing portfolio of products includes Evercode Whole Transcriptome, Evercode TCR, Gene Capture, and a software tool for data analysis. Headquartered in Seattle, Washington's vibrant South Lake Union district, Parse Biosciences recently opened a 34,000 square foot headquarters and state-of-the-art laboratory.

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4721 Calle Carga
Camarillo, CA 93012
USA
www.pbsbiotech.com

PBS Biotech is a leading manufacturer of single-use bioreactor systems and provider of process development services. PBS bioreactors utilize proprietary Vertical-Wheel® technology to create homogeneous and scalable mixing conditions for a variety of sensitive cell therapy products and cell culture applications. PBS Biotech's vision is to become the world's standard manufacturing platform for allogeneic cell-based therapies.

SEKISUI AMERICA CORPORATION

6659 Top Gun Street
San Diego, CA 92121
USA
www.sekisui-cell.jp/en

Sekisui America Corporation is the North American regional HQ for Sekisui Chemical. We work on developing solutions for the life science industry, with a focus on cell and gene therapy. Our products include cell culture consumables such as media and scaffolds. Our chemically defined scaffold (CDS) can be used for the serum-free culture of pluripotent stem cells (iPSCs, ESCs, MSCs). It is a synthetic polymer that can be stored at room temperature, pre-coated on a variety of surfaces and can be adapted to automated culture equipment. We are looking for partners in the field of regenerative medicine such as academic organizations, pharmaceutical companies and automated bioreactor manufacturers, who can jointly develop and adapt our products for their pipeline.

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amsbio» Accelerate Your Discovery

Need help with your stem cell cultures?

We're With You Every Step of the Way

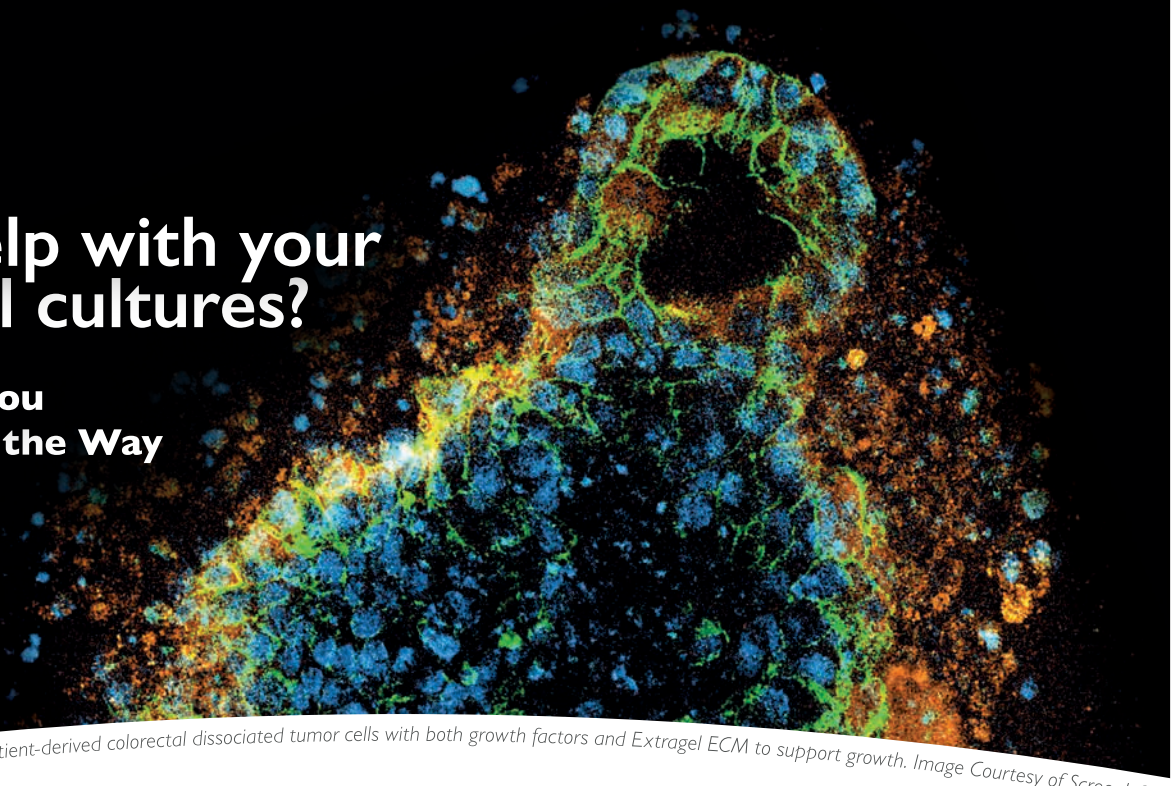

Image: Organoids derived from AMSBIO patient-derived colorectal dissociated tumor cells with both growth factors and Extracel ECM to support growth. Image Courtesy of ScreenIn3D.

Struggling to find **diverse** cell sources or **user-friendly** reprogramming kits?

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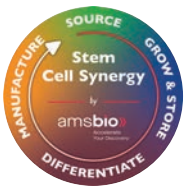
Talk to our experts



»

Find out more on our Expanded Stem Cell Synergy Solution here!

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SPEAKER ABSTRACTS

All times are listed in Central European Summer Time (CEST)

WEDNESDAY, 2 OCTOBER 2024

9:00 AM – 10:05 AM
OPENING KEYNOTE SESSION

Sponsored by Medicon Valley Alliance

9:00 AM – 9:02 AM

WELCOME REMARKS

Alm, Keith

CEO, International Society for Stem Cell Research (ISSCR)

9:02 AM – 9:05 AM

OPENING REMARKS

Fruebis, Joachim

Novo Nordisk, Denmark

9:05 AM – 9:15 AM

PROGRAM INTRODUCTION & OVERVIEW

Carpenter, Melissa K.

Carpenter Consulting Corporation, USA

9:15 AM – 9:40 AM

KEYNOTE ADDRESS 1:
CMC CONSIDERATIONS FOR PSC-DERIVED CELL THERAPIES—A CBER PERSPECTIVE

Lombardi, Heather

OTP, CBER, FDA, USA

ABSTRACT NOT AVAILABLE AT TIME OF PUBLISHING

9:40 AM – 10:05 AM

KEYNOTE ADDRESS 2:
HOW THE DANISH MEDICINES AGENCY FACILITATES THE ENTRY INTO THE REGULATORY LANDSCAPE FOR ADVANCED THERAPY MEDICINAL PRODUCTS

Nissen, Lotte Dahl

The Danish Medicines Agency, Denmark

As the EU regulatory landscape for the advanced therapy medicinal products is evolving, the Danish Medicines Agency is becoming more transparent about the work done to facilitate the entry into clinical trials and the way towards a centralized marketing authorization. The homepage provides a central entry for the regulation of advanced therapy medicinal products in the EU, with regulatory information and relevant contact points for the i.e. scientific and regulatory advice, classification, GMP certification and clinical trials. Regulation of advanced therapies in the EU (ATMP) (laegemiddelstyrelsen.dk). Lotte Dahl Nissen addresses recommendations for the format and content of the regulatory documentation, for applications for clinical trials (under the new clinical trial regulation). Lotte is a non-clinical assessor in the clinical trial department at the Danish Medicines Agency, with international regulatory experience, also regarding CMC, and particular interest in the innovative therapies.

10:30 AM – 12:00 PM
CLINICAL TRIAL UPDATES: PSC-DERIVED THERAPIES OF THE EYE

10:30 AM – 11:00 AM

LONG-TERM FOLLOW-UP OF PARTICIPANTS IN A PHASE 1/2A CLINICAL TRIAL OF AN ALLOGENEIC BIO-ENGINEERED HESC-DERIVED RPE IMPLANT FOR GEOGRAPHIC ATROPY: PLANS FOR A PHASE 2B CLINICAL TRIAL

Clegg, Dennis

Regenerative Patch Technologies and UC Santa Barbara, USA

An open-label phase 1/2a clinical trial assessing the safety and preliminary efficacy of a polarized monolayer of allogeneic human embryonic stem cell-derived RPE cells (CPCB-RPE1) on a biosynthetic substrate was conducted in subjects with geographic atrophy (GA) secondary to dry age-related macular degeneration (AMD). The CPCB-RPE1 implant was delivered to the worst-seeing eye of 15 participants during outpatient surgery. No attempts were made to HLA match subjects. All subjects received low-dose tacrolimus immunosuppression for 68 days in the peri-implantation period. The median age was 78 years (range 69–85). The treated eyes of all subjects were legally-blind with a baseline best corrected visual acuity (BCVA) of ≤20/200. There were no unexpected serious adverse events. Four subjects in cohort 1 had serious ocular

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adverse events including: retinal hemorrhage, edema, focal retinal detachment, or RPE detachment. This was mitigated in cohort 2 using improved hemostasis during surgery. There was no clinical or serologic evidence of inflammation suggestive of immune response directed towards the donor implant in any subject. At 1 year, a larger proportion of treated eyes experienced >5 letter gain in best corrected visual acuity when compared to the untreated eye (27% vs 7%) and a larger proportion of nonimplanted eyes demonstrated >5 letter loss (53% vs 33%). This trend was maintained as of final follow-up (mean 36.9 months, range 12–54 months) where a larger proportion of treated eyes experienced >5 letter gain when compared to the untreated eye (27% vs 7%) and a larger proportion of nonimplanted eyes demonstrated >5 letter loss (80% vs 47%). One subject died from causes unrelated to the study two years after surgical implantation of CPCB-RPE1. Postmortem histopathology demonstrated the presence of donor mature RPE cells by immunoreactivity for RPE65, Na/K ATPase and Bestrophin. Colocalization of rhodopsin with RPE65 positive donor RPE also suggest the presence of phagosomes within functional donor RPE. In summary, subretinal implantation of the CPCB-RPE1 is feasible and well-tolerated. Sub-retinally implanted, allogeneic, RPE cells survive, express functional markers, and do not elicit clinically detectable intraocular inflammation or serologic immune responses even without long-term immunosuppression. Plans for the Phase 2b clinical trial will be presented.

11:00 AM – 11:30 AM

OPREGEN®: A SUSPENSION OF ALLOGENEIC RETINAL PIGMENT EPITHELIAL (RPE) CELLS IN PATIENTS WITH GEOGRAPHIC ATROPHY (GA) SECONDARY TO AGE-RELATED MACULAR DEGENERATION (AMD)

Culley, Brian

Lineage Cell Therapeutics, USA

OpRegen—a suspension of allogeneic RPE cells—delivered subretinally has demonstrated acceptable safety and preliminary evidence of activity through Month 24 in patients with GA secondary to age-related macular degeneration (AMD) (Ph 1/2a; NCT02286089). In an open-label, single arm, multicenter PhI/IIa clinical study, patients in cohorts 1–3 (n=12, BCVA ≤20/200) and cohort 4 (n=12, BCVA 20/64-20/250) received up to 200,000 OpRegen cells by either vitrectomy/retinotomy (n=17) or suprachoroidal access using the Orbit™ subretinal delivery system (n=7). OpRegen has been well tolerated in all cohorts through Month 24. Most adverse events were mild, and there have been no reported cases of rejection following OpRegen delivery. Patients in Cohort 4 (less advanced disease) continued to show an improvement in mean BCVA compared with baseline in OpRegen-treated eyes through Month 24 (Month 12 [n=12]: +7.6 letters; Month 24 [n=10]: +5.5 letters). The 5 patients with extensive bleb coverage of GA showed greater gains in BCVA in their study eye that persisted at Month 24 (Month 12 [n=5]: +12.8 letters; Month 24 [n=5]: +7.4 letters) compared with those with limited bleb coverage (Month 12 [n=5]: +3.9 letters; Month 24 [n=5]: +3.6 letters). Patients with extensive bleb coverage also demonstrated greater improvements in RPE and ELM area by optical coherence tomography (OCT) persisting through Month 24 (mm2; RPE, +2.63±2.91 gain in study eye vs −2.79±3.34 loss in fellow eye; ELM, +0.81±1.17 gain vs −1.85±1.70 loss; [n=4]) compared to patients with limited bleb coverage (mm2; RPE, −1.46±1.81 loss vs −4.75±3.59 loss in fellow eye; ELM, −2.97±1.54 loss vs −3.67±1.43 loss; [n=5]). Restorative approaches are needed for patients with GA who have lost significant vision and have experienced significant damage to their retina. These data suggest that OpRegen may support retinal health and function in GA, with the potential to slow, stop or reverse disease progression and possibly improve visual function.

11:30 AM – 12:00 PM

SUSTAINABLE REGENERATIVE MEDICINE

Takahashi, Masayo

Vision Care, Japan

Our goal is to develop cell and gene therapies for diseases of the outer retina. We initiated the first clinical study of iPSCs as a retinal pigment epithelial (RPE) cell transplant in 2013. Since then, we have been improving the formulation to achieve safe and effective treatment. First, we performed an autologous transplantation of iPS-derived retinal pigment epithelial cell sheet from

SPEAKER ABSTRACTS

2013 to demonstrate the safe use of iPS cells. The cell sheet is still functioning and maintaining the patient's visual function. The allogeneic transplantation clinical study from 2017 confirmed that immune rejection can be controlled with only topical administration of steroids if HLA mismatches are avoided, leading to a pipeline using HLA partial KO iPS cells. Currently, the target disease has been expanded to RPE impairment diseases, and RPE strips are developed that can be easily transplanted through a small hole. It has been found that RPE strips spread as a single-layer cell sheet beneath the retina after transplantation, and this has the potential to produce effects that cannot be obtained with drugs. Such formulations have made it possible to provide safe and effective treatment. In addition, replacing photoreceptors is an important issue for reconstructing the outer layer of the retina. The results of transplanting retinal organoids were as expected. Since replacement therapy is a surgical treatment, there is a gap between the end product and the treatment. It is necessary to select the appropriate case for each treatment method. Otherwise, unnecessary patients will undergo harmful surgery and the cell therapy becomes an expensive gamble. From our experiences, we believe that we should prepare the appropriate treatment method for each case of retinal outer layer disease. We need to strictly select the cases for each treatment method and prepare other than cells such as clinical tests and surgical procedures. In addition, although all the equipment in the facility is usually validated for cell manufacturing, the cell culture technology is not validated, so the state of the cells varies depending on the culture technology. To address this issue, we have introduced humanoid robots that perform the same operations as our tacit technical staff. With the strict purification step and this robot make the protocol robust and we can always obtain stable cell quality. Furthermore, we are constructing various systems necessary for early standardization of treatment, AI software to support doctors and patient registration registries. Now we start working with the Japanese Society for Regenerative Medicine, the Japanese Ophthalmological Society, and the government to prepare for the introduction of private insurance to build an appropriate medical system. A business model for cell therapy that is good for patients, hospitals, and industry has not yet been established, and in order to make it a sustainable treatment, it is important to built whole system, the development way and medical system including renversement, appropriate.

1:30 PM – 3:15 PM
CLINICAL TRIAL UPDATES: PSC-DERIVED THERAPIES OF THE BRAIN

1:30 PM – 2:00 PM

PHASE 1 TRIAL OF HUMAN EMBRYONIC STEM CELL-DERIVED MIDBRAIN DOPAMINE NEURONS FOR PARKINSON'S DISEASE

Studer, Lorenz

Memorial Sloan Kettering Cancer Center and BlueRock Therapeutics, USA

ABSTRACT NOT AVAILABLE AT TIME OF PUBLISHING

2:00 PM – 2:15 PM

PRECLINICAL SAFETY AND EFFICACY OF DOPAMINE NEURON PROGENITOR CELLS DERIVED FROM PD DONOR-INDUCED PLURIPOTENT STEM CELLS

Bratt-Leal, Andres[1], Barken, Derren[1], Hills, Rachel[2], Mossman, Jim[1], Tran, Ha[1], Chandrasekaran, Chandee[1], Williams, Roy[1], Beauvais, Genevieve[1], Lane, Emma[2], Lelos, Mariah[2], Zhang, Xiaokui[1]

[1]Aspen Neuroscience, USA, [2]Cardiff University, UK

At the time of diagnosis, it is estimated that a Parkinson's disease (PD) patient has already lost the majority of dopaminergic neurons in the substantia nigra and their projections in the putamen, resulting in the motor symptoms of the disease. Several groups have begun clinical investigation of a cell replacement therapy using pluripotent stem cell-derived dopaminergic progenitor cells. Current technology enables ex vivo generation of patient-specific dopaminergic neurons by reprogramming somatic cells into induced pluripotent stem cells (iPSCs), then further differentiating iPSCs to dopamine neuron progenitor cells (DANPCs) and neurons. Autologous cell therapy offers the potential advantage of not requiring immune suppression, which is costly and may not be well

SPEAKER ABSTRACTS

tolerated in an elderly population. We have established the GMP-compliant manufacturing process to produce DANPCs from PD donor iPSCs and characterize these cells using a battery of genomic and phenotypic quality control assays. Instead of testing each cell line in animal models, we have generated large in vitro and in vivo reference data sets and have developed bioinformatic assays to predict the ability of any given lot of DANPCs to engraft in a rodent brain, including their ability to innervate and release dopamine. Furthermore, these DANPCs from multiple PD donors were tested for efficacy in a 6-OHDA medial forebrain bundle lesion rodent model of PD. A separate study was conducted in unlesioned immuno-deficient Rowett nude rats under Good Laboratory Practices (GLP) to characterize the safety, biodistribution and tumorigenicity of the DANPCs. All tested lots were efficacious in restoring dopaminergic signaling and reducing amphetamine-induced rotations. Cell migration was limited to the site of injection and no adverse safety events related to the cellular product were observed in the rodents. These preclinical studies demonstrate the ability to manufacture patient-specific DANPCs which are safe and demonstrate robust efficacy in support of clinical investigation in PD. Based in part on this data, Aspen Neuroscience has initiated a first-in-human clinical trial to evaluate the safety and tolerability of two escalating doses of autologous iPSC-derived DANPCs in subjects with moderate to advanced PD and expects to present initial safety data by the end of 2024.

2:15 PM – 2:45 PM

IPS CELL-BASED THERAPY FOR PARKINSON'S DISEASE: A KYOTO TRIAL

Takahashi, Jun

Kyoto University and CiRA, Japan

Human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease (PD). Toward the clinical application of iPSCs, we have developed a method for (1) scalable DA neuron induction on human laminin fragments and (2) sorting DA progenitor cells using a floor plate marker, CORIN. The grafted CORIN+ cells survived well, functioned as midbrain DA neurons in the 6-OHDA-lesioned rats, and showed a minimal risk of tumor formation. In addition, we performed a preclinical study using primate PD models. Regarding efficacy, human iPSC-derived DA progenitor cells survived and functioned as midbrain DA neurons in MPTP-treated monkeys. Regarding safety, cells sorted by CORIN did not form any tumors in the brains for at least two years. Moreover, we found that MRI and PET imaging were helpful in monitoring the survival, expansion, and function of the grafted cells as well as the immune response by the host brain. Based on these results, we started a clinical trial to treat PD patients at Kyoto University Hospital in Kyoto, Japan, in 2018. The trial evaluated the safety and efficacy of transplanting human iPS cell-derived DA progenitors into PD patients' putamen. Using a stereotaxic surgical technique, we implanted approximately 5 or 10 million cells into the bilateral putamen of the patients. The target was seven patients, and we observed each of them for two years. I will summarize the results of the trial.

Funding Source: Grants from the Research Project for Practical Application of Regenerative Medicine of the Japan Agency for Medical Research and Development (AMED)

2:45 PM – 3:15 PM

HUMAN INHIBITORY INTERNEURON CELL THERAPY FOR DRUG-RESISTANT EPILEPSY

Nicholas, Cory R., Banik, Gautam, Bershteyn, Marina, Blum, David, Bulfone, Alessandro, Feld, Brianna, Fuentealba, Luis, Hixson, John, Lee, Seonok, Madrid, Sheri, Maury, Yves, Priest, Catherine, Watson, Michael

Neurona Therapeutics, USA

Despite over three dozen approved anti-seizure drugs on the market, 56% of adults with epilepsy continue to have uncontrolled seizures and lack effective therapeutic options. Mesial temporal lobe epilepsy (MTLE) is common in adults and characterized by focal-onset seizures that originate in the hippocampus. Surgical resection or ablation of the temporal lobe may be an option for some with drug-resistant MTLE; however, these surgical options can result in serious neurocognitive impairment due to the destruction of

SPEAKER ABSTRACTS

brain tissue. Here, we have developed an alternative approach: a regenerative cell therapy candidate called NRTX-1001 that is designed to restore balanced activity to the hyperactive circuits in the epileptic hippocampus. NRTX-1001 comprises allogeneic hPSC-derived post-mitotic inhibitory (GABAergic) interneurons of a specific cortical lineage for single-dose intracerebral administration. Two open-label multicenter Phase 1/2 trials are ongoing to evaluate NRTX-1001 safety and efficacy in adults with drug-resistant MTLE (NCT05135091, NCT06422923). Long-term durability data from the first patients to receive NRTX-1001 will be presented. Clinical results thus far indicate that NRTX-1001 is well-tolerated and has the potential to control seizure activity. Neurocognitive deficits have not been detected, and some patients have shown improved memory and quality of life scores. In addition, unpublished preclinical data will be presented from extensive single-cell RNAseq profiling of NRTX-1001 after transplantation into the mouse brain, demonstrating a consistent composition of interneuron subtypes with high fidelity to endogenous cortical interneurons from the medial ganglionic eminence.

Funding Source: CIRM (DISC2-10525; TRAN1-11611; CLIN2-13355)

3:45 PM – 4:45 PM
CLINICAL TRIAL UPDATES: PSC-DERIVED THERAPIES OUTSIDE OF THE CNS

3:45 PM – 4:15 PM

TISSUE ENGINEERED HEART REPAIR—FROM NON-HUMAN PRIMATES TO PATIENTS WITH ADVANCED HEART FAILURE

Zimmermann, Wolfram

Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany and German Center for Cardiovascular Research (DZHK), partner site Lower Saxony, Germany

Tissue-engineered heart repair has evolved over the past 30 years from a scientific concept to clinical stage investigations in patients with advanced heart failure. Engineered Heart Muscle (EHM) are constructed for this purpose from induced pluripotent stem cell-derived cardiomyocytes and stromal cells in collagen. Key to clinical translation has been the simulation of tissue engineered heart repair in a homologous Rhesus macaque model. Safety and efficacy data obtained in healthy macaques and in macaques with chronic heart failure after myocardial infarction informed the design of the first-in-patient “Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure (BioVAT-HF)” Phase I/II clinical trial (NCT04396899). Key findings in macaques and early experience from BioVAT-HF will be discussed.

4:15 PM – 4:45 PM

THE DEVELOPMENT OF OFF-THE-SHELF iPSC-DERIVED CAR T-CELL THERAPY TO ELIMINATE A BROAD SPECTRUM OF PATHOGENIC CELLS IN HEMATOLOGICAL DISEASES IN THE ABSENCE OF CONDITIONING CHEMOTHERAPY

Valamehr, Bob

Fate Therapeutics, USA

In this presentation I will discuss the clinical translation of FT819, an off-the-shelf, anti-CD19 CAR T-cell product candidate derived from a renewable master iPSC line and offer insight from preclinical and clinical data in how it is an ideal therapeutic candidate for broad patient access in the management of B cell malignancies and autoimmune disease. I will also discuss the development of next-generation iPSC-derived CAR T cells which represent a promising off-the-shelf approach to cell therapy for the treatment of autoimmune disorders and B cell malignancies, with the unique potential to elicit durable elimination of an array of aberrant immune cells, to avoid toxicities associated with intense conditioning chemotherapy treatment, and to maximize patient access and reach.


