INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

BlueRock Therapeutics

From Stem Cell Biology to New Therapies

INTERNATIONAL 2019 SYMPOSIA

TORONTO
CANADA

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ISSCR

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Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR), and in partnership with BlueRock Therapeutics, we welcome you to the 2019 ISSCR Toronto International Symposium "From Stem Cell Biology to New Therapies." Toronto is a hub of innovative biotechnology, where world-leading academic stem cell research intermingles with a growing cell therapy industry, making it an ideal location for ISSCR's first meeting dedicated exclusively to the clinical translation of stem cells.

As our understanding of stem cell biology advances, new investigational therapies are rapidly emerging. After decades of robust basic research, tangible and real applications of stem cell technologies to improve human health are seemingly within reach. However, substantial challenges, both scientific and regulatory in nature remain to move exciting and promising research towards and through clinical trials. We have organized this symposium with the goal of addressing these challenges through the exchange of new ideas and data and in doing so, move the field a step closer to realizing the clinical potential of stem cells.

Over the next three days, you will be hearing from world-renown experts in the clinical translation of stem cells for a wide range of diseases. The development of experimental stem cell therapies for the treatment of diabetes, cardiovascular disease, Parkinson's disease, Huntington's disease, ALS, spinal cord injuries, glial diseases, vision loss, and autoimmune diseases, as well as new cancer immunotherapies and strategies to escape the immune system, will be discussed, among others.

Please take advantage of the more intimate setting of this international symposium to strengthen ongoing collaborations and network to forge new ones that will help accelerate your research. This symposium presents an opportunity to exchange knowledge and technologies as we strive to fulfill the hope that stem cells symbolize for patients around the world. Thank you for your continued support of the ISSCR, and we hope that you enjoy the symposium.

Sincerely,

The Toronto Organizing Committee

Gordon Keller, Committee Chair, *McEwen Stem Cell Institute, Canada*
Nissim Benvenisty, *Hebrew University, Israel*
Robert Deans, *BlueRock Therapeutics, Canada*
Michael Laflamme, *University Health Network, Canada*
Jane Lebkowski, *Regenerative Patch Technologies, USA*
Lorenz Studer, *Memorial Sloan Kettering Cancer Center, USA*
Sally Temple, *Neural Stem Cell Institute, USA*

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

ABOUT THE ISSCR

Mission Statement

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

What We Do

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

Contact Us

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BlueRock Therapeutics

ABOUT BLUEROCK

The convergence of cell biology and genetic engineering is creating fundamental new ways to impact disease. Founded in 2016 to capitalize on these technological breakthroughs, we are advancing our novel *CELL+GENE*™ platform to develop, manufacture, and deliver an entirely new generation of authentic and engineered cell therapies across three therapeutic areas: neurology, cardiology, and immunology.

Our foundational science harnesses the ability to create and then direct the differentiation of universal pluripotent stem cells into authentic, functional cells that can be used as allogeneic cellular therapies to treat a broad array of diseases. We can also further engineer these cells, enabling them to produce enzymes, antibodies, and other proteins for additional therapeutic benefit.

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General Information

VENUE

From Stem Cell Biology to New Therapies, an ISSCR 2019 International Symposium, takes place at the John Bassett Theater at the Metro Toronto Convention Centre, 255 Front Street West, Toronto, ON M5V 2W6, Canada.

Registration can be found by the entrance of the John Bassett Theatre on Level 100 of the Metro Toronto Convention Centre. Right by the John Bassett Theater is the Exhibit and Poster Hall (Room 104), in which you will find refreshment breaks, scheduled lunches, poster session, receptions and exhibits.

REGISTRATION AND BADGE PICKUP

Pick up your attendee name badge at the ISSCR Registration Desk found outside of the John Bassett Theatre. Name badges are required for admission to all sessions, poster presentations, and social events. Since the meeting badge serves as proof of participation, all attendees, speakers and exhibitors are required to wear their badges at all times. Access to events may be refused if the meeting badge is not displayed.

REGISTRATION DESK AND BADGE PICKUP HOURS

Wednesday, 6 November	07:30 – 17:00
Thursday, 7 November	08:00 – 17:00
Friday, 8 November	08:00 – 15:30

INTERNET ACCESS

Complimentary access to the Metro Toronto Convention Centre public Wi-Fi is available within the public spaces of the convention centre during the symposium.

As a courtesy to speakers, please be sure to silence any mobile phones and devices and refrain from using the internet during sessions. Please note that the bandwidth of this connection might be limiting.

REFRESHMENT BREAKS

Refreshment breaks will be available in the Exhibits & Poster Hall (Room 104) of the venue. Complimentary coffee and tea will be served during the following days and times (subject to change):

Wednesday, 6 November	09:45 – 10:15
	15:00 – 15:30
Thursday, 7 November	10:30 – 11:00
	14:30 – 15:00
Friday, 8 November	10:30 – 11:00
	14:30 – 15:00

General Information

POSTER SET-UP AND TAKEDOWN

Poster presenters are responsible for displaying their poster at the appropriate times and removing them at the end of their presentation hour in the Exhibit and Poster Hall (Room 104). Push pins will be available.

All posters are to be displayed on Thursday, 7 November by 16:00, and can remain up until Friday, 8 November, until 15:00.

Posters not removed by Friday, 8 November at 15:00 will be discarded by the organizer.

POSTER PRESENTATIONS

Poster presenters will be at their posters for discussion at these times:

EVEN numbered posters presented Thursday, 7 November from 16:00 to 17:00

ODD numbered posters presented Thursday, 7 November from 17:00 to 18:00

RECORDING POLICY

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

Thank you for your cooperation.

ISSCR CODE OF CONDUCT

The ISSCR is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination. All participants are expected to treat others with respect and consideration, follow venue rules, and alert staff or security of any dangerous situations or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics. These policies apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the meeting and related events.

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

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Lorenz Studer
Memorial Sloan Kettering Cancer Center, USA

Sally Temple
Neural Stem Cell Institute, USA

Program Schedule

Wednesday, 6 November

PRE-MEETING ETHICS AND POLICY FORUM — JOHN BASSETT THEATRE

FROM GERMLINE EDITING TO CLINICAL TRIAL DESIGN – THE ETHICAL ISSUES AND PRACTICAL CONSIDERATIONS OF MOVING CELL THERAPIES TO THE CLINIC
Chair, **Sally Temple,** *Neural Stem Cell Institute, USA*

8:30 – 9:45 SESSION 1: ETHICAL & REGULATORY CONSIDERATIONS FOR GERMLINE GENOME EDITING — JOHN BASSETT THEATRE

08:30 – 08:55	**George Q. Daley,** *Boston Children's Hospital, USA* **GENOME EDITING: PROMISE AND PERIL**
08:55 – 09:20	**R. Alta Charo,** *University of Wisconsin, USA* **ETHICS AND REGULATION OF GENOME EDITING**
09:20 – 09:45	**Leslie Thompson,** *University of California, USA* **GENOME EDITING FOR HUNTINGTON'S DISEASE**

9:45 – 10:15 REFRESHMENT BREAK — THEATRE FOYER

10:15 – 11:30 SESSION II: ETHICAL & REGULATORY CHALLENGES OF DESIGNING CELL-BASED CLINICAL TRIALS — JOHN BASSETT THEATRE

10:15 – 10:40	**Charles Murry,** *University of Washington, USA* **Title TBA**
10:40 – 11:05	**Kapil Bharti,** *National Institutes of Health (NIH), USA* **REGULATORY CHALLENGES IN DEVELOPING AN AUTOLOGOUS IPS CELL-BASED THERAPY**
11:05 – 11:30	**Matthew Majewski,** *Charles River Associates, USA* **INNOVATIVE PRICING AND REIMBURSEMENT MODELS FOR CELL AND GENE THERAPIES**

Program Schedule

Wednesday, 6 November

13:00 — 14:00 OPENING KEYNOTE SESSION — JOHN BASSETT THEATRE

13:00 — 13:10 **Welcoming Remarks**
Gordon Keller, *McEwen Stem Cell Institute, Canada*
Nancy Witty, *ISSCR, CEO*

13:10 – 14:00 **Keynote Address**
George Q. Daley, *Boston Children's Hospital, USA*
CURRENT STATE AND CUTTING EDGE OF STEM CELL BASED THERAPY

14:00 – 15:00 DIABETES — JOHN BASSETT THEATRE

Chair: **Gordon Keller,** *McEwen Stem Cell Institute, Canada*

14:00 – 14:30 **Felicia Pagliuca,** *Semma Therapeutics, USA*
DEVELOPMENT OF A STEM CELL DERIVED ISLET CELL THERAPY FOR THE TREATMENT OF DIABETES

14:30 – 15:00 **Kevin D'Amour,** *VIACYTE, USA*
DEVELOPMENT OF STEM CELL DERIVED, ISLET REPLACEMENT FOR TYPE 1 DIABETES

15:00 – 15:30 REFRESHMENT BREAK — EXHIBIT & POSTER HALL, ROOM 104

15:30 – 17:00 CARDIOVASCULAR DISEASE — JOHN BASSETT THEATRE

Chair: **Robert Deans,** *BlueRock Therapeutics, USA*

15:30 – 16:00 **Michael Laflamme,** *University Health Network, Canada*
REMUSCULARIZATION OF INFARCTED HEARTS WITH HUMAN PLURIPOTENT STEM CELLS

16:00 – 16:30 **Mauro Giacca,** *King's College London, UK*
MICRORNA THERAPY TO INDUCE CARDIAC REGENERATION

16:30 – 17:00 **Charles Murry,** *University of Washington, USA*
Title TBA

17:00 – 19:00 WELCOME RECEPTION — EXHIBIT & POSTER HALL, ROOM 104

Thursday, 7 November

09:00 – 10:30 NEURODEGENERATIVE DISEASES I — JOHN BASSETT THEATRE

Chair: **Lorenz Studer,** *Memorial Sloan Kettering Cancer Center, USA*

09:00 – 09:30 **Elena Cattaneo,** *University of Milan, Italy*
DECODING THE UNIQUE GENE SIGNATURE OF THE DEVELOPING HUMAN FETAL STRIATUM FOR STUDIES ON HUNTINGTON'S DISEASE

09:30 – 10:00 **Leslie Thompson,** *University of California, Irvine, USA*
HUMAN NEURAL STEM CELLS AS A THERAPEUTIC CANDIDATE FOR HUNTINGTON'S DISEASE

10:00 – 10:30 **Steven Goldman,** *University of Rochester Medical Center, USA*
STEM CELL-BASED MODELING AND TREATMENT OF HUMAN GLIAL DISEASES

10:30 – 11:00 REFRESHMENT BREAK — EXHIBIT & POSTER HALL, ROOM 104

11:00 – 12:00 NEURODEGENERATIVE DISEASES II — JOHN BASSETT THEATRE

Chair: **Sally Temple,** *Neural Stem Cell Institute, USA*

11:00 – 11:30 **Hideyuki Okano,** *Keio University, School of Medicine, Japan*
IPSCS-BASED REGENERATIVE MEDICINE AND DRUG DEVELOPMENT FOR CNS DISEASES

11:30 – 12:00 **Lorenz Studer,** *Memorial Sloan Kettering Cancer Center, USA*
DEVELOPING A PLURIPOTENT BASED CELL THERAPY FOR THE TREATMENT OF PARKINSONS DISEASE

12:00 – 13:30 LUNCH — EXHIBIT & POSTER HALL, ROOM 104

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Program Schedule

Thursday, 7 November

13:30 – 14:30 VISION LOSS I — JOHN BASSETT THEATRE

Chair: **Sally Temple,** *Neural Stem Cell Institute, USA*

13:30 – 14:00 — **Kapil Bharti,** *National Institutes of Health (NIH), USA*
AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

14:00 – 14:30 — **Rachael Pearson,** *University College London, UK*
NOVEL MECHANISMS OF INTERCELLULAR COMMUNICATION UNDERLIE RESCUE BY PHOTORECEPTOR TRANSPLANTATION IN MURINE MODELS OF PROGRESSIVE RETINAL DEGENERATION

14:30 – 15:00 REFRESHMENT BREAK — EXHIBIT & POSTER HALL, ROOM 104

15:00 – 16:00 VISION LOSS II & DIVERSE APPLICATIONS — JOHN BASSETT THEATRE

Chair: **Jane Lebkowski,** *Regenerative Patch Technologies, USA*

15:00 – 15:30 — **Graziella Pellegrini,** *University of Modena and Reggio Emilia, Italy*
EPITHELIAL STEM CELLS, A REAL TOOL FOR EFFECTIVE REGENERATIVE MEDICINE TREATMENTS

15:30 – 16:00 — **Erin Kimbrel,** *Astellas Institute for Regenerative Medicine, USA*
DEVELOPMENT OF PLURIPOTENT STEM CELL (PSC)-BASED THERAPIES TO TREAT RETINAL DEGENERATIVE DISEASES

16:00 – 18:00 NETWORKING AND POSTER RECEPTION

16:00 – 17:00 — EVEN numbered poster presentations

17:00 – 18:00 — ODD numbered poster presentations

Friday, 8 November

09:00 – 10:30 IMMUNOTHERAPY — JOHN BASSETT THEATRE

Chair: **Nissim Benvenisty,** *Hebrew University, Israel*

09:00 – 09:30 **Daniel Shoemaker,** *Fate Therapeutics, USA*
NEXT GENERATION INDUCED PLURIPOTENT STEM CELL DERIVED CELLULAR IMMUNOTHERAPIES FOR TREATING PATIENTS WITH CANCER

09:30 – 10:00 **Harold Atkins,** *The Ottawa Hospital, Canada*
RESTORING IMMUNE TOLERANCE FOR PATIENTS WITH AUTOIMMUNE DISEASE OR ALLOGRAFT REJECTION USING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

10:00 – 10:30 **Sjoukje van der Stegen,** *Memorial Sloan Kettering Cancer Center, USA*
NEW DIRECTIONS IN CAR T CELL ENGINEERING

10:30 – 11:00 REFRESHMENT BREAK — EXHIBIT & POSTER HALL, ROOM 104

11:00 – 12:00 ESCAPING THE IMMUNE SYSTEM — JOHN BASSETT THEATRE

Chair: **Gordon Keller,** *McEwen Center for Regenerative Medicine, Canada*

11:00 – 11:30 **Sonja Schrepfer,** *University of California, San Francisco/Sana Biotechnology Inc., USA*
ESCAPING THE IMMUNE SYSTEM

11:30 – 12:00 **David Russell,** *Universal Cells, Inc., USA*
UNIVERSAL DONOR STEM CELLS

12:00 – 13:30 LUNCH — EXHIBIT & POSTER HALL, ROOM 104

Program Schedule

Friday, 8 November

13:30 – 14:30 PANEL DISCUSSION "REGULATORY CHALLENGES" — JOHN BASSETT THEATRE

Moderator: **Michael Laflamme,** *University Health Network, Canada*

Participants: **Doug Sipp,** *RIKEN, Japan*

Erin Kimbrel, *Astellas Institute for Regenerative Medicine, USA*

Joy Cavagnaro, *Access BIO, USA*

14:30 – 15:00 REFRESHMENT BREAK — EXHIBIT & POSTER HALL, ROOM 104

15:00 — 16:00 CLOSING KEYNOTE SESSION — JOHN BASSETT THEATRE

15:00 – 15:50 **Jane Lebkowski,** *Regenerative Patch Technologies, USA*
CLINICAL TRANSLATION OF STEM CELL BASED THERAPIES: TODAY'S ADVANCES AND TOMORROWS CHALLENGES TO DELIVER SAFE AND EFFICACIOUS THERAPIES FOR PATIENTS WITH UNMET MEDICAL NEEDS

15:50 – 16:00 **Final Remarks**
Robert Deans, *BlueRock Therapeutics, USA*

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Since 2001, SCN has injected over $100M into stem cell and regenerative medicine research, training and outreach across Canada, with the goal of translating science from the lab to clinical applications, commercial products and public policy. SCN works in partnership with industry, health charities, government and NGOs to build on Canada's leadership in the sector. Tomorrow's health is here. stemcellnetwork.ca

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115+ Members & Partners, **11** Countries

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Tomorrow's health is here.