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SPEAKER ABSTRACTS

THURSDAY, 3 OCTOBER 2024

9:00 AM – 10:50 AM
PRECLINICAL CONSIDERATIONS & INNOVATIONS

9:00 AM – 9:30 AM

STEM CELL DERIVED KIDNEY TISSUE; THE CHALLENGES OF CLINICAL DELIVERY

Little, Melissa H.

Murdoch Children's Research Institute, Australia

It is a decade since the first protocols for directing the differentiation of human pluripotent stem cells to kidney tissue were developed. Since that time, these models of the early human kidney have been investigated for their accuracy, capacity to model human renal disease and more recently as potential tools for drug development. The more challenging objective is to generate stem cell-derived tissues for the treatment of end stage renal disease. This presentation will discuss the progress and challenges of such a complex multicellular product.

9:30 AM – 9:45 AM

DEVELOPING A PSC-DERIVED THERAPY FOR HEARING LOSS: PRECLINICAL EFFICACY AND BIOSAFETY OF OTIC NEUROPROGENITOR CELLS

Rivolta, Marcelo[1], Abbas, Leila[2], Cacciabue, Daniela[2], Clark, Jenna-Rae[2], Papasavva, Zoi[2], Fell, Adam[2]

[1]University of Sheffield, UK, [2]Centre for Stem Cell Biology, School of Biosciences, UK

Disabling hearing loss has reached almost epidemic proportions, with nearly 500 million people affected worldwide. Whilst the sensory hair cells are fundamental for hearing, a growing body of research has shown that often the main cause of deafness lies in the loss of hair cell innervation and auditory neurons. One of the most frequent causes of hearing loss, presbycusis, is associated with a significant loss of innervation that is almost three times larger than the loss of hair cells. Hearing loss can also arise from auditory neuropathy, a condition characterised by loss of neurons while maintaining normal hair cell function. Despite the huge 'burden of disease' from presbycusis and auditory neuropathy, there are currently no disease modifying therapeutics available. Deafness is usually treated with devices such as hearing aids and cochlear implants; the latter can, to an extent, replace hair cell function. However, there is no effective therapeutic option for the loss of cochlear neurons. To date, deafness remains an intractable condition. To address this, we are using human PSCs to target the auditory nerve. We have shown that we can restore hearing in a deafened gerbil model of auditory neuropathy, by transplanting hESC-derived otic neuroprogenitors (hONPs) into the cochlear nerve. Adaptations to the manufacturing process for hONPs have not impacted efficacy, as different batches display a similar functional profile. To model cochlear implantation, we used a fully-implantable rodent stimulator in which the electrode is activated by a magnetic field—with this, we demonstrated functional integration between the transplanted cells and the implant. To monitor for safety, we have carried out long-term studies exploring the distribution of transplanted cells. Animals were followed for up to a year, with whole-body MRI scans performed at termination to identify any potential lesions. Additionally, biodistribution studies for human-specific DNA sequences were performed by QPCR. No tumours attributed to the test article were found and there was no detection of cells spreading systemically. Similar safety studies are being undertaken using the Rag2/Il2rg (SRG) double knock-out rat. These findings demonstrate that the application of hONPs is efficacious and safe, and support their translation into clinical trials.

9:45 AM – 10:00 AM

MULTIDISCIPLINARY PURSUIT OF GI ORGANOID THERAPY IND

Cobb, Beth, Helmrath, Michael A.

Cincinnati Children's Hospital and Medical Center, USA

As many as 11 percent of Americans have gastrointestinal (GI) tract acute inflammatory conditions. A small yet significant percentage do not respond to existing therapies resulting in severe chronic conditions. GI organoid therapies are a disease agnostic approach that may provide clinically feasible solutions to regenerate all regions of the GI tract, dramatically

SPEAKER ABSTRACTS

improving quality of life and resolving morbidities. To realize the goal to use iPSC derived (GI) tissues to transform clinical care, we have initiated preclinical work that includes establishing methods to generate GMP GI organoids and a preclinical transplant model. A multidisciplinary team of clinical, basic science, translational and engineering academicians and industry experts is needed to (1) define criteria for patient enrollment and optimal route of application; (2) generate cGMP autologous iPSCs that will provide the greatest benefits for patients; (3) develop GMP protocols to pattern human GI organs; (4) implement automation to scale production; and (5) obtain an Investigational New Drug (IND) clearance. We have established a clinical advisory team to define criteria for patient enrollment and optimal route of application of cell therapy product, and to establish clinical protocols and objective measures of outcome. We have validated the use of cGMP-compliant iPSC lines by confirming the formation of GI organoids and appropriate tissue patterning upon transplantation into the animal model. Further, we have developed analytical tests to assess biodistribution, off target effects and tumorgenicity of transplanted organoids in our preclinical model. We have introduced a comprehensive automation system to standardize organoid culture and reduce variability. With support of an external advisory board and regulatory consultancy, we are preparing to submit an IND for first-in-human GI iPSC cell therapy in 2025. Combining in-house developed GI organoid technology with excellence in patient centered care provides a unique opportunity to accomplish this work. iPSC and organoid therapies will revolutionize 21st century medicine. With safety defining our mission, Cincinnati Children's is poised to be THE place where GI organoid therapy happens first and where it is done the best.

Funding Source: Farmer Family Foundation and Cincinnati Children's Research Foundation

10:00 AM – 10:20 AM

INNOVATION SHOWCASE

STEM CELLS AND 3D: EXPANSION AND UPSCALING

Presented by Thermo Fisher Scientific

Akenhead, Michael L.

Thermo Fisher Scientific, USA

Scalable and efficient expansion systems for pluripotent stem cells (PSCs) are crucial for PSC-derived allogeneic cell therapies. Three-dimensional (3D) suspension culture supports large-scale production of high-quality PSCs but faces barriers, particularly the lack of regulatory-compliant culture media. To address this, Gibco™ Cell Therapy Systems (CTS) StemScale™ PSC Medium has been developed. This Xeno-free medium enables single cells to self-aggregate into 3D spheroids and is related to the research-use only (RUO) StemScale Medium, with both showing similar performance. CTS StemScale Medium supports the growth of both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), achieving 5x–10x expansion per passage, depending on the cell line. It allows PSCs to scale up in various vessel sizes, from small-scale (<100 mL) to large-scale (>1 L) systems. Up to 5 billion cells can be harvested from spheroids grown in a 3 L bioreactor. These cells can be cryopreserved at high densities for future use in large-scale studies or downstream applications requiring high cell quantities. Cells grown in CTS StemScale Medium can differentiate as 3D spheroids, dissociate into single cells for downstream applications, or be cryopreserved for later use.

10:20 AM – 10:50 AM

NEXT GENERATION OF PRECLINICAL MODELS FOR CELL BASED THERAPIES AGAINST LIVER DISEASES

Vallier, Ludovic

Berlin Institute of Health (BIH) at Charite, Germany

The liver sustains reserves of iron, vitamins and minerals and detoxifies alcohol, drugs and other chemicals. The liver has also a function in synthesis by producing albumin which represents the most abundant protein in the plasma, and blood clotting factors. Finally, the liver has an essential metabolic function by storing glycogen and lipids. Most of these activities are managed by

SPEAKER ABSTRACTS

one cell type, the hepatocyte. However, additional cell types such as cholangiocytes also play essential roles in organ repair, tissue homeostasis and diseases. Diseases targeting the liver are life threatening and the only treatment for end-stage disease is organ transplantation. Such therapy entails high risk of surgical complications and indefinite immunosuppression associated with severe side effects. Furthermore, lack of organ donors greatly limits the number of patients who can benefit from this therapy. Thus, cell therapy using primary cells, Hepatocytes or cholangiocytes, have been proposed as an alternative for organ transplantation. However, this approach has been limited since adult primary hepatocytes can be grown only for a limited time in vitro without losing their functional activity. For this reason, production of hepatocytes from human induced pluripotent stem cells (hiPSCs) represent an advantageous option. Here, we will provide an update of the technologies available to produce liver cells in vitro and the different approach currently available for improving their functionality. We will also describe the use of ex-vivo perfused human organs for proof-of-concept applications in regenerative medicine.

Funding Source: ERC, Einstein Foundation, Berlin Institute of Health

11:15 AM – 12:15 PM

REGULATORY INTERACTIONS & UNIFIED STANDPOINTS PANEL DISCUSSION I

Sponsored by Burroughs Wellcome Fund

Moderator: Bharti, Kapil, *NEI/NIH, USA*

Barry, Jacqueline

CGT Catapult, UK

Parmar, Malin

Lund University, Sweden

Lombardi, Heather

OTP, CBER, FDA, USA

Dahl, Lotte

Nissen, DKMA, Denmark

Takahashi, Jun

Kyoto University, Japan

1:15 PM – 2:15 PM

REGULATORY INTERACTIONS & UNIFIED STANDPOINTS PANEL DISCUSSION II

Sponsored by HOYA Consulting

Moderator: Carpenter, Melissa K., *Carpenter Consulting Corporation, USA*

Zimmermann, Wolfram

University Medical Center Göttingen & Repairon, Germany

Clegg, Dennis

Regenerative Patch Technologies & UC Santa Barbara, USA Masayo Takahashi, Vision Care, Japan

Hidalgo-Simon, Ana

reNEW & LUMC, The Netherlands

Rowbottom, Rachel

Simon-Kucher, UK

Takahashi, Masayo

Vision Care, Japan

2:15 PM – 2:45 PM

CURRENT BEST PRACTICE FOR THE TRANSLATION OF PSC-BASED THERAPIES—AN UPDATE

Barry, Jacqueline[1], Bharti, Kapil[2]

[1]CGT Catapult, UK, [2]NEI/NIH, USA

As the stem cell field is maturing and basic stem cell discoveries are advancing from the laboratory into clinical trials the ISSCR has assembled global subject matter experts in the preclinical, translational, and commercial space to develop a set of internationally relevant best practices for the development of PSC-based therapies. The document, currently in draft form, leverages the ISSCR's strength in scientific rigor and puts forward key principles and processes from starting materials through clinical trials.

SPEAKER ABSTRACTS

3 October 2024

3:15 PM – 5:00 PM
MANUFACTURING AND AUTOMATION IN CELL THERAPY DEVELOPMENT

3:15 PM – 3:45 PM

NAVIGATING THE BRIDGE FROM LAB TO CLINIC IN STEM CELL-BASED THERAPIES

Carlsen, Thomas Hassing Ronøe

Novo Nordisk Foundation Cellerator, Denmark

Cell therapy is a promising field of biomedicine that uses living cells to treat or cure various diseases and disorders. However, many hurdles hinder the progress of cell therapy candidates from research laboratories to clinical trials, such as the scarcity of high-quality raw materials and cell lines, the complexity of designing flexible and scalable facilities, the gap in workforce skills and expertise, the lack of robust and standardized processes and analytics, and the difficulty of interpreting and meeting quality and regulatory requirements. These challenges result in high costs, long timelines, and low success rates for cell therapy products. Approaches to address these hurdles involve the selection or production of appropriate cell lines that meet quality and regulatory standards, bridging the gap between the discovery and research phases to clinical product manufacturing, applying Chemistry Manufacturing and Control principles to assist research initiatives throughout their development journey, and product tailoring of analytical assays, quality control, and release processes. Additionally, gathering and employing data can aid in creating platforms to standardize and enhance process development and regulatory submission, while an informed approach to intellectual property matters for cell lines and technologies will increase the chances of successful drug product development throughout commercialization. The Novo Nordisk Foundation Cellerator is a platform that aims to enable and accelerate cell therapy development and translation by providing early engagement with research groups, offering multi-purpose and multi-product facilities, pre-licensed pluripotent stem cell lines and gene editing technologies, clinically relevant manufacturing scale, efficient scalability approaches and models, and optimized quality control and release packages. Cellerator also focuses on future perspectives on the use of data and automation strategies to establish standardized platforms and minimize hands-on work, reduce the risk of contamination and mistakes, and to create the path for a time- and cost-efficient process development, highly compliant with regulatory requirements for cell therapy manufacture. By offering these services and solutions, Cellerator seeks to bridge the gap between cell therapy research and clinical trials, and ultimately, to bring cell therapy products to the patients in need.

3:45 PM – 4:15 PM

GMP MANUFACTURING AND RELEASE ASSAYS FOR INDUCED PLURIPOTENT STEM CELLS DERIVED RETINAL PIGMENT EPITHELIUM “PATCH” FOR AUTOLOGOUS CELL-BASED THERAPY FOR AGE-RELATED MACULAR DEGENERATION

Sharma, Ruchi, Bharti, Kapil

National Institutes of Health (NIH), USA

Age-related Macular Degeneration (AMD) is the primary cause of blindness in elderly individuals in the US. Patients lose their central vision due to the death of light-sensitive retinal cells, which precede the degeneration of the Retinal Pigment Epithelium (RPE). RPE is a single layer of cells with four essential characteristics: polarized, phagocytic, polygonal, and pigmented. With these properties, RPE performs critical functions that support the health of photoreceptors on the top side and choroidal vasculature on the bottom side. The dry form of AMD has not been treatable to date. However, the development of Induced Pluripotent Stem Cells (iPSCs) has opened the possibility of cell-based replacement therapies for end-stage degenerative diseases. iPSC-derived RPE provides a source of cells for replacing lost RPE in AMD patients. The generation of RPE from iPSC for cell therapy requires a viral-free iPSC source, xeno-free reagents, a manufacturing process, and thorough testing of the final clinical product for functionality, safety, and efficacy. We used CD34+ cells for iPSC generation, and the karyotyping and deep exome sequencing of iPSC confirmed a stable genome and the absence of tumorigenic mutations. The pluripotent expression

SPEAKER ABSTRACTS

markers for OCT-4 and TRA-1-81 show more than 80% positivity. Xeno-free differentiation methods were used to differentiate iPSCs into RPE progenitors (intermediate stage) and immature RPE. The RPE cells were seeded onto biodegradable scaffolds to create a mature RPE monolayer. After five weeks of maturation, we conducted release assays on the final iPSC-RPE patch to confirm its maturity, purity, sterility, morphometry, and functionality. Maturity and purity were confirmed using a FLOW-based assay for RPE-specific markers (BEST1, TYRP1, CRALBP) and pluripotent purity markers (TRA-1-81 and OCT-4). The quality release criteria were set at an expression level above 85% for CRALBP and BEST1 and above 97% for TYRP1 and PMEL17. Morphometric analysis was performed using AI-based software to determine the number of neighbors and hexagonality. Functionality was assessed by measuring the trans-epithelial resistance, set at 400 ohms.cm2. In conclusion, we have optimized the manufacturing process of the iRPE patch using release assays to confirm its maturity and functionality.

4:15 PM – 4:25 PM

INNOVATION SHOWCASE

OPTICAL BIOPROCESSING: AN AI- AND LASER-BASED BIOMANUFACTURING TECHNOLOGY TO SCALE PRODUCTION OF PERSONALIZED iPSC-BASED CELL THERAPIES

Presented by Cellino

Madrid, Marinna

Cellino Biotech, USA

Autologous induced pluripotent stem cell (iPSC)-based therapies have the potential to address a wide range of chronic degenerative diseases without the need for immunosuppression or donor matching, making them well-suited to impact an increasingly diverse and aging population. Today, autologous cell therapy manufacturing relies on manual processes, highly-skilled experts for in-process decision making, and the use of a separate cleanroom for each patient. This does not scale, limiting patient access. Cellino is building an advanced biomanufacturing technology to enable consistent, scalable production of high-quality autologous iPSC-derived cell therapies. The technology is based on an optical bioprocess that reduces the need for human interventions, automates unit operations, and enables small-footprint, closed cassette-based parallelized manufacturing. Here we present an AI (artificial intelligence)-driven optical biomanufacturing technology for consistent, scalable production of autologous iPSC-derived therapies. Cells are imaged, AI-driven algorithms analyze images for in-process cell and colony characterization, and laser-generated bubbles selectively remove cells as needed. Here we present several cell culture processes that have been replaced by AI-driven optical bioprocesses, including: estimation of well confluence, clonalization of a stem cell population, and passage-free culture of proliferating cells. We demonstrate that the optical bioprocesses are capable of generating high-quality cells at increased scale by increasing the output of an individual expert operator. The optical bioprocesses shown here are compatible with closed biomanufacturing, which is necessary to enable parallelization while minimizing the risk of cross-contamination. We present prototypes of fluidic cassettes for closed cell culture for clinical-grade manufacturing. Together, this work enables scalable and consistent manufacturing of personalized regenerative medicines.

4:25 PM – 4:30 PM

INNOVATION SHOWCASE

GMP-COMPLIANT MANUFACTURING OF iPSCS AND iPSC-DERIVED CELL THERAPIES

Presented by NecstGen

van Pel, Melissa

NecstGen, Netherlands

The presentation “GMP-Compliant Manufacturing of iPSCs and iPSC-Derived Cell Therapies” will focus on the rigorous processes involved in producing induced pluripotent stem cells (iPSCs), their use in Cell Therapy development, and Good Manufacturing Practice (GMP)-compliant manufacturing. It will explore the critical steps of establishing and maintaining GMPMaster Cell Banks (MCBs) and Working Cell Banks (WCBs) to ensure the consistency, safety, and potency of iPSC lines. Additionally, the presentation will discuss the use of bioreactors for the scalable and controlled expansion

SPEAKER ABSTRACTS

of iPSCs, addressing the challenges of maintaining cell quality, pluripotency, and preventing contamination. Emphasis will be placed on the regulatory requirements, quality control measures, and process validation necessary for translating iPSC-based cell therapies from the laboratory to clinical applications. Attendees will gain a comprehensive understanding of the complexities and best practices in GMP-compliant manufacturing, aimed at advancing the development of safe and effective iPSC-derived cell therapies.

4:30 PM – 4:32 PM

MYTOS iDEM: A FLASK-BASED AUTOMATION PLATFORM, TO MOVE FAST FROM LAB TO MANUFACTURING

Afshar, Ali[1], Weng Jiang, Xian[2], Weston, Stephen[1], Costa, Joana[1], Assarian, Borna

[1]Mytos, UK, [2]Mytos, Spain

Pluripotent stem cell (PSC) culture and differentiation is critical for regenerative medicine. But today, culture protocols get developed manually in flasks, before having to be redeveloped in scalable, GMP-compliant formats such as 3D suspension. The Mytos iDEM platform is a faster route to scale, without having to redevelop manual protocols. iDEM fully automates PSC culture in a closed flask-based cartridge, enabling manually-developed flask-based protocols to be directly translated to large automated production for PD and manufacturing. Herein we tested iDEM's performance across iPSC expansion and maintenance, cardiomyocyte differentiation, and DA neuron differentiation. Rigorous testing across various iPSC lines and conditions included assessments of cell morphology, genomic integrity, and pluripotency markers. Results demonstrated that iPSCs cultured using the Mytos system matched the quality of manually grown cells, with no differences in TRA-1-81, Nanog, and SSEA4 markers, and passed Karyostat+ and PluriTest. Differentiated cardiomyocytes across 3 iPSC lines showed healthy morphology, started beating after 2 weeks and were found to have a high %CTnT+ cells. In summary, iDEM was shown to versatile across a range of lines and differentiations, and offers a promising fast-track to scaling differentiations.

4:32 PM - 5:00 PM

MANUFACTURING AND AUTOMATION IN CELL THERAPY DEVELOPMENT

Moderator: Barry, Jacqueline, *CGT Catapult, UK*

Hassing Ronøe Carlsen, Thomas

Cellerator, Denmark

Sharma, Ruchi

NEI/NIH, USA

Madrid, Marinna

Cellino Biotech, USA

van Pel, Melissa

NecstGen, Netherlands

Afshar, Ali

Mytos, UK

Learn more at cellinobio.com

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SPEAKER ABSTRACTS

FRIDAY, 4 OCTOBER 2024

9:00 AM – 10:10 AM
PSC CLINICAL TRIAL CONSIDERATIONS: DRUG PRODUCT, CELL DELIVERY, TRIAL DESIGN, AND CLINICAL ENDPOINTS I

9:00 AM – 9:15 AM

OPENING REMARKS

Fruebis, Joachim

Novo Nordisk, Denmark

9:15 AM – 9:45 AM

PRACTICAL CONSIDERATIONS IN SPINAL CORD STEM CELL TRANSPLANTATION

Boulis, Nicholas

The Emory Clinic, USA

Throughout the latter half of the aughts extending until 2014, our team focused on the development of devices and strategies to enable spinal cord stem cell transplantation. This work was initially conducted in collaboration with Clive Svendsen PhD, who provided fetal stem cell derived neural progenitors to our laboratory used in a series of gottingen minipig spinal cord transplantation efforts targeting the ventral horn. Initial work utilized an injectrode that allowed for extracellular recording to confirm injections in the ventral horn. The injectrode was positioned with a platform mounted to individual animals' spinous processes. Microelectrode recording proved to be time consuming and unacceptably morbid, leading to revision of the system to utilize anatomical targeting. Further revision of the system reduced the size of the necessary incision by mounting the frame to percutaneous laminar posts. In addition, a "floating cannula" system was introduced to compensate for the movement of the spinal cord occurring within the spinal canal with ventilation. This cannula reduced cell reflux and improved safety. This system was ultimately used to provide the preclinical safety data to enable neural progenitor transplantation for ALS in the NeuralStem Trials, as well as the Vescovi/Mazzini trials conducted in Italy demonstrating the safety and feasibility of multiple injections into the lumbar and cervical spinal cord. The system was utilized for a trial investigating the application of the NeuralStem spinal cord derived fetal neuroprogenitors to chronic spinal cord injury at UCSD. The system is currently in use for transplantation of Glial Restricted Precursors (Q Therapeutics) into the spinal cord for Transverse Myelitis (PI: Benjamen Greenberg, UTSW). The presentation will review the development of the system as well as clinical trial data. It will further present data on the survival of the transplanted cells, as well as efforts to explore nanoparticle cell tracking as well as the development of an interventional MRI approach to minimally invasive spinal cord stem cell transplantation.

9:45 AM – 9:55 AM

INNOVATION SHOWCASE

INNOVATIVE SYNTHETIC PEPTIDE-BASED GROWTH FACTOR: OVERCOMING CHALLENGES IN STEM CELL MEDICINE PRODUCTION

Presented by PeptiGrowth

Brownlee, Robert

PeptiGrowth Inc., UK

Traditional recombinant protein-based growth factors and cytokines used in regenerative and cell therapy products face several challenges, including lot-to-lot quality variation, contamination with animal-derived components, low stability, and extremely high costs. To address these issues, we have developed a novel class of "Growth Factor Alternative Peptides" with comparable or superior activity to traditional growth factors and cytokines, utilizing our proprietary Peptide Discovery Platform System (PDPS). Our growth factor alternative peptides are cyclic peptides synthesized through complete chemical reaction, allowing for high-quality, cost-effective production and exceptional stability. To date, we have successfully developed peptides that have equivalent or superior activity to the functions of HGF, BDNF, Noggin, VEGF, Wnt3a, EGF, and TPO. This presentation will provide an overview of our development of these growth factor alternative peptides and will include new data on the recently completed development of an FGF2 (bFGF) alternative peptide.

SPEAKER ABSTRACTS

4 October 2024

9:55 AM – 10:10 AM

CELL THERAPY SAFETY IS A MULTIFACETED TARGET

Nagy, Andras

Lunenfeld Tanenbaum Research Institute, Sinai Health, Canada

Cell therapy holds immense promise, offering potential solutions for various diseases. However, ensuring the safety of transplanted cells is paramount. Our FailSafe cell system is designed to mitigate the multifaceted risks associated with cell therapy, including harmful genetic mutations, epigenetic changes, pluripotent cell contamination, and the possibility of oncogenic transformation at any stage, from cell source generation to post-transplantation of therapeutic cells. The FailSafe system incorporates robust genetic editing to prevent cancer development in therapeutic cell grafts regardless of the cause while maintaining their desired functional properties. This solution offers a critical step towards realizing the clinical translation of cell therapy, ensuring patient safety and maximizing therapeutic benefits. Our reliable safety system's dominance allows the development and implementation of inherently dangerous additional genetic changes, such as hiding allogeneic cells from the recipient's functional immune system. Our solution involves the forced expression of eight immune modulatory genes, which proved to be sufficient to protect allogeneic cell transplants across major histocompatibility (MHC) gene differences without the need for immune suppression. The system, referred to iACT (induced Allogeneic Cell Tolerance), not only enables cells to evade the immune system but also allows the cells to form an artificial immune-privileged tissue for potential additional therapeutic applications. The combination of FailSafe and iACT technologies will pave the way for the production of a safe and universally usable cell source for cell-based medicine, serving all humans.

10:40 AM – 11:10 AM
PSC CLINICAL TRIAL CONSIDERATIONS: DRUG PRODUCT, CELL DELIVERY, TRIAL DESIGN, AND CLINICAL ENDPOINTS II

10:40 AM – 11:10 AM

UNIQUE ASPECTS OF CELL THERAPY FOR NEUROLOGICAL DISEASE

Svendsen, Clive

Cedars-Sinai Medical Center, USA

iPSC technology allows the generation of all types of neural tissue and serves as an ideal source for cell therapy approaches to treat neurological diseases. This talk will focus on lessons leant from two ongoing clinical trials using fetal derived neural progenitors in some cases engineered to release GDNF. Unlike normal drug trials with pills, cell therapy trials include developing complex manufacturing processes, deciding on immune suppression regimes, how to get the cells to their target, patient enrolment to complex neurosurgical trials, sham surgery requirements, spread of the disease to the transplant and deciding on outcome measure options. The benefits of cell therapy trials include one time delivery of the cell product, the ability to do unilateral assessment of patient outcomes (using the other side as a within patient control) and the unique ability to replace damaged cells within the nervous system. As we transition these trials from fetal derived neural progenitors to iPSC derived neural progenitors unique opportunities arise for manufacturing and cell composition—these will be discussed.

SPEAKER ABSTRACTS

11:10 AM – 12:00 PM

PSC-DERIVED DRUG PRODUCT, CELL DELIVERY, AND CLINICAL TRIALS DISCUSSION PANEL DISCUSSION

Moderator: Fruebis, Joachim, *Novo Nordisk, Denmark*

Boulis, Nicholas

The Emory University, USA

Takahashi, Masayo

Vision Care, Japan

Nicholas, Cory

Neurona Therapeutics, USA

Svendsen, Clive

Cedars-Sinai Medical Center, USA

Bratt-Leal, Andres

Aspen Neuroscience, USA

Nagy, Andras

Lunenfeld Tanenbaum Research Institute, Sinai Health, Canada

1:15 PM – 2:45 PM IMMUNOSUPPRESSION & IMMUNE EVASION

1:15 PM – 1:45 PM

ALLOGENEIC HYPOIMMUNE CELL THERAPEUTICS AS UNIVERSAL MEDICINES

Deuse, Tobias

University of California San Francisco, USA

Novel cell therapeutics are currently being developed for both immuno-oncology and regenerative medicine indications as the era of living medicines dawns. The one critical issue preventing the widespread use of allogeneic cells is immune rejection. Allogeneic cell products so far have inferior persistence and efficacy when compared to autologous alternatives. Therefore, successful engineering of hypoimmune cells that fully overcome allorejection would solve this issue and would probably make autologous products obsolete. We have developed hypoimmune (HIP) B2M-/- CIITA-/- CD47 transgenic iPSCs that can evade all innate and adoptive allogeneic immune cell responses. This engineered immune evasiveness is based on MHC class I and II deletion to escape T cell recognition and on activating the CD47-SIRPalpha immune checkpoint in innate myeloid and lymphoid immune cells. Such HIP iPSCs can be differentiated into functionally active, hypoimmune cell types to treat diseases in allogeneic recipients without any immunosuppression. We show engraftment and survival of several allogeneic HIP cell types in fully MHC-mismatched mice and non-human primates. Human hypoimmune anti-CD19 CAR T cells and human pancreatic islets survived in fully allogeneic humanized mice and controlled cancer and alleviated diabetes, respectively. Novel synthetic molecules add to the toolbox of transgenes that can additionally provide protection against cytotoxic IgG antibodies and utilize so far inaccessible immune checkpoints. Our studies support the development of universal HIP cell products for regenerative medicine and immuno-oncology for cost-effective treatments of major diseases.

1:45 PM – 2:00 PM

ISLET-LIKE CELLS GENERATED FROM HLA-E EXPRESSING HUMAN EMBRYONIC STEM CELLS LACKING B2M AND RFXANK AVOID ALLOREJECTION IN VITRO AND IN VIVO

Nostro, Maria Cristina[1], Misra, Paraish[1], Lee, Jong Bok[2], Zhang, Li[1], Wang, Jinguo[1], Russel, David W.[3]

[1]McEwen Stem Cell Institute, University Health Network, Canada, [2]University of Calgary, Canada, [3]University of Washington, Seattle, USA

Initial results from the clinical trial using human embryonic stem cell (hESC)-derived islet-like cells for type 1 diabetes (T1D) treatment indicate that insulin independence can be achieved, but patients must use induction and maintenance immunosuppression to prevent allorejection. Eliminating the need for immunosuppression could significantly improve the risk-benefit profile of this therapy and thus increase the number of people eligible for it. Therefore, several approaches are currently being undertaken to prevent graft rejection using physical barriers or by genetically modifying the cell product. HLA I hyperexpression in human beta cells is a defining feature of T1D, while their expression of HLA Class II remains controversial. Human islets do not express HLA Class II under normal

SPEAKER ABSTRACTS

conditions, but it has been shown that HLA II can be induced in vitro in response to IFN-gamma and TNF-alpha treatment. Consistently, we show that prolonged IFN-gamma stimulation can induce HLA-II expression in hESC-derived islet-like cells. This suggests that inflammation may induce expression of HLA class II on transplanted islet cells, potentially causing anti-donor sensitization and adversely impacting islet transplant outcomes. Therefore, to advance our understanding of the protective effects of HLA editing, we sought to generate islet-like cells from hESC lacking surface expression of HLA class I and II, but expressing the inhibitory ligand HLA-E (B2MEdimer/-RFXANK-/-hESCs). We show that these "triple HLA-edited" hESCs effectively generate functional islet-like cells that do not express HLA-I and HLA-II under inflammatory stress in vitro, but physiologically respond to IFN-gamma by upregulating HLA-E expression. Additionally, we demonstrate that triple HLA-edited hESC-derived islet-like cells effectively evade T cell allorecognition in vitro and are not rejected following transplantation into a humanized mouse model (NSG-MHC-DKO reconstituted with human Peripheral Blood Mononuclear Cells). While these findings need to be validated by additional studies, these early results suggest that islet-like cells derived from HLA-edited hESCs may be a superior candidate for clinical use in T1D.

Funding Source: CIHR-JDRF—Accelerating Stem Cell-Based Therapies for Type 1 Diabetes Team Grant

2:00 PM – 2:15 PM

ESTABLISHED NEW IMMUNODEFICIENT LARGE ANIMAL MODEL FOR RECEIVING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED HEMATOPOIETIC GRAFTS

Brunet Manquat, Mathias[1], Linard, Christine[2], Petit, Laurence[3], Lapillonne, Hélène[3], Jaffredo, Thierry[3], Chapel, Alain[2], Guyonneau-Harmand, Laurence[4]

[1]Etablissement Français du Sang, France, [2]IRSN/ PSE-Sante/SERAMED/LRMED, France, [3]Sorbonne Université, France, [4]EFS Ile de France

Hematopoietic cell transplantation (HCT) is the first lifelong cell therapy for many blood disorders, including leukemia, sickle cell disease, and autoimmune diseases. The demand for clinical-grade HSCs has increased significantly in recent decades, creating challenges in patient treatment. To address this, we propose large-scale production of hematopoietic stem cells from human induced pluripotent stem cells (hiPSC) at an Advanced Therapy Medicinal Production (ATMP) core facility. We also plan to establish a new large animal model for human HCT. We have previously developed a robust, transgene-free protocol for the differentiation of hiPSCs into bona fide transplantable HSCs. This involves a 17-day culture period with morphogens and cytokines. We collaborated with Atlantic Bio's GMP (ABG) ATMP core facility for technology transfer and scale-up. The large-scale HSCs underwent quality controls, including cytometric validation against pre-defined markers and transplantation into irradiated NSG mice. In parallel we worked with the Aachen mini-pig model: a 6-week-old Aachen pig weighs approximately 6 kg, which is compatible with most pediatric oncology cases. Establishing a specific conditioning regimen involved defining radiation doses and immunosuppressive drugs, monitoring the animals, and analyzing blood samples. We successfully scaled up HSC production with ABG, validating the produced cells by FACS analysis and successful transplantation into mice. Aachen conditioning was a challenge, but we addressed their innate immunity using Cobra Venom Factor to protect human cells by inhibiting porcine complement. With the protocols in place, the HCT large animal trial can now begin. This proof of concept in a large animal will be an important step towards hiPSC-derived transplants and thus improved access to treatment for patients.

2:15 PM – 2:45 PM

UNIVERSAL CLOAKING OF ALLOGENEIC T CELL THERAPIES AGAINST NATURAL KILLER CELLS VIA CD300A AGONISM

Cowan, Chad

Century Therapeutics, USA

Immunogenicity limits the persistence of off-the-shelf, allogeneic cell therapies and transplants. While ablation of human leukocyte antigen (HLA) removes most T cell and humoral alloreactivity, no solution has enabled universal protection against the resulting natural killer (NK) cell response. Here, we engineered

SPEAKER ABSTRACTS

Trans Antigen Signaling Receptors (TASR) as a new class of NK inhibitory ligands and discovered CD300a, a previously inaccessible receptor, as a functional target. CD300a TASR outperformed leading alternative strategies in focused screens, including CD47 and HLA-E, and was solely capable of universally protecting allogeneic T cells against a large human cohort (45/45 donors), spanning diverse demographics and NK cell phenotypes. When combined with an anti-CD19 Chimeric Antigen Receptor (CAR) using multiplexed non-viral integration, allogeneic primary T cells exhibited enhanced B cell killing potency under allogeneic immune pressure. Additionally, the expression of CD300a TASR on iPSC-derived T cells provided protection against alloreactive NK cells. CD300 TASR represents a universal solution to NK alloreactivity, broadening the population that could be effectively treated by next-generation allogeneic cell therapies, including iPSC-derived CAR T cells.

3:15 PM – 4:55 PM
NEXT GENERATION TECHNOLOGIES AND PLATFORMS

3:15 PM – 3:45 PM

BIOENGINEERING HUMAN KIDNEYS TO TREAT KIDNEY FAILURE

Chen, Alice

Trestle Biotherapeutics, USA

Chronic kidney disease (CKD) is a global health crisis affecting 850 million people worldwide. In the US alone, nearly 1 in 7 adults has CKD, 650,000 patients depend on dialysis for survival, and 100,000 patients need a kidney transplant. Unfortunately, the treatment paradigm for patients with end stage renal disease (ESRD) has been stagnant for 70 years, since the first kidney transplant and first demonstration of dialysis. While organ transplantation remains the only cure, there is a widening gap between the number of donated organs and patients awaiting a kidney transplant. Moreover, dialysis—a bridge to transplantation with a 5-year survival rate below 40%—is expensive, time consuming, and exceptionally challenging for both patients and their families. Trestle is advancing a program which integrates stem cell biology, 3D biofabrication, and principles of developmental biology to bioengineer novel therapeutic tissues for patients with kidney failure. These implantable tissues aim to supplement renal function, take patients off dialysis, delay the need for transplantation, and become the foundation from which we develop long-term solutions to address the organ shortage. In this talk, I will discuss our current efforts in generating human kidney tissues with increased anatomical complexity, including building connections between developing nephrons and the ureteric epithelium, and the exploration of strategies for fabricating longer, centralized structures for excretion. Further, I will highlight our efforts in promoting tissue vascularization and maturation, in vitro. This work serves as the foundation from which we step towards the next generation of treatments for kidney failure through the development of bioengineered tissues and organs.

3:45 PM – 3:55 PM

INNOVATION SHOWCASE
MOVING IPSC BASED CGT INTO A GMP SETTING

Presented by CCRM

Rothberg, Janet

CCRM, Canada

Induced pluripotent stem cell (iPSC)-based therapies have the potential to offer broad clinical applicability and cost-effective scalable manufacturing to meet global patient demand. However, there are challenges in moving these therapies from the research lab into a manufacturing setting. CCRM has over a decade of expertise in iPSC expansion and differentiation, including reprogramming, gene editing, and scale up, downstream drug product. Working with our spin-out company LineaBio, we are producing GMP-grade iPSC lines to enable the community to address crucial challenges in clinical manufacturing. Along with OmniaBio, we are also continuing to evolve our capabilities in gene editing and scale up so that one day PSC-derived therapies can be revolutionary new treatment options for patients worldwide.

SPEAKER ABSTRACTS

3:55 PM – 4:10 PM

DEVELOPING MANUFACTURING PLATFORMS AND TARGETED PRECISE DELIVERY OF ADVANCED THERAPIES

Baptista, Ricardo

SmartCella, Sweden

SmartCella is a Swedish innovative biotechnology company combining developing and manufacturing novel stem cell- and RNA-based therapies and methods for administering such treatments via targeted and precise delivery into organs and tumours through a unique medical device. In this talk, we aim to present development data of our priority program, within a long-term collaboration with Astra Zeneca, of our human ventricular progenitor cardiac cell product derived from human pluripotent stem cells to treat heart failure aiming for first-in-human trials in 2025. In addition, we present the SmartCella product pipeline, our GMP manufacturing capabilities, and our strategies to develop scalable manufacturing platforms of advanced therapies within the concept of Industry V4.0 and the de-risking approach of "keeping the end product in mind from the start." Lastly, we aim to introduce the audience to the capabilities of our SmartWise Extroducer® for the targeted and precise delivery of any therapeutic payload into tissues and organs directly. We believe that with our capabilities from early research, industrialisation development and GMP manufacturing, to delivery of advanced therapies, we can change how to treat some of the most incurable diseases and support placing the Nordics region at the forefront of ATMP clinical and business development.

4:10 PM – 4:25 PM

FATEVIEW™: NON-INVASIVE LONGITUDINAL AND FUNCTIONAL CHARACTERISATION OF STEM CELLS POWERED BY AI

Pedone, Elisa[1], Sjogren, Rickard[2], Berecz, Tunde[1], Mohamad, Saad[1], Vandijk, Robert[1], Ren, Edward[1], Slaykovskiy, Vladimir[1], Carazo-Salas, Rafael[1]

[1]CellVoyant, UK, [2]CellVoyant, Sweden

In the field of human pluripotent stem cell (hPSC) research, significant challenges such as intra- and inter-cell line variability, poor prediction performance of cell potency, and batch effects hinder progress in regenerative medicine and disease modelling. Traditional stem cell characterisation techniques often rely on end-point measurements, which require invasive or destructive labelling of cells. We have developed FateView™, a novel technology powered by label-free real-time imaging and advanced AI algorithms that makes it possible to monitor and predict hPSC dynamic behaviour in a non-invasive, non-destructive way. FateView™ accurately identifies differentiation stages and cell types using only label-free imaging signals, eliminating the need for disruptive chemical or genetically-encoded labels. FateView™ not only matches the accuracy of traditional end-point methods but goes significantly beyond by allowing real-time, non-invasive monitoring of hPSC cultures—effectively overcoming the need for end-point testing and enabling longitudinal, functional characterisation of stem cells through time. We show that FateView™ can quantitatively reveal intra- and inter-cell line variability, improve prediction of cell line differentiation propensity and help mitigate batch effects, thereby significantly enhancing the efficiency and scalability of hPSC line production. By leveraging advanced AI FateView™ resolves critical challenges in hPSC research—such as enabling better, more cost effective oversight and optimisation of cell differentiation processes and ensuring higher quality and consistency in therapeutic applications—and sets the stage for future innovations with high potential to help accelerate the development of better, more cost effective cell therapies therapies, aligning with the ISSCR's commitment to advancing stem cell science and its applications.

4:25 PM – 4:55 PM

PRODUCTION OF ALLOGENEIC CAR-T CELLS AND CD4+ T CELLS FROM iPSCS TO ADVANCE IMMUNOTHERAPIES

Zandstra, Peter

The University of British Colombia, Canada

Engineered T cells offer immense potential for treating cancer, autoimmune diseases, and aiding transplant engraftment. These therapies have already transformed

SPEAKER ABSTRACTS

patient care for certain hematological malignancies, inspiring the development of next-generation engineered cell therapies for a broader range of conditions. The challenge now lies in cost-effective manufacturing of precision cell products to overcome various process and clinical design hurdles. Pluripotent stem cell (PSC)-derived engineered T cells present a promising solution. Producing allogeneic 'off-the-shelf' T cells from induced PSCs (iPSCs) could drastically reduce costs, improve therapy access, and enable the creation of advanced therapeutic functionalities. Our group recently developed clinically relevant, scalable methods for producing cytotoxic (CD8+) T cells from PSCs. We have also engineered transgenes, including chimeric antigen receptors (CARs), and performed gene knock-outs in PSCs, followed by single-cell cloning to generate fully gene-edited starting material for multi-edited CD8 T cell manufacturing. We have demonstrated that gene-edited, iPSC-derived CD8 T cells can be efficiently manufactured in scalable, stirred-tank bioreactors, exhibiting robust antigen-induced proliferation and persistence both in vitro and in vivo. These advancements pave the way for a potent and uniform clinical cell supply for allogeneic CAR-Ts, where a single manufacturing batch could suffice for early-phase clinical studies. Moving beyond CD8 cells, robust production of helper (CD4+) T cells from iPSCs has remained elusive. Producing these cells is crucial due to their diverse and critical immune functions, potentially enhancing the anti-cancer potency of cytotoxic (CD8+) T cells. Here, we employ in vitro niche engineering to facilitate the production of mature CD4+ T cells from iPSCs. By tuning Notch and TCR stimulation, we can control the CD4+ to CD8+ T cell ratio, achieving almost pure CD8 cells, almost pure CD4 cells, or targeted ratios in between. We further demonstrate the functionality and polarization of these cells. In conclusion, our work demonstrates the ability to precisely control the differentiation of iPSCs into mature human immune cell types, opening new opportunities for the immuno-engineering of allogeneic T cell therapies.

Funding Source: Wellcome Leap HOPE Program, the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), Notch Therapeutics

NecstGen
a non-profit CDMO

Your Non-Profit Partner in **Advancing iPSC-derived Therapies**

We empower therapy developers to turn ideas into clinical reality, making sure new therapies reach the patients in need. We specialise in iPSC technology, accomodating all development stages from early development to GMP manufacturing in our state-of-the-art facilities, led by our expert team.

Development

iPSC Reprogramming
Differentiation
Expansion & Characterisation
Target Cell Isolation
Gene Editing

Manufacturing

Cell Line Generation
Master Cell Bank
Working Cell Bank
Clinical Batch Manufacturing

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POSTER ABSTRACTS

2 October 2024

All times are listed in Central European Summer Time (CEST)

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Poster Session III Abstracts are listed on pages 65 – 77

WEDNESDAY, 2 OCTOBER 2024

4:45 PM – 5:30 PM
POSTER SESSION I

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AUTOMATIO-ASSISTED DERIVATION OF MESENCHYMAL STEM CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS POSSESS MULTIPOTENCY AND IMMUNOMODULATORY PROTERITIES

Shen, Chia-Ning[1], Hsiao, Chih-Chiang[2], Cheng, I-Fen[2], Chien, Chiao-Yun[2]

[1]Genomics Research Center, Academia Sinica, Taiwan, [2]Academia Sinica, Taiwan

The major barrier for the application of Induced pluripotent stem cells (iPSCs) is unable to supply uniform quality of target cells from iPSCs due to the complexities associated with iPSC manufacturing process. Establishment of an automated iPSC cell culture system can possibly solve these complexities. Although mesenchymal stem cells (MSCs) can be isolated from various tissue sources exhibit multiple differentiation potency and have shown promising therapeutic effects in a broad range of diseases, however, these applications can be hindered by their limited expansion ability and variation across donors. Thus, there is an increasing need for reproducible large-scale production of MSCs, with minimal variation. Here, programmable all-in-one Panasonic automated culture machine was utilized to derive MSCs from human iPSCs. Initial characterization had been conducted that revealed MSCs derived either from automatic or manual process possess similar characteristics and multipotency. To further uncover the cellular homogeneity in MSCs derived either from automated or manual process, FACS and single-cell analysis using BD Rhapsody system were performed. Initial characterization revealed that automatic and manual derived MSCs were positive for CD29, CD44, CD73, CD90, CD105, HLA-ABC, and negative for CD45, and HLA-DR. To further validated the interaction between MSCs and immune cells, PAPPA+ and HIST1H4C+ subpopulations were co-cultured with human primary natural killer (NK) cells which revealed MSC subpopulations derived from automatic process primed NK cells to produce increased levels of IFN-γ in response to IL-12 and IL-18. In contrast to cytotoxic CD56dim NK cells, CD56bright NK cells primed by MSC subpopulation derived from automatic process expressed higher levels of CCR2 which were sensitive to CCL2. The current work elucidated the possibility of derivation of functional MSCs with superior immunomodulatory properties utilizing automated production method.

Funding Source: NBRP core facility grant provided by Academia Sinica

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ENHANCING OF HEMATOPOIETIC STEM CELLS DIFFERENTIATION TO ERYTHROCYTES USING SMALL MOLECULE TARGETING HIPPO-YAP PATHWAY

Damkham, Nattaya[1], Issaragrisil, Surapol[2], Lorthongpanich, Chanchao[2], Klaihmon, Phatchanat[2], Kheolamai, Pakpoom[2]

[1]Mahidol University, Thailand, [2]Faculty of Medicine Siriraj Hospital, Thailand

Blood shortage is becoming a problem worldwide due to the lack of donors. Yes-associated protein (YAP) is a key transcription co-activator of Hippo signaling pathway which play a major role in regulating organ size and cell proliferation. Previously, we found that YAP plays a crucial role in erythrocyte maturation. We therefore interested to use small molecules to modulate YAP activity to increase erythroid differentiation. Human hematopoietic stem cells (HSCs) from mobilized peripheral blood were isolated and differentiated to erythrocytes using in vitro liquid culture system for 18 days. YAP activator: Lysophosphatidic acid (LPA) or YAP inhibitor: Dobutamine hydrochloride (DH) was added during erythroid differentiation. While activation of YAP by LPA did not change erythroid production yield but inhibition of YAP by DH for the entire period of culture significantly decreased erythroid differentiation

POSTER ABSTRACTS

2 October 2024

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and enucleation. To further identify the most affected differentiation step upon DH treatment, we then added small molecules at various differentiation time points. Surprisingly, inhibition of YAP by DH for a short period of time, during differentiation day 8–11, could increase the number of mature erythrocytes at the end of differentiation. Moreover, we found that inhibition of YAP by DH promoted gamma-globin switching. We also studied the effect of LPA and DH on the differentiation capacity of CD34+ HSC using colony-forming unit (CFU) assay. We found that DH increased the number of BFU-E colony and % of erythroid cells. This result suggested that inhibition of YAP in CD34+ HSC by DH could drive HSCs differentiation to erythroid lineage. However, at terminal differentiation stage, YAP activity needed to be re-activated to complete erythroid maturation and enucleation. Here, we demonstrated that targeting YAP activity at specific time point of differentiation could increase erythroid production yield in vitro.