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Ethics and Policy Forum

From Germline Editing to Clinical Trial Design – the Ethical Issues and Practical Considerations Moving Cell Therapies to the Clinic

WEDNESDAY, 6 NOVEMBER 2019

Ethical & Regulatory Considerations for Germline Genome Editing

GENOME EDITING: PROMISE AND PERIL

Daley, George Q.
Boston Children's Hospital, Boston, MA, USA

Rapid advances in gene editing techniques have raised the prospect for novel treatments of genetic disorders ranging from sickle cell anemia to rare forms of congenital blindness. Based on the remarkable capacity of bacteria to recognize and inactivate invading viruses, gene editing entails reengineering of the RNA-directed nuclease of the CRISPR/Cas9 system for applications in mammalian cells. Unlike the cumbersome methodologies of the past, the ease of the CRISPR/Cas9 system make gene editing far more feasible, and have enabled consideration of editing of human embryos in the context of in vitro fertilization, prompting an urgency for deliberations on the ethical and social implications of this new competence for manipulating human heredity. A framework for understanding the current state of the science of gene editing—both the efficiency and fidelity—as well as practical and ethical considerations of potentially permissible applications in preventing the transmission of human genetic disease will be presented.
Keywords: Genome Editing, CRISPR/Cas9, Human Embryo

ETHICS AND REGULATION OF GENOME EDITING

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Bringing genome editing from bench to clinical trial and, ultimately, to clinical practice will require surmounting hurdles both ethical and regulatory. For somatic editing, ongoing debates about whether and where to draw the line on uses that improve upon already functional conditions (i.e. "enhancement") will reflect anxieties about exacerbating existing social inequities, even though the use of medical technology for non-genetic enhancements are largely accepted outside the realm of sports competitions. For germline editing, even therapeutic applications will raise concerns about the wisdom of inducing multi-generational changes, and regulators will face the challenge of constructing and enforcing longterm monitoring regimes.
Keywords: CRISPR, editing, germline

GENOME EDITING FOR HUNTINGTON'S DISEASE

Thompson, Leslie
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Huntington's disease is an autosomal dominant, fatal and devastating neurodegenerative disease that typically strikes in the prime of life. Because the single gene mutation, a CAG repeat expansion in the coding region for the protein Huntingtin, is known, this disease serves as a candidate for germline gene editing. It also serves as a paradigm for the ethical and practical considerations that underlie this approach. Significant advances have recently been made supporting the feasibility of somatic and germline genome editing approaches to date largely focused on gene silencing. These include using CRISPR-Cas technology to selectively inactivate the allele containing the CAG-repeat expansion in HD patient fibroblasts and patient iPS cells based on unique nucleotide polymorphisms. Permanent suppression and attenuated early neuropathology of the Huntintin protein was achieved in vivo in a mouse model of HD through global targeting of the gene. As more information emerges relating to the haplotypes associated with expanded HTT alleles, other allele specific strategies can be developed. However, an additional consideration in both allele specific and global strategies involves the aberrant production of an exon 1 protein fragment arising from incomplete splicing in the presence of the expanded repeat. These advances and the knowledge of the gene mutation make germline gene editing a potential reality for HD. However, it is known that the Huntingtin protein is required during development and in particular critical for neuronal development raising critical considerations around germline Huntingtin gene silencing approaches. Further, the impact on human development of allele selective Huntingtin gene silencing it is also not known. Gene correction approaches are in development, however these approaches are complex and are weighed against the availability of preimplantation genetic diagnosis available to HD families. Given these are permanent changes and standards are not yet in place for these therapies that remain at the experimental stage, ethical considerations will be discussed.
Keywords: Genome Editing, Huntington's disease, allele specific

Speaker Abstracts

Ethical & Regulatory Challenges of Designing Cell-Based Clinical Trials

Murry, Charles
University of Washington, WA, USA

Abstract TBA

REGULATORY CHALLENGES IN DEVELOPING AN AUTOLOGOUS IPS CELL-BASED THERAPY

Bharti, Kapil
National Institutes of Health (NIH), Bethesda, MD, USA

iPS cells are a promising source of personalized cell therapy. These cells can provide immune-compatible autologous replacement tissue for the treatment of potentially all degenerative diseases. We are preparing a phase I clinical trial using iPS cell derived ocular tissue to treat age-related macular degeneration (AMD), one of the leading blinding diseases in the US. AMD is caused by the progressive degeneration of retinal pigment epithelium (RPE), a monolayer tissue that maintains vision by maintaining photoreceptor function and survival. We are developing a autologous iPS cell derived RPE patch to replace degenerating RPE in AMD patients. Some of regulatory challenges in developing an autologous cell therapy are: developing a validated manufacturing process that can reproducibly generate functional tissue; consistency of the autologous transplant produced from multiple patients; safety of the transplant; and efficacy of the transplant. We derived clinical-grade iPSC-RPE patch from three patients for IND-enabling preclinical studies and from three additional patients to support technology transfer to the GMP manufacturing facility. Functional iPSC-RPE-patch was successfully generated from three individual iPS cell clones from each patient – validating the manufacturing process and its reproducibility. All RPE-patches showed tissue resistance, cell shape metrics and polarized cytokine secretion within our pre-defined release criteria – confirming transplant consistency. Safety of the RPE-patch was confirmed by lacking 'actionable' mutations in iPS cells used to derive the RPE tissue, by lacking iPS cells in the final product, and no tumor/teratoma/ectopic tissue formation in preclinical animal models. Finally, efficacy was confirmed in a pig model with laser-induced RPE damage, where the human RPE-patch was able to rescue pig retinal degeneration. This work has supported a phase I IND application to test an autologous cell therapy in AMD patients.
Keywords: iPS cells, Stem cell therapy, Autologous cell therapy

INNOVATIVE PRICING AND REIMBURSEMENT MODELS FOR CELL AND GENE THERAPIES

Majewski, Matthew
Charles River Associates, New York, NY, USA

In the last decade, few healthcare interventions have made a greater impact than gene and cell therapies, which offer curative benefits for debilitating diseases. Current pharmaceutical reimbursement structures in the USA and around the world have not been designed to accommodate expensive one-time treatments, particularly when clinical trial data do not include long-term outcomes. The problem is two-fold, (1) how do manufacturers support the initial value of the product if it does have proven durable outcomes and (2) how do manufacturers develop contingent offerings that support that value until it can be proven definitively. To address both these problems we need to consider alternative payment models for cell and gene therapies that accommodate the high cost burden and inherent risk in providing potentially curative and paradigm shifting treatments. During this presentation we will explore a variety of analogues such as Novartis' Zolgensma, in which the price is based on a 5-year SOC-based healthcare cost for SMA and then that value proposition is paired with an annuity-based payment model to lessen the budget impact in any given year to payer organizations. Similarly, novel treatments like Sparks' gene therapy, Luxturna, have utilized payer agreements granting coverage on the condition of generating long-term evidence and outcomes. In our experience, manufacturers are willing to be creative in leveraging innovative value- based pricing and payment models in order to support novel treatment options; however, the policies supporting the current reimbursement systems are not always flexible enough to accommodate these innovations. We will explore these creative pricing and reimbursement models throughout this presentation.
Keywords: Pricing and reimbursement modeling, Cell and gene therapy valuation, Pricing considerations for cell therapy development

Speaker Abstracts

International Symposium Opening Keynote: From Stem Cell Biology to New Therapies

CURRENT STATE AND CUTTING EDGE OF STEM CELL BASED THERAPY

Daley, George Q.
Boston Children's Hospital, Boston, MA, USA

The derivation of induced pluripotent stem cells (iPSCs) has revolutionized stem-cell research. Like embryonic stem cells (ESCs), iPSCs can propagate in unlimited fashion and differentiate into essentially any specialized cell type. iPSCs are generated from somatic cells, obviating ethical debates and providing patient-derived models of disease for studies of pathogenesis and drug screening and a source of cells for experimental transplantation therapies. I will present a critical analysis of the current state of the art and describe advances in and challenges for the development of stem-cell–based therapies, focusing on the skin, heart, eye, skeletal muscle, neural tissue, pancreas, and blood.
Keywords: Stem Cell therapy, iPSC, ESC

Diabetes

DEVELOPMENT OF A STEM CELL DERIVED ISLET CELL THERAPY FOR THE TREATMENT OF DIABETES

Pagliuca, Felicia
Semma Therapeutics, Boston, MA, USA

Recent advances in the directed differentiation of pluripotent stem cells into functional human pancreatic islets have set the stage for development of a novel cell therapy for the treatment of diabetes. Type 1 diabetes results from the destruction of the insulin-producing beta cells in the pancreatic islet. The development of replacement sources of beta cells, combined with effective methods of delivery back into the patient's body, has the potential to provide a functional cure for this disease. In order to facilitate clinical translation, further optimization and innovation in differentiation technologies, manufacturing process and scale-up, and characterization of stem cell derived islets in preclinical studies have been performed. In parallel, innovative encapsulation solutions using novel materials and device configurations have been developed to solve the challenge of protecting these therapeutics from immune destruction. Together these technological advances set the stage for the first clinical tests of stem cell-derived islets.
Keywords: Diabetes, Pancreatic islet, Cell therapy

DEVELOPMENT OF STEM CELL DERIVED, ISLET REPLACEMENT FOR TYPE 1 DIABETES

D'Amour, Kevin
VIACYTE, San Diego, CA, USA

ViaCyte Inc. is a clinical stage company developing stem cell-based islet replacement therapies for treatment of patients with diabetes. The therapies are combination products comprised of pancreatic endoderm cells encapsulated within retrievable delivery devices. After implantation, progenitor cells differentiate into glucose-responsive, insulin-secreting cells. The renewable starting material for cell product manufacturing is pluripotent stem cells that are directed to differentiate into the pancreatic endoderm cells using scalable processes. The bio-stable delivery devices are designed to contain cells and facilitate formulation and delivery of the products to subcutaneous tissue sites.
Keywords: diabetes, islet replacement, clinical

Speaker Abstracts

Cardiovascular Disease

REMUSCULARIZATION OF INFARCTED HEARTS WITH HUMAN PLURIPOTENT STEM CELLS

Laflamme, Michael
University Health Network, Toronto, ON, Canada

After myocardial infarction (MI), damaged myocardium is replaced by non-contractile scar, often setting the patient on a course toward progressive heart failure. Human pluripotent stem cells (hPSCs) represent an essentially inexhaustible source of cardiomyocytes that may have application in the "remuscularization" of infarcted hearts. The transplantation of hPSC-derived cardiomyocytes has been reported to improve functional outcomes in rodent and non-human primate MI models, and our group has recently reported their successful transplantation in a more translationally relevant porcine MI model. Engraftment of hPSC-derived cardiomyocytes in pigs results in the formation of large (centimeters-scale) human myocardial implants within the infarct scar that show progressive maturation over time, form vascular networks with the host, and evoke minimal cellular rejection. That said, a number of important barriers to translation remain, including concerns about the immature and heterogeneous phenotype of hPSC-derived cardiomyocytes, their incomplete electromechanical integration following transplantation, and the risk of graft-related arrhythmogenesis. In this presentation, I will summarize our recent efforts to: 1) develop economic, scalable methods to promote maturation of hPSC-derived cardiomyocytes in vitro; 2) validate new optical mapping tools to probe the electrical integration and function of graft myocardium; and 3) determine structural, electrocardiographic and contractile outcomes after hPSC-derived cardiomyocyte transplantation in the porcine MI model.

Keywords: Pluripotent stem cells, Cardiac regeneration, Myocardial infarction

MICRORNA THERAPY TO INDUCE CARDIAC REGENERATION

Giacca, Mauro
King's College London, UK

There is an impelling need to develop innovative therapies that promote cardiac repair in patients with myocardial infarction and heart failure. In contrast to other species that can regenerate the heart throughout their entire life, post-natal damage to the myocardium in mammals is repaired through fibrosis and scarring. Copious evidence nonetheless indicates that the capacity for myocardial renewal, albeit limited, also exists in adult individuals. Over the last years, my laboratory has become deeply interested in developing methods to search for factors able to foster this endogenous cardiac regenerative capacity. In particular, we performed a high throughput screening for human microRNAs stimulating cardiomyocyte proliferation from a whole genome microRNA library using high content microscopy. We identified at least 8 microRNAs that increase neonatal and adult cardiomyocyte proliferation in mice, rats and pigs, as well as proliferation of human cardiomyocytes from fetal hearts or hES cells. All these miRNAs activate YAP-mediated transcription, nuclear localization of active YAP and increased expression of YAP responsive genes. In particular, miR-199a-3p, one of the most effective miRNAs, directly downregulates two mRNA targets impinging on the Hippo pathway, the upstream inhibitory kinase TAOK1and the E3 ubiquitin ligase beta-TrCP. Most of the miRNAs that promote proliferation also modulate the dynamics of the cardiomyocyte actin cytoskeleton. We found that delivery of miR-199a in pigs with myocardial infarction using an AAV vector stimulates repair through cardiac regeneration. One month after treatment, the treated animals showed marked improvements in both global and regional contractility, increased muscle mass and reduced scar size. These functional and morphological findings correlated with cardiomyocyte de-differentiation and proliferation. Together, these results show that achieving cardiac repair through the stimulation of endogenous cardiomyocyte proliferation using miRNAs as drugs is attainable in large mammals, however this therapy needs to be tightly dosed, since uncontrolled expression of these proliferative miRNAs can lead to undesired arrhythmic events in the long term.

Keywords: Cardiac regeneration, Myocardial infarction, microRNA

Murry, Charles
University of Washington, WA, USA

Abstract TBA

Speaker Abstracts

THURSDAY, 7 NOVEMBER

Neurodegenerative Diseases I

DECODING THE UNIQUE GENE SIGNATURE OF THE DEVELOPING HUMAN FETAL STRIATUM FOR STUDIES ON HUNTINGTON'S DISEASE

Cattaneo, Elena
University of Milan, Italy

Conventional approaches to classify the different cell populations of the striatum have been limited to exploring relatively few markers and therefore have provided a narrow characterization of any given cell type. Accordingly, the available protocols for differentiation of stem cells into MSN that are lost in Huntington's Disease (HD) suffer from the lack of information about their normal development. Taking these aspects under consideration, we performed single-cell RNA-seq to decode an unambiguous gene signature of the striatum and reveal how neural progenitors of this domain are able to differentiate at the single cell level. This map should help improve protocols for differentiation into distinct MSN states and therefore increase our understanding of diseases affecting the striatum together with advancing the possibility of cell therapy treatments for HD.

Keywords: Huntington's disease, human striatum, single-cell RNA-seq

HUMAN NEURAL STEM CELLS AS A THERAPEUTIC CANDIDATE FOR HUNTINGTON'S DISEASE

Thompson, Leslie, Reidling, Jack, Cepeda, Carlos, Holley, Sandra, Meshul, Charles, Levine, Michael
University of California, Irvine, CA, USA

Huntington's disease (HD) is a devastating and fatal neurodegenerative disease that typically strikes individuals in midlife and progresses over 15-20 years before patients succumb to disease1. HD is caused by an autosomal dominant mutation, a CAG repeat expansion in the huntingtin (HTT) gene2. Neuropathologically, the disease most overtly impacts the striatum, with progressive loss of medium spiny neurons, and causes atrophy of the corte1. At a molecular level, the disease is accompanied by a progressive loss of neuronal proteins, including the neurotrophic factor BDNF that supports the survival of striatal neurons. Further, aberrant accumulation of aggregated huntingtin (HTT) protein species correspond to disease pathogenesis. Stem cell based approaches are promising as a treatment option for HD with great potential to modulate pathology in a complex tissue such as brain. we have developed and evaluated a GMP-compliant human embryonic stem cell (ESC)-derived neural stem cell (NSC) product, ESI-017 hNSCs, for transplantation into the striatum of HD patients, with the intent of slowing or preventing the progression of the disease. The transplanted ESI-017 hNSCs have been tested in three mouse models of HD. Cells engraft and differentiate to neuronal populations, express BDNF and reduce mutant HTT accumulation. Further, host tissue appears to form synaptic contacts with transplanted cells as evidenced by EM, suggesting they may provide new and functional circuitry to reduce the aberrant cortical excitability that occurs in human HD. These molecular and histological improvements correlate with improvement in behavioral and electrophysiological deficits in HD mice. We are currently moving forward with IND enabling safety studies in preclinical models of HD.

Keywords: neural stem cells, Huntington's disease, transplantation

Speaker Abstracts

STEM CELL-BASED MODELING AND TREATMENT OF HUMAN GLIAL DISEASES

Goldman, Steven
University of Rochester Medical Center, Rochester, NY, USA

Diseases of glial cells, which include the myelin disorders as well as those referable to astrocytic and oligodendrocytic dysfunction, are among the most prevalent conditions in neurology. This talk will cover the potential use of pluripotent stem cell-derived glial progenitor cell transplantation as a means of treating both the diseases of myelin, and those neurodegenerative disorders with significant astrocytic involvement. It will also focus on the development of humanized models for glial disease, which result from the implantation of human glial progenitor cells into the developing mouse brain. In these animals, the human glial progenitors out-compete those of the mouse to dominate – and ultimately replace – the host glial cells. By generating mice using patient-derived hiPSC-derived glial progenitors, we can therefore investigate the contributions of glia to human brain disease, by producing disease-specific human glial chimeras. These chimeric mice provide us new model systems by which to study the effects of human glial pathology in vivo, not only for the myelin disorders, but for the entire range of neurodegenerative and neuropsychiatric diseases in which glial cells may causally participate. These include disorders as diverse as Huntington disease, schizophrenia, and frontotemporal dementia, as well as the leukodystrophies, all of which are based at least in part on glial pathology, and each of which we have now modeled in disease-specific human glial chimeras. These studies have revealed common themes in the nature of the glial dysfunction contributing to these diseases, suggesting that a core set of glial-targeted therapeutic and replacement strategies may prove effective for a broad variety of otherwise distinct neurological disorders.