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PRECLINICAL INVESTIGATION OF HUMAN EMBRYONIC STEM CELL-DERIVED DOPAMINERGIC PROGENITORS FOR THE TREATMENT OF PARKINSON'S DISEASE

Shiri, Zahra[1], Naderi, Somayeh[1], Salahi, Sarvenaz[1], Mollamohammadi, Sepideh[1], Badri, Motahareh[1], Taei, Adeleh[1], Hosseini, Parastoo[1], Zarghami, Maryam[1], Rahimi, Golnoosh[1], Soroori, Farzaneh[1], Shakerian, Farideh[1], Zare, Meysam[1], HajiNasrollah, Mostafa[1], Mirsadeghi, Ehsan[1], Bitarafan Rajabi, Ahmad[2], Hassanzadeh, Leila[2], Hassani, Seyedeh Nafiseh[1], Dehaqani, Mohammad Reza[3], Javan, Mohammad[4], Baharvand, Hossein[1]

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Cell therapy has shown great promise for impeding the progression of neurodegenerative diseases. We previously established large-scale production of cryopreserved clinical-grade human embryonic stem cell-derived dopaminergic progenitor cells (DAPs) for the treatment of Parkinson's disease (PD). Here, we present safety and efficacy assessments of DAPs to meet local regulatory requirements. The clinical-grade DAPs were extensively characterized in vitro prior to conducting animal studies. DAPs were transplanted to 200 immunosuppressed rats, 50 immunocompromised mice, and 5 non-human primate models of PD. Toxicity, tumorigenicity, and biodistribution were assessed over 26-weeks following intra-striatal transplantation of DAPs in rats and showed no adverse effects. Tumor formation assay was performed over a 39-week period and no tumors formed following subcutaneous injection of DAPs in immunocompromised nude mice. Moreover, intra-striatal transplantation of DAPs in 6OHDA-lesioned rats showed a significant functional recovery in the amphetamine-induced rotation test. Finally, MPTP-lesioned rhesus monkeys underwent intra-putaminal transplantation of DAPs to demonstrate scalability of our intervention. Our extensive preclinical testing showed no adverse effects attributable to the grafted DAPs, no distribution outside of the brain, and most importantly no tumor formation. Our non-human primate study confirmed feasibility, safety, and efficacy of DAPs transplantation. Overall, the presented data serve as a green light for considering first in-human clinical trials.

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PRECLINICAL STUDY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS FOR PERIPHERAL ARTERY DISEASE

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Peripheral artery disease (PAD) affects approximately 230 million people globally and chronic limb-threatening ischemia (CLTI) can lead to limb amputation. Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) offer a promising source for PAD treatment. However, to date, regulatory criteria for the clinical application of hiPSC-ECs have not been established yet,

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and there have been no reports on preclinical studies involving hiPSC-ECs. This study aims to address this gap by investigating the feasibility, safety, and efficacy of clinical-grade hiPSC-ECs through a preclinical proof-of-concept analysis. Clinical-grade hiPSC lines were established from the blood of three PAD patients using episomal plasmids. Their pluripotency was confirmed by assessing their expression of pluripotency markers through qRT-PCR and immunostaining, as well as their pluripotency in a teratoma assay. Furthermore, PAD-hiPSCs exhibited normal karyotypes. Subsequently, we differentiated PAD-hiPSCs into ECs, all of which displayed a cobble-stone EC morphology and expressed EC markers as confirmed by qRT-PCR and immunostaining. PAD-hiPSC-derived ECs also expressed CDH5 at a minimum of 98.4±0.2% and VWF at 94.4±1.3% by flow cytometry. PAD-hiPSC-ECs maintained their normal karyotypes and copy number variation across the genome, as determined by CGH array. Additionally, they exhibited endothelial characteristics such as tube formation in Matrigel and intracellular nitric oxide production. Administration of PAD-hiPSC-ECs into ischemic hindlimbs of both female and male mice led to improved blood flow recovery (~3.3 fold), reduced risk of limb loss (~8.8±0.6%), and increased vascular density (~2.7 fold) compared to control groups. These engrafted hiPSC-ECs exhibited vessel-forming capacities, thereby contributing to neovascularization. More importantly, we evaluated the toxicity, biodistribution, and tumorigenic potential of these cells in immunodeficient nude mice over one year. The results demonstrated non-detection of tumorigenic cells in twelve organs and adverse events. With all the data, the use of hiPSC-ECs was approved in Koreafor a clinical trial to treat PAD. Our preclinical proof-of-concept findings demonstrate, for the first time, the clinical compatibility of hiPSC-ECs derived from PAD patients for autologous cell therapy for PAD. Our study further suggests regulatory guidance for the clinical development of hiPSC-ECs for treating PAD patients.

Funding Source: R61/R33 HL154116/HL/NHLBI NIH HHS/United States 2023ER130201/KNIH/Korea 2024ER130200/KNIH/Korea 20016564/MOTIE/Korea RS-2022-TI016657/MSS/Korea

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INDUCING MATURATION OF AMELOBLASTS FOR TOOTH REGENERATION AND DISEASE MODELING OF AMELOGENESIS IMPERFECTA

Patni, Anjali P.[1], Mout, Rubul[2], Moore, Rachel[1], Alghadeer, Ammar[3], Daley, George Q.[2], Baker, David[4], Mathieu, Julie[1], Ruohola-Baker, Hannele[1]

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Tooth enamel, the hardest material in the human body is required to protect our living organ, tooth. However, over 90% of adults have lost or damaged enamel and cannot regenerate the protective structure due to lack of the cells secreting enamel, ameloblasts. In addition, a birth defect Amelogenesis Imperfecta (AI) results in enamel loss already during fetal development. We have used single-cell sequencing of human fetal ameloblasts (AM) to develop a novel iPSC-based AM differentiation protocol. These cells mature into secretory AM (isAM) when co-cultured in close proximity with odontoblasts (OB). My goal is to identify the critical signals emanating from OB for this maturation process. To dissect the signaling pathways crucial for isAM maturation, we utilized a pathway analysis (TopPath) and identified Notch pathway as a potential candidate involved in OB and AM interaction resulting in maturation. Our analysis of Notch and Delta gene expression patterns supports the hypothesis that Delta from OB can activate Notch in AM. To test if Notch pathway activation is required and sufficient for AM maturation, we utilized small molecules and novel AI-based designed protein scaffolds. Our results show that Notch inhibition (DAPT) affects enamelin (ENAM) secretion in the co-culture, indicating that Notch is required for AM maturation. We next developed a designed scaffold that can

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activate Notch in solution presumably due to force generated by interactive cells. Notch activation using this computationally designed scaffold enhanced the expression of mature isAM markers, ENAM, and MMP20, with or without coculture with OB cells. These data show that Notch pathway activation is critical for AM maturation and designed Delta scaffold can replace OB in the process. Our regenerative dentistry approach holds promise for addressing tooth loss and congenital disorders like AI.

Funding Source: ISCRM

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LEVERAGING ADVANCED ANALYTICS FOR DETAILED PROCESS CHARACTERISATION IN iPSC-NK CELL THERAPY GENERATION

Lee-Reeves, Charlotte[1], Jayawardena, Mudith[1], Oddy, Joseph[1], O'Grady, Chris[1], Nair, Aishwarya[1], Braam, Mitchell[1], Henry, Marianne[1], Karels, Vera[1], Guenat, Juline[1], Tregidgo, Molly[1], Statham, Patrick[1], Karachaliou, Antonia[1], Zingaro, Simona[1], Podovei, Robert[1], Marginean, Dragos[1], Ullmo, Ines[2], Brady, Hugh[2], Tarunina, Marina[3], Ponomaryov, Tanya[3], Mata, Marcia[1], Hasan Jahid[1]

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Utilisation of large-scale bioprocessing for allogeneic iPSC-derived cell therapy development requires comprehensive process understanding for the implementation of robust, biologically relevant controls during long-term cell differentiation. Following the successful demonstration of seamless end-to-end iNK generation in a stirred-tank reactor (STR) system in collaboration with a therapy developer, Cell and Gene Therapy Catapult (CGTC) applied high-content analytical methods including transcriptomics, proteomics and metabolomics to identify key biomarkers and critical process parameters (CPPs). By integrating these multi-omics technologies, indicators of high-quality iNK differentiation may be identified and utilised for future in-process monitoring to ensure consistent cell quality and functionality during manufacture. Single cell transcriptomics (employing Parse Evercode™ technology) was used to elucidate the dynamic gene expression profiles associated with critical stages of iPSC-NK differentiation and map the cell trajectory over a 35-day process. This was coupled with extensive flow cytometry and cancer cell cytotoxicity outputs to provide a detailed overview of cell phenotype, maturity and end-product functionality, which can help to infer the potency and quality of cells produced by this process. The data presented here highlights an intrinsic relationship between bioprocessing parameters and the yield of functional iNK cells, with a direct impact of cell density on key biological characteristics. Using these insights, with deeper investigations into the mechanisms that drive high productivity, future work can look to optimise production of highly cytotoxic iNKs at clinically relevant doses. Offline analytics can additionally be used to inform future development of online methodologies for providing real-time monitoring of differentiation.

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NON-VIRAL DELIVERY OF CRISPR ACTIVATION TOOLS FOR DIRECTED DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS

Jayawardena, Mudith, Awonusi, Ofure, Perez, Salvador, Esse, Ruben, Krishnan, Siddharth, Braam, Mitchell, Lopes Tiburcio, Marta, Zucchelli, Ele, Di Cerbo, Vincenzo

Cell and Gene Therapy Catapult, UK

The differentiation of induced pluripotent stem cells (iPSC) offers a promising solution for allogeneic cell therapies. To enhance their potential, cell engineering strategies have been applied to modify their genome or to promote their differentiation via viral and non-viral methods. However, the potential for insertional mutagenesis and genotoxicity of viral methods for both autologous and allogeneic therapies could hinder clinical translation. Additionally, the collection and manufacture of haematopoietic lineage cells from patient donor material is often constrained in autologous therapies. Yet, traditional methods of deriving haematopoietic progenitor cells (HPC) from iPSCs pose their own challenges where complex, highly variable

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protocols are used. To support the development of next generation allogeneic cell therapies, we aim to establish an iPSC directed differentiation workflow based on non-viral delivery of CRISPR-mediated transcriptional activation (CRISPRa) tools, targeting the activation of four factors that favour differentiation of iPSCs into HPCs. Following a technology evaluation and protocol optimisation, we have established an iPSC electroporation protocol with 95% transfection efficiency of a dead mutant Cas9 (dCas9)/CRISPRa complex multiplexed with four guide RNAs (gRNA) for the selected transcription factors. In addition, we developed a flow cytometry and droplet digital PCR analytics to evaluate gene activation. We also demonstrate the iPSCs' ability to form aggregates following gRNAs electroporation, establishing the impact of gRNAs delivery to follow-on HPC differentiation. This work encapsulates our efforts on benchmarking non-viral delivery technologies, including lipid nanoparticles, as an alternative and potentially cost-effective strategy to iPSC differentiation. We foresee this work will represent a baseline that can be repurposed to fit alternative targets for new differentiation processes for additional iPSC-derived cell types.

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IN-USE STABILITY OF CellThRPE1, A GMP-COMPLIANT HESC-RPE DRUG PRODUCT FOR THE TREATMENT OF DRY AGE-RELATED MACULAR DEGENERATION

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Dry age-related macular degeneration (dAMD) is one of the major causes of blindness in the developed world. Impairment of the retinal pigment epithelium (RPE) cells in dAMD leads to the degeneration of photoreceptors in the macula and loss of central high-acuity vision. While recent pharmacological advances have indicated promising results in halting disease progression, no clinical interventions have been approved to cure dAMD. Pluripotent stem cell-based replacement cell therapy emerges as an attractive strategy to halt progressive vision loss. Here, we present the 2-year mark of our 5-year stability programme of a cryopreserved allogeneic embryonic stem cell (ECS)-derived RPE drug product (CellThRPE1), manufactured to Good Manufacturing Practice (GMP) standards. FACS-based assays were developed to assess product purity and lingering pluripotent contaminants, while drug product-specific functional in vitro assays were established to determine the epithelial integrity of the product, included in a panel of critical quality attributes with well-defined acceptance criteria. In addition to reporting 48 months of stability for our clinical batch, we validate a 4-hour shelf-life of our dose at room temperature to facilitate the transport from the formulation facility to administration in the operating room (OR). These data support long-term functional cryopreservation and in-use stability and will supplement future clinical translation through a first-in-human trial at St. Erik Eye Hospital in Stockholm, Sweden.

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FULL-LENGTH LAMININS ARE CRUCIAL FOR RECREATING THE PLURIPOTENT STEM CELL NICHE IN VITRO

Kele, Malin, Xiao, Zhijie, Fereydouni, Noah, Eleuteri, Boris, Kallur, Therese

BioLamina, Sweden

Laminins are an extracellular matrix (ECM) family of 16 different protein isoforms. The expression of the laminins are temporally and spatially specific, being essential for tissue formation and homeostasis. The laminins are within all tissues of the body, particularly concentrated in the formation and maintenance of the basement membrane (BM). Intact laminins are essential since mutations in genes encoding laminins can cause a wide spectrum of disorders called "laminopathies". Mutations in different domains of the laminin protein result in BM weakness which can result in tissue-specific dysfunctions, affecting for example muscle, kidney, nerve, skin, and eye. Examples, of such congenital

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disorders caused by laminin chain mutations, are Pierson syndrome and Epidermolysis bullosa. In vivo, most laminin receptors are integrins and non-integrins including dystroglycan, syndecan, and Lutheran blood group glycoprotein. Multiple essential growth factors (GF) can bind with high affinity to the complex laminin molecules. Interactions between GFs and ECM are essential for controlling GF release kinetics Intact laminins (full-length). Laminins are large trimeric proteins of about 800 kDa, with all multiple binding sites which, crucial for building the correct network and capable of self-assembly which provide a key structure element of BMs. Thus, the intact trimeric laminin complex is necessary for the bioactivity levels of the protein. Truncated laminin proteins are not produced by healthy tissue and lack essential components for healthy tissue homeostasis. We have compared the survival, proliferation, and migration of human PSCs, on truncated laminins to intact full-length laminin protein isoform 521. We can demonstrate a robust and enhanced survival, proliferation, and migration, on the full-length laminin. It even allows for single-cell seeding and omits the need for ROCKi. The cells are highly migratory on full-length laminin-521, reaching a 100% closure compared to commercial truncated laminin products, which reach a maximal closure of 50%. In conclusion, full-length laminin-521 mimics the natural cell microenvironment for PSCs in vitro crucial for PSC culture and the development of successful differentiation protocols, predictable disease models, and effective gene editing.

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RECOMBINANT FULL-LENGTH LAMININS SUPPORTING CELLS IN ALL CULTURE FORMATS

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BioLamina, Sweden

Recombinant full-length human laminins, Biolaminin, can improve the hPSC culture at different scales, from microtiter plates for gene editing, to upscaling with hollow-fibers and microcarriers. Due to high cellular yield and attachment, 34% savings in matrix and material costs could be achieved. The cell culture environment must be carefully designed to mimic the natural cell niche, including relevant medium and substrate, to achieve standardized, reproducible, and biorelevant cells. Developing recombinant and scalable protein matrices for efficient expansion and differentiation of hPSC has been a milestone for emerging cell therapies. Laminins are an extracellular matrix (ECM) protein family of 16 different isoforms and essential components of the basement membranes through the human body, vital for tissue development and homeostasis. The extracellular matrix (ECM) protein, laminin-521 is the major ECM protein expressed in the inner cell mass of the pre-implanted embryo and, therefore the most biologically relevant matrix for hiPSCs and hESCs, directly involved in their survival and self-renewal, independent of platform size or material. In microtiter plates, Biolaminin 521 proved to be an excellent substrate for gene editing, automated workflows, and imaging analysis. High content image analysis system assessed the best hiPSC confluence and single-cell cloning efficiency on LN521, compared to other ECMs. For CRISPR/Cas9, Biolaminin 521 coating supported single hPSC seeding at a very low density. For imaging on glass surfaces, the purity and adhesive properties of Biolaminin 521 proved to be a major advantage. In large-scale expansion systems for PSCs, Biolaminin 521 coating significantly increased the cell numbers and population doublings, on both porous hollow fibers and microcarriers. Biolaminin 521 coating resulted in a 7.5-fold increase in cell expansion with 90% viability for PSCs by providing survival, proliferation, and migration signals. This influenced improving attachment (87%) and surface area usage (85% spreading). Thus, cutting the overall matrix and material costs by 34% due to the increased culture efficiency. In conclusion, by re-creating the natural cell niche, with recombinant full-length human laminins in vitro, human ESC and iPSC culture can be supported from clonal stages to stable long-time expansion, independent of the platform or level of automation.

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TRANSPLANATION OF AN EFFICIENT DIFFERENTIATED AND CLINICAL GRADE HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS IN A MACULAR DEGENERATION MODEL

Barati, Mojgan, Taei, Adeleh, Satarian, Leila, Khalaj-Asadi, Zahra, Sabahi Moosavi, Narges, Samadian, Azam, Salahi, Sarvenaz, Shiri, Zahra, Vahedi, Amirreza, Soroori, Farzaneh, Hajizadeh, Ensieh, Hassani, Seyedeh-Nafiseh, Baharvand, Hossein

Royan Institute, Iran

Human embryonic stem cell-derived retinal pigment epithelial cells (hESC-RPE) have increasingly been the focus of clinical translational studies for the treatment of age-related macular degeneration (AMD). One of the intriguing methods to increase the therapeutic potential of hESC-RPE is to increase protocol efficiency. Here we present an improved method for developing pure and functional hESC-RPE cells under GMP-compliant conditions. hESC-RPE cells were generated using BMP4 in a stepwise manner under GMP-compatible conditions and were subjected to extensive in vitro characterization and evaluated for safety and efficacy by transplantation into the subretinal space of Royal College of Surgeons (RCS) rats. The cells were cryopreserved in BSS+ and their long-term stability was monitored. Subsequently, hPSC-RPE were transplanted into the subcutaneous or subretinal space of nude mice or Royal College of Surgeons (RCS) rats respectively, to evaluate the safety and efficacy of these cells. The differentiated cells demonstrated a high purity and showed phagocytic potential and polarized VEGF and PEDF secretion. Cryopreserved cells remained viable and retained their cellular characteristics. Subretinal injection of hESC-RPE in RCS rats showed functional rescue of photoreceptors and prevented vision loss in 6 months evaluations. hESC-RPE transplantation showed no tumor formation, no biodistribution in the host body, and no systemic toxicity. These findings highlight the potential of our protocol for differentiation and cryopreservation of hPSC-RPE cells under GMP compliant conditions, emphasizing their safety and stability for future research and therapeutic development.

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GENOMIC INTEGRITY OF THE CRISPR-CAS9 EDITED PLURIPOTENT STEM CELLS

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Several studies showed that human pluripotent stem cells including iPSCs lost immunogenicity when MHC class I and II genes were inactivated by CRISPR-Cas9 editing. To reduce alloreactive immunogenicity of these cells, we have generated B2M-/-CIITA-/- knockout iPSCs and selected single cell clones, which do not express HLA-class I and II molecules on the cell surface. Specifically, we investigated the whole genome integrity of these CRISPR-Cas9 edited human pluripotent stem cells to uncover possible structural alterations, particularly those that may not be detected by short-read sequencing. We performed 10x linked-read sequencing by 10x Genomics and optical genome mapping by Bionano. After analysis of the entire genome structure and sequences, we identify unexpected large chromosomal deletions (>90 kb) at atypical non-homologous off-target sites without sequence similarity to the sgRNA in approximately 18% edited lines, in addition to the previously reported structural variants at on-target sites. These observed large structural variants induced by CRISPR-Cas9 editing in dividing cells may thus result in the loss of important active genes, thus causing pathogenic consequences in latter year. Whether these unexpected large chromosomal changes can limit the usefulness of the CRISPR-Cas9 editing system for disease modeling and gene therapy is one unsolved question. But, in this work, our whole genomic analysis may provide a valuable strategy to ensure genome integrity after genomic editing to minimize the risk of unintended effects in research and clinical applications.

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DETERMINING THE IDENTITY OF TRANSPLANTED CORNEAL ENDOTHELIAL CELLS THAT CONTRIBUTE TO IN VIVO REGENERATION

Bonelli, Filippo, Bailey, Tom, Dickman, Mor, LaPointe, Vanessa

MERLN Institute for Technology-Inspired Regenerative Medicine, Netherlands

Corneal endothelial failure is a leading cause of blindness. The current treatment, Descemet membrane endothelial keratoplasty (DMEK), replaces the diseased endothelium with donor tissue, but suitable donor tissue is scarce. Recently, injecting allogeneic corneal endothelial cells (CEnCs) expanded in vitro has emerged as a promising alternative. This method involves removing the diseased endothelium, injecting a cell suspension into the anterior chamber, and having the patient maintain a face-down position for three hours to facilitate cell engraftment. Two prominent culture protocols have been developed for the in vitro expansion of CEnCs, and their efficacy has been demonstrated in various in vivo studies, including a phase I clinical trial. However, the identity of the transplanted cells and their contribution to in vivo outcomes remain poorly understood. Our aim is to provide a deeper characterization of the two protocols, with particular emphasis on the qualitative evolution of the two cellular products over multiple passages. Our immunofluorescence findings highlight distinct differences in the expression profiles of positive markers (CD166, ZO-1, and ATP1A1) and negative markers (CD44, CD10, and ACTA-2) between the two protocols, with these differences becoming more pronounced over time. Quantitative analysis via flow cytometry corroborated the immunofluorescence results. Additionally, morphological analysis revealed significant differences in cell size, which were related to the differing proliferative capacities of cells cultured in the two media. Through this work, we aim to enhance our understanding of the importance of various markers in vitro to identify key factors contributing to successful regeneration. Our findings will contribute to advancing the standardization and success of this therapeutic approach.

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DEVELOPMENT OF SMT-M01 FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Brown, Kristy[1], Yan, Lu[1], Lee, ChangHee[1], Gunnlaugsson, Hilmar[1], Djeffal, Yannis[2], Bouchekioua, Amine[2], Rodriguez-de-la-Rosa, Alejandra[2], Morris, Carl A.[1], Klein, Allon[1], Pourquie, Olivier[1]

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Somites are transient structures in developing embryos that give rise to diverse cell types, including skeletal muscle, dermis, bone, cartilage, tendons, and brown adipose tissue. Somite Therapeutics is developing cell replacement therapy products to treat diseases affected by the degeneration or loss of somite-derived lineages. Our lead program, SMT-M01, is initially being developed for the treatment of Duchenne muscular dystrophy (DMD), a rare X-linked genetic disorder characterized by progressive muscle loss. This product is expected to have broader applications for other muscle degenerative diseases and trauma. We have developed a stepwise myogenic induction protocol that recapitulates the developmental trajectories of skeletal muscle, resulting in the efficient production of myofibers and satellite cells (PAX7+ muscle stem cells) from human pluripotent stem cells (iPS) in vitro. This protocol has been optimized using cues obtained from bioinformatic analyses of single cell RNA sequencing datasets of the developing myogenic lineage in the embryo. Key developments include: (1) replacement of growth factors with chemicals for directed differentiation; (2) optimized harvest protocol that enriches for PAX7+ cells; and (3) reduction of the fibrogenic potential. SMT-M01 produced with the optimized protocol has been characterized by scRNA-seq, in vitro characterization of muscle fibers and in vivo efficacy, monitoring muscle graft size and percent of human cells. The improved process results in a cell product that is ~50% muscle stem cells, 35% muscle lineage cells, 12% fibroblast and 3% neuronal subpopulations. When grafted to irradiated and cardiotoxin injured NOG mouse TA muscles, the new protocol results in improved efficacy with 25% increased TA muscle mass, increased

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cross sectional area (~3x) and higher percentage of human cells (>70% of nuclei) using the new protocol compared to the previous protocol. SMT-M01 is advancing toward the clinic with IND-enabling studies currently underway. The initial clinical study will evaluate the safety and potential efficacy of SMT-M01 in non-ambulant male DMD patients. The treatment approach involves dosing the thenar muscles, which has the potential to reverse muscle weakness that limits DMD patients' ability to fully use computer screens and operate wheelchairs which is critical for maintaining quality of life.

Funding Source: All funding is provided by Somite Therapeutics

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GENERATION AND CHARACTERISATION OF 'UNIVERSAL' LOW-IMMUNOGENIC HUMAN PRIMARY CHOLANGIOCYTE ORGANOIDS FOR TREATMENT OF BILE DUCT DISORDERS

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Other than complex surgery or transplantation, there are no current curative therapies for bile duct diseases/cholangiopathies affecting the intra- or extrahepatic biliary tree. We have previously shown that human bile duct epithelial cells can be cultured as 3D organoids to generate mature human primary cholangiocyte organoids (PCOs) for the treatment of cholangiopathies. Since the generation of autologous PCOs is likely to remain logistically and economically prohibitive for the foreseeable future, immune rejection of allogeneic PCOs remains a key outstanding barrier to their clinical translation. We thus aimed to develop 'universal' low-immunogenic cholangiocyte organoids for regenerative medicine applications. After systemic testing of numerous conditions, human leukocyte antigen (HLA) I and II double knock out (DKO)-edited PCOs (ePCOs) were generated using CRISPR-Cas9 by dissociating PCOs into single cells, electroporation with the guide-Cas9 complex and sorting for the specific double negative cells. Assessment comparing to parental wild-type cells was carried out by flow cytometry, functional readouts, co-culture with human peripheral blood mononuclear cells (PBMC) in vitro, and by engraftment under kidney capsule of immunodeficient mice subsequently humanised. Mutational load and CRISPR-driven off-target genetic mutations of parental vs ePCOs was quantified using whole genome sequencing and Nanoseq techniques. The HLA I and II DKO ePCOs generated maintained a mature PCO phenotype demonstrated by flow cytometry and functional analyses. Immune characterization in vitro by co-culture with PBMC experiments and in vivo with humanised mice, showed that ePCOs have reduced PBMC cell activation, reduced local immune infiltration and increased graft survival. Additionally, off-target analysis and mutation burden of parental vs ePCOs did not show CRISPR-driven off-target sites nor excess mutation in ePCOs. Overall, human PCOs lacking HLA I and HLA II can be efficiently generated using a CRISPR-Cas9 approach without CRISPR-driven off-target effects. Additionally, ePCOs retain the phenotypic characteristics of mature PCOs and show reduced immunogenicity when co-cultured with PBMC and in humanised mouse models compared to parental cells.

Funding Source: This work was supported by awards from Medical Research Council (MRC) UK Regenerative Medicine Platform (MR/S020934/1) and MRC Confidence in Concept (G116517)

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DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TOWARDS BASAL FOREBRAIN CHOLINERGIC PROGENITORS AS A REGENERATIVE THERAPY FOR DEMENTIA

Salvador, Alison[1], Rågård Christiansen[1], Josefine[2], Schörling, Alrik L.[2], Kazaku, Nina Lydia[2], Piilgaard Petersen, Louise[2], Gustaffsson, Emma[3], Kajtez, Janko[2], Casado Sainz, Agata[2], Bjorklund, Anders[3], Kirkeby, Agnete[2]

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Dementia with Lewy bodies (DLB) and Parkinson's Disease with dementia (PDD) are common neurodegenerative diseases, prevalently caused by the presence of alpha-synuclein (asyn) intracellular neurotoxic aggregates in the brain. In the basal forebrain (BF), this leads to degeneration of acetylcholine (ACh)—releasing neurons and cholinergic denervation in the hippocampus and pre-frontal cortex. The extension of basal forebrain cholinergic neurons (BFCNs) loss has been found to correlate with the level of cognitive impairment. In fact, current available treatments for dementia are mainly ACh esterase inhibitors (e.g. rivastigmine) which aim to increase ACh levels in the brain, providing a temporary symptomatic relief in the initial phases of the disease. However, their effect is greatly reduced or absent towards later stages of the disease when the cholinergic neurons of the baasal forebrain are completely degenerated. In this context, the development of a cholinergic cell replacement therapy is a promising approach to replace the damaged BFCNs, restoring the innervation of hippocampal and cortical areas, and therefore the cognitive functions. Proof-of-concept studies have already shown the potential of a BFCN cell replacement therapy, transplanting rat foetal-derived cholinergic progenitors in a rat model for dementia. In this study, we optimized the differentiation of human pluripotent stem cells towards authentic BFCN progenitor cells, suppressing the differentiation of non-BFCN neuronal populations with similar developmental origin, such as the GABAergic interneurons. Moreover, we validated the terminal maturation to a bona fide BFCN fate by transplanting cholinergic progenitors into the rat CA1 hippocampal region and pre-frontal cortical area, which lead to mature grafted cells and integration in the layered structure of the hippocampus. We also explored two approaches to generate a rat model of dementia with Lewy bodies, either by using a targeted toxin or by injecting in the basal forebrain a combination of adenovirus carrying alpha synuclein and sonicated preformed fibrils. The outcome of this project is to assess the clinical potential of such cell therapy for DLB and PDD through PoC efficacy studies in an animal model of cholinergic deficiency.

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MODELING FRAGILE X SYNDROME USING MULTI-REGION HUMAN BRAIN ORGANOIDS

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Human tissue-based models serve as crucial intermediate tools to investigate the mechanisms underlying human disease and to validate drugs. Fragile X syndrome (FXS) is among the most prevalent forms of intellectual disability, caused by monogenetic mutation. In FXS patients, hyper-methylation at the promoter region of the fragile X mental retardation 1 (FMR1) gene results in reduced or absent expression of fragile X mental retardation protein (FMRP), a multifunctional RNA-binding protein. Recent studies employing FXS cortical organoids have elucidated cellular and molecular mechanisms leading to FXS phenotypes. However, these investigations have lacked interneurons, which are essential for studying FXS phenotypes. The primary objective of this study is to establish the ganglion eminence-cortex (GE-Cx) fusion organoid system as a more accurate model for FXS, incorporating both excitatory and inhibitory neural

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networks for drug screening and validation. Cortical organoids were generated from FXS patient lines and preliminary findings indicate an increased number of SOX2+ neural progenitors and a reduced count of TBR1+ neurons in D98 FXS organoids, suggesting premature neuronal differentiation. Currently, we are generating FXS GE-Cx fusion organoids to investigate inhibitory neuron migration and alterations in axo-dendritic morphology in interneurons. This alternative human-based model system, the GE-Cx fused organoids, offers an unprecedented opportunity to explore FXS excitatory and inhibitory imbalance and would be a better platform for drug validation prior to clinical trials.

Funding Source: NIH R00 FRAXA Foundation CIRM COMPASS Scholarship (EDUC5)

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BR-DA02: A NEXT-GENERATION CELL REPLACEMENT THERAPY FOR PARKINSON'S DISEASE

Paladini, Carlos A., Xu, WenJin, Barboza, Luendreo, Petko, Alyssa, Smith, Ryan, Wilkinson, Dan, LoSchiavo, Deven, Conner, Kate, Harvey, Lucas, Clark, Haley, Bernal, Sonia, Jones, Melissa, Ebel, Mark, Srinivas, Maya, Tomishima, Mark, Irion, Stefan

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Transplantation of human induced pluripotent stem cell (hiPSC)-derived dopamine (DA) neuron progenitors represents a promising treatment option for those afflicted with Parkinson's Disease (PD), with numerous advantages over other available modalities. With the recent advent of several PD stem cell therapies, the next generation cell replacement therapy must provide additional benefits to end users at a scale and robustness sufficient to reach a large patient population. Here, we establish a repertoire of preclinical characteristics associated with identified next-generation attributes for PD amelioration. Multiple batches of BR-DA02 yielded reproducible cell type compositions, gene expression profiles, neural activity characteristics, and DA production. When grafted into the rodent brain, the same BR-DA02 batches reproducibly conferred efficacy in the amphetamine-induced rotation, and stepping, tests. Additionally, we measured functional synaptic integration with the host brain in the same animals. By demonstrating the robustness of our product, manufacturing at scale becomes more reliable, allowing for wider patient reach with a next-generation cell therapy.

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BENCHMARKING OF DOPAMINERGIC PROGENITOR SURFACE MARKERS WITH SEMI-AUTOMATED ANALYSIS OF FLOW CYTOMETRIC DATA

Schörling, Alrik L.[1], Salvador, Alison[1], Hänninen, Erno[1], Holm, Amalie[1], Rifes, Pedro[2], Rathore, Gaurav S.[3], Christiansen, Josefine R.[1], Jensen, Simone M.[4], Niclis, Jonathan C.[4], Gustafsson, Emma[5], Aldrin-Kirk, Patrick[6], Nelander, Jenny[5], Zhang, Yu[7], Parmar, Malin[5], Kirkeby, Agnete[1]

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Human pluripotent stem cells are a promising source of dopaminergic (DA) progenitor cells that are used in cell replacement therapy for Parkinson's Disease. However, heterogeneity in cell composition is inevitable in the cell differentiations. Cell surface markers predictive of functional maturation of DA progenitors to ventral midbrain (VM) DA neurons have utility as a convenient quality control and to enrich for the target cell type. By performing single-cell RNA sequencing of our in vitro model of the developing neuronal tube, we identified a candidate surface marker of DA progenitors. With a semi-automated analysis of flow cytometric data in Python, we benchmarked the specificity of this marker to DA progenitor populations compared previously published markers. Our marker outperformed other markers and correlated to genes indicative of a VM DA fate. Cells positive for our marker, sorted from a mix of neuronal progenitor cells, yielded behavioural recovery in a Parkinsonian rat model, but cells negative for the

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marker did not. Taken together, our new DA surface marker outperforms previously published markers, and could be utilized in upcoming clinical trials exploring cell therapy as Parkinson's Disease treatment.

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SURVIVAL OF TRANSPLANTED HUMAN SOMATIC NUCLEAR TRANSFER STEM CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS IN A HUMAN RECIPIENT FOR 12 MONTHS

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We would like to present the histological and genetic findings from the first clinical application of somatic cell nuclear transfer (SCNT) stem cell-derived retinal pigment epithelium (RPE) in a human subject. SCNT is a cloning technique where the nucleus of a somatic cell is transferred to the cytoplasm of an enucleated oocyte. SCNT is potentially valuable for generating genetically matched stem cells for research and therapeutic purposes. One Asian female participant with advanced dry age-related macular degeneration (AMD) was recruited for this study. SCNT was established from the patient's skin fibroblasts. The Korean Ministry of Food and Drug Safety authorized the clinical trial. We transplanted SCNT-RPE cells into the subretinal space of the patient following a standard pars plana vitrectomy. Anatomically, subretinal and preretinal areas exhibited dark brown pigmentation without any definitive signs of rejection, adverse proliferation, or ectopic tissue formation. At two weeks postoperatively, an epiretinal membrane (ERM) with dark brown pigmentation developed. The ERM enlarged, causing minimal thickening of the underlying retina, and the preretinal pigmentation area increased and darkened by 52 weeks. Best corrected visual acuity (BCVA) decreased at 43 weeks, and a cataract with NO4 NC3 grade developed in the treated eye. At 52 weeks, the ERM was removed following standard pars plana vitrectomy. The cataract was also extracted via phacoemulsification, and an intraocular lens was implanted in the posterior chamber. Two weeks after surgery, BCVA improved to baseline level, and retinal thickening was significantly reduced. Histological sections revealed a thin fibrous membrane containing dark brown pigment under low-power magnification. High-power views showed two distinct cell types. Most cells were spindle-shaped with abundant collagen, consistent with a typical ERM. The other type, epithelioid cells containing dark brown melanin pigment, was attached to the ERM. These cells did not exhibit any anaplasia, including mitotic figures, pleomorphism, or a high nuclear/cytoplasmic ratio. To determine the origin of the pigmented cells, we performed mitochondrial DNA genotyping. Intriguingly, the pigmented epithelial cells contained the same mitochondrial DNA sequence as the oocyte donor, which differed from the recipient's somatic cell sequence (rs2853826, m. 10398 A>G). Since the SCNT was established using the donor's enucleated oocyte and the recipient's cell nucleus, we concluded that the pigmented epithelial cells originated from the SCNT-RPE. These findings demonstrate that SCNT-derived cells can survive in humans for 12 months without anaplasia and may participate in the formation of ERM.

Funding Source: This research was supported by a grant of the Research Driven Hospital R&D project, funded by the CHA Bundang Medical Center (grant number: BDCHA R&D 2015-15)

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RAPID INDUCTION OF HIGH PURITY SPINAL MOTOR NEURON PROGENITOR CELLS THROUGH NEUROMESODERMAL PROGENITOR FROM PLURIPOTENT STEM CELLS

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It has recently been suggested that the differentiation of spinal cord neural stem cells(scNSCs) occurs through neuromesodermal progenitors (NMPs). In this study, we have discovered a method for generating high purity thoracic scMNPs from pluripotent stem cells

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(ES or iPSC). We were able to efficiently and sequentially produce NMPs, scMNPs, and pan-motor neurons (pMNs) within 12 days. Each corresponding stage showed the following results when immunocytochemistry was performed: CDX2+/SOX2+/SOX1- (>95%/>95%/<5%), OLIG2+/NKX2.2- (>90%/<5%), and HB9+(>85%). Moreover, it appears that we can regulate thoracic spinal cord motor neuron subtypes, which was suggested only in rodent models, by controlling the duration of differentiation. We regulated the duration of the pMNs differentiation stage to isolate motor neurons from the MMC/HMC, and PGC columns. Our studies will provide high purity, rapid induction protocol of scMNPs and in vitro generation of motor neuron subtypes using human PSCs. This finding will serve as a model for producing neuron subtypes matching specific muscles, which can be applied to cell therapy in the future.

Funding Source: This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health & Welfare) (23A0204L1)

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THE COMPARISON OF PROPERTIES OF iPSC-DERIVED CARDIOMYOCYTE SPHEROIDS BETWEEN VARIOUS FORMATION METHODS

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Heart failure is the leading cause of death all over the world. Although cardiac transplantation is an effective therapy, it is limited by a shortage of donors. Thus, cell therapies using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a promising treatment especially for severe heart failure. In in vivo study, it is known that transplantation of hiPSC-CM spheroids formed in a microwell plate have a better engraftment rate than single cells. For futural practical therapy, large-scale production of hiPSC-derived cardiac spheroids (hiPSC-CSs) is required, and one of them is, for example, the production in bioreactor. However, there has been no understanding of how different spheroid formation methods affect the properties of hiPSC-CSs as far as we know. Therefore, in this research, we compared the properties of hiPSC-CSs formed in microwell plates, bioreactors, and CAT system, a spheroid-forming material we are developing, in terms of their quality as hiPSC-CSs for transplantation. The CAT system consists of cell-adhesive materials and anti-adhesion coatings, which allows for self-organization of cells, spheroid size control, and massive scale production. As a result, there were significant differences in a diameter deviation of hiPSC-CSs between various methods. In particular, hiPSC-CSs formed in bioreactors had a wide size distribution and tended to collapse due

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to physical stress easily. We also compared the gene expression of cardiomyocyte markers and beating rate. These data suggest that the properties of hiPSC-CSs could be changed depending on the method for producing spheroids. To realize a mass-production of hiPSC-CSs with suitable properties for transplantation, now we develop a reliable, large-scale, and closed culture system by using the CAT system.

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SCALABLE MANUFACTURING OF HUMAN HEMATOPOIETIC STEM CELLS USING STIRRED TANK BIOREACTORS: ENSURING CONSISTENCY FOR CELL THERAPY AND REGENERATIVE MEDICINE APPLICATIONS

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Hematopoietic stem cells (HSCs) are essential for blood and immune cell development, offering significant potential for cell therapy and treating various haematological conditions. Consistent, large-scale manufacturing of HSCs from induced pluripotent stem cells (iPSCs) is crucial but challenging due to the complex differentiation process. Ncardia, a leader in iPSC innovation, has developed robust HSC differentiation protocols using stirred tank bioreactors to ensure consistency and scalability. Ncardia adapted 2D HSC differentiation processes to 250 mL stirred tank bioreactors, aiming to scale up to 1 L and 3 L bioreactors. We employed a Design of Experiments (DoE) approach to systematically evaluate critical process parameters (CPPs) such as dissolved oxygen, agitation speed, and inoculation density. Parallel testing of multiple protocols established proof of concept. In-process monitoring and comprehensive data analysis provided a robust understanding of critical quality attributes (CQAs), ensuring optimized and consistent differentiation. The stirred tank bioreactor protocols developed by Ncardia yielded high-quality HSCs with self-renewal and differentiation capabilities into various blood and immune cell types. These iPSC-derived HSCs exhibited typical morphology and expressed key markers (>80% CD34, CD45, and CD43). They demonstrated potential for differentiation into multiple immune cell lineages, including natural killer (NK) cells, microglia and macrophages, underscoring their relevance in cell therapy and immunotherapy. Stirred tank bioreactors showed higher cell yield (100×106 cells/bioreactor) and viability (>90%) compared to 2D adherent and suspension methods. Ncardia's innovative approach using stirred tank bioreactors for HSC differentiation addresses the critical need for large-batch consistency and scalability in cell therapy. By integrating stem cell biology expertise with advanced bioreactor technology, we ensure high-quality, reproducible HSC production compliant with Good Manufacturing Practice (GMP) standards. This capability aims to accelerate cell therapy development and increase the potential for generating diverse immune cell types for therapeutic applications.

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JUMP START ALLOGENEIC CELL THERAPIES USING cGMP-GRADE TARGATT MASTER iPSCS

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Induced pluripotent cells (iPSCs) for site-specific DNA-fragment knock-in are essential for developing and manufacturing the next generation of cell therapeutic products. Traditional genome editing technologies, such as CRISPR/Cas9 systems, can be used to establish stable knock-in cell lines; however, transgene size can be limited, and substantial licensing fees are required to move the engineered cell line into product development and manufacturing. To generate a ready-to-use cGMP-grade iPSC line for site-specific gene of interest (GOI) knock-in, we adapted the TARGATT integrase system by integrating an attB landing pad into the Hipp11 locus (H11) by MAD7 nuclease in a cGMP-grade iPSC line, and isolated desired clones harboring the landing pad, which were then characterized and banked. The established cGMP-grade TARGATT iPSC line was tested for site-specific insertion of a single copy of

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RFP construct at the H11 locus to verify efficiency of knock-in (insertion) as well as expression robustness and the ability of engineered iPSCs to be differentiated into many different cell types. Our data demonstrates that the TARGATT iPSC system can be particularly useful for developing allogeneic cell products such as CAR-iNK. For example, unique therapeutic genes (such as CAR constructs) can be inserted in the cGMP TARGATT Master iPSC line for differentiating into NK cells or T cells that could potentially be utilized to fight various serious human diseases. Furthermore, all gene editing is done without using the CRISPR/Cas9 method, therefore allowing a clear IP path for commercialization. In summary, the cGMP TARGATT iPSC platform provides off-the-shelf, ready-to-use cGMP-grade iPSC as a starting material for allogeneic cell products of many cell types, allows large cargo insertion up to 20 kb with non-viral transfection, and accelerate cell product development at lower cost.

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FLUID DYNAMICS PROMOTE THE INDUCTION OF LESION-SPECIFIC SPINAL CORD ORGANOIDS FOR TRANSPLANTATION

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Amyotrophic lateral sclerosis (ALS) is an intractable disease caused by a progressive loss of motor neurons in spinal cords and motor cortex. Cellular therapy has been expected as ALS treatment, including the transplantation of stem cell-derived neural progenitors and glia to support the damaged motor neurons. However, as it is still in the earlier stage of development, several difficulties such as the optimization of cells for transplantation and sufficient number of cells need to be overcome. In addition, the importance of interneurons has been recently demonstrated in ALS. We have succeeded in generating a neural organoid using bioreactor with vertical mixing. In this study, we developed this system to generate spinal cord organoids with an enriched number of motor neurons, interneurons, and glia. In addition, lesion-specific cell production was evaluated by single-cell RNA-seq analysis. This technology using fluid dynamics may produce the opportunity of developing practical materials for transplantation strategies.

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OFF-TARGET CARTILAGE CAN BE REMOVED FROM iPSCS-KIDNEY ORGANOIDS BY MODULATING PROTEIN KINASE A AND NOTCH 1

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IPSC-derived kidney organoids can replicate many of the features of a human kidney. Therefore, they are used as 3D in vitro models to investigate kidney diseases, drug screening and toxicity, and to study developmental biology of the organ. However, several drawbacks need to be overcome to optimize their use for these applications, including insufficient maturation and the presence of non-renal cell populations, such as cartilage. Protein kinase A (PKA) and Notch pathways are known to play an important role in renal function and development. The aim of this work was to reduce the appearance of cartilage appearance during kidney organoid development via the modulation of the PKA and Notch pathways. We modified the culture protocol, first described by Takasato et al., by adding a PKA activator (DbcAMP) or inhibitor (PKI) and/or a Notch activator (Jagged1) or inhibitor (ASR-490). Organoids were harvested at day 7+25, when their development reaches a plateau in the original protocol, and renal structures were evaluated by immunostaining. The activation of PKA with DbcAMP increased the number of nephrons (NPHS1) in the organoids. By comparison, the inactivation of PKA with PKI increased the number of tubules (LTL), but also induced more cartilage. A sequential treatment of PKI followed by DbcAMP improved the nephron–tubule balance in the organoids with engulfment of the nephrons in the proximal tubules and no detectable off-target cartilage. Moreover, organoids treated with DbcAMP showed higher CD31

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and aquaporin 2 expression, indicating increased vascularization and a more mature phenotype. The inhibition of Notch induced the appearance of more cartilage, while its activation reduced it. These results demonstrate improved maturation and shape of kidney organoids cultured at the air–liquid interface and show a potential role for PKA in the development of renal structures and a potential role of Notch in the appearance of off-target cartilage. Understanding the dynamic of these two pathways could help produce higher-quality kidney organoids that can be maintained longer in culture for further in vitro and in vivo work.