Keywords: glial progenitor cell, schizophrenia, myelin disease

Neurodegenerative Diseases II

IPSCS-BASED REGENERATIVE MEDICINE AND DRUG DEVELOPMENT FOR CNS DISEASES

Okano, Hideyuki
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There is an increasing interest in the induced pluripotent stem cells (iPSCs)-based regenerative medicine for various diseases. We have been developing regenerative medicine of spinal cord injury (SCI) by the transplantation of neural stem/progenitor cells (NS/PCs)-transplantation for many years. In a series of our previous efforts, we have addressed the issues of safety and tumorigenesis using iPSCs-derived NS/PCs (iPSCs-NS/PCs). The first-in-human clinical study of iPSC-based cell therapy for subacute SCI was approved by the government on February 18, 2019 as class I regenerative medicine protocol, which will be provided for under Japan's Act on the Safety of Regenerative Medicine. I will also talk about our new clinical trial for ALS using a drug identified in the iPSCs-based phenotypic screening of ALS patients-derived motor neurons with FDA-approved drug library (Fujimori et al., Nat Med, 2018). Using iPSC-technology, we have established a large number of in vitro cellular models of familial ALS (FALS) and sporadic ALS (SALS). We therefore developed a system for case clustering capable of subdividing these heterogeneous SALS models by their in vitro characteristics. We further evaluated multiple-phenotype rescue of these sub-classified SALS models using agents selected from non-SOD1 FALS models, and identified ropinirole (ROPI: known as D2 receptor agonist) as a potential therapeutic candidate. Furthermore, we found that i) ROPI's anti-ALS action is mostly D2R-independent, by improving mitochondrial function, and suppressing ROS production, ii) ROPI's anti-ALS action exceeds the effects of pre-existing anti-ALS drugs (Riluzole, Edaravone) in a dish, iii) ROPI is effective about 70% of SALS patients (16/22) as assayed in our in vitro system and iv) localization of ALS related RNA-binding proteins (TDP-43 and FUS) potentially acts as biomarker to predict Responders vs Non-Responders. Based on these findings on the potential anti-ALS action of ROPI, we started A Phase I/IIa, to verify the safety and tolerability of "ROPI" in subjects with ALS (ROPALS trial) from December, 2018. Notably, we will also generate iPSCs from patients and compare in vitro and in vivo effects of ROPI on ALS phenotypes.

Keywords: iPS cells, ALS, Spinal cord injury

Speaker Abstracts

DEVELOPING A PLURIPOTENT BASED CELL THERAPY FOR THE TREATMENT OF PARKINSONS DISEASE

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Human pluripotent stem cells (hPSCs) present a powerful new avenue for studying human disease and for developing cell-based therapies in regenerative medicine. Our group has developed strategies to coax human PSCs into specific neurons on demand and at scale. Our work demonstrates that insights from developmental biology can guide the rationale design of human stem cell differentiation towards a myriad of CNS and PNS lineages. Here, I will present an update on our work geared specifically towards the translation of hPSC technology for treating Parkinson's disease (PD). I will discuss our progress in developing a cell-based therapy that is at the verge of clinical translation with a first in human clinical trial scheduled to start this year. Those studies involve a multidisciplinary effort involving neurologist, neurosurgeons, cell manufacturing specialists, rodents and non-human primate experts and experts in regulatory affairs and working closely with the FDA towards human applications. Those studies also provide a template for translating additional hPSC-based cell products for CNS and PNS disease including our efforts to develop cell-based therapies for enteric neuron disorders. After nearly two decades of human PSC research the field is at an exciting stage where human testing is imminent with PD being one of the main therapeutic targets.

Keywords: Parkinson's disease, cell therapy, brain repair, pluripotent stem cells, directed differentiation, neural transplantation

Vision Loss I

AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

Bharti, Kapil
National Institutes of Health (NIH), Bethesda, MD, USA

Age-related macular degeneration (AMD) affects more than 30 million individuals worldwide. In the advanced AMD stage called Geographic Atrophy (GA) RPE cell death precedes photoreceptor death that leads to vision loss and blindness. RPE replacement has been suggested as a potential treatment of GA. Here we developed an autologous replacement therapy for AMD using an RPE-tissue developed from patient-specific induced pluripotent stem (iPS) cells. Combining developmental biology with tissue engineering we have developed clinical-grade iPS cell derived RPE-patch on a biodegradable scaffold. This patch performs key RPE functions like phagocytosis of photoreceptor outer segments, ability to transport water from apical to basal side, and the ability to secrete cytokines in a polarized fashion. We confirmed the safety and efficacy of this replacement patch in animal models as part of a Phase I Investigational New Drug (IND)-application. Approval of this IND application will lead to transplantation of autologous iPS cell derived RPE-patch in patients with the advanced stage of AMD. Success of NEI autologous cell therapy project will help leverage other iPS cell-based trials making personalized cell therapy a common medical practice.

Keywords: iPS cell, Cell therapy, Autologous phase I trial

NOVEL MECHANISMS OF INTERCELLULAR COMMUNICATION UNDERLIE RESCUE BY PHOTORECEPTOR TRANSPLANTATION IN MURINE MODELS OF PROGRESSIVE RETINAL DEGENERATION

Pearson, Rachael
University College London, UK

Retinal degenerations resulting in photoreceptor loss are the leading cause of untreatable blindness in the Industrialised world. Photoreceptor cell replacement therapy offers the potential to reverse sight loss and recent advances in stem cell differentiation make the generation of bona fide photoreceptors from a renewable source a realistic clinical prospect. Studies have shown it is possible to rescue visual function in mouse models of stationary and progressive blindness following mouse photoreceptor transplantation. This rescue was understood to be mediated by a mechanism of donor cell migration into and integration within the

Speaker Abstracts

remaining retinal circuitry. However, in 2016, we and two other groups made the surprising discovery that in progressive degenerations where some host photoreceptors remain, rescue is predominantly mediated by a novel process of intercellular communication that we termed material transfer. This permits the exchange of proteins and/or RNA and renders the acceptor neurons functional. Several potential mechanisms have been proposed for mediating this surprising phenomenon, including extracellular vesicles, phagocytosis, cell fusion and tunnelling nanotubules. Here, we show that developing photoreceptors generate and release extracellular vesicles that can alter function in target cells. However, these do not underlie material transfer between photoreceptors. Instead, communication is mediated by direct physical contact via nanoscale tubulovesicular processes. Our findings reveal a novel and robust form of horizontal intercellular communication between sensory neurons that can alter acceptor cell function. This is of significance to regenerative cell transplantation strategies, but also offers important new insights into non-synaptic intercellular communication between neurons.
Keywords: Transplantation, Photoreceptor, Embryonic Stem Cells

Vision Loss II & Diverse Applications

EPITHELIAL STEM CELLS, A REAL TOOL FOR EFFECTIVE REGENERATIVE MEDICINE TREATMENTS

Pellegrini, Graziella
University of Modena and Reggio Emilia, Modena, Italy

Regenerative medicine has generated many efforts to explore new therapeutic potentials of both somatic and pluripotent stem cells with many possibilities envisaged for therapeutic applications. Hematopoietic and epithelial cells are extensively adopted for tissue regeneration, due to their high proliferative capacity and their accessibility. 30 years ago, the method for producing epidermis was discovered by cultivation from a small skin biopsy, allowing life-saving treatment of thousands severely burned patients in the following years. The importance of stem cell content was proven for tissues or organs in different pathologies. For instance, recent developments in cell-based therapy for ocular burns provided support for improvement and standardization of the cure for this disabling disease causing depletion of limbal stem cells. Indeed, biopsies taken from the healthy eye, or other autologous source as oral mucosa in bilateral blindness, can be used for their content of stem cells. Few of these therapies overcame the hurdles related to medicinal product regulation and became available to patients. The combined use of cell and gene therapy represents a further scientific approach for the treatment of congenital diseases. This approach was proven on hematopoietic cells and has recently been established using genetically modified epidermal cells for life-saving treatment on severe genetic diseases, as epidermolysis bullosa.
Keywords: Regenerative medicine, Tissue regeneration, Ocular burns

DEVELOPMENT OF PLURIPOTENT STEM CELL (PSC)-BASED THERAPIES TO TREAT RETINAL DEGENERATIVE DISEASES

Kimbrel, Erin
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Retinal degenerative diseases involve the progressive loss of specific cell types within the stratified layers of the retina and may be genetic in nature or arise through age-related changes in ocular health. Diseases such as age-related macular degeneration (AMD) are a major cause of visual impairment worldwide and may lead to blindness if left untreated. Through their ability to differentiate into a wide range of cell types, PSCs serve as the basis for therapies that may (a) provide trophic support for sick or dying retinal cells and/or (b) functionally replace host cells that have already been lost due to degeneration. The first wave of clinical trials involving the use of PSC-derived retinal pigment epithelium (RPE) has suggested that PSC-based therapies can be used safely in patients with retinal degeneration yet efficacy studies are still underway. As these trials continue, additional PSC-based therapies are being developed to treat not only AMD but also diseases such as retinitis pigmentosa, glaucoma, and diabetic retinopathy. Efforts are underway to differentiate PSCs into cells that mimic those natively found within the retina while various engineering approaches are being utilized to endow these PSC-derivatives with enhanced therapeutic properties that go above and beyond what normal retinal cells may do. We will discuss PSC-based approaches that are in development as well as the challenges that lie ahead in the clinical translation of these exciting new therapies.
Keywords: differentiation of pluripotent stem cells into ocular cell types, retinal degeneration, trophic support, functional replacement

Speaker Abstracts

FRIDAY, 8 NOVEMBER

Immunotherapy

NEXT GENERATION INDUCED PLURIPOTENT STEM CELL DERIVED CELLULAR IMMUNOTHERAPIES FOR TREATING PATIENTS WITH CANCER

Shoemaker, Daniel
Fate Therapeutics, San Diego, CA, USA

CAR-T cell therapy is a remarkably promising treatment for cancer patients and represents one of the biggest breakthroughs since the introduction of chemotherapy. However, several obstacles currently limit the broad use of this promising new technology, including the inherent variability and cost of manufacturing cellular populations along with the absolute requirement for precise genetic editing to ensure safety and efficacy. We have developed a unique approach to create master pluripotent stem cell lines, clonally derived to contain precisely edited events and the conversion of these master cell lines into uniform populations of highly efficacious engineered T and NK cells. Analogous to master cell lines used to manufacture biopharmaceutical drug products such as monoclonal antibodies, clonal master iPSC lines are a renewable source for manufacturing cell therapy products which are well-defined and uniform in composition, can be mass produced at significant scale in a cost-effective manner, and can be delivered off-the-shelf for patient treatment. In this presentation, I will discuss how we are leveraging this powerful platform to generate a pipeline of highly-edited iPSC derived CAR-NK and CAR-T products for treating patients with cancer.
Keywords: iPSC, CAR-T, Immunotherapy

RESTORING IMMUNE TOLERANCE FOR PATIENTS WITH AUTOIMMUNE DISEASE OR ALLOGRAFT REJECTION USING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Atkins, Harold
The Ottawa Hospital, Ottawa, ON, Canada

Nearly 30 years ago it was noted that autoimmune diseases could remit in leukemia patients undergoing hematopoietic stem cell transplantation (HSCT). Base on this observation, 127 autoimmune disease patients have undergone autologous HSCT in clinical trials or for clinical care at the Ottawa Hospital. The most common disease indications are multiple sclerosis (MS, n=64), scleroderma (n=18) and myasthenia gravis (MG, n=11). Chemotherapy and antibody containing conditioning regimens are used to destroy pre-existing immunity and purified autologous hematopoietic stem cells are used to regenerate blood and immune elements. Durable elimination of acute inflammatory disease activity has been seen in all patients with MS and MG but responses are less complete in other diseases. Patient, treatment and disease factors that influence response are just beginning to be understood. Unlike other treatments which must be chronically administered, and which result in global suppression of the immune system, HSCT regenerates an immune system which is tolerized to self while being able to protect against pathogens. Associated changes include deletion of pre-existing cellular and antibody repertoire, changes in lymphocyte subpopulations and evidence of improved peripheral immunoregulation. Intriguingly in some patients, damaged organs; including the brain, skin and liver, have been observed to remodel following HSC resulting in histological and functional improvement. The biological basis of this change is uncertain. Harmful effects of the conditioning regimen remain an important barrier to the widespread application of this procedure however over time, the recognition of toxicity has been mitigated by Improvements in prophylactic supportive care measure. With increasing recognition of the impact HSCT has on autoimmune disease, the number of patient and the range of diseases treated are growing.
Keywords: Hematopoietic stem cell transplantation, Multiple sclerosis, Immune Tolerance

Speaker Abstracts

NEW DIRECTIONS IN CAR T CELL ENGINEERING

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Immunotherapy with second-generation chimeric antigen receptor (CAR) T cells has changed the treatment paradigm for hematologic malignancies. However, durable remissions are not achieved in all eligible patients, with relapses occurring within the first year in a fraction of patients. Recent developments, including targeted integration of the CAR, and titration of the activation signaling have resulted in significant improvements of CAR efficacy in preclinical models. Incorporation of these changes in to a T cell-derived induced pluripotent stem cells (TiPSC) provides an attractive platform for an off-the-shelf CAR T cell future. CARs are typically transduced into patient T cells using γ-retroviral or other randomly-integrating retroviral vectors (RVs), which may result in variegated CAR expression and transcriptional silencing. Site-specific CAR integration into the T cell receptor (TCR) alpha chain (TRAC) locus resulted in efficient and uniform CAR expression, enhanced T cell potency, delayed differentiation and exhaustion, vastly outperforming conventionally generated CAR T cells in an Acute Lymphoblastic Leukemia (ALL) mouse model. Combining TRAC-targeting with titration of CAR signaling strength through modulation of the immunoreceptor tyrosine-based activation motifs (ITAMs) further augmented functionality through prolonged persistence at the tumor site and an increase in the central memory population. Currently, the autologous nature of CAR T cell therapy renders some patients ineligible for CAR T cell treatment. TiPSCs may provide a powerful source for unlimited production of specific T cells. We previously described that TiPSCs engineered to express 1928z CAR could effectively be differentiation into CAR T cells, with in vitro and in vivo efficacy. However, they were characterized by an innate CD8aa phenotype, lacking the capacity for long-term memory formation as well as in vivo persistence, in contrast to CD8ab T cells. T cell lineage development is tightly orchestrated through Notch and TCR signaling. A thorough assessment of their individual roles and the titration of their signaling strengths is needed to enable the development of mature CD8ab CAR T cells.

Keywords: Immunotherapy, Chimeric Antigen Receptor, Induced Pluripotent Stem Cells

Escaping the Immune System

ESCAPING THE IMMUNE SYSTEM

Schrepfer, Sonja
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Autologous induced pluripotent stem cells (iPSCs) constitute an unlimited cell source for patient-specific cell-based organ repair strategies. However, extended iPSC culture increases the risk for amplifying neoantigenic SNPs on a large scale. This acquired immunological barrier may become an inherent shortcoming of autologous iPSC-based regenerative strategies. In addition, their generation and subsequent differentiation into specific cells or tissues entail cell line-specific manufacturing challenges and form a lengthy process that precludes acute treatment modalities. These shortcomings could be overcome by using prefabricated allogeneic cell or tissue products, but the vigorous immune response against histo-incompatible cells has prevented the successful implementation of this approach. Here we show that both mouse and human iPSCs lose their immunogenicity when major histocompatibility complex (MHC) class I and II genes are inactivated and CD47 is over-expressed. These hypoimmunogenic iPSCs retain their pluripotent stem cell potential and differentiation capacity. Endothelial cells, smooth muscle cells, and cardiomyocytes derived from hypoimmunogenic mouse or human iPSCs reliably evade immune rejection in fully MHC- mismatched allogeneic recipients and survive long-term with- out the use of immuno-suppression. These findings suggest that hypoimmunogenic cell grafts can be engineered for universal transplantation.