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5′-END MODIFICATION SSODNS IMPROVE CRISPR-MEDIATED HOMOLOGY DIRECT REPAIR IN SITU GENE CORRECTION EFFICIENCY

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Recently, the FDA has approved the first CRISPR sickle cell gene therapy to repair the mutation in the adult hemoglobin (HBB) gene. The single base mutation is mainly corrected by homology-directed repair (HDR) pathway. Briefly, CRISPR/Cas9 induces double-stranded breaks (DSBs) at specific genomic loci, and HDR can achieve precise genome modifications utilizing its homologous DNA copy or supplied DNA donors. Unfortunately, cytotoxicity, donor stability, and delivery chanllenges associated with repair template DNA can limit HDR efficacy. Here, we demonstrate that one simple 5′-end chemical modification to a single-stranded DNA-repair template(ssODN) consistently increases the frequency of precision editing in the cultured human iPSCs and human blastoid/heart organoids(hHO) model without compromising iPSCs pluripotency. To improve HDR efficacy, we incorporated several modifications into the donor molecules. Among all kinds of modifications, we found that a simple 5′-end chemical modified donor increased the efficiency of templated repair by 2- to 5-fold with a striking reduction in cytotoxicity compared to an unmodified donor, and it can be easily delivered to the DNA repair site. This approach has been verified in three different human iPSC cell lines, targeting HBB(NC_000011.10:g.5227002T>A, base subsition)/ GLP1R(NM_002062.3;c.402+3delG, base insertion)/ engineered GFP mutant(fragment deletion) gene. Additionally, we attempted direct in situ gene correction in human blastoid and hHO models. Blastoid is a stem cell-based embryo model that resembles the human blastocyst, and hHO recapitulates the complexity of the heart in vitro. 5′-end modification of the donor achieved more than 2-fold gene correction improvement with minimal detrimental effects. Mechanismly, the chemically attached modification is beneficial for the targeting and matching of the modified ssODN. In addition, the modification improves DNA stability, strengthens supramolecular interactions, and results in more stable configurations. Interestingly, we also discovered that H1.0 linker histone could promote 5′-end modified ssODNs HDR efficiency. In summary, the 5′-end modified ssODNs hold tremendous potential for broader applications, including therapeutic approaches to correct disease-causing mutations.

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THERAPEUTIC ASSESSMENT OF CYCLIN DEPENDENT KINASE INHIBITION IN iPSC-DERIVED NEURONS FROM C9ORF72 CARRIERS

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia are fatal neurodegenerative disease that have no cure. The GGGGCC (G4C2) repeat expansion in the chromosome 9 open reading frame (C9ORF72) constitute the most common form of familial ALS and frontotemporal dementia. G4C2 repeat expansions can generate five different dipeptide repeat proteins (DPRs) by non-canonical translation. Arginine containing DPRs, have shown to be highly toxic in several in vitro

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and in vivo models. Previous work demonstrate that genome instability is a major contributor to C9ORF72 pathogenesis and that DPRs are the main drivers of this phenotypes. In our experimental design we used high-yield, post-mitotic, iPSC-derived motor neuron cultures from three C9ORF72 carriers and three controls. These iPSC lines were generated and extensively characterized, as was the differentiation protocol that yields around 90% motor neurons. Our in vitro preliminary data revealed an age-dependent increase in cell division markers Geminin, ki67, cyclins and cyclin dependent kinases as well as an increased number of neurons in S phase in induced pluripotent stem cell (iPSC) derived neurons from C9ORF72 carriers as compared to neurons from healthy controls. Since our neuronal cultures are post-mitotic, and it has been shown that genotoxic damage can induce entry into the cell cycle that primes apoptotic death. We tested inhibition of aberrant cell cycle progression with palbociclib, an FDA approved CDK4/6 inhibitor, we found that palbociclib can inhibit the expression of cell cycle markers, showing efficient target engagement in C9ORF72 iPSC-derived motor neurons. After we established conditions to inhibit CDK4/6 with Palbociclib, we performed survival and functional assays in iPSC-derived neurons, and we found that CDK inhibition can rescue neuronal death and function. Our results suggest that CDK inhibition could be a potential therapeutic target for C9ORF72-related FTD/ALS.

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INCREASED T CELL POTENTIAL OF iPSCS THROUGH NOVEL HEMATOPOIETIC TRANSCRIPTION FACTOR mRNA DIFFERENTIATION SYSTEM

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Engineered T cells are a promising avenue to treat advanced cancers and autoimmune diseases; however, modification of autologous T cells is often limited by disease status or prior therapies, necessitating alternative T cell sources. Human induced pluripotent stem cells (iPSCs) represent a scalable source of off-the-shelf T cells for therapy; however, current differentiation protocols rely on engineered murine feeder cells limiting their translational utility. Here, we report a novel 2D monolayer differentiation protocol incorporating transient expression of hematopoietic transcription factors ERG, HOXA5, HOXA9, HOXA10, and RUNX1 by mRNA delivery (TF-mRNA) that results in the production of iPSC-hematopoietic progenitor cells (iHPCs) with increased in vitro T cell potential. Using both fibroblast and T cell-derived iPSC lines (F-iPSC and T-iPSC, respectively), we demonstrate TF-mRNA significantly increases the frequency of CD34+CD45+ iHPCs compared to cells treated with GFP-mRNA (F-iHPC 59% vs 43%; T-iHPC 76% vs 61%). To further demonstrate the effectiveness of the TF-mRNA, we removed hematopoietic cytokines (VEGF, SCF, IL-3, IL-6, TPO, SR-1) from the iHPC differentiation protocol and again observed greater CD34+CD45+ expression in the TF-mRNA Minimal Cytokine (MC) condition compared to the GFP-mRNA MC condition (F-iHPC 50% vs 27%; T-iHPC 70% vs 41%). Remarkably, the TF-mRNA MC condition resulted in increased expression of CD34+CD117+ cells compared to the TF-mRNA All Cytokine condition (F-iHPC 51% vs 4%; T-iHPC 68% vs 5%). In feeder-free T cell differentiation culture, iHPC conversion into CD45+CD5+CD7+ T cell progenitors was highest in the TF-mRNA MC condition. Both TF and GFP-mRNA MC conditions were further differentiated into iT cells. We observed a higher frequency of CD4+CD8+CD3+TCRαβ+ iT cells in the TF-mRNA condition compared to GFP-mRNA (F-iT 8% vs 0.4%; T-iT 15% vs 2%). To further improve CD4+CD8+CD3+TCRαβ+ expression we included the WNT activator CHIR99021 during the first 14 days of T cell differentiation which markedly improved the phenotype of both lines (F-iT 8% to 32%; T-iT 15% to 64%). These findings demonstrate the effectiveness of transient TF-mRNA delivery to enhance hematopoietic differentiation and provide a new method for the production of iHPCs with enhanced T cell potential.

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DRIVING PLASTICITY AND FUNCTIONAL INTEGRATION OF HUMAN STEM CELL-DERIVED NEURAL GRAFTS THROUGH EXERCISE IN A RODENT MODEL OF PARKINSON'S DISEASE

Moriarty, Niamh

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The ability of dopamine (DA) neurons within human pluripotent stem cell (hPSC)-derived neural transplants to structurally and functionally integrate in the brain in preclinical Parkinson's disease (PD) models provided necessary evidence for current clinical trials. However, these studies highlighted the low proportions of DA neurons within grafts and inferior plasticity, compared to fetal donor tissue. Exercise has been shown to enhance survival and plasticity of neurons and hence warranted investigation in the context of influencing hPSC-derived grafts in rodent PD models. We show that housing animals in an environment with access to voluntary exercise (wheel running) significantly increases graft plasticity. This exercise-induced plasticity accelerated the reversal of motor deficits in animals receiving ectopic intra-striatal, but not homotopic intra-nigral placed neural grafts, suggestive of a threshold requirement. Graft plasticity was accompanied by significantly increased phosphorylated-ERK expressing cells in the graft (and host), reflective of MAPK-ERK signaling, a downstream target of GDNF and BDNF, proteins which were also increased. Animals additionally showed increased cFos+ postsynaptic striatal neuron numbers, indicative of improved graft circuitry integration. These findings have direct implications for the adoption of physical therapy-based approaches to support neural transplantation outcomes in future PD clinical trials.

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RECOMBINANT ANTIBODY ENGINEERING: A NEW FRONTIER IN ADVANCING STEM CELL RESEARCH

Sharma, Neha, Karuppuchamy, Thangaraj, Almeida, Rimple, Shyamprasad, Nanditha, Khan, Abrar, Kaliyamoorthy, Kannadasan, Ketkar, Alhad, R., Sangeetha, Dey, Isha, Balasubramanian, Sudha, Sridharan, Haripriya, Palanivelu, Dinesh

Thermo Fisher Scientific, India

Advances in the field of stem cell therapy are critically dependent on the availability of highly specific antibodies that have been validated in biologically relevant model systems. Additionally, it is vital that the antibodies are sensitive and reproducible. Recent advancements in the field of Molecular biology have significantly influenced the evolution of recombinant antibodies, rendering them the most sought-after reagents for scientific research. In addition to offering advantages of reproducibility, sustained availability, and animal-free production, recombinant antibodies offer yet another unique benefit, of being amenable to rational engineering, such as FcR mutations and re-formatting. At Thermo Fisher Scientific, we have harnessed this potential of engineering in our recombinant research-use antibodies and have developed exceptional quality engineered antibodies that demonstrate desired functional enhancements such as excellent sensitivity, specificity, and multiplexing ability. These engineered antibodies show greater than two-fold increase in signal-to-noise ratios, with lower limits of detection, and demonstrate functionality in multiple molecular biology applications. The recombinant antibodies are also offered in multiple formats, primary conjugates and specie switched phenotypes to further aid multiplexing without affecting the paratope-epitope interactions. Here, we showcase our high-quality stem cell recombinant antibodies using extensively validation strategies across multiple applications, utilizing a holistic understanding of the target protein biology; thereby demonstrating the detection of the target in the appropriate cellular developmental subtypes. We propose that our recombinant antibodies, generated

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using our proprietary engineering technology, delivers antibodies of exceptional quality, which can serve as efficient reagents to help advance research and regenerative medicine.

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THE ROLE OF STEM CELL-DERIVED EXTRACELLULAR VESICLES IN THE REGENERATION OF INJURED NERVOUS TISSUE IN VITRO

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Spinal cord injury (SCI) is a severe condition characterized by complex pathology and permanent consequences. Although there have been recent improvements in diagnosis and survival rates, effective treatment is still unavailable. One promising approach is the use of stem cells, which have demonstrated neuroprotective and immunomodulatory benefits in SCI. However, the precise mechanisms behind these beneficial effects remain unclear. We suggest that the positive effect is mediated by small extracellular vesicles (sEVs) derived from stem cells, and especially by miRNA—short non-coding RNA molecules contained in these vesicles. Therefore, the focus of this research is to evaluate neuroprotective and antiapoptotic potential of sEVs derived from different types of stem cells on an in vitro rat SCI model. Three stem cell lines were cultivated—two neural stem cell (NSC) cultures: immortalized spinal fetal cell line (SPC-01) and neural precursors derived from a clone of human iPSCs (iMR-90), and one mesenchymal stem cell (MSC) line: human adipose tissue derived MSCs (hAT-MSC). Characterization of isolated sEVs was performed, including size distribution measurement and detection of expressed exosomal markers—Alix, TSG101, CD9 and CD81. SPC-01-derived vesicles contained several miRNAs with neuroprotective potential, namely miR-20a-5p, miR-320a-3p, miR-24-3p and miR-21a-5p. Therapeutic potential of sEVs treatment was evaluated and its effect on astrogliosis, axonal sprouting and nerve tissue regeneration investigated. sEVs suspension was applied on the injured nervous tissue, with organotypic tissue sections (OTS) prepared form rat spinal cord utilized as an in vitro model. The results showed a decrease in the levels of proteins involved in pathophysiological and apoptotic processes (GFAP, pSTAT3, cleaved PARP a cleaved caspase -3 and -7) compared to injured tissue. When applied to an in vitro model, NSC-derived sEVs have shown stronger neuroprotective effect compared to MSC-derived sEVs. Based on this, we conclude that sEVs derived from stem cell culture media have neuroprotective and antiapoptotic effects when applied to an in vitro SCI model, and represent a new promising therapeutic approach for the SCI treatment.

Funding Source: This study was supported by GAUK 409222 and CZ.02.01.01/00/22_008/0004562

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HPSC-CARDIOMYOCYTE PRODUCTION: STANDARDIZATION AND PROTEIN-FREE MEDIUM FOR UPSCALING TO 2000 ML

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To fully unlock the potential of human pluripotent stem cell-derived cardiomyocytes (CMs) for regenerative medicine, cost-effective large-scale production is essential. This includes the development of standardized processes utilizing chemically defined, low-cost media of consistent quality. Recently, we have addressed the challenge of hPSC-CM mass production from two perspectives: (1) Aiming at the simplified GMP-compliant production, single-use stirred spinner flasks were applied. A novel process based on SOP-like handling was established, including prolonged

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maintenance of the differentiated hPSC-CM aggregates, enabling the uncoupling of cardiac differentiation and quality control measures from the cells' application. Distinct quality control steps were established at different stages emphasizing GMP compliance. The process allowed for the production of ≥100×106 CMs per 300 mL scale batch at ≥90 % CM purity within 10 days of differentiation. Live-dead staining on intact aggregates, flow cytometry for CMs and "bystander lineages", and confocal microscopy of the 3D aggregate structure promoted the in-depth analysis of the generated cell product. (2) Further, we have focused on differentiation media-related challenges, particularly high protein concentrations that hamper the secretome analysis and lead to batch-dependent alterations in media quality. This has led to the development of entirely protein-free media formulations (PFMFs). These formulations, which include components such as polyvinylalcohol to replace albumin, allow the production of CM yields and purity equivalent to established, protein-containing differentiation media. The applicability of our novel media formulations is demonstrated across numerous culture platforms, including Erlenmeyer flasks (20 mL volume), followed by process upscaling to 150 mL in stirred tank bioreactors (STBRs) and ultimately into 2000 mL STBR scale. At the 2000 mL scale, we achieved the production of ≥1.3×109 CMs per process batch. Our extensive quality control measures, including assessing electrophysiological and contractile characteristics, provide a robust assurance of the sustained quality of CMs generated in PFMFs.

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PRODUCTION OF FUNCTIONAL AND SCALABLE KERATINOCYTES AND FIBROBLASTS FROM iPSC-DERIVED SKIN ORGANOIDS AS A PLATFORM FOR TRANSLATING iPSC THERAPIES FOR RDEB TREATMENT

Pavlova, Maryna[1], Flores, Jocelyn C.[1], Balaiya, Velmurugan[1], McGrath, P. Sean[1], Han, Chann Makara[1], Rozhok, Andrii[1], Butterfield, Kiel C.[1], Bruckner, Anna L.[2], Roop, Dennis R.[1], Bilousova, Ganna[1], Kogut, Igor[1]

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Induced pluripotent stem cells (iPSCs) provide a platform for developing therapies for inherited skin disorders such as recessive dystrophic epidermolysis bullosa (RDEB), which is caused by mutations in the COL7A1 gene. The iPSCs can be generated from RDEB patients, genetically corrected, differentiated into new skin stem cells, and administered back to the same patient as an autograft. To date, we have addressed all major challenges in developing this iPSC-based therapy for RDEB. One of the major challenges was the inconsistent differentiation of iPSCs into high-quality keratinocytes (iKs) and fibroblasts (iFs). This issue was addressed by differentiating iPSCs via a human skin organoid approach. Additionally, we developed a protocol for scaling up the production of organoid-derived skin cells in 2D conditions and adapted this protocol to cGMP manufacturing. Our organoid-based differentiation allows us to generate both genetically corrected RDEB iKs and iFs in one differentiation procedure. This simplifies and reduces the cost of manufacturing our iPSC-based therapy for RDEB. For the delivery of corrected skin cells, we developed a novel preclinical model using a composite grafting approach. This involved suspending human skin cells in a fibrin-based gel formulation and applying it onto immunocompromised mice. As of today, we have successfully generated genetically corrected iPSCs from three RDEB patients using our previously developed combined gene editing and reprogramming approach, which we have adapted to cGMP conditions. We

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differentiated all our corrected RDEB iPSCs into iKs and iFs via skin organoids and expanded them. The purity of the genetically corrected RDEB-iK and -iF populations was estimated based on flow cytometry and single-cell RNA sequencing data. Using these cells, we successfully reconstituted corrected human skin on a mouse model. We also demonstrated that frozen-then-thawed corrected RDEB-iKs and -iFs can be successfully grafted by utilizing a fibrin-based gel, significantly simplifying the future application of these cells in the clinic. We are currently generating safety and efficacy data for the FDA. If successful for RDEB, our iPSC-based therapy could then be easily expanded to other diseases.

Funding Source: DOD, NIH, EBRP, EBMRF, Cure EB, DEBRA Austria, Gates Frontiers Fund, Gates Grubstake Fund

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NOVEL MULTIPLE TISSUE-ADAPTIVE HUMAN MUSCULOSKELETAL STEM CELLS INDUCED FROM HUMAN PLURIPOTENT STEM CELLS FOR REGENERATING SPECIFIC MUSCULOSKELETAL TISSUES

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A valuable and reliable source of stem cells for musculoskeletal repair remains unavailable. Although human mesenchymal stem cells (hMSCs) can differentiate into musculoskeletal tissues under strictly controlled in vitro conditions in the presence of chemicals and growth factors, they engraft poorly and do not differentiate well in vivo. In this study, we induced novel human musculoskeletal stem cells (hMuSSCs) from human pluripotent stem cells under chemically defined culture conditions by modulating the key developmental pathways involved in mesodermal specification. Molecular and functional analyses showed that hMSSCs exhibit marker expression and differentiation potential distinct from those of hMSCs. Single-cell transcriptomics and cell marker analyses showed that hMSSCs are a homogenous cell population. hMuSSCs express the hMSC positive markers CD73, CD105 and CD44 except CD90, and lack the hMSC negative markers CD11b, CD19, CD34, CD45, and HLA/DR. In vivo differentiation capacity studies showed that hMuSSCs differentiate into the musculoskeletal tissues muscle, tendon, cartilage, and bone. When transplanted into the bone, tendon, cartilage, and muscle, hMuSSCs undergo adaptive differentiation into the respective tissues, adjusting their cell fates to match their surrounding tissues. hMuSSCs showed substantial therapeutic efficacy in osteoarthritis and sarcopenia disease models, where they directly contributed to cartilage regeneration in osteoarthritis mice and muscle regeneration in sarcopenic mice, relieving the symptoms. Our results indicate that hMuSSCs represent a breakthrough cell source for treating musculoskeletal disorders, including osteoarthritis and sarcopenia.

Funding Source: This research was fully supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) (2017M3A9B4065302) (To MKH)

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CELL-CYCLE-SENSITIVE CRISPR/CAS9 SCREEN IDENTIFIES JARID2 AMONG ESSENTIAL FACTORS DETERMINING TROPHECTODERM DIFFERENTIATION

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The relationship between embryonic stem cells' (ESCs) cell cycle dynamics and their differentiation outcomes remains elusive. In our investigation, we utilized a cell-cycle-sensitive CRISPR/Cas9 genomic screen to uncover genes associated with the transition from pluripotency to differentiation by subjecting pre-sorted G1 and G2/M mouse ESCs to a temporal differentiation process. We identified a range of genes displaying cell cycle dependency implicated in early differentiation stages. Next, we focused on Jarid2, a critical subunit of PRC2.2, which emerged as a pivotal G2/M-dependent differentiation factor in our screen. Our study elucidates

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the selective recruitment of RNA polymerase Ser-5 by Jarid2, during G2/M phase, to genes associated with the transition to extra-embryonic, XEN, and trophectoderm states. We show that although Jarid2 is associated with promoters, lack of Jarid2 mainly affects the proper activation of enhancers. Moreover, in direct XEN differentiation, Jarid2 knockout ESCs activate trophectodermal gene signatures rather than XEN genes, suggesting dysregulated activation of enhancers. Overall, we show that lack of Jarid2 delays the exit from pluripotency, and allows cells that have already committed to a differentiated state to revert and reactivate their pluripotent program. Our screening has the potential to highlight functional aspects of iPSCs and improve the efficiency and quality of dedifferentiation for better clinical outcomes.

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OPTIMIZATION OF BIOPROCESS PARAMETERS TO GENERATE A ROBUST β-CELL DIFFERENTIATION PROTOCOL FOR GENE-EDITED HUMAN PLURIPOTENT STEM CELLS

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Type 1 diabetes (T1D) is a lifelong disease impacting around 10% of the global population where the pancreas is depleted of β-cells that are responsible for producing insulin. Currently, patients rely on continuous blood glucose monitoring and regular exogenous insulin injections to avoid severe hyper- and hypoglycemic events. Transplantation of stem cell-derived insulin producing β-cells into patients could represent a more sustainable and long-term solution in restoring glucose homeostasis. However, transplantation requires upscaling and optimization of conventional stem cell cultivation and differentiation to achieve enough stem cell-derived β-cells. In this study, human pluripotent stem cells (hPSC) and gene-edited hPSC (GE hPSC) designed for improved engraftment in patients were differentiated to stem cell-derived β-cells in suspension culture vessels ranging from 250 mL to 1 L, using an already established protocol. Initial results showed that the GE hPSC displayed a 2x higher kinetic growth during the expansion phase, compared to the wildtype. Since initial cell culture conditions can greatly affect β-cell differentiation the parameters such as seeding density and agitation speed of the GE hPSC compared to the wildtype hPSC were investigated. The results indicated that a lower seeding density fully compensated for the faster growth rate of the GE hPSC. In addition, a 20% increase in the agitation during cluster formation of the GE hPSC gave rise to desirable cluster sizes allowing for optimal exchange of oxygen and nutrients during β-cell differentiation. Implementation of these parameters resulted in stem cell-derived β-cells from GE hPSC that were more than 45% double positive for standard β-cell markers such as Isl1 and Nkx6.1 and a yield of approximately 100E+06 cells/mL, comparable to previous experiments from wildtype hPSC. These results highlight the importance of strict control of the physical parameters such as seeding density and agitation speed in addition to the differentiation media.

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USE OF A NEW XENO-FREE SUSPENSION CULTURE MEDIUM TO PROMOTE THE LARGE-SCALE EXPANSION OF 3D PLURIPOTENT STEM CELL SPHEROIDS

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Scalable and efficient pluripotent stem cell (PSC) expansion continues to limit PSC-derived allogeneic cell therapies. By growing PSCs as three-dimensional (3D) aggregates or spheroids, suspension culture has the potential to enable large-scale production of high-quality PSCs. However, the adoption of suspension culture to assist in workflows is limited by the lack of commercial options for PSC suspension culture media. To address this, we have developed the new GMP manufactured Gibco™ Cell Therapy Systems (CTS) StemScale™ PSC Suspension Medium. CTS StemScale is a xeno-free formulation that promotes the self-aggregation of single cells into 3D spheroids. Both

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induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are capable of forming spheroids, with cell-line dependent growth in the range of 5x–10x expansion per passage. When grown over multiple consecutive passages, these spheroids have been demonstrated to maintain pluripotency, genomic stability, and trilineage differentiation potential to the three germ layers. Furthermore, spheroid expansion enables easy scale-up in small scale (<100 mL) and large-scale (>1 L) suspension culture systems, including bioreactors. Notably, we used this PSC culture medium to grow spheroids inside a 3 L bioreactor and expanded 450 million cells to 5 billion cells over a 5 day period. With this high cell yield, we then were able to cryopreserve these cells at high densities (50–100 million cells/mL) to minimize the seed train when scaling back to large vessels from frozen cell banks. Cells thawed from these high density cryovials showed high viability and were able to nucleate into spheroids which expanded at normal rates over multiple passages. Ultimately, cells grown in CTS StemScale have the flexibility to differentiate as 3D spheroids, dissociate into single cells and be utilized in downstream applications, or be cryopreserved as single cells for future use.