Keywords: Immunogenecity, pluripotent stem cells, fetomaternal tolerance

UNIVERSAL DONOR STEM CELLS

Russell, David
Universal Cells, Inc., Seattle, WA, USA

The clinical use of pluripotent stem cell (PSC)-derived products is limited by allogeneic rejection, primarily due to differences in the diverse human leukocyte antigen (HLA) genes, and the use of autologous induced PSCs or the establishment of HLA-typed PSC banks are problematic due to the large number of cGMP-grade cell lines that must be prepared, characterized, and approved by regulatory agencies. I will describe an approach for generating universal donor PSCs, which allows a single PSC-derived cell product to be used in multiple recipients. Gene editing with recombinant adeno-associated virus vectors is used to efficiently alter genes involved in HLA expression, without the use of potentially genotoxic nucleases. Through this process, we eliminate cell surface expression of polymorphic HLA class I and class II molecules, which prevents peptide presentation to T cells and recognition by anti-HLA antibodies. Gene editing is also used to reintroduce a non-polymorphic class I molecule and thereby prevent lysis by Natural Killer cells. These HLA-engineered universal donor cells resist allogeneic responses of NK, B and T cells, both in vitro and in vivo in humanized mouse models. Universal donor PSCs can be differentiated into diverse therapeutic cell products that are compatible with all recipients, and they allow the production of off-the-shelf cellular therapy products for many indications.
Keywords: Stem cell, Gene editing, HLA

Closing Keynote

CLINICAL TRANSLATION OF STEM CELL BASED THERAPIES: TODAYS ADVANCES AND TOMORROWS CHALLENGES TO DELIVER SAFE AND EFFICACIOUS THERAPIES FOR PATIENTS WITH UNMET MEDICAL NEEDS.

Lebkowski, Jane
Regenerative Patch Technologies, Portola Valley, CA, USA

There are numerous considerations that must be addressed in the development of a stem cell-based therapy. These key points range from: 1) the starting materials, reagents, process, and facility used to manufacture the therapeutic cells; 2) the procedures to characterize the composition, functionality, stability, and adventitious agent profile for release of the therapeutic product; 3) determination of the delivery devices, dose, and route of administration of the cells; 4) the pre-clinical safety and efficacy testing of the therapeutic cells; 5) the initial and final targeted patient population for testing the product in clinical trials; 6) the outcome assessments to be used during clinical development and their acceptability to regulatory bodies; 7) the distribution routes of the cell product to healthcare providers; and 8) the scale and costs of manufacturing that can allow cost coverage by healthcare providers. Specific therapeutic designs must be conceptualized and revisited by scientists, engineers, and physicians during the development of a cell-based therapy to ensure that manufacturing, safety, clinical, and other needs are addressed to provide a safe, efficacious, and cost-effective product to patients. This presentation will address these key considerations in developing a stem cell-based therapy, and will include specific historical examples of developments and challenges that have faced the field. Finally, the challenges for the future: including development of robust, scaled manufacturing systems; facile delivery modalities; demonstration of clinical safety and efficacy; and widespread distribution of such therapies will be addressed. The field of stem cell-based therapies is maturing attracting new and innovative multi-disciplinary teams to help realize the full potential of these therapeutic approaches.
Keywords: Stem Cell-Bases Therapies, Advances, Challenges

Poster Abstracts

Even numbered posters presented Thursday, 7 November from 16:00 to 17:00.

Odd numbered posters presented Thursday, 7 November from 17:00 to 18:00.

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NON-INVASIVE MRI ENGRAFTMENT LOCALIZATION OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE THERAPY

Cook, Moses P.[1], Barry, Jennifer[2], Ghugre, Nilesh[2], Wright, Graham[2], Laflamme, Michael[3], Qi, Xiuling[2], Qiang, Beiping[3], Romagnuolo, Rocco[3], Weyers, Jill[4]

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Myocardial infarction (MI or a heart attack) remains the most common cause of heart failure (HF) worldwide (with ~70,000 Canadians suffering an MI each year). Recent advances have improved survival during the early phase post-MI, but ~25% of survivors go on to develop HF, a condition with a mortality rate of ~50% over 5 years. According to the Heart and Stroke Foundation, there are currently 600,000 Canadians living with heart failure, the majority of which are MI-related. There is no cure for HF; the only clinically effective way to restore function in end-stage HF is through heart transplantation, which offers hope for only 10% of viable candidates. Thus, cardiac regenerative therapy using human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) presents a revolutionary mechanism to restore heart function in MI-related HF. Two hallmarks of an effective stem cell-based therapy have been proposed, including an increase in viable myocardial volume in the infarcted area and structural integration of the new myocardium with the host tissue. In this context, MRI presents an attractive non-invasive method of corroborating histological analysis. Our group has recently used a novel MRI protocol to assess hPSC-CM engraftment viability in a porcine model of MI to quantify the above milestones longitudinally. Non-invasive scar delineation using high resolution 3D Late Gadolinium Enhanced (3D LGE) images may provide the detail necessary to detect graft in vivo. 3D LGE has proven to be a superior method of detecting graft size in vivo when compared to the more traditionally used 2D LGE. Ex vivo histological analysis demonstrated that 3D LGE was able to successfully localize the engraftment in vivo. 3D LGE-MRI currently offers the only method of non-invasive graft detection in vivo. High resolution MRI thus serves as an new option for validating graft localization following cell transplantation.

Keywords: Magnetic Resonance Imaging; Transplantation; Therapy

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HUMAN PLURIPOTENT STEM CELL-DERIVED AVN PACEMAKER CELLS FOR BIOLOGICAL PACEMAKER APPLICATIONS

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The Atrioventricular Node (AVN) is the secondary pacemaker of the heart and responsible for propagating action potentials from the atria to the ventricles. AVN-block due to aging, congenital heart disease or surgery results in a slow heartbeat and requires treatment with an electronic pacemaker (EPM). In Canada 21,000 EPMs are implanted each year. EPMs are associated with a relatively high complication rate (16%). Pediatric patients especially suffer from this treatment due to recurrent battery replacements every 5-10 years and the lack of adoption to growth that require lead refitting surgeries. The ability to replace damaged AVN tissue with human pluripotent stem cell (hPSC) derived AVN-like pacemaker cells (AVNLPCs) that can function as a biological pacemaker would represent a promising alternative. Currently, no protocols exist to efficiently differentiate hPSCs into AVNLPCs. To establish such a protocol, we generated a double knock-in NKX2.5:GFP TBX3:tdTomato hPSC reporter line (HES3). AVNLPCs can be distinguished from NKX2-5- sinoatrial node like pacemaker cells and TBX3- working cardiomyocytes by flow cytometry. Differentiating this reporter line with a standard cardiac differentiation protocol detected a population of NKX2-5+TBX3+ cells that express high levels of AVN genes (TBX3, TBX2, MSX2), show pacemaker action potentials and fast beating rates compared to ventricular-like cardiomyocytes. These data suggest that NKX2-5+TBX3+ cells represent AVNLPCs. We next aimed to optimize the differentiation protocol based on insights form developmental biology. Segregation of the different cardiomyocyte lineages occurs at the primitive streak, as a result of varying levels of BMP and Nodal/Activin A. We therefore varied the concentration of BMP4 and ActivinA during the mesoderm induction in our cultures. The largest proportion of AVNLPCs was obtained from mesoderm induced with 5ng/ml BPMP4 and 4ng/ml Activin A. This mesoderm did not express RALDH2 nor CD235a – markers that were previously shown

to be expressed on sinoatrial and ventricular mesoderm respectively, suggesting that AVNLPCs develop from a different mesoderm. We will use these insights to optimize the differentiation protocol and generate cultures highly enriched in AVNLPCs that can be used in future biological pacemaker applications.

Funding Source
Canada First Research Excellence Fund, Medicine by Design, University of Toronto; New Ideas Grant, Ontario Institute for Regenerative Medicine, and BlueRock Therapeutics LP
Keywords: Human Pluripotent Stem Cells; Biological Pacemaker; Atrioventricular Node

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MODELING CARDIAC ARRHYTHMIAS USING IPSC-DERIVED CARDIOMYOCYTES

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Treatments such as implantable devices and surgery are now available for some cardiac arrhythmias, however, knowledge of the underlying biology and available drugs have not kept up with these technical advances. Most anti-arrhythmic drugs also have proarrhythmic risks, toxicity and low tolerability since most agents were developed with incomplete understanding of their mechanism of action (MOA). Recently, deeper understanding of human genetics and functional genomics suggest that the pathophysiology of cardiac arrhythmias may be tractable at molecular level. Moreover, the introduction of cardiomyocytes (CMs) differentiated from induced pluripotent stem cells (iPSCs) offer a human-relevant model system that is amenable for studying various arrhythmias, such as atrial fibrillation, Long-QT syndrome, and catecholaminergic polymorphic ventricular tachycardia. Here, we combined iPSC-CMs with two phenotyping platforms, Multi-Electrode Array (MEA) and Traction Force Microscopy (TFM), to evaluate how unknown genes control CM conductance and contraction. Commercial iPSC-CMs treated with benchmark compounds displayed stereotypical responses in the MEA and TFM assays, demonstrating the robustness of these platforms. We then used siRNAs to perform a focused loss-of-function phenotypic screen for unknown targets of interest. We modified published protocols to differentiate human iPSC into atrial-like CMs, which makes for a more relevant system for testing genes involved in atrial remodeling. The completion of this project will establish an integrated platform for testing compounds in an atrial relevant system and understanding the MOA of potential drug targets of arrhythmias.

Keywords: Arrhythmias; IPSC; Cardiomyocyte

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REGENERATING THE INFARCTED PIG HEART USING HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

Romagnuolo, Rocco[1], Qiang, Beiping[1], Masoudpour, Hassan[1], Porta-Sanchez, Andreu[2], Laskary, Andrew[1], Masse, Stephane[2], Magtibay, Karl[2], Valdman Sadikov, Tamilla[1], Gomez-Garcia, Juliana[1], Rothberg, Janet[3], Titus, Emily[3], Li, Ren-Ke[4], Zandstra, Peter[3], Wright, Graham[5], Nanthakumar, Kumaraswamy[2], Keller, Gordon[1]

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) have shown substantial promise in rodent and small macaque models of myocardial infarction (MI), in which their transplantation has been found to remuscularize a significant portion of the infarct scar and mediate beneficial effects on left ventricular contractile function. To determine the structural and functional consequences of hESC-CM transplantation in hearts with a size and physiological properties more closely matched to humans, we conducted transplantation studies in a porcine subacute MI model. To induce MI, we used balloon angioplasty to occlude the mid left anterior descending artery for either 90 or 120 minutes to create small (group A) or large (group B) infarcts. At 3-weeks post-MI, a left lateral thoracotomy was performed, and either 10^9 hESC-CMs (cardiac purity of 82.3 ± 3.0%; group A, n=10; group B, n=5) or vehicle alone (group A, n=7; group B, n=10) was directly injected into the infarct zone. Recipients were monitored by telemetric ECG recording and serial cardiac MRI for up to 8-weeks post-transplantation, with a small cohort of animals undergoing electroanatomical mapping (10 days post-transplantation; n=4 for vehicle and n=3 for hESC-CMs recipients). Overall, hESC-CM transplantation resulted in substantial myocardial implants within the infarct scar that matured over time and formed vascular networks with the host. While arrhythmias were rare in infarcted pigs receiving vehicle alone (n=17), hESC-CM recipients (n=15) experienced frequent monomorphic ventricular tachycardia before reverting back to normal sinus rhythm by ~4-weeks post-transplantation. Electroanatomical mapping and pacing studies implicated focal mechanisms for these graft-related tachyarrhythmias as evidenced by an abnormal centrifugal pattern with earliest electrical activation in

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histologically-confirmed graft tissue. These findings demonstrate the suitability of the pig model for the preclinical development of a hESC-based cardiac therapy and provide new insights into the mechanistic basis of electrical instability following hESC-CM transplantation.

Funding Source
OIRM, McEwen Stem Cell Institute, Peter Munk Cardiac Centre, Canadian Foundation for Innovation, Medicine by Design/Canada First Research Excellence Fund initiative and BlueRock Therapeutics.
Keywords: Heart regeneration; Cardiomyocytes; Arrhythmias

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CHORION-DERIVED MESENCHYMAL STEM CELL IS A PREFERENTIAL OPTION FOR STEM CELL THERAPY FOR ISCHEMIC HEART DISEASE

Kim, In Beom[1], Park, Yong Soo[1], Kim, Myungshin[2], Park, Hun-Jun[3]

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Stem cell therapy for ischemic heart disease has emerged as a new treatment method to reduce progression of the heart failure, after myocardial infraction (MI). However, the effect of stem cell therapy is still controversial, and needs further optimization. In our previous report (Kwon et al. (2016) Sci Rep 6:23544), chorion-derived MSCs (C-MSCs) showed a more pronounced ability in vitro to differentiate into cardiomyocyte. Thus, this study was designed to test whether the specific differentiation potency of C-MSCs produces more effective outcome in in vivo ischemic heart disease model than ones with other MSCs. We applied C-MSC, umbilical cord-derived MSCs (UC-MSCs), and bone marrow-derived MSCs (BM-MSCs) in a rat ischemic heart injury model and compared their effects. We used 8-week-old Fischer 344 rats, of which left anterior descending (LAD) artery was permanently ligated to induce myocardial infarction. Cells were labeled by Dil and injected in the border zone of the infarcted myocardium right after ligation. Echocardiogram was performed at 1, 2, 4, 8, and 12 weeks after ligation and measured left ventricle ejection fraction (LVEF) and fraction shortening (FS). Heart tissues were embedded in paraffin and sectioned in 4 μm thickness. Three different cardiac markers, anti¬human alpha-sarcomeric actin (α-SA), cardiac troponin-T (cTnT) and connexin 43 (Cx43) were immunostained to evaluate degrees of differentiation into cardiomyocyte. In functional assessment, echocardiograms showed no differences in LVEF and FS among control injured group and all MSC-injected groups by 4 weeks after LAD ligation (ANOVA, $p>0.05$). In 8 and 12 weeks groups, however, both LVEF and FS were significantly increased in C-MSC injected group ($p<0.05$), compared to control and UC-MSC and BM-MSC injected groups. In tissue sections containing Dil-labled MSCs were apparently observed. Although in all MSC injected groups, Dil-labeld MSCs expressing a-SA, cTnT and CX43 immunoreactivities were observed, number of C-MSCs showing cardiomyocyte differentiation markers were statistically more than those of UC-MSCs and BM-MSCs ($p<0.05$). These functional and histological results suggest that C-MSC could be better candidate for stem cell therapy for ischemic heart disease than other MSCs including BM-MSCs.

Funding Source
This work was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C3076).
Keywords: Chorion-derived mesenchymal stem cell; Myocardial infarction; Cardiomyocyte differentiation

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IN VITRO MATURED HESC-DERIVED CARDIOMYOCYTES FORM INTRA-CARDIAC GRAFTS WITH ENHANCED STRUCTURE AND IMPROVED ELECTROMECHANICAL INTEGRATION IN INJURED HEARTS

Dhahri, Wahiba[1], Sadikov Valdman, Tamilla[1], Masoudpoor, Hassan1, Wulkan, Fanny[2], Ceylan, Eylul[3]

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The immature and pro-arrhythmic phenotype of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) represents a major barrier to their successful translation in ischemic heart disease. Multiple strategies have been described to promote hESC-CM maturation, but most have limited scalability. The present study was designed to test two hypotheses: 1) the maturation of hESC-CMs by culture on polydimethylsiloxane (PDMS) membranes can be upscaled

for transplantation studies; and 2) PDMS-matured hESC-CMs will efficiently engraft in injured hearts and will form grafts with enhanced structural and functional properties relative to immature hESC-CMs. We generated transgenic hESC-CMs that stably expressed ASAP1, a fluorescent voltage-sensitive protein, and phenotyped these myocytes after in vitro maturation for 20 and 40 days on tissue culture plastic (TCP) or PDMS. At both time-points, hESC-CMs on PDMS exhibited increased cardiac gene expression (MYH7, MYL2, TNNI3, PLB, CKM and COX6A2) and more mature structural and electrophysiological properties in vitro relative to TCP controls. Injured guinea pig (GP) hearts were then transplanted with day 20 or 40 TCP vs PDMS ASAP1+ hESC-CMs (n=6-9 per group) and analyzed by histology and ex vivo optical voltage mapping at 14 days post transplantation. While all four cell populations resulted in similarly sized grafts, those formed with PDMS-matured hESC-CMs exhibited far better alignment, increased sarcomere lengths, enhanced expression of connexin-43 and cardiac maturation markers such as cardiac troponin I (cTnI) and the ventricular isoform of myosin light chain 2 (MLC2v), and reduced expression of immature markers like the slow skeletal isoform of troponin I (ssTnI). During optical mapping studies based on ASAP1 fluorescence, grafts formed with PDMS-matured hESC-CMs showed greatly improved electromechanical integration. Our study demonstrates that hESC-CMs matured on soft PDMS can be produced in large quantities (scale of 10e8 to 10e9 CMs). Furthermore, PDMS-matured myocytes form large intramyocardial grafts with enhanced cardiac structure and improved electrical integration, establishing that CM maturation prior to transplantation improves outcomes in vivo.

Funding Source
McEwen Centre for Regenerative Medicine, the Peter Munk Cardiac Centre and the University of Toronto's Medicine by Design/Canada First Research Excellence Fund initiative.
Keywords: Human embryonic stem cells; Cardiomyocyte maturation; Electrophysiology

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FUNCTIONAL ANALYSIS OF PATIENTS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC) USING IPSC-DERIVED CARDIOMYOCYTES

Wulkan, Fanny[1], Steichen, Clara[2], Dhahri, Wahiba[1], Sadikov, Tamilla V.[1], Williams, Kenneth[3], Biaggi, Diogo[2], Olivetti, Natalia S.[4], Sacilotto, Luciana[4], Darrieux, Francisco C.[4], Scanavacca, Mauricio I.[4], Krieger, Jose E.[2], Nunes, Sara S.[3], Laflamme, Michael A.[1], Pereira, Alexandre C.[2]

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic cardiomyopathy characterized by the replacement of myocardial cells by fibro-fatty tissue and a large number of mutations in 12 different genes have already been described as pathogenically related to the disease. The use of patient-specific induced pluripotent stem cells (iPSCs) and their differentiation into target cells enables in-vitro modeling of human cardiac disorders, including ARVC. Here we describe the use of patient-specific cardiomyocytes derived from iPSCs (hiPSC-CM) as a tool to determine how different genetic mutations impact disease phenotype. Forty-seven unrelated probands with clinical diagnosis of ARVC (81% male, mean age 40.2 ± 15.56 years), were submitted to next-generation sequencing using a cardiomyopathy-related gene panel. Urine progenitor cells (UPCs) and fibroblasts were isolated from two patients with a known genetic background, and hiPSCs lines were generated by episomal transfection. The resultant hiPSCs were characterized for pluripotency and subsequently differentiated into cardiomyocytes (hiPSC-CMs). Relative to control hiPSC-CMs, ARVC hiPSC-CMs showed significantly reduced expression of desmosomal proteins, alterations in desmosome structure, accumulation of lipid droplets, increased expression of the pro-adipogenic transcription factor PPAR-γ, prolonged field potential and action potential duration (APD90), slower conduction velocity and significantly reduced active contractile force. Interesting, while the ARVC lines were from two patients with different ARVC mutations, clones from both gave rise to myocytes with a very similar phenotype in our cellular model. In addition, our results suggest that the prolonged action potential duration and reduced force generation of ARVC-CMs may be important hallmarks of the disease at the cellular level.

Funding Source
Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – São Paulo Government)
Keywords: Cardiomyopathies; Induced pluripotent stem cells; Disease modeling

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ASSESSMENT OF IN UTERO THERAPY OF HEMOPHILIA MICE BY AMNIOTIC FLUID STEM CELLS ENGRAFTMENT

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Hemophilia is a genetic disease. There is blood coagulation dysfunction congenitally with patients. There are three types in hemophilia, A, B, and C type, and it is caused by clotting factor VII (FVII), factor IX (FIX) and factor XI (FXI) deficiency respectively. Besides, the number of hemophilia patients are about 180,000 patients in the world and its increases year by year. Thus, a brand-new gene therapy method treatment is essential for these people. The amniotic fluid mesenchymal stem cells (AFMSCs), are suitable for gene therapy because AFMSCs are multipotent that can differentiate into a variety of cell types and are immune tolerance which can reduce the rejection effect. Furthermore, the source of AFMSCs is more stable compared with other MSC, like bone marrow MSC. In utero transplantation (IUT) is a novel approach to treat the inherited genetic disease due to the immune system of the fetus are not completely developed. Therefore, the AFSCs can participate in the development of the fetus and it might restore the genetic problem of the fetus. This study aims to evaluate whether the coagulation disorder can be recovered after using the AFMSCs to treat the hemophilia A mice by IUT. The hemophilia A animal model is FVIII knockout mice. The C57BL/6 mice are normal control and untreated FVIII knockout mice are negative control. To establish the IUT animal model, we used the ICR mice, which is more suitable for surgery than other strain. First, we conducted the laparotomy to the mice, the pregnant date is 14.5 days. Then, injected 5 × 10^4 AFSCs with fluorescence to each fetus. Finally, until three weeks after birth, the treated mice were sacrificed and the AFMSCs in several organs was detected by flow cytometry, frozen section, IVIS and PCR. As well, the results indicated that not only the liver but intestine of mice can detect the fluorescent signal. The next step, we expect to use the gene-modified AFMSCs to treat the hemophilia A mice and achieve gene correct effect in coagulation problem.

Keywords: Hemophilia A; Amniotic fluid mesenchymal stem cells; In utero transplantation

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AUTOLOGOUS CELL-BASED THERAPY TO AUGMENT CARDIAC DEVELOPMENT IN INFANTS WITH SEVERE CONGENITAL HEART DISEASE

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Hypoplastic Left Heart Syndrome (HLHS) is a rare, severe form of congenital heart disease that mandates a three-stage surgical palliation that creates a systemic pump from the right ventricular (RV) chamber. The optimized clinical outcome in the US is only 61% transplant-free survival within the first 6 years of life. HLHS has been characterized by a decreased proliferative capacity that may prevent optimal long-term outcomes. Therefore, early augmentation of cardiac regeneration within the RV may strengthen the heart muscle in hopes of delaying/preventing heart failure. Cell-based therapies may augment the innate regenerative capability of the infant heart and maximize the number of RV cardiomyocytes available for the long-term physiological demands. Our preclinical data demonstrates that autologous umbilical cord blood derived mononuclear cells (UCB-MNC) are safe and sufficient to significantly increase the proliferation of cardiomyocytes. Clinical safety and feasibility data of the initial 23 treated HLHS children enrolled in our Phase I study reported no serious adverse events related to the product or product delivery to establish the safety and feasibility of this approach. The Phase I clinical trial provided the first experience worldwide with a direct injection of autologous UCB-MNC into the infant's RV during surgery and suggested early benefit to cardiac function that informed clinical endpoints for Phase IIb study design. The ongoing Phase IIb study is a multi-center, prospective, open-label, non-randomized, observational study designed to evaluate the efficacy and safety of UCB-MNC delivered into the RV myocardium of 50 subjects with HLHS or HLHS variants with RV dependent single ventricular CHD at the time of a planned Stage II surgical repair. Data collected in the treatment group will be compared to the equivalent data collected in the control group. Endpoints are focused on cardiac function by MRI and have been informed

by Phase I study results and the physician/scientific guidance from members of the 8-site HLHS Consortium, and a nation-wide patient advocacy group. This cross-functional team approach from basic cellular biology to preclinical and clinical trials aims to accelerate the translation of cell-based therapies for children with congenital heart disease.

Funding Source
Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome
Keywords: stem cells; congenital heart disease; HLHS

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USING PRE-VASCULARIZATION STRATEGIES FOR STEM CELL THERAPY FOR THE TREATMENT OF TYPE 1 DIABETES

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Type 1 Diabetes (T1D) is an autoimmune disease characterized by destruction of the beta cells and impaired insulin production. Using the Edmonton protocol, donor-derived islets perfused into the portal vein successfully restore glycemia in 58% of T1D patients, however donor scarcity and poor engraphment (60-70% loss imemdiately post transplantation) limit therapeutic application. To overcome these challenges, human stem cell-derived pancreatic progentitors (PP) are being tested in clinical trials. Transplantation of PPs in the kidney capule of immunodeficient mice leads to formation islet-like structures that secrete human insulin. While insulin-producing cells appear 6 weeks after transplantation, restoration of normoglycemia occurs ~5 months later, suggesting poor connection to the host vasculature. Moreover, pancreas developmental studies indicate that interactions with islet capilaries trigger dorsal pancreas budding, beta cell maturation, glucose sensing and insulin secretion. Our goal is to accelerate PP cell functionality and glucose normalization by increasing vascularization. We tested endothelial cells (ECs) or ready-made microvessels (MVs) from adipose tissue; which were mixed with PPs and transplanted subcutanously in a T1D mouse model. The PP + MV grafts inosculated with host vasculature in the first week post-transplantation resulting in higher rate of PP proliferation, while PP + EC grafts had significantly lower perfusion and proliferation rates. Furthermore, recipents of PP + MVs, reached normoglcemia 8 weeks post-transplantation, responded to glucose stimulated insulin secretion and displayed significantly higher human insulin levels (c-peptide) than PP + ECs. Our future studies will focus on characterizing the mechanisms through which vascularization improves PP performance in vivo.

Keywords: Pancreatic progenitors; Microvessels; Type 1 diabetes

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HUMAN ALPHA-1 ANTITRYPSIN OVEREXPRESSING MESENCHYMAL STEM CELLS DELAY THE ONSET OF TYPE 1 DIABETES

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Islet/β cell death and dysfunction caused by immune-mediated injury are major features of type 1 diabetes (T1D). The ideal T1D intervention should both protect pancreatic β cells from death while effectively establishing homeostasis of the immune system. Currently no single intervention can provide these benefits. The use of mesenchymal stem cells (MSCs) as a therapeutic tool represents a promising new intervention since increasing evidence shows that MSC therapy can effectively suppress autoimmune responses and target several injury pathways in a variety of autoimmune and inflammatory diseases. Based on the ability of alpha-1 antitrypsin (AAT) to decrease B-cell activating factor, decrease anti-insulin auto-antibodies, promote tolerogeneic dendritic cells and promote Treg function, we generated human AAT (hAAT) engineered MSCs (hAAT-MSCs) and studied thier effect in the prevention of T1D onset. hAAT-engineered MSCs were generated by infecting human bone marrow-derived MSCs using the pHAGE CMV-a1aT-UBC-GFP-W lentiviral vector encoding hAAT with a green fluorescent protein (GFP) as a reporter. AAT overexpression was confirmed by presence of GFP. A dramatic amount of hAAT was consistently secreted into the cell culture medium. To assess the impact of AAT-overexpression to MSCs, we measured expression of 84 genes involved in maintaining pluripotency and self-renewal status of MSCs using the Human Mesenchymal Stem Cell PCR Array, and found out that 35 genes were upregulated and 5 genes were downregulated in hAAT-MSCs. Upregulated genes include those critical for the stemness of MSCs

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(i.e, WNT3A, KDR), migration (ICAM-1 and VICAM-1), and cell survival (IGF-1). Pathway analysis showed that changed genes are related to growth factor activity, positive regulation of cell migration, and positive regulation of transcription. Indeed, hAAT-MSCs migrated much faster than the control MSCs when measured by the migration assay. We next injected those cells into the non-obese diabetic (NOD) mice. Mice receiving AAT-MSCs showed a dramatic delay in diabetes onset compared to those receiving vehicle or control MSCs (n=25-29 in each group). Taken together, hAAT overexpression of improves the capabilities of MSC used for cellular therapy in treatment of T1D.

Funding Source
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Keywords: Human α1-antitrypsin; Mesenchymal stem cells; Type 1 diabetes

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SCALABLE GENERATION OF MESENCHYMAL STEM CELLS AND ADIPOCYTES FROM MULTIPLE HUMAN PLURIPOTENT STEM CELL LINES

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Human pluripotent stem cells (hPSCs) can provide unlimited supply for mesenchymal stem cells (MSCs) and adipocytes that can be used for therapeutic applications. In this study, our aim was to enhance the generation of hPSC-derived MSCs and subsequently differentiate them into large number of adipocytes. Therefore, we modified previously published protocols by using different concentrations of retinoic acid (RA) at early stage of MSC differentiation. Embryoid bodies (EBs) derived from several hESC and hiPSC lines were treated with three different concentrations of RA from day 2 to day 5 of differentiation. On day 7 of differentiation, treatment of hPSCs with RA enhanced the generation of EBs by 3.3-fold (low concentration, RA-low) and 6.8-fold (high concentration, RA-high) in comparison to the untreated condition. This increase in the number of EBs was associated with decreased apoptosis without any effect on cell proliferation. At day 16 of differentiation, most RA-untreated EBs formed fibroblast-like cells and 56% of them expressed the MSC specific marker CD73, while RA-treated EBs maintained their undifferentiated phenotype and only 9% of the cells expressed CD73. However, the dissociation of RA-treated EBs on day 12 induced their rapid differentiation into fibroblast-like cells and 90% of them expressed the MSC marker CD73 on day 16 of differentiation indicating the importance of cell dissociation for MSC differentiation of RA-treated cells. At day 25, all three RA-treatment conditions showed elongated MSC-like cells expressing the MSC markers CD44, CD73 and CD90. The number of these cells was significantly increased by 3.8-fold and 18-fold when the hPSC-derived EBs were treated with RA-low and RA-high, respectively. Interestingly, RA-high effect on iPSC-derived EBs was more dramatic than on hPSC-derived EBs; allowing 314 to 10700-fold increase in comparison to untreated cells. Importantly, the MSCs derived from RA-high treated EBs showed 3-fold increase in their proliferation rate and 2-fold increase in their differentiation potential towards adipocytes. These results indicate that short-term EB treatment with high RA concentration dramatically enhances the yield of differentiation towards highly proliferative MSCs with enhanced adipogenic differentiation potential.

Funding Source
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Keywords: hPSCs; MSCs; adipocytes

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A SUBCUTANEOUS NOVEL ECTOPIC TISSUE FOR HUMAN AND MOUSE PANCREATIC BETA CELL TRANSPLANTATION

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According to the International Diabetes Federation (IDF) estimation in 2017, 451 million adults currently have diabetes and an additional 318 million are at risk of developing diabetes. A cell therapy for insulin-dependent diabetes requires two main components: 1) glucose-responding, insulin-secreting β-cells and 2) a niche to host transplanted β-cells that can provide nutrients, oxygen, and the vascularization needed to distribute the secreted insulin to the rest of the body. With the development of the Edmonton protocol, transplantation of pancreatic islets has become a promising cell therapy strategy to treat T1D. However, only 50% of these patients remain insulin independence after five years. One reason for the failure is that the standard site of transplantation is poorly

hospitable for islets. In general, using biomaterial-coated devices often fails because of the inflammatory reaction against foreign bodies Here, using an engineered, safe ES (SC-ES) cell line, that contains a TK-activated suicide system for elimination of the proliferating cells, we generated a stable and pre-vascularized artificial tissue under the skin of diabetic mice that can provide an optimal niche for hosting human and mouse β-cells. To that end, (SC-ES) cells were subcutaneously injected into C57BL/6 or NOD-Scid Gamma (NSG) mice to form artificial safe tissues, that could be rendered dormant at 500-600 mm3 by activation of the suicide gene. After induction of hyperglycemia using STZ, mice and human islets were transplanted into this artificial tissue. Our data demonstrated that about 78% of diabetic mice (n=15) that received the islets into these artificial tissues returned to normoglycemia. This was similar to the rate observed after transplantation of islets underneath the kidney capsule, and more efficient than when using the fat pad as a niche. These mice secreted insulin into the blood in a glucose-responsive manner. When the human or mouse islet-containing artificial tissue was removed, the mice reverted back to hyperglycemia, proving the efficacy of this artificial site as a niche to host functional beta cells. These results demonstrate that our artificially generated ectopic tissue can provide an optimal niche to host the transplanted human and mouse β-cells and reverse hyperglycemia in diabetic mice.

Funding Source
Grant support to AN from CIHR foundation scheme, Canadian Research Chair and Banting and Best Diabetes Centre.
Keywords: Diabetes; Cell therapy; Niche

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TOWARDS A SAFE BETA CELL REPLACEMENT THERAPY USING AN INTEGRATED BIOMATERIAL AND FAIL-SAFE SYSTEM APPROACH

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In-vitro generated insulin secreting β-like cells from pluripotent cells have shown promise to treat Type 1 diabetes (T1D); however, these immature cells present substantial risk of tumor formation with unregulated insulin secretion. The main objective of our study is to generate a fail-safe (FS) insulin-secreting β-like cell line incorporated into an immune-isolating hydrogel system for the treatment of T1D. The proliferative β-like cells were generated using the interrupted reprogramming of pancreatic β-cells. To establish FS β-like cells, Thymidine Kinase (TK) suicide gene was transcriptionally linked to CDK1 using CRISPER/Cas9 knock-in strategy to allow for the ablation of the dividing cells by the pro-drug Ganciclovir (GCV). We fabricated, optimized and used a microfluidic system resembling blood-tissue cross-interaction to validate the insulin secretion and safety of the FS β-like cells in a 3D dynamic microenvironment. We encapsulated the insulin-secreting FS β-like cells in immune-isolating alginate-poly-L-lysine-alginate (APLA) microspheres and characterized them in-vitro and in-vivo for reversing hyperglycemia in diabetic mice. Our microfluidic-based assay revealed that GCV could ablate the proliferating FS β-like cells and lead to surviving (non-dividing) β-like cell populations. Also, our microfluidic system confirmed a regulated insulin secretion by the FS GCV-treated β-like cells in response to different glucose concentrations. The cell-impregnated APLA microcapsules were mechanically stable, prevented the β-like cells from protrusion, and supported insulin secretion from the FS GCV-treated β-like cells in-vitro. Our in-vivo study has suggested that the ALPA-encapsulated FS β-like cells could restore normoglycemia in diabetic mice.