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iPSCS AS A MODEL TO REVEAL AMYOTROPHIC LATERAL SCLEROSIS ORIGINS AND NATURE

Sgromo, Chiara[1], Tosi, Martina[1], Olgasi, Cristina[1], Cucci, Alessia[1], Venturin, Giorgia[1], Piola, Beatrice[1], Favero, Francesco[1], De Marchi, Fabiola[2], D'Alfonso, Sandra[1], Mazzini, Letizia[2], Follenzi, Antonia[1]

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the loss of neuronal function, primarily affecting central and peripheral motoneurons, with phenotypic and genetic heterogeneity. Mutations were found in familial and sporadic cases, including mainly C9ORF72, SOD1, TARDBP and FUS, and over 30 genes associated to rare ALS form. Although these mutations affect different molecular pathways, all of them result in motor neuron degeneration, highlighting the need to understand the molecular basis of these disruptions for developing targeted therapies. For this study, we used Induced Pluripotent Stem Cells (iPSCs) as a cellular model to investigate transcriptomic differences associated with various ALS genetic mutations and identify pathways related to ALS. iPSCs were generated from 4 patients carrying mutations in C9orf72 (G4C2 expansion), TARDBP (c.1144G>A), and KIF5A (c.2753+1G>A) genes and 2 healthy donors by reprogramming peripheral blood CD34+ cells with the Sendai virus system. The iPSCs showed typical morphology and expression of stem-cell markers at RNA and protein levels, without residual of Sendai virus. Embryoid bodies were generated, demonstrating the expression of ectoderm, mesoderm, and endoderm markers. iPSCs transcriptomic analysis has been performed, identifying several differentially expressed genes (DEGs) ($|log2FC|>2$; $padj \leq 0.05$) in each patient compared to the healthy control. Subsequently, an enrichment analysis has been conducted by Metascape. Fifty-two genes are shared across all patients, belonging to protein and intracellular transport regulation pathways. No common GO terms were found across all mutations, while genes belonging to extracellular matrix (ECM) (GO:0031012) or extracellular matrix organization (GO:0031012) have been found to be enriched in all the patients, underlying a potential role of ECM in the pathological process. In conclusion, iPSC technology is a powerful tool for modelling ALS, enabling the study of disease-specific mutations and their effects on cellular function. The identified transcriptomic differences provide valuable insights into ALS progression and genetic variability, supporting the development of novel targeted therapies.

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REVERSING TYPE I DIABETES IN NOD MICE BY HUMAN HIGH-PLASTICITY STEM CELLS

Hu, Min[1], Li, Shaowei[1], Liu, Bowen[1], Liu, Eric[1], Huang, Jessica[1], Song, Lanying[1], Chen, Xi[2], Xin, Jingxue[2], Huang, James[1], Wong, Wing Hung[2], Lakey, Jonathan[3]

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Type 1 diabetes (T1D) occurs when the immune system mistakenly attacks and destroys the insulin-secreting islet β cells. Current treatments include insulin therapy and islet or β cell transplantation. Most recently islets cells derived from pluripotent stem cells demonstrated efficacy, but they require prior in vitro terminal differentiation. To prevent graft rejection of the transplanted islet cells, immunosuppression or encapsulation devices are required, which can result in serious complications and risks. Confronting such challenges in cell therapy for T1D, we have developed a new source of adult stem cells that have high plasticity in vivo to adapt to the host environment and significantly improve islet cell function and relief the insulitis. These human adult high-plasticity stem cells (AHPSCs) are derived from cellular communication between blood-derived cells and human umbilical cord-derived mesenchymal stromal cells (UC-MSCs) and demonstrated unique transcriptomic profile, differentiation potential across germ layers, significant regenerative capability, as well as high immune modulatory capability. Human AHPSCs combined with a biodegradable and injectable hydrogel were subcutaneously injected in NOD mice after diabetic onset. In the absence of exogeneous insulin, AHPSC/hydrogel treatment led to reversal of blood glucose to non-diabetic level within 6 weeks, exhibiting significantly higher plasma insulin and c-peptide levels compared to control groups (hydrogel, or UC-MSC/hydrogel). AHPSC/hydrogel treated mice exhibited clear relief of typical diabetic symptoms. Histological analysis demonstrated significantly larger average area of pancreatic islets in AHPSC/hydrogel treated NOD mice compared to that in control mice. In addition, AHPSC/hydrogel treated NOD mice showed significantly lower levels of plasma triglycerides and total cholesterol than that in diabetic controls, indicating lipid modulation effects. Further, AHPSCs demonstrate high safety profile (non-tumorigenicity and low immunogenicity). Therefore, human AHPSCs offer a promising approach to cure T1D through a less invasive procedure, without requiring immune suppression.

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PRECLINICAL DEVELOPMENT OF A THERAPEUTICALLY HIGHLY EFFECTIVE CELL PRODUCT FOR PARKINSON'S DISEASE

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Cell therapies based on the transplantation of mesencephalic dopaminergic (mDA) neurons derived from human pluripotent stem cells (hPSCs) offer a potential restorative treatment for Parkinson's disease (PD). The field has reached the exciting clinical evaluation stage, but despite this progress, first-in-class technologies show an unexpectedly low yield of therapeutic mDA neurons after transplantation in preclinical animal models. Consequently, most cells in grafts constitute undesired non-therapeutic cell types, which arise in part from prolonged cell proliferation post-transplantation and contribute to enlarged graft volumes without adding therapeutic benefit. We have developed methods that utilize retinoic acid signaling to direct hPSCs to adopt a dopaminergic fate during differentiation. After optimizations, our methodology shows promising results in preclinical transplantation studies in mice and rats. Cryopreserved RA-specified preparations, produced under a xeno-free and scalable format, generate a notably high proportional yield of mDA neurons after transplantation. The vast majority of stem cell-derived TH+ neurons also express defining transcription factors characteristic of endogenous mDA neurons, including LMX1A, FOXA2, EN1, PITX3, and NURR1. Grafted cells stop dividing shortly after transplantation, resulting in small, mDA neuron-rich grafts with reduced abundance of undesired cell types.

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After transplantation into parkinsonian rats, functional recovery is observed after four months, indicating good therapeutic efficacy. Our technology addresses certain limitations associated with first-in-class methods and provides potential to develop a clinical-grade cell product for PD with high therapeutic efficacy.

Funding Source: This work was supported by grants from Swedish Research Council, Hjärnfonden, Parkinson Fonden, Novo Nordisk Foundation

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AN INNOVATIVE APPROACH FOR CONDUCTING 3D ELECTROPHYSIOLOGICAL RECORDINGS WITHIN INTACT ORGANOIDS

Roth, Bastian[1], Stumpp, Tom[2], Mirsadeghi, Sara[3], Hosseini, Ali[4], Mierzejewski, Michael[2], Stumpf, Angelika[2], Chang, Haein[2], Kraushaar, Udo[2], Giugliano, Michele[4], Jones, Peter O.[2], Hsieh, Jenny[3]

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Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 headstage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth, and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

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A 3D MODEL TO CHARACTERIZE THE MATURATION OF AN iPSC-DERIVED MDA PROGENITOR CELL THERAPY

Bernal, Sonia, Clark, Haley, Jones, Melissa, Wilkinson, Dan, Ebel, Mark, Michael, Kevin, Adams, Nathaniel, Carpenter, Lilia, Harvey, Lucas, Barboza, Luendreo, LoSchiavo, Deven, Xu, Jim, Srinivas, Maya, Smith, Ryan, Paladini, Carlos

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Human pluripotent stem cells (hPSCs) can be leveraged to generate midbrain floorplate progenitor cell therapies to replace the dopaminergic (DA) neurons lost to Parkinson's Disease (PD). The efficacy of these therapies is dependent on the successful differentiation of the floorplate progenitor cells into adult DA neurons that acquire physiological maturity over several months in the in vivo environment. To study this protracted transition from progenitors to neurons, it is important to have in vitro culture systems that support long-term neuronal maturation and mimic the in vivo environment. Conventional in vitro models have relied on monolayer (2D) cell culture systems, which do not accurately recapitulate the architectural complexity of the in vivo environment and can be confounded by cell detachment. Here we describe a 3D cell culture model developed and leveraged to characterize the maturation of an hiPSC-derived midbrain dopaminergic progenitor cell therapy. Ventral midbrain DA (mDA) progenitors were cultured long-term in 2D and 3D in vitro environments in parallel and submitted for molecular (qPCR, flow cytometry, single nuclei RNA sequencing) and immunohistochemical assays at various timepoints across maturation to understand cell fate and function. To assess the relevance of the 3D modeling environment, results were compared to the immunohistochemical characterization of iPSC-mDA cells grafted into the 6-OHDA rodent model of PD. These findings provide insights into the transition of midbrain floorplate progenitors to DA neurons in the context of a cell therapy for PD and demonstrate the potential of a 3D culture system for modeling the dynamics of iPSC-derived cell therapies upon transplantation.

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ROBUST LARGE-SCALE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS IN CGMP ANIMAL ORIGIN-FREE CULTURE MEDIUM AND COMPATIBILITY WITH STEMdiff™ DIFFERENTIATION PROTOCOLS

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Translation of human pluripotent stem cell (hPSC)-based therapies to the clinic is reliant on demonstrating safety of the cell product and of the components in contact with the product throughout the manufacturing process. Equally critical is the capacity to efficiently expand hPSCs for applications requiring a large number of high-quality cells for production of cell banks and directed differentiation. The development of large-scale multilayer tissue culture plastics and stabilized hPSC maintenance media have led to improved methods for 2D expansion of hPSCs. We have an optimized protocol for the expansion of hPSCs in a 10-layer cell factory using TeSR™-AOF, Y-27632 and CellAdhere™ Laminin-521. TeSR™-AOF is manufactured under relevant cGMPs, developed with animal-free raw materials with traceability to at least the secondary level of manufacturing, and was optimized to improve plating efficiency and expansion of hPSCs compared to low-protein formulations. Typically, plating efficiency in TeSR™-AOF was enhanced by 27.1±4.71% (mean±STDEV; n=3 cell lines); however, in select hPSC lines with historically low plating efficiency in low-protein media formulations, the plating efficiency was improved by 80 to 140% (n=2 cell lines) in TeSR™-AOF. This enhanced expansion results in cell yields between 1.2×109–2.5×109 cells in only 12 days utilizing our optimized defined protocol starting from 1–2 wells/6-well plate, transferred

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to a T225 flask and finally expanded to a 90–95% confluent 10-layer cell factory. We further assessed the regenerative potential and differentiation efficiency of hPSCs maintained in TeSR™-AOF, we assessed compatibility with several clinically-relevant directed differentiation protocols. hPSCs expanded in TeSR™-AOF efficiently differentiated to megakaryocytes using the STEMdiff™ Megakaryocyte Differentiation Kit, with 79.2±5.76% CD41+/CD42+ double-positive cells (n=2 cell lines), and into natural killer cells using the STEMdiff™ NK Cell Kit, with 89.9±7.63% CD56+ cells (n=2 cell lines). In summary, TeSR™-AOF was designed with quality and safety in mind, and formulated to improve attachment efficiency, consistency, and reproducibility. TeSR™-AOF enables efficient scale-up and versatile workflows to support high-quality hPSCs in large-scale and long-term culture.

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OPTOGENETIC REGULATION OF INSULIN SECRETION IN HPSC-DERIVED PANCREATIC ISLET-LIKE ORGANOIDS

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Optogenetic technology is widely employed to understand diverse cellular functions because it has some advantages such as non-invasiveness, spatio-temporality, and reversibility. Among various optogenetic tools, monSTIM1 (monster-opto-Stromal interaction molecule (1) is a synthetic protein designed to spatio-temporally modulate the intracellular calcium ion level ([Ca2+]i) in several biological model systems. In this study, we represent a novel optogenetic regulatory system for insulin secretion in human pluripotent stem cell (hPSC)-derived pancreatic islet-like organoids (PIOs) using monSTIM1. The monSTIM1 transgene was inserted into the AAVS1 locus of human embryonic stem cells (hESCs) by CRISPR-Cas9-mediated genome editing. Homozygous monSTIM1+/+-hESCs could induce intracellular Ca2+ concentration ([Ca2+]i) transients via the endogenous CRAC channel by light stimulation. The monSTIM1+/+-hESCs could differentiate to endocrine cells, and then develop to PIOs. Some β-cells of the monSTIM1+/+-PIOs displayed reversible and reproducible [Ca2+]i transient dynamics upon light stimulation. Furthermore, the monSTIM1+/+-PIOs secreted human insulin in response to photoexcitation even in the low glucose condition. Interestingly, light-induced insulin secretion was observed in monSTIM1+/+-ND-PIOs developed from neonatal diabetes (ND) patient-derived induced pluripotent stem cells (iPSCs). Moreover, diabetic mice transplanted with monSTIM1+/+-PIOs produced human c-peptide in the blood under LED illumination. Our results are the first cellular model for the optogenetic control of insulin secretion using hPSCs, with the potential to be applied to the amelioration of hyperglycemic disorders.

Funding Source: This work was supported by a grant (21A0402L1-12) of Korean Fund for Regenerative Medicine (KFRM)

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ENHANCING SHELF-LIFE OF HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STROMAL CELL THERAPY PRODUCTS WITH SUB ZERO NON-FROZEN STORAGE

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The preservation of viable cellular therapeutic products remains a significant roadblock to the development and widespread distribution of efficacious therapies. Cryopreservation is often used yet freezing and thawing protocols are difficult to employ and often do not produce consistently high cellular viability. One possible solution is the use of media which depresses the freezing point to extend the viable life of cells when stored below 0 °C under ambient pressure with no danger of freezing. We tested a proprietary preservation media (Woolii™Cool) developed by CryoStasis Inc. to assess viability and function of

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umbilical cord derived mesenchymal stromal cells (UC-MSCs) held in suspension at -1 °C for up to 17 days. UC-MSC cell bank was free from viral, bacterial, mycoplasma contamination and tested positive (>95%) for CD73, CD90, CD105 and negative (<2%) for CD14, CD19, CD34, CD45 and HLA-DR. UC-MSCs were viable (>80%), highly proliferative (24h population doubling times) and potent (IDO+ >210 in IFN-treated). UC-MSCs were thawed, cultured for 3 to 4 days, harvested, counted and 2.5×106 cells/mL were resuspended in saline (control) or Woolii™Cool. Cell aliquots were removed at regular intervals from 1 to 17 days and measured for viability, recovery, identity, potency, attachment, and proliferative capacity. UC-MSCs stored in Woolii™Cool had complete viable cell recovery (100%±20%) as well as >80% viability as measured by Trypan Blue and verified by Annexin V and propidium iodide staining for up to 14 days when maintained at 4 °C (direct comparison to saline) or -1 °C. In comparison, cells stored in saline maintained >80% viability for 2 days with viability dropping to <50% after day 7. Additionally, UC-MSCs stored in Woolii™Cool at both temperatures demonstrated enhanced attachment and maintained proliferation capacity equivalent to freshly cultured cells (doubling times of 24h) for up to 7 days. Comparatively, cryopreserved UC-MSCs had doubling times of 36–48 post-thaw due to variable recovery and attachment. Furthermore, UC-MSCs maintained identity as measured by flow cytometry and were potent (enhanced IDO expression) for up to 7 days. In comparison, cells stored in saline were unable to attach and proliferate after 2 days in suspension. These findings indicate the Woolii™Cool media from CryoStasis Inc. increases useful shelf-life of UC-MSCs in suspension over conventional media when stored at 4 °C or -1 °C, suggesting non-frozen preservation of regenerative medicine products may be a feasible alternative for storage.

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INTEGRIN ALPHA 2 (ITGA2) ENHANCES THERAPEUTIC EFFICACY OF MESENCHYMAL STROMAL CELL IN SEPTIC SHOCK THERAPY

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Septic shock, characterized by a 30–50% mortality rate, leads to life-threatening organ dysfunction due to a dysregulated host response to infection. Human mesenchymal stromal cells (MSCs) show promise as a treatment for inflammatory diseases like sepsis because of their anti-inflammatory and immunomodulatory properties. However, their clinical efficacy is limited by poor survival and limited adaptability after transplantation. In this study, we discovered that culturing MSCs in a functional polymer-based 3D niche, which simulates the in vivo microenvironment, significantly increased the expression of Integrin alpha 2 (ITGA2) compared to traditional 2D cultures, as revealed by RNA-seq analysis. Subsequently, ITGA2-overexpressing MSCs were administered intravenously to an animal model of septic shock induced by intratracheal LPS injection to evaluate immunomodulatory gene expression and therapeutic efficacy. Blood tests revealed that the ITGA2-overexpressing MSC group experienced significantly less tissue damage and lower IL-6 levels compared to the control group. Additionally, CD206 expression was highest in the ITGA2-overexpressing MSC group, which was associated with the activation of M2 macrophage polarization, contributing to inflammation reduction and tissue repair. Finally, MSCs with ITGA2 overexpression demonstrated enhanced survival and adaptability when intravenously injected into mice, as indicated by red fluorescence staining. These results suggest that ITGA2 overexpression creates a favorable microenvironment for MSCs, enhancing their immunomodulatory functions and offering a promising strategy for MSC-based cell therapy for sepsis.

Funding Source: This work was financially supported by a National Research Foundation of Korea(NRF) grant funded by the Korean government (Grant nos. 2023R1A2C2006894 and 2020R1A2C2011617)

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DIFFERENTIALLY EXPRESSED GENES AND PATHWAYS IN MYELODYSPLASTIC SYNDROME: INSIGHTS FROM A COMPREHENSIVE META-ANALYSIS

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Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and risk of progression to acute myeloid leukemia. To gain deeper insights into the molecular pathogenesis of MDS, we conducted a meta-analysis of gene expression profiles from multiple publicly available microarray datasets. We identified and integrated four relevant GEO datasets (GSE30195, GSE19429, GSE58831 and GSE30201) comprising a total of 414 samples (366 MDS and 48 healthy controls). Differentially expressed genes (DEGs) were identified using the limma package with an adjusted p-value ≤0.01. Gene ontology and pathway enrichment analyses were performed on the DEGs to elucidate key biological processes and signaling pathways dysregulated in MDS. Our analysis revealed no significantly upregulated and 3 downregulated hub genes in MDS compared to controls. downregulated genes included CMTM8, CXCR4, PDE4B for which pathway enrichment highlighted aberrations in Intestinal immune network for IgA production, Morphine addiction and Viral protein interaction with cytokine and cytokine receptor. This comprehensive meta-analysis aims to provide an integrated view of transcriptomic alterations in MDS, identifying key genes and pathways that may serve as potential biomarkers or therapeutic targets. The findings offer new insights into MDS pathobiology and may guide future experimental studies and clinical investigations aimed at improving diagnosis, prognosis, and treatment of this challenging hematological disorder.

Funding Source: This study was totally self-funded

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ACCELERATING HIGH-QUALITY RETINAL PIGMENT EPITHELIUM DIFFERENTIATION FROM hiPSC USING PIC-OCT

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Producing high-quality retinal pigment epithelial (RPE) cells suitable for transplantation is crucial to developing effective treatments for degenerative retinal diseases like age-related macular degeneration (AMD). RPE cells derived from human pluripotent stem cells (hiPSC) can potentially replace damaged cells in the retina, offering a promising therapeutic approach. Current protocols for inducing RPE differentiation typically take around 100 days to achieve fully mature and functional RPE cells. Our research, however, offers a more efficient approach. We explored the impact of a small polyphenolic molecule, PIC-OCT, on RPE maturation. Our goal was to identify a molecule capable of enhancing the differentiation process, yielding high-quality RPE cells more efficiently, which are crucial for successful transplantation. Previous studies within our group have demonstrated that PIC-OCT activates SIRT1, reducing intracellular reactive oxygen species levels and protecting against retinal degeneration in vitro and in vivo. To assess the efficacy of PIC-OCT in promoting RPE maturation, we employed a well-established iPSC differentiation protocol outlined in the literature (Plaza-Reyes et al., Nat Comm, 2016). Beginning on day 50 of the differentiation process, we supplemented the culture medium with three doses of PIC-OCT (100, 250, and 500 nM) for 30 days until day 80 of differentiation. Subsequently, we conducted a comprehensive characterization of the RPE cells, evaluating functional parameters such as transepithelial electrical resistance (TEER), phagocytosis of photoreceptor outer segments (POS) labelled with FITC using flow cytometry, and polarized release of the anti-angiogenic factor pigment epithelium-derived factor (PEDF) via ELISA. Additionally,

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we assessed the mRNA expression levels of crucial RPE maturation genes, including MITF, BEST1, and PAX-6, through RT-PCR. Furthermore, we analyzed the expression of surface markers, including TRA-1-60, CD140b, CD56, GD2, and CD184, using flow cytometry to gauge the degree of RPE maturity. Our findings indicate that PIC-OCT enhances TEER, POS phagocytosis, and PEDF secretion from RPE cells on day 80 compared to untreated RPE. Moreover, we observed increased expression of mature RPE markers at both mRNA and surface marker levels in RPE cells treated with PIC-OCT. We have identified PIC-OCT, an acylated resveratrol derivative, as a potent inducer of RPE differentiation, with the potential to significantly expedite the maturation process. This discovery opens up exciting possibilities for the field of regenerative medicine. We are considering incorporating this molecule into RPE differentiation media at earlier stages to evaluate its potential to significantly expedite the differentiation process. This could offer significant optimization of cell culture protocols for cell therapy applications. Ultimately, including this novel molecule in RPE generation protocols may pave the way for preparing high-quality RPE transplants to treat retinal degenerative diseases such as AMD.

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BIOREACTOR-PRODUCED iPSC-DERIVED DOPAMINERGIC NEURON CONTAINING NEURAL MICROTISSUES INNERVATE AND NORMALIZE ROTATIONAL BIAS IN A DOSE-DEPENDENT MANNER IN A PARKINSON RAT MODEL

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A breadth of preclinical studies is now supporting the rationale of pluripotent stem cell-derived cell replacement therapies to alleviate motor symptoms in Parkinsonian patients. Replacement of the primary dysfunctional cell population in the disease, i.e. the A9 dopaminergic neurons, is the major focus of these therapies. To achieve this, most therapeutical approaches involve grafting single-cell suspensions of DA progenitors. However, a considerable number of cells die during the transplantation process, as cells face anoïkis. One potential solution to address this challenge is to graft solid preparations, i.e. adopting a 3D format. Cryopreserving such format remains a major hurdle and is not exempt from causing delays in the time to effect, as observed with the use of cryopreserved single-cell DA progenitors. Here, we used a high-throughput cell-encapsulation technology coupled with bioreactors to provide a 3D culture environment enabling the directed differentiation of hiPSCs into neural microtissues. The proper patterning of these neural microtissues into a midbrain identity was confirmed using orthogonal methods including qPCR, RNAseq, flow cytometry and immunofluorescent microscopy. The efficacy of the neural microtissues was demonstrated in a dose-dependent manner using a parkinsonian rat model. The survival

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of the cells was confirmed by post-mortem histological analysis, characterised by the presence of human dopaminergic neurons projecting into the host striatum. The work reported here is the first bioproduction of a cell therapy for Parkinson's disease in a scalable bioreactor, leading to a full behavioural recovery 16 weeks after transplantation using cryopreserved 3D format.