Funding Source
This study was supported by Banting & Best Diabetes Centre Postdoctoral Fellowship award to M.I. and the Canadian Institutes of Health Research (CIHR) Foundation Grant to A.N.
Keywords: Safe cell therapy; Biomaterials and microfluidic systems; Diabetes

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INTEGRATIVE SCREENING FOR IDENTIFICATION OF CELL FATE DRIVER FOR BETA CELLS WITH ENDOCRIEN PROGENITOR CELL LINE TEC-3P AND TRACING MODEL OF DUAL-LABELED NGN3-EGFP/INS-DSRED MICE

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Stem cell therapy has been expected as novel approaches by replacement of islet transplantation and insulin therapy for curing type 1 diabetes. However, it remains to be solved to avoid cancer risk, allogenic immunosuppression and massive cost based on long-term quality control of stem cell-derived functional insulin-producing cells. In the context of potential future therapy for all T1D patients, cell reprogramming of somatic cells could be still insufficient production although several findings with direct conversion of somatic cells introduced by three transcription factors, MafA, Neurog3 and Pdx1, into beta cells seem to be fascinating solutions.

Poster Abstracts

We found, however, that no induction of insulin in murine embryonic fibroblasts (MEFs) by above 3 factors. Here we propose a hypothesis in which other cell fate key driver might be involved in MEFs-derived cell reprogramming. To address this, we have established a novel cell line, Tec-3p which is very likely immature endocrine progenitors. We have also developed the lineage-tracing model from endocrine progenitors to beta cells in dual-labeled transgenic mice expressing eGFP and DsRed2 driven by the Ngn3 and insulin promoters. Integrative screening analyses with our unique models has performed to identify potential key factors such as growth factors facilitating beta cell differentiation from progenitors mediated by Notch signaling. Moreover, we found that these factors accelerated the differentiation through the downregulation of Neurog3 gene expression epigenetically regulated by DNMT/HDAC3 complex. These data demonstrate that dynamic regulation of DNA methylation and chromatin remodeling mediated by cooperation of the Notch pathway with DNMT and HDAC 3 are require for progenitor maintenance and pancreatic endocrine cell fate for the useful of cell reprogramming in concert with potent cell fate drivers identified in the screening system.

Funding Source
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Keywords: differentiation; reprogramming; diabetes

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HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS FOR NEW ONSET TYPE 1 DIABETES

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Effective long-term therapy for islet/β cell death in type 1 diabetes (T1D) will require restoration of β cell mass and treatment of autoimmune injury. Currently no single intervention can provide these benefits and insulin therapy is required. The possibility of using mesenchymal stem cells (MSCs) as a therapeutic tool to effectively suppress autoimmune responses and restore β cell function has been explored in a few small studies. Herein, we report a funded, prospective, randomized human clinical trial for new onset T1D that has begun at the Medical University of South Carolina in Charleston. The study objective is to determine the efficacy of MSC infusion in addition to standard of care (SOC) in diabetes control. To qualify for the study, aged 12-30 participants must have new onset (within 3 months) T1D. Safety evaluation requires that the first 6 patients be adults. Inclusion criteria require a new diagnosis of T1D based on the ADA criteria, positivity for at least one T1D-associated autoantibody, such as GAD, IA-2 or ZnT8 autoantibodies, residual β cell function with a stimulated peak C-peptide >0.2 nmol/l during a 2 hour MMTT, and willingness to comply with intensive diabetes management. Exclusion criteria include evidence of retinopathy, presence of malignancy, abnormally high lipid levels for LDL cholesterol or triglycerides, abnormal blood pressure at time of consent, Body Mass Index < 14 or >35, severe infection, breast feeding or pregnancy, severe medical comorbidities, HgbA1c >12%, and/or fasting blood glucose >270 mg/dL. Participants qualified for the study will be randomized into intravenous umbilical cord autologous MSC/kg (n=25) or placebo control group (n=25) at day 0. Patients will be followed at 1 and 2 weeks and then at 1, 3, 6, and 12 months after treatment, and blood samples will be collected at each visit for mechanistic studies. MMTT tests will be performed at 6 months and 12 months post therapy and the 12-month C-peptide AUC will be used as the primary efficacy endpoint. Adverse events will be monitored and a panel of reactive autoantibodies, metabolic panels, and mechanistic indicators will be measured at each visit and used as safety endpoints. Travel reimbursement, a clinical trial website www.stemcelldiabetes.com, and listing on clinicaltrials.gov (NCT NCT04061746) are available to assist referral.

Funding Source
This study was in part supported by the National Institute of Health/National Institute of Diabetes and Digestive and Kidney Diseases grants # R01DK118529; 1R01DK105183, DK120394 and the NCATS MUSC CTSA award UL1 TR001450.
Keywords: Mesenchymal stem cells; Type 1 diabetes; Autoantibodies

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SYSTEMIC MODULATION OF THE IMMUNE RESPONSE TO ALLOGENEIC CELL TRANSPLANTS

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Immune rejection is a critical barrier to successful organ transplantation and stem cell therapies. In this project, we investigated the targeted modulation of the immune system to induce immune tolerance and alter the immune response to allogeneic grafts. Learning from the cancer immunology and immune regulatory network, we expressed a combination of immunomodulators (IMs) in allogeneic cells in order to induce local immune tolerance and prevent allogeneic immune rejection after transplantation. Human embryonic stem cells with a fail-safe system (H1-FS) were used to generate IM-expressing cells (H1-FS-8IMs). The in-vitro cell interaction between human immune cells and H1-FS-8IMs cell derivatives showed suppressed immune proliferation and proinflammatory cytokine production. Immune profiling results by mass cytometry also demonstrated the dynamic changes in human immune cells co-cultured with H1-FS-8IMs cell derivatives. We observed the shift to naïve status in the immune cell homeostasis, the weakened homing ability of naïve T cells and dendritic cells, indicating the expressed immunomodulators induced immune suppression. Further in vivo analysis is needed to assess the immune response and immune tolerance induced by H1-FS-8IMs-derived allogeneic cell grafts.

Keywords: Immune Rejection; Immune Tolerance; Stem-cell therapy

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INTRAVENOUS CELL THERAPEUTIC APPROACHES FOR PSYCHIATRIC DISORDERS: DAMPENING PERIPHERAL INNATE IMMUNE ACTIVATION TO PROTECT THE BRAIN

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Prolonged or severe psychosocial stress is a major risk factor linked to the development of mood disorders, including major depressive disorder (MDD) and anxiety. Recently, clinical and animal studies have demonstrated a pronounced, stress-induced, innate immune response detectable in circulation via quantification of pro-inflammatory cytokines and monocytes. Novel approaches targeting inflammatory processes may provide improved or adjunctive therapeutic opportunities in the treatment of MDD. Mesenchymal stromal cells (MSC), isolated from diverse sources, have demonstrated immunomodulatory capabilities in the context of infection and injury but have yet to be tested in stress-based preclinical models of psychiatric disorders. We sought to test the ability of MSC, to modulate stress-induced inflammation and depressive behaviors in a stress-based murine model of MDD / anxiety known as repeated social defeat (RSD). MSCs decreased stress-induced circulating pro-inflammatory cytokines and monocytes. MSCs also reduced neuroinflammation and reduced depressive and anxiety-like behaviours. However, biodistribution and IHC analyses of pre-labelled cells revealed that tail vein infused MSCs distributed entirely within peripheral organs without homing to the brain. Fate studies indicated that infused MSCs provoked rapid, transient recruitment of recipient neutrophils and monocytes to the lungs, even in immunocompromised animals. Infused MSCs and recruited neutrophils were subsequently cleared by macrophages which accumulated in the lungs and spleen. Clearance of both MSCs and recruited recipient neutrophils, promotes a phenotypic switch towards anti-inflammatory macrophages and ultimately, resolution of systemic inflammation, associated with detectable increases in circulating anti-inflammatory mediators. This effect may provide downstream protection to distal organs in preclinical disease models in which peripherally generated innate immune cells contribute to pathogenesis, including RSD. These data represent a novel avenue for translational MSC research and potentially identify unexpected targets in the periphery towards improved treatment of psychiatric disorders with an inflammatory component.

Keywords: Cell therapy; Immunomodulation; Psychiatric Disorders

Poster Abstracts

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COMBINED GENE AND CELL THERAPY FOR THE TREATMENT OF NEUROLOGICAL DISEASES

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Cell-based regenerative medicine is being intensively investigated for treatment of neurological diseases, however concerns about the risk of teratoma formation and immune rejection of allogeneic grafts still remain. Employing genome-editing strategies, we addressed these issues through developing the SafeCell (CS) and induced-allograft tolerance (iACT) systems. The SC system inserts a suicide gene into a cell division essential locus, allowing selective elimination of proliferative cells through the administration of a pro-drug, whilst also protecting the suicide gene from inactivation. The iACT system incorporates combined overexpression of immunomodulatory genes involved in immune-tolerance to permit long-term allograft survival after subcutaneous transplantation. Here, we sought to demonstrate the application of the SC and iACT technologies in neurological diseases, by using bioluminescence imaging to monitor the survival and proliferation of gene-edited mESCs injected into the mouse brain. We show that SC mESCs engraft and proliferate to form teratomas in isogenic and allogeneic recipient mice. Teratoma growth could be prevented by delivery of the pro-drug at the time of transplantation. Delayed treatment enabled elimination of the proliferative component of the teratoma, leaving the non-proliferative component intact. We are currently using a model of central nervous system autoimmunity to assess whether the iACT system enables survival of allogeneic transplants following infiltration of peripheral immune cells. If validated, our approach harnessing the SC and iACT technologies will allow the development of safe, immune tolerated and more effective cell therapies for treatment neurological disease.

Funding Source
This work is supported by Multiple Sclerosis Research Australia

Keywords: Gene editing; Cell therapy; Immune evasion

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DEVELOPMENT OF A FEEDER-FREE PSC CULTURE SYSTEM ENABLING TRANSLATIONAL AND CLINICAL RESEARCH

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Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-origin-free alternatives for growth factors contained within the Essential 8™ Medium and incorporate ISO13485 manufacturing for the recombinantly expressed, truncated human Vitronectin (rhVTN-N), producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with existing cGMP-manufactured passaging reagents: Versene Solution for clumped cell passaging and CTS™ TrypLE™ Select combined with RevitaCell™ Supplement for single cell passaging. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

Keywords: stem cell medium; GMP; cell therapy

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FACILITATED GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELLS TO SUPPORT CELL LINE GENERATION FOR THERAPY DEVELOPMENT

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Human induced pluripotent stem cells (hiPSCs) have been globally recognized as a multipurpose research tool for modeling human disease and biology, screening and developing potential therapeutic drugs, and implementing cell and gene therapies. The ability to differentiate human iPSCs into any cell type supports the generation of large amounts of specialized cells that ultimately could be used for cell-based treatments in patients. The emergence of genome editing tools, including the CRISPR/Cas9 system or TALENs, enable genetic modification of these cells; such as introduction of single base changes or inserting expression cassettes to correct disease causing mutations or to enhance cellular function respectively. Given the challenges with genome editing efficiency, mainly cell survival and clonal isolation, we have developed a number of reagents and processes to dramatically improve the success rate and timelines for a genome editing in hiPSCs. Key areas of the genome editing workflow that have been addressed include the genome editing tools themselves, the delivery methods and the maintenance of healthy hiPSC cultures during these stressful manipulations. Using the introduction of single nucleotide (SNPs) changes to explore and identify the best workflow for the genome editing process in hiPSC, we built a reliable approach that reproducibly supports the generation of hiPSC lines. In addition, using a small number of loci, we found that using the optimized workflow introduction of large DNA donors into specific loci was significant enough to allow for the clonal isolation of the edited cells, yet was dramatically lower compared to the introduction of SNPs. In summary, we detail advances with tools, reagents and protocols that facilitate the genome editing workflow in hiPSC and demonstrate that the use of such tools can be readily implemented to generate hiPSC cell lines that can be used for cell therapy research.

Keywords: Genome Editing; Human Induced Pluripotent Stem Cells; Therapy Development

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INTRACELLULAR CALCIUM HOMEOSTASIS MEDIATES EXIT FROM NAÏVE PLURIPOTENCY

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Progression through states of pluripotency is required for cells in early mammalian embryos to transition away from heightened self-renewal and toward competency for lineage specification. This transition is referred to as the exit from naïve pluripotency and coincides with embryo implantation, which can be studied in vitro using mouse embryonic stem cells (ESC). To identify novel genes required for exit out of naive pluripotency, we used a CRISPR knockout mutagenesis screen targeting the mouse genome with ~90k unique sgRNAs. The screen yielded 30 high confidence candidates (FDR<5%) in both expected and unexpected aspects of cell biology. Unexpectedly, we identified a role for intracellular Ca2+ homeostasis during exit out of the naïve state of pluripotency. Mutation of a plasma membrane Ca2+ pump encoded by Atp2b1 increased intracellular Ca2+ such that it overcame effects of intracellular Ca2+ reduction, which is required for naïve exit. Persistent self-renewal of ESC was supported in both Atp2b1-/- Tcf7l1-/- double knockout ESC passaged in defined media alone (no LIF or inhibitors) and in wildtype cells passaged in media containing only calcitonin and a GSK3 inhibitor. These new findings suggest a central role for intracellular Ca2+ in safeguarding naïve pluripotency, which may impact the derivation of therapeutic cellular products from pluripotent cells.

Funding Source

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Keywords: Pluripotency; CRISPR; Calcium

Poster Abstracts

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GENERATION OF NOVEL SINGLE CELL-DERIVED ENGINEERED MASTER PLURIPOTENT CELL LINES AS RENEWABLE SOURCES FOR OFF-THE-SHELF CANCER IMMUNOTHERAPIES

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Adoptive transfer of immune cells is hindered by expensive and complex manufacturing, limited applicability and inherent variability. Induced pluripotent stem cells (iPSCs) are a promising renewable source for mass manufacture of widely applicable off-the-shelf (OTS) immune cell therapies that can be banked and validated in advance. Here, we describe the use of iPSCs to generate two distinct investigational multi-engineered OTS cell immunotherapies. FT819 is a first-of-kind OTS chimeric antigen receptor (CAR) T cell therapy for hematologic malignancies derived from a clonal master iPSC line with bi-allelic disruption of the T cell receptor (TCR) and a novel CD19 CAR inserted into the TCR α constant (TRAC) locus to provide antigen specificity and avoid graft versus host disease. FT538 is an OTS NK cell therapy for multiple myeloma derived from a clonal master iPSC line with three engineered modalities: CD38 knockout to confer resistance to CD38 antibody-mediated fratricide, expression of an IL-15 receptor alpha fusion (IL-15RF) protein to enhance persistence, and expression of a high-affinity non-cleavable CD16 (hnCD16) to enhance and sustain antibody-dependent cellular cytotoxicity. iPSCs were generated, engineered and clones were screened for precise engineering, lack of random integration, loss of reprogramming plasmids and maintenance of stable pluripotent phenotype. Multiplexed testing before banking enabled efficient screening of hundreds of clones for desired attributes. For FT538, targeting a large bicistronic cassette constitutively expressing IL-15RF and hnCD16 into the CD38 locus was inefficient, but engineered clones were readily selected by sorting and screening for expressed IL-15RF by flow cytometry with an overall success rate of 15%. For FT819, clones with biallelic CAR integration into the TRAC locus with no random integration were readily obtained (9.2% of screened clones). Clones were evaluated again after banking for genomic stability, off-target editing, differentiation propensity, clone identity, and sterility. Our data demonstrate that iPSCs can be efficiently engineered and cloned from single cells to create master cell banks of renewable pluripotent cell lines for the mass manufacture of OTS cell immunotherapies.
Keywords: Pluripotent; Immunotherapy; CAR

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SCALABLE MANUFACTURE PLATFORM OF HUMAN IPS-NK CELLS FOR IMMUNOTHERAPIES

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NK cells are cytotoxic cells critical for innate immune system. CAR-T therapy requires either autologous or MHC matching cells to avoid graft-verse-host disease (GVHD), whereas allogeneic CAR-NK therapy have shown promising antitumor activities without GVHD. NK cells, therefore, are regarded as the ideal Off-The-Shelf products for immunotherapy. Unsatisfactory cells source, however, has been hindered the progress of NK cell based therapy. Human pluripotent stem cells (PSCs) offer unlimited sources for the manufacture of NK cells. Previously process of NK cell generation from PSCs used feeder cells and multiple steps, hence limiting industrial production for clinical application. Here we described a novel 3D-bioreactor platform that can continuously generate highly pure NK cells with strong cytotoxic activity from PSCs. First, human iPS cells were cultured as 3D-spheres with mesoderm inducing conditions, which efficiently converted iPS cells into hemogenic endothelial progenitors (HEP). By switching to NK cell differentiation conditions, these HEP spheres continuously released NK cells into the culture media which can be collected daily up to 50 days. These iPS-NK cells can be further expanded and cryopreserved for future usage. These iPS-NK cells display a distinctively homogenous morphology and ≈95% of them express CD56, NKG2D, NKp44 and NKp46; Approximately 30-50% of CD56+ iPS-NK cells also express KIR2DL/DS1 and KIR2DS4 receptors. These cells do not express TCR and CD3, and CD19, which are specific markers for T and B cells, respectively. Surprisingly, we discovered that over 80% iPS-NK cell are CD56+CD8+ suggesting strong cytotoxic effector phenotype. Indeed, strong cytotoxic activity was observed when iPS-NK cells and K562 cells were incubated together, with over 80% of K562 cells killed within hours as demonstrated both by FACS

analysis and time-lapse movie. We are currently introducing chimeric antigen receptors (CAR) into iPS cells to establish master iPS-CAR cell lines, which will provide inexhaustible cell sources for the generation of truly off-the-shelf CAR-NK cells suitable for treatment of a large numbers of patients, which can revolutionize the immuno-oncology field.
Keywords: iPS-NK cells; 3D suspension culture; Immunotherapy

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AN IN VITRO 3D STEM CELL NICHE SCREENING PLATFORM FOR PRE-EMPTIVE DETECTION OF CHEMO-IMMUNOTHERAPY-INDUCED MYELOSUPPRESSION

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Immune checkpoint inhibitors in combination with conventional chemotherapy have the potential to establish a new paradigm as first-line cancer treatments. Chemo-immunotherapy combinations however can induce myelosuppression toxicity, with approximately 10% of patients impacted and forced to discontinue therapy. Myelosuppression side-effects arise due to toxicity of hematopoietic stem and progenitor cells (HSPC) residing in the bone marrow. Toxicity testing on HSPC currently uses 2D cell culture methods, however, 3D niche conditions that permit extended culture conditions (>72h) remain undefined. Here we report the development of an in vitro 3D stem cell niche system based on porous natural extracellular matrix components mimicking the bone marrow. With this new culture method requires the development of dissociation approaches to optimize maximum recovery of cells from the 3D porous niches. Cells were seeded onto 3D porous scaffolds composed of collagen in 96 well plates subjected to mechanical, enzymatic and a combination of mechanical-enzymatic to yield cell recovery ranging from 20-40%. Ongoing experiments are testing recovery rates from collagen, hydroxyapatite and elastin scaffolds. Furthermore, volumetric, high-throughput flow cytometry in 96 well plate format was used for quantification of phenotype and cell number between niche conditions. This high-throughput 3D stem cell niche system is amendable to screening of drug combinations, including chemo-immunotherapy, on HSPCs to identify toxic conditions.
Keywords: Stem Cell Niche; Myelosuppression; Hematopoietic Stem Cell

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BARCODING OF PATIENTS SAMPLES FOR DECONVOLUTION OF BIOLOGICAL RESPONSE VARIATION: AN EFFICIENT SCREENING PLATFORM TO ACCELERATE IMMUNOTHERAPY DRUG DISCOVERY

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Immunotherapy agents, like most drugs, demonstrate varying biological responses between human patients ranging from responders to non-responders. A high-throughput cellular barcoding technique for simultaneously testing drug response on multiple patient samples can facilitate deconvolution of biological response variation and promote biomarker and patient stratification. Fluorescent protein-based reporters have been used for long-term cell-tracing experiments; however, these systems inherently transform primary cells which can perturb biological responses while subjecting to lengthy transfection procedures. Therefore, the development of a broad emission colour spectrum, to allow for rapid patient sample barcoding with low cytotoxicity provides a powerful tool for analyzing variations in biological responses. Here we show the use of carbocyanine lipophilic cell membrane dyes, DiD, DiI, and DiO (far-red, red, and green emission, respectively) to barcode human myeloid leukemia cells (HL60), acute myeloid leukemia (AML), and hematopoietic stem and progenitor cell (HSPCs) as a method to simultaneously deconvolute biological responses using volumetric flow cytometry. Utilization of these lipophilic stains not only provides strong, uniformly stained cells but also allows for parallel measurements in biological populations under the same experimental environments. These 3 lipophilic stains permitted barcoding of 8 separate samples while minimizing cytotoxicity. The biological response of anti-leukemic stem cells agents was simultaneous tested in barcoded leukemia and HSPC samples to reveal selective anti-leukemic toxicity while sparing HSPC populations. Importantly, this method ensures that cell surface markers remain free and untouched by antibodies, thereby permitting investigations which probe receptors of interest with immunotherapy agents, small molecules, or cytokines. These investigations can permit deeper insight into cell signaling pathways to aid in the development of biomarker and patient stratification for safer and more effective immunotherapy treatments.
Keywords: Barcoding; Drug Discovery; Leukemia

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GENERATION OF OFF-THE-SHELF UNIVERSAL HAEMATOPOIETIC CELLS FOR ALLOGENEIC CELL THERAPIES

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The future of regenerative medicine and cell-based therapies is incredibly promising. Many of these therapies will utilize hematopoietic cells, which given their vast range of functions could be used in cancer treatment, autoimmunity, or tissue regeneration. However, the widespread use of hematopoietic cells remains hindered because they are expensive and labor intensive to generate, usually involving the isolation and manipulation of autologous cells in a patient-specific manner. Furthermore, even autologously derived blood cells pose a safety risk, which can stem from in vitro transformations or aberrant activity after they are engrafted in vivo. What is desperately needed are hematopoietic progenitors that are engineered with safety elements to overcome these risks, as well as systems that allow them to survival in allogeneic recipients so they do not need to be derived autologously. Our lab has engineered two separate technologies to address these challenges. The first is the SafeCell (SC) system, which consists of a TK-based suicide gene transcriptionally linked to an endogenous locus that is essential for cell division. The second is the induced allogenic cell tolerance (iACT) system, a collection of immunomodulatory transgenes that allows pluripotent cells to escape immune rejection in foreign hosts. Here we show that our SC/ iACT murine ESCs can differentiate into alveolar-like macrophages, a mature and functional immune cell. We also show that human ESCs that contain the SC and iACT systems can differentiate into CD34+ hematopoietic like progenitor cells. Moving forward, we will test if these human CD34+ cells can differentiate into NK-, T cells and macrophages. In summary, our goal is to show that these technologies allow for the creation of functional, universal hematopoietic cells, so as to overcome the most important biologic and economic hurdles to their widespread clinical use.

Funding Source
Ontario Research Fund
Keywords: Hematopoiesis; immunotherapies; immune evasion

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SCALED EXPANSION OF PLURIPOTENT STEM CELLS IN SUSPENSION CULTURE FOLLOWED BY DIRECT NEURAL DIFFERENTIATION FROM 3D CELL AGGREGATES

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Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While two-dimensional (2D) feeder-free expansion of PSCs is well established, the scale is limited. Here we describe a new system for highly scalable expansion of PSCs as three-dimensional (3D) spheroids in suspension as self-assembled aggregates followed by neural induction and differentiation starting from the expanded PSC aggregates or spheroids. While expansion potential is an important parameter for assessing a fit for purpose medium system (i.e., 2D vs. 3D), another important consideration is compatibility with downstream differentiation protocols. In recent years, 3D aggregate cell culture has been gaining traction as an enhanced culture technique which provides more physiologically relevant cell-cell interactions over the traditional 2D cell culture protocols. When determining whether to move from 2D culture environments to 3D culture environments, a number of considerations need to be made; including the quantity of desired cell type(s) required for downstream applications, compatibility of reagents and experimental endpoints designed for 2D, and importantly, how neurons derived using 2D and 3D methodologies compare and contrast to each other. Here, we demonstrate the feasibility of directing expanded PSC 3D aggregates to neurons using differentiation reagents designed originally for monolayer applications. Key parameters and consideration for both PSC expansion and neural differentiation are presented and discussed, which include scalability, expansion rates, and differentiation efficiency. Notably, 3D differentiation resulted in significantly higher expansion rates of progenitor cells and similar marker expression compared to standard 2D methods. Finally, the impact of 2D vs 3D neural differentiation and expansion on neuronal maturation will be presented.

Keywords: Human Pluripotent Stem Cells; 3 Dimension; Neural differentiation

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ENHANCED MATURATION OF FAST-SPIKING INTERNEURONS DRIVEN BY MTOR ACTIVATION

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The inhibitory interneurons of the cerebral cortex, differentiated from human pluripotent stem cells, have therapeutic potential for pathological conditions associated with dysregulation of fast-spiking interneurons. However, the use of stem cell derived interneurons for cell-based therapies is limited by their protracted maturation. Enhanced maturation of stem cell derived interneurons could overcome this limitation, providing a resource for novel therapeutics. Here, we present an approach for accelerating the post-mitotic maturation of human stem cell derived interneurons. We first modified a human embryonic stem cell line with a fluorescent reporter for transcription of Lhx6, which is expressed from cell cycle exit in MGE-derived interneurons through postnatal development, and is critical for their development. Previous reports have suggested the regulation of synaptic maturation and neuronal connectivity by PTEN signaling. To explore the role of PTEN signaling and its downstream effectors in interneuron maturation, we devised strategies for inducible activation of mTOR pathway, which regulates cellular proliferation, growth, and maturation. The PTEN gene in post-mitotic, fate committed human interneuron-like cells was inactivated through genetic manipulation by Cre-Lox recombination. PTEN deletion resulted in accelerated neuronal maturation, measured by enhanced dendritogenesis and synaptogenesis as well as maturational changes in electrophysiological characteristics. Importantly, these effects were reversed by rapamycin, an inhibitor of mTOR signaling. We observed similar effects using doxycycline-inducible activation of Akt. We thus present an inducible, reversible approach of accelerating the maturation of human stem cell derived interneurons. This work presents a novel approach and a model system for studying the effects of persistent activation of mTOR signaling on interneuron-related disorders, including epilepsy and autism.

Funding Source
NIH Grant R01MH110185
Keywords: Interneuron; mTOR; maturation

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POPULATION-SCALE PHARMACOGENOMICS IN-A-DISH: MAPPING ALLELES TO STRATIFY PATIENT RESPONSE TO THERAPEUTIC INTERVENTION USING IPSC-DERIVED MASSIVELY MOSAIC EXPERIMENTAL SYSTEMS

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A fundamental need in biomedical research is to understand how human allelic variation shapes cellular phenotypes. Our goal is to be able to genetically dissect any phenotype of interest to measure its heritability, and discover how genetic variation converges to affect biological processes, vulnerability to illness, and response to therapeutics. We developed population-in-a-dish experimental systems in which cells from hundreds of donors can be simultaneously phenotyped and subjected to genetic analyses. Co-culturing cell lines in this way provides orders-of-magnitude increases in scalability allowing statistically meaningful genotype-phenotype correlations, and minimizes aspects of variability that would otherwise plague comparisons of individual cell lines. As a model phenotype, we focused on discerning the effects of common variation on expression of Survival Motor Neuron (SMN), encoded by paralogues SMN1 and SMN2. We show that the power and scalability of our system affords the opportunity to rapidly and inexpensively correlate SMN phenotypic variation (as measured by flow cytometry) with underlying SMN copy number (CN), markedly surpassing genome-wide significance ($p = 8.72 \times 10\text{-}22$). We then asked whether our approach could illuminate the genetic foundation for patient variation in drug response and map the basis of this pharmacogenetic effect. We used flow cytometry to detect changes in SMN expression following treatment with the splicing enhancer LMI070. We show that LMI070 response correlates strongly with SMN2 CN (but not SMN1, $p = 6.55 \times 10\text{-}10$) confirming the hypothesis that LMI070 increases SMN levels by specifically modulating splicing of SMN2. We questioned why some donors with the same SMN2 CN showed variation in response to LMI070 and performed an in-depth re-analysis of the genomic architecture of the SMN locus. We discovered a novel drug-nonresponsive allele of SMN that lacks exons 7 and 8 (including drug binding site), rendering these donors unable to enhance SMN protein production in response to LMI070 treatment. These results provide a therapeutic guideline for genomics-informed

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stratification of patients with Spinal Muscle Atrophy, and illuminate the power of population-in-a-dish systems for connecting human genetic variation with disease-relevant biological processes.
Keywords: Pharmacogenomics; Genetic neuroscience; Patient stratification

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NEUROINFLAMMATORY AND INTEGRATED STRESS RESPONSE SIGNATURES PERSIST IN A HUMAN IPSC TRI-CULTURE MODEL OF HIV INFECTION DESPITE ANTIRETROVIRAL TREATMENT

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HIV-Associated Neurocognitive Disorders (HAND) affect 55% of HIV-infected individuals worldwide. While antiretroviral treatments (ART) have reduced the severity of HAND, the prevalence has increased due to increased life expectancy. The full, underlying mechanism is unknown partly since there is no in vitro model to study the direct interactions among HIV-infected microglia, neurons, and astrocytes. We developed a human-induced pluripotent stem cell (HiPSC) based model; whereby, we separately differentiate HiPSCs into neurons, astrocytes, and microglia and create a tri-culture of the three cell types with or without HIV-infection and ART. scRNAseq analysis revealed inflammatory signatures and integrated stress response in all three cell types; however, the microglia were most affected during HIV-infection. Further, ART mostly resolved these signatures, but did not completely quell inflammation in the microglia and neurons. Analysis also revealed activation of Fcγ-mediated phagocytosis in infected iMg. However, we discovered a reduction in synaptic phagocytosis by the infected microglia, revealing that enhanced phagocytosis by HIV-infected microglia is unlikely to account for the synaptodendritic damage observed in the central nervous system (CNS) of HIV-infected patients. Pathway analysis also revealed increased RhoGDI and CD40 signaling in the HIV-infected microglia exposed to ART. This signal activation was associated with a persistent increase in TNFa expression in the infected with ART condition. This work establishes a novel, all human tri-culture that recapitulates key features of HIV infection in the CNS and provides a new model to examine the effects of HIV infection and its treatment with antiretroviral compounds in a cellular context.
Keywords: HiPSC; neuroinflammation; HIV

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IMPLICATION OF LNCRNA TUNA IN NEUROGENESIS: BALANCE BETWEEN DIFFERENTIATION AND PLURIPOTENCY

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Embryonic development consists of a succession of cell state changes, leading to the specialization of cells and formation of the different functional tissues of an organism. The establishment of these cellular states, for instance during neurogenesis, is regulated by the expression of complex gene networks. Their disruption impairs cell fate and may lead to developmental and degenerative disorders. To date, most studies on regulation of these cell states focused on coding genes. However, the emergence of high throughput sequencing techniques revealed the existence of thousands of long non-coding RNAs (lncRNAs) in the mammalian genome. Like mRNAs, they can be capped, spliced and polyadenylated, but they do not code for any protein. They are known to be implicated in gene expression regulation and control of cell fate. Here we focus on the lncRNA Tuna (Tcl1 upstream neuron-associated lncRNA) which is strictly expressed in the central nervous system (CNS) during embryo development and is required for commitment of embryonic stem cells (ESCs) towards a neural lineage. Tuna is associated with neurological function in vertebrates and its expression is linked to neurological conditions such as Huntington's disease. We found that Tuna was highly expressed in basal ganglia tissue and more specifically in GABAergic neurons of the ventral midbrain, suggesting a role for Tuna in ventral brain development and in neurological conditions involving these brain regions. We also identified two isoforms, sht.Tuna and alt.Tuna, that are differentially expressed during ESC neuronal differentiation. Alt.Tuna is the result of alternative splicing of exon 1 of Tuna and is specifically expressed in both human and mouse ESCs. This suggests a conserved processing of the RNA between mouse and human cells, and that alternative splicing of particular lncRNAs may lead to distinct functions depending on cell state. Moreover, Tuna contains a highly conserved sequence in which site-directed mutagenesis affected its function in ESCs. Investigating the role of alternative splicing and sequence conservation of Tuna lncRNA during neuronal differentiation will allow us to better understand its function in embryonic development and its implication in pathological conditions, such as neurodegenerative diseases.
Keywords: Long non-coding RNAs; Neurogenesis; Pluripotency

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SMART NEURAL STEM CELL THERAPY TO DEGRADE SCAR AND OPTIMIZE REGENERATION AFTER CHRONIC CERVICAL SPINAL CORD INJURY

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Human iPS neural stem cells (NSC) are an exciting regenerative approach for traumatic spinal cord injury (SCI). Unfortunately, most individuals are in the chronic phase of their injury where dense perilesional chondroitin sulfate proteoglycan (CSPG) scarring significantly impairs regeneration. Scar-modifying enzymes can enhance recovery, however, intrathecal catheter injection increases the risk of off-target CNS effects. We aimed to generate a novel, genetically-engineered line of hiPS-NSCs capable of locally expressing a scar-degrading enzyme to enhance recovery. A proprietary scar-degrading enzyme was genetically integrated into hiPS-NSCs under an inducible promoter with CRISPR. Enzyme expression was extensively characterized in vitro by biochemical and culture assays. To assess efficacy in vivo, T-cell deficient rats with chronic C6-7 injuries were randomized to (1) NSCs, (2) enzyme-expressing NSCs, (3) vehicle control, or (4) sham surgery. Animals were assessed with weekly neurobehavioural tests of locomotion, forelimb recovery, and sensation for 13 weeks post-transplant. A monoclonal line was established and found to retain key human NSC characteristics including the capacity to form neurospheres and differentiate along all three neuroglial lineages. The enzyme expressed by the NSCs degrades CSPGs in vitro and allows hiPS-NSC-derived neurons to extend into CSPG-dense scar-like areas after 7 days in culture. While blinded histological analyses are ongoing, an interim analysis of several animals shows that grafted human cells are extending remarkably long (cervicomedullary junction to mid-thoracic) axons through rodent white matter. Interim behavioural analyses also show one group scoring 3 points higher on BBB at 22 weeks post-injury. This work provides exciting proof-of-concept data that genetically-engineered SMaRT cells can degrade CSPGs in vitro and that human NSC grafts can form long axonal processes in the typically inhibitory chronic cervical SCI niche.