Funding Source: This study was funded by Treefrog Therapeutics. This study received financial support from the French government in the framework of the Deeptech, FEDER (European fund) and AAP France 2030 programs

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MYTOS iDEM: A FLASK-BASED AUTOMATION PLATFORM, TO MOVE FAST FROM LAB TO MANUFACTURING

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Pluripotent stem cell (PSC) culture and differentiation is critical for regenerative medicine. But today, culture protocols get developed manually in flasks, before having to be redeveloped in scalable, GMP-compliant formats such as 3D suspension. The Mytos iDEM platform is a faster route to scale, without having to redevelop manual protocols. iDEM fully automates PSC culture in a closed flask-based cartridge, enabling manually-developed flask-based protocols to be directly translated to large automated production for PD and manufacturing. Herein we tested iDEM's performance across iPSC expansion and maintenance, cardiomyocyte differentiation, and DA neuron differentiation. Rigorous testing across various iPSC lines and conditions included assessments of cell morphology, genomic integrity, and pluripotency markers. Results demonstrated that iPSCs cultured using the Mytos system matched the quality of manually grown cells, with no differences in TRA-1-81, Nanog, and SSEA4 markers, and passed Karyostat+ and PluriTest. Differentiated cardiomyocytes across 3 iPSC lines showed healthy morphology, started beating after 2 weeks and were found to have a high %CTnT+ cells. In summary, iDEM was shown to versatile across a range of lines and differentiations, and offers a promising fast-track to scaling differentiations.

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DEVELOPING IMAGING-BASED METHODS TO ASSESS STEM CELL-DERIVED KIDNEY TISSUE

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We have previously developed methods to produce functional, scalable kidney tissue from human stem cells, providing a foundation to develop new therapies for renal failure. Kidney organoid tissue is produced through a stepwise directed differentiation protocol, giving rise to intermediate mesoderm-derived progenitors that form patterned nephrons, the functional units of the kidney, as well as surrounding stroma. Batch variability in kidney organoids is a major challenge and may manifest as changes in the proportions, identity and maturity of the heterogeneous mix of cells present, including multiple classes of 'off-target' cells. Understanding how this variability influences the capacity to produce functional tissue is a further challenge and depends on being able to identify both the desirable and undesirable attributes of in vivo engrafted tissue. Focussing on imaging-based approaches, we have developed methods to routinely quantify 3D tissue morphology during in vitro culture, and after subsequent engraftment in an immunocompromised mouse model. Applying AI-based tools, we can efficiently and accurately extract tissue metrics that can be used to compare outcomes between batches. By correlating these metrics to transcriptional profiling, we can begin to establish a systematic description of variability and identify priorities for quality control development.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, is supported by a Novo Nordisk Foundation grant number NNF21CC0073729

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CONTINUOUS PHYSIOLOGICAL OXYGEN TENSION DECREASES VENTRICULAR SPECIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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The human heart is unable to replace cardiomyocytes (CMs) lost following myocardial infarction, a major cause of death worldwide. In standard care, curative treatments for MI lack, but recent advances in generating CMs from induced pluripotent stem cells (iPSC-CMs) has renewed hope for a feasible therapy of MI patients. Despite this progress, current iPSC-CMs fail to fully reproduce the physiology of primary adult CMs, and one contributor might be a sub-optimal laboratory environment, including exposure to atmospheric O2 tension rather than the physiological O2 tension experienced by stem cells residing in the body. At present, we tested three different O2 settings to investigate the effect of physiological O2 tension on CM derivation as compared to iPSC-CM derived under atmospheric O2. For future manufacturing purposes, we exploited a highly specialized GMP-compliant quad SCI-TIVE glovebox compartment with sensitive surveillance, enabling accurate monitoring of gas composition and temperature to ensure continuous and stable specified O2 tensions. We found that iPSC-CMs derived under theoretical physiological O2 levels displayed less ventricular specification, higher ploidy, and a lower binucleation rate as compared to iPSC-CMs derived at atmospheric O2. Moreover, single cell transcriptomics confirmed that iPSC-CMs at physiological O2 were more atrial-like, suggesting that O2 tension affects CM subtype specification. Ongoing studies comparing large scRNAseq datasets of atrial and ventricular CMs against our iPSC-CMs, will determine CM subtype status of the iPSC-CMs generated under different oxygen settings. In perspectives, these data are expected to provide new biological insights to be used when designing manufacturing conditions for iPSC-CM cell products that are to be used in clinical interventions after MI.

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CAR-EXTRACELLULAR VESICLES: A PROMISING ALTERNATIVE TO CELL-BASED THERAPIES

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Chimeric antigen receptor (CAR)-cell-based therapies have been approved for treatment of hematological malignancies, however, several challenges and limitations remain. CAR extracellular vesicles (CAR-EVs) may represent safer and more effective allogeneic therapies than their CAR-cell counterparts. The potential advantages of CAR-EVs include reduced immunogenicity, lower risk of cytokine release syndrome RS, increased tumor infiltration, opportunity for combination therapies and simplified manufacturing and storage. In this study, we evaluated the cell-targeting and cytotoxic effects of EVs derived from CAR-cells on both blood and solid cancer cells in vitro. CAR-T cells targeting EGFR were cultured in xeno-free conditions, with CAR expression confirmed by microscopy and flow cytometry analyses. CAR-EVs were isolated from cell-conditioned media, enriched by ion-exchange chromatography (EXO-ACE™) and sterilized by filtration. Nanoparticle tracking analysis (NTA, Zetaview) was performed to determine EV yield, concentration and size distribution. MCF-7 breast adenocarcinoma and K-562 chronic myeloid leukemia

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cells were treated over 2 days in 3 independent experiments with increasing concentrations (0–500,000 particles/cell) of CAR-EVs, naïve EVs or a PBS control. EV cytotoxicity was assessed by MTS assay. Flow cytometry confirmed CAR expression. Isolated EVs were of an expected size distribution (140-160 nm), as assessed by NTA. A dose-dependent decrease in MCF7 (71% reduction, $p \leq 0.05$) and K-562 (37% reduction, $p \leq 0.01$) cell proliferation and viability was observed following exposure to CAR-EV for 2 days. In contrast, cell viability was not significantly decreased following treatment with naive EVs. EVs released by CAR-T cells in vitro and isolated by ion-exchange chromatography display similar anticancer effects as their genetically-engineered parent cells. CAR-EVs but not EVs released by naïve cells were effective in killing breast and leukocytic cancer cells. The production of CAR-EVs that target specific cell populations and the development of rapid and scalable methods for their isolation affords opportunity to improve both the safety and efficacy genetically engineered allogeneic therapies.

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MANUFACTURING OF MULTILINEAGE COMPETENT HEMATOPOIETIC PROGENITOR CELLS

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Pluripotent stem cells (PSC) are a promising source of in vitro generated specialized cells for disease modeling, drug discovery, and cell therapy. However, large-scale cell differentiation is necessary to achieve this potential. Hematopoietic stem cells (HSCs) sustain lifelong blood cell production by balancing self-renewal and differentiation. Primary HSCs are widely used as therapeutics to provide a healthy supply of blood cell types for patients with hematological cancers and inherited blood disorders. As an alternative to primary HSCs, iPSC-derived hematopoietic progenitor cells (HPCs) have gained interest from researchers seeking a donor-independent source of cells that are easier to gene editing. Using an unbiased approach, High-Dimensional Design of Experiments (HD-DoETM), we extracted the critical process parameters of a novel differentiation protocol capable of generating iPSC-derived HPCs. Additionally, we developed a manufacturing process for hematopoietic progenitor differentiation yielding above 100M HPCs depending on the day of harvest. Cell viability after cryopreservation was maintained at higher than 90%. CD34 levels were high during the hematopoietic differentiation protocol. Expression of HLF, MECOM, SPINK2 and other hematopoietic progenitor markers was observed. Bulk RNAseq analysis revealed that the expression of HSC genes is dynamic and rapidly changes over time. The hematopoietic potential of HPCS was measured in vitro by using colony forming unit (CFU) assay, which demonstrated robust colony formation comparable to iPSC-derived HPCs, with both multilineage and lineage-specific progenitors. Additionally, HPCs produced on a large scale could be differentiated into erythroid, monocytes, and neutrophils using directed differentiation protocols. Although limited, lymphoid potential in a serum and feeder-free system was observed. Our recent findings shed light on the strength of using an unbiased systems biological approach to develop novel protocols and process understanding of large-scale production of blood cells.

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NEXT-GENERATION ELECTROPHYSIOLOGY FOR FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS

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MaxWell Biosystems AG, Switzerland

Human induced pluripotent stem cell (hiPSC)-derived neural models have emerged as invaluable tools for studying neurological disorders, such as epilepsy, Alzheimer's, and Parkinson's disease. Real-time, label-free measurement of electrical activity in self-organizing in vitro cellular models provides critical insight into the complexity of their neuronal networks.

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High-density microelectrode arrays (HD-MEAs) enable non-invasive electrophysiological recordings from various electrogenic samples, including iPSC-derived neurons, retinal explants, brain slices, and neural organoids. In this study, we used MaxOne and MaxTwo high-density MEA platforms (MaxWell Biosystems AG, Switzerland), with 26,400 electrodes per well to record extracellular action potentials in neural organoids at different scales, ranging from cell population networks to single-cell resolution and subcellular levels. We showcased the flexible selection of electrodes for recording neural activity, increasing the reproducibility and statistical power of the data collected. Key metrics such as firing rate, spike amplitude, and network burst profile were extrapolated in a parallelized manner to capture even the smallest neuronal signals. Furthermore, we characterized axonal function and structure using the AxonTracking Assay, which allows measurement of action potential conduction velocity, latency, axonal length, and branching. This automated assay facilitates high-throughput characterization of disease models targeting axon initial segments, axonal branching, development, and conduction. MaxWell Biosystems' HD-MEA platforms, along with automatically generated plots and extracted metrics, provide a unique, user-friendly approach to identifying and isolating functionally active regions in 3D cultures. These powerful platforms enable long-term in vitro disease modeling and compound testing in acute recordings and/or longitudinal studies.

Funding Source: This work is funded by the HyVIS project, GA 964468, NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission

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BIOLOGICAL CHARACTERIZATION OF NOVEL NEUROGENIC MICRONEUROTROPHIN MIMETICS USING STEM CELL MODELS OF ALZHEIMER'S DISEASE

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Neural stem cell (NSC) proliferation and differentiation sharply decline postnatally in mammals. However, neurogenic niches remain in the adult cortex and hippocampus across species, including rodents, primates, and humans. Adult NSC differentiation mechanisms resemble those in development and impairments in adult neurogenesis are linked to Alzheimer's Disease (AD). Addressing these impairments offers a promising therapeutic avenue, though in-vivo models pose challenges due to scarce adult NSCs and differences between species. Our study uses both mouse and human stem cell models to overcome these issues, focusing on the brain-derived neurotrophic factor (BDNF) pathway, crucial for neuronal growth and differentiation via its receptor TrkB. We characterize new steroidal DHEA derivatives and BDNF mimetics on their neurogenic and neuroprotective actions, focusing on mouse primary adult hippocampal NSCs and embryonic cortical NSCs. To link to human cells, we also use NPCs derived from human induced pluripotent stem cells from healthy and AD donors, showing selected candidates can promote proliferation and prevent cell death in human NPCs after Aβ exposure. Finally, we also use RNA-seq profiling to demonstrate that top candidates operate through a core gene network similar to BDNF. Our work introduces novel BDNF mimetics with potential for neurogenic and neuroprotective actions in AD, validated via stem cell-based screening, demonstrating the efficacy of stem cell systems in identifying promising therapeutic candidates for further development.

Funding Source: This work was supported by grants from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (No 765704)

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OVEREXPRESSION OF WİLD TYPE ASS1 GENE RESCUES DISEASE PHENOTYPE IN iPSC DERIVED CITRULINEMIA MODEL

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Citrullinemia is a rare metabolic disorder classified under urea cycle disorders (UCD) that results from a deficiency in the enzyme argininosuccinate synthetase 1 (ASS1). This deficiency leads to the accumulation of citrulline and ammonia in the blood, which can cause severe neurological damage, coma, and even death if left untreated. Citrullinemia typically manifests in the neonatal period or early childhood, necessitating urgent medical intervention. Current treatment options are primarily limited to protein-restricted diets and pharmacological interventions that aim to reduce ammonia levels. However, these strategies often prove insufficient in preventing long-term complications and maintaining a satisfactory quality of life for patients. Given these limitations, there is a pressing need for more effective therapeutic approaches that target the underlying genetic and biochemical abnormalities associated with Citrullinemia. In this study, we first established and characterized a Citrullinemia specific inducible pluripotent stem cell derived endodermal hepatic organoid model, shortly named eHEPO. Then we overexpressed wild type of ASS1 gene in eHEPO- Citrullinemia model and showed significant decrease in the ammonia elimination capacity in vitro. Thus, we concluded that overexpression approach may offer new insights into potential treatment strategies in Citrullinemia.

Funding Source: This research was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) via projects SBAG-115S465 and SBAG-213S182

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A NEW ADHERENT PSC CULTURE MEDIUM DEVELOPED TO ENABLE ROBUST PERFORMANCE IN CELL THERAPY WORKFLOWS

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Thermo Fisher Scientific, USA

Culture systems for human pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells capable of differentiation to all three germ lineages with potential for cell-based regenerative therapies. To advance PSC therapy research to the clinic, there is a need for high-quality ancillary materials with GMP-compliant manufacturing, quality and safety testing, raw material traceability and supporting regulatory documentation. To ease this transition, we have developed Gibco™ Cell Therapy Systems (CTS™) StemFlex™ Medium based on research use StemFlex™ Medium, but without raw materials directly of human or animal origin. CTS StemFlex Medium supports expansion of high-quality PSCs as well as challenging applications in the PSC workflow. Here we demonstrate PSCs cultured long-term in CTS StemFlex Medium maintain normal PSC characteristics including morphology, pluripotency, trilineage differentiation potential and karyotype. This new medium allows for versatility in culture conditions: a flexible feed schedule with a weekend-free option and compatibility with multiple defined matrices and passaging reagents. CTS StemFlex Medium is shown to provide critical support of stressful PSC applications including single-cell passaging and challenging parts of the gene editing workflow. This new medium supports PSC expansion post electroporation-based delivery of the CRISPR Cas9-gRNA complex as well as downstream clonal expansion of edited PSCs. Adherent PSCs cultured in CTS StemFlex Medium can be transitioned to 3D culture using CTS StemScale™ PSC Suspension Medium resulting in high-quality spheroids with consistent expansion and maintenance of pluripotency.

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KEY CONSIDERATIONS FOR SCALABLE MANUFACTURING OF ALLOGENEIC CELL THERAPY PRODUCTS

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Curing serious diseases using allogeneic cell therapies is poised to become the next biopharmaceutical revolution. Along with demonstrating cell product efficacy and safety for humans, one of the most difficult hurdles to overcome is developing a scalable manufacturing process that is robust and reproducible across stages, from R&D to clinical trials to commercial production. Selection of a manufacturing platform with the right mixing and culture conditions to accommodate sensitive human cells is the key to unlocking scalability. While single-use bioreactors can suspend cells that grow as aggregates, on microcarriers, or as single cells, the ideal platform must be able to solve various process challenges during scale up. These include maintaining optimal hydrodynamic conditions, minimizing medium exchange times for multiple differentiation steps, and providing sufficient oxygen at larger volumes. In particular, hydrodynamic conditions such as energy dissipation rate (EDR) and fluid shear forces can have a significant impact on the yield and quality of the cells grown in bioreactors. Vertical-wheel (VW) bioreactors represent a potential solution for a variety of therapeutic cell types and culture applications. Hydrodynamic conditions modeled in VW bioreactors show narrow EDR distributions which result in homogeneous size-distribution of pluripotent stem cell (PSC) aggregates that greatly benefit the yield and quality of cells for both expansion and differentiation. By incorporating processes for perfusion-based medium exchange and external oxygenation with VW bioreactors from 0.5 L to 50 L scale, linear scalability of PSC production was achieved with yield of ~4 million cells/mL, comparable aggregate size distributions, and consistent pluripotency markers. For expansion of mesenchymal stem cells (MSCs) grown on microcarriers, 0.5 million cells/mL cell yield was consistently achieved when scaled up from 0.1 L to 3 L, 15 L, and 50 L scales. Production of chondrocytes was also shown to be highly consistent across a range of VW bioreactors, from 0.5 L up to 80 L. The continuing development and refinement of processes and techniques for scalable manufacturing of therapeutic cells will allow allogeneic treatments to benefit patients worldwide.

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TESTING MAD7 NUCLEASE IN A GMP WORKFLOW TO PRODUCE HYPOIMMUNE iPSC FOR CLINICAL APPLICATION

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Induced pluripotent stem cell (iPSC)-based therapies have the potential to offer broad clinical applicability and cost-effective scalable manufacturing to meet global patient demand. However, there are several challenges on the path to clinical manufacturing of allogeneic iPSC lines, including complicated licensing terms for nucleases such as Cas9 that limits freedom to operate, as well as manufacturing difficulties for generating multiple edits without compromising iPSC quality, editing efficiency, or functionality. To successfully develop iPSC-based therapies and meet patient demand, universal iPSC lines need to become commercially available. To address these challenges and enable hypoimmune iPSC for use in iPSC derived cellular therapeutics, CCRM has developed a GMP compatible workflow using the IP friendly MAD7 nuclease to perform multiple gene edits of knock-out and knock-in for immune cloaking. The GMP workflow was designed and optimized to allow single cell sorting of edited iPSC with the proof of clonality that can be used in a regulatory package for IND submission. This optimized strategy was tested with MAD7 nuclease using sequential and multiplex editing approaches to knock out HLA-I/II complexes, and MAD7 showed comparable editing efficiency to Cas9. Further, assays were developed to test the functional

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efficacy of the knockouts in iPSC for enhanced product characterization. Both MAD7 and Cas9 mediated HLA-I/II complex knockout iPSC lines were characterized using the developed HLAI/II functional tests as well as standard iPSC characterization. These gene edited iPSC lines are commercially available for researchers and therapeutic developers to test in their respective differentiation processes with unedited parental lines as a control. Access to these iPSC lines enables groups to evaluate how standard hypoimmune edits may affect various applications, such as their specific downstream differentiation process, in vivo model assays or downstream drug product. These iPSC lines offer a solution to address crucial challenges in clinical manufacturing while commercial availability to different research groups will broaden the application of hypoimmune iPSC derived cell therapies. The long-term goal of this work is to produce off-the-shelf GMP hypoimmune iPSC for therapeutic developers.

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DIFFERENTIATION OF iPSCS TO FUNCTIONAL INK CELLS IN SUSPENSION AND APPLICATIONS IN CANCER RESEARCH

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Pluripotent stem cells (PSCs) are a renewable cell source that could be used to generate cell therapies to treat many diseases including cancer. To realize the promise of PSCs significant challenges to commercial manufacturing must be overcome to ease the transition from discovery to therapeutic, including the availability of compatible instruments and reagents, and establishing scaled-up workflows. Recently we developed CTS™ StemScale™ PSC suspension culture medium to enable the large-scale culture of PSCs in clinical manufacturing settings. Natural killer (NK) cells are innate, cytotoxic lymphoid immune cells that can kill cancer cells and are a major focus for allogeneic therapy development. NK cell therapy clinical trials indicate that ~106–108 NK cells per dose may be required for effective treatments. However, requirements such as donor sourcing and successful expansion impede the ability to efficiently generate large quantities of functional NK cells. Here, we describe a feeder-free method to produce PSC-derived NK (iNK) cells, from CTS StemScale suspension cultures enabling the generation of highly enriched, functional iNKs in a scalable culture format. PSCs grown in suspension as spheroids were induced using growth factor cocktails to differentiate into CD34+CD90+ hematopoietic progenitor cells and subsequently to CD56+ iNK cells that could be cryopreserved. Subsequent recovery and culture of iNK cells in CTS™ NK-Xpander™ led to significant enrichment of CD56+CD3- and CD56+CD16+ phenotypes. The cytolytic potential of these iNKs was further demonstrated by their ability to kill K562 cancer cells as well as patient-derived 3D colon tumoroids. In summary, the use of CTS-StemScale highlights the potential for feeder-free PSC suspension cultures to be differentiated into cytolytic iNKs at scale.

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DEFINING THE ROLE OF GLYCOSAMINOGLYCAN INSTRUCTIVE CUES ON THE NEURONAL STEM CELL NICHE AND THEIR IMMUNOMODULATORY POTENTIAL

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A major challenge for exploiting the full potential of stem cells (SCs) is the limitations imposed by lack of defined matrix substrates for cell growth and directed differentiation. In nature cells develop from specialized SCs in an environment called a “niche”, yet the full range of controlling cues remain unexplored. Glycosaminoglycans (GAGs), especially heparan sulfates (HS), are a structurally diverse class of sulphated sugars found in the matrix of the SC niche that are master regulators of SCs via interactions with multiple growth/differentiation factors. The key challenge we will address is to show that unique HS cues can be exploited to create tunable fully defined and clinically compatible matrix (bio-instructive)

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substrates to control cell growth and fate decisions by SCs. GAGs have been under-exploited due to technical barriers to study their structure and function but can now be tackled for the first time by integrating advances in GAG synthesis, analytical methods and high throughput stem cell screening. This work focuses on developing and establishing protocols compatible with clinical grade manufacturing of biomaterials for regenerative medicine applications, specifically neuronal re-generation by; utilizing established model systems and high throughput screening to define the role of heparan GAGs on neuronal regeneration and their immunomodulatory potential. Preliminary data has demonstrated that heparan sulfate glycosaminoglycans can increase neu-rite outgrowth in murine neural stem cells and SH-SY-5 cell line at doses from 2–500ug/mL with no toxicity observed, heparin was used as a positive control and at higher doses of heparin the neural stem cells displayed a distinct lack of neuronal outgrowth and increased the numbers of dead cells. This was confirmed in the SH-SY-5 cell line demonstrating again increased outgrowth and increased cell yields in all doses treated with the compounds. The immunomodulatory potential of these compounds was also assessed using a validated THP-1 activation model, the compounds had a distinct effect on the growth, proliferation and resulting levels of repair associated macrophages (M2) confirmed by specific cell surface markers via flow cytometry. To further explore this effect, the secretome will be characterized by quantitative cytokine analysis of a panel of 32 cytokines, in order to understand how GAGs are able to alter the cytokine profile of immune cells. The next steps of this work will focus on further confirmation of preliminary findings by iPSC derived neuronal stem cells (Axiol Biosciences, Cambridge, UK) and with the utilization of clinical grade biomaterials. Moving forward, this work will provide a deeper understanding of how heparan derived glycosaminoglycans can be using to improve and modulate tissue engineering constructs, by incorporating multiple GAGs to elucidate their synergistic effects on stem cell fate and their composite potential for tissue regeneration, whether the form is as functionalized, crosslinked GAGs alone or GAGs immobilized or crosslinked into a gel composed of another polymer.

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