Keywords: Neural stem cell; Spinal cord injury; Bioengineering

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THE LOW RATE OF RESULTS REPORTED FOR CLINICAL TRIALS EXAMINING REGENERATIVE MEDICINE FOR NEURODEGENERATIVE DISEASES REGISTERED IN CLINICALTRIALS.GOV

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Clinical trial results are rarely registered in clinical trial registries; however, their results can be retrieved if they are published with an abstract containing an associated Study ID. Using the clinical trial registry, we have previously reported trend analyses of clinical studies in seven areas of regenerative medicine. In the process, we found a low rate of reporting results of clinical trials in regenerative medicine, even after combining results reported in trials in the registry and in literature. In this study, we analyzed the rate of result-reporting for completed studies registered in ClinicalTrials.gov for regenerative medicine in the four areas of neurodegenerative diseases (Stroke, Spinal Cord Injury (SCI), Traumatic Brain Injury (TBI), and Parkinson Disease (PD)). We used the number of studies with 'Drug' in the intervention column as a control; the reporting rates for the four diseases were 41–43% in control studies. In contrast, the reporting rates of clinical trials for regenerative medicine were as low as 21% for stroke, 27% for SCI, and 0% for TBI. In addition, this rate was 50% for PD, which was higher than the overall drug reporting rate; however, all the results for PD were from gene therapy trials, not cell therapy trials. For investigating the causes of lower reporting rates of regenerative medicine trials than of drug trials, we compared completion rates for each drug group and regenerative medicine group. The percentages for the completion rate of the regenerative medicine group/Drug group were 65% for stroke, 81% for SCI, 66% for TBI, and 45% for PD. Based on the low completion rate of the regenerative medicine group, we speculated that one of the causes of this low reporting rate was time required for completing the trial. However, other issues, for example, PD gene therapy products and cell therapy products, are also involved. Moreover, analyzing other factors, including publication bias, that might influence the reporting rate has to be done. Clinical trial outcomes are valuable resources that should be

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shared so that subjects enrolling for new trials can avoid known dangers and unnecessary duplication of research is prevented. Clinical researchers, therefore, should report their findings as an obligation.

Funding Source
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Keywords: ClinicalTrials.gov; Stroke, SCI, TBI, and Parkinson Disease; result-reporting rates for clinical trials

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MULTIWELL MICROELECTRODE ARRAY TECHNOLOGY FOR THE DEVELOPMENT, OPTIMIZATION, AND VALIDATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTE AND NEURON MODELS

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The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of cardiomyocyte and neuron electrophysiology to be used in screening applications in drug discovery and safety. Importantly, stem cells also hold great promise for cell-based clinical therapies. The translation of stem cell technologies, for screening or therapy, relies on the continued improvement and optimization of the commercial cell products, including the differentiation protocol, the manufacturing process, and the consumables required for development. Here, we present data supporting the use of multiwell microelectrode array (MEA) technology as an efficient approach for the development, optimization, maturation, and validation of human iPSC-derived neuron and cardiomyocyte models. A planar grid of microelectrodes embedded in the substrate of each well interfaces with cultured networks, such that the electrodes detect the raw electrical activity from the cells. Recent advances in MEA technology afford the quantification of the shape and propagation of the cardiomyocyte action potential, as well as the mechanical contraction of the cardiomyocytes. By comparison, the organization of the cellular activity across neurons within a network and across time can be described by measures of activity, synchrony, and oscillations to quantify phenotypes of network electrophysiology. For both neurons and cardiomyocytes, the discrete nature of the MEA technology easily adapts to the localized cell populations associated with advanced preparations like spheroids. Example case studies are presented to illustrate how quantitative assessment of the cell function facilitates model development, quality control, and preclinical validation. These results support the continued development and use of human iPSC-derived cardiomyocyte and neural assays on multiwell MEA technology for development, optimization, and validation towards cell-based clinical therapies.

Keywords: Disease-in-a-Dish; Electrophysiology; In Vitro Assay

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ARGINYL-DIOSGENIN ANALOG RESTORES COGNITION, HIPPOCAMPAL INFLAMMATION, AND NEUROGENESIS IMPAIRED BY LIPOPOLYSACCHARIDES IN MICE

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Microglia-mediated neuroinflammatory responses are well known to inhibit neurogenesis in the dentate gyrus (DG) of the adult hippocampus, and growing evidence indicates that therapeutic intervention to suppress microglial activation could be an effective strategy for restoring the impaired neurogenesis and memory performance. In the present study, we investigated the effects of water-soluble arginyl-diosgenin analog (Arg-DG) on the adult hippocampal neurogenesis using a central LPS-induced inflammatory mice model. Arg-DG attenuates LPS-impaired neurogenesis by ameliorating the proliferation and differentiation of neural stem cells (NSCs). The impaired neurogenesis in the hippocampal DG triggered the cognitive function, and that treatment of Arg-DG led to the recovery of cognitive decline. Arg-DG also suppressed the production of LPS-induced pro-inflammatory cytokines in hippocampal DG by blocking microglial activation. In in vitro study, Arg-DG inhibited the production of nitric oxide (NO), cyclooxygenase-2 (COX-2) expression, and prostaglandin D2 production (PGD2), as well as the pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, and

tumor necrosis factor alpha (TNF-α). The anti-inflammatory effect of Arg-DG was regulated by NF-κB and MAPK JNK signaling both in vivo, and in LPS-stimulated microglial BV2 cells. Taken together, these results suggest that Arg-DG might have the potential to treat various neurodegenerative disorders resulting from microglia-mediated neuroinflammation.

Funding Source
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Keywords: Arginyl–diosgenin; Adult neurogenesis; Neuroinflammation

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GUIDING IN VIVO CELL FATE OF TRANSPLANTED CELLS WITH BIOMATERIALS

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Stem cell transplantation holds the potential to replace lost neurons and promote functional repair following central nervous system injury; however, their survival and differentiation are only poorly controlled once transplanted, leading to extensive cell death and uncontrolled differentiation, mainly into glial cells. Biomaterials can advance cell therapies by providing local, sustained release of survival and differentiation factors. Here, we modified an injectable hydrogel comprised of hyaluronan (HA) and methylcellulose (MC) with a series of peptide sequences to improve the survival and neuronal differentiation of human pluripotent stem cell-derived motor neuron progenitor cells. Using PHSRN-GRGDS-modified HAMC, 30,000 cells/µl were transplanted one week after a moderate, 23.5g clip compression spinal cord injury at level T2 at four points rostral and caudal to the lesion site (8 µl in total) or directly into the lesion site (3µl total). Control animals received cells in unmodified HAMC. Induced pluripotent stem cells successfully differentiated into caudal neural stem cell and motor neuron progenitor cells, as indicated by the downregulation of NANOG, OTX2 and the expression of NESTIN, SOX1, HOXB4, HOXC9, OLIG2, TUBB3, HB9, CHAT, and ISL1. The peptide sequence PHSRN-GRGDS improved cell-substrate interactions and survival seven days after seeding. One week after transplantation cells migrated towards the injury site when transplanted rostral and caudal, and many cells expressed nestin, DCX, and Ki67. In stark contrast, at four weeks only cells injected with the modified HAMC survived, filling out the lesion site with higher survival when transplanted directly into the lesion site. They differentiated mostly into neurons with long axons extending rostral and caudal. Approximately 30% of the cells expressed the mature neuronal marker NeuN, and many cells also expressed the motor neuron marker choline acetyltransferase (ChAT). In conclusion, the modified hydrogel promoted the survival and neuronal differentiation of transplanted motor progenitor cells, especially when cells were transplanted directly into the lesion site. Ongoing studies are examining tissue and functional repair.

Funding Source
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Keywords: Spinal cord injury; Biomaterials; stem cell-derived motor neuron progenitors

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GENERATION OF FUNCTIONAL GABAERGIC INTERNEURONS FOR EPILEPSY TREATMENT

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Epilepsy is a neurological condition affecting 1% of the world's population. Available pharmacological therapies, providing solely symptomatic relief, are effective in only 70% of patients. The remaining 30% of patients, the majority suffering from mesial temporal lobe epilepsy (mTLE), have very few therapeutic alternatives and often continue to experience uncontrollable seizures. Therefore, there is a need for novel treatment strategies. One of these novel approaches that may prove to be effective is cell therapy. Studies in rodent epilepsy models suggest that GABAergic interneuron progenitor grafts can reduce hyperexcitability and seizures in mTLE. In this study we focused on the generation and characterisation of GABAergic interneurons derived from human embryonic stem cells (hESCs) modifying a published protocol by Yang et al. 2017. By overexpressing 2 transcription factors, Dlx2 and Ascl1, we successfully generated a neuronal population in vitro. With immunocytochemistry we confirmed cell identity using GABAergic markers as well as markers for various interneuron subtypes. Furthermore, to confirm functional maturation, we performed whole-cell patch-clamp electrophysiological recordings focusing on intrinsic properties and synaptic events of these cells in

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culture. To confirm that our generated cells are able to form functional synapses with host cells, we transduced our cells with channelrhodopsin-2 and cocultured them with human primary neurons. By performing whole-cell patch-clamp combined with optogenetics we confirmed the presence of functional synaptic connections from our cells onto the primary "host" cells. With these results in vitro we proceeded to transplant generated interneuron precursors in vivo in the hippocampus of athymic rats. We observed a graft with surviving cells 4 weeks post-transplantation, with cells being positive for neuronal markers, able to migrate and extend their processes throughout the hippocampus. In summary, we successfully generated functional GABAergic inhibitory neurons from hESCs which are able to form functional synaptic connections in vitro and survive grafting in vivo. These cells will be further used for transplantation in rodent epilepsy models and are a promising resource for potential future therapeutic use in refractory epilepsy.

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Keywords: human embryonic stem cells; cell differentiation and transplantation; GABAergic interneurons

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GENERATION OF PHOTORECEPTOR PROGENITORS FROM HUMAN PLURIPOTENT STEM CELLS USING A SCALABLE 3D SPHERE CULTURE SYSTEM

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An essential requirement for the development of cell-based therapies is the establishment of robust manufacture process that allow the derivation of large quantities of highly pure transplantable cells from renewable sources, which recapitulate the characteristics of the endogenous cell types intended to replace. Retinal neurons including photoreceptors precursor cells (PRPCs) generated in vitro from human pluripotent stem cells (hPSCs) are potential cell source for regenerative therapies, drug discovery and disease modeling. However, numerous approaches to differentiate hPSCs into retinal neurons and PRPCs for the purpose of cell replacement therapy produced undesirable results in terms of efficiency, purity, homogeneity and scalability. This study describes a robust, defined and scalable 3-dimension (3D) sphere culture system for the generation of highly enriched retinal neurons at different developmental stages from hPSCs, including early and late committed retinal neuron progenitors (CRNP), PRPCs as well as photoreceptor-like cells by synchronizing the differentiation process, which can be easily adapted to current general manufacture practice (cGMP) protocol. This novel protocol starts with hPSCs as 3D spheres, which are directly induced to differentiate into early CRNPs, late CRNPs, PRPCs and photoreceptor-like cells by a combination of small molecules with continuous sphere dissociation/reaggregation and sphere reformation approach in bioreactors under matrix-free conditions. This well controlled 3D sphere system overcomes numerous limitations, especially the scalability, facing conventional adherent 2D culture and traditional embryoid body as well as organoid systems. Our novel approach routinely generates 3-4.5 x 108 PRPCs starting with 3 x 106 hiPSCs with a purity of approximately 95%. Multiple levels of analyses, including immunofluorescence staining, flow cytometry, and quantitative gene expression by RT-qPCR confirmed the identities of early and late CRNPs, PRPCs and photoreceptor-like cells. This novel 3D sphere platform is amenable to the development of an in vitro GMP-compliant retinal cell manufacturing protocol from multiple renewable hPSC sources for future preclinical studies and human cell replacement therapies.

Keywords: iPS lineage-specific differentiation; Photoreceptor Progenitors; 3D Suspension Manufacture

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FIBRONECTIN SCALFOLD AND GROWTH COCKTAIL PROMOTED IPSC-DERIVED NEURAL STEM CELLS TO AMELIORATE MOTOR DEFECTS

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Patient-specific induced pluripotent stem cells (iPSCs) have great therapeutic potential for neurodegenerative diseases such as Parkinson'disease (PD). However, the therapeutic efficacy and mechanisms of dopamine neuronal conversioin from iPSCs have not been clearly determined. In this study, we developed a new method to increase conversion of neural stem cells to dopamine neurons from iPSCs of PD patients by addition of the growth factor cocktail containing noggin, CHIR99021, SHH, FGF8, TGFβ, GDNF, and BDNF. In

combination with the fibronectin and growth cocktail, the iPSC-derived NSCs were transplanted to striatum of of 6-OHDA lesioned rats. As a result, iPSC-derived NSCs showed an efficient DA neuronal conversion in vivo. Both the grafted iPSC-NSCs and the differentiated dopaminergic neurons survived and integrated into the host rat brains 16 weeks after transplantation. The iPSC-NSCs-derived neural cells displayed the same electrophysiological profile as DA neurons in vivo. More importantly, rats with transplanted iPSC-NSCs showed progressive improvements in motor behaviors compared to controls from weeks 4 to 16 post-grafting. These results demonstrated the efficacy and clinical application of combined transplantation of growth factor cocktail and iPSC-NSCs in 6-OHDA lesioned rats and provided experimental evidence for cell-based therapy of PD.

Funding Source
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Keywords: Induced pluripotent stem cell; Parkinson's disease; dopamine neuron

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DERIVATION OF OLFACTORY PLACODAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Olfactory dysfunction, or loss of smell, is estimated to occur in 5.8 to 11.6 million people in North America. The olfactory sensory neurons are the receptors in the nose that detect odors from the environment and transmit odorant information to the brain. Loss of smell can occur from degeneration of olfactory sensory neurons and is often the first symptom experienced in neurodegenerative diseases. Cell based therapies have the potential to treat olfactory dysfunction through replacement of olfactory progenitors, also known as olfactory placodal cells, however, such therapies are not yet available due to the lack of protocols available for the generation of these placodal cells. Human pluripotent stem cells (hPSCs) can be differentiated into any cell type in the body; therefore, they are an invaluable tool for cell replacement therapies. The first step towards a cell-based therapy is to create a strategy to differentiate hPSCs into olfactory placodal cells through a series of molecular cues that recapitulate characteristics of their natural development. To this end, we are utilizing a combination of various growth factors and small molecules to generate olfactory placode. The treatment has included timed exposure to cells using a bone morphogenetic protein (BMP) inhibitor, wingless/integrated protein (WNT) inhibitor, epidermal growth factor (EGF), and transforming growth factor alpha (TGFα). Our results show spontaneous differentiation of placode-like structures that positively express key early placodal genes from day 6 to day 12 of hPSC differentiation. Our key findings indicate that addition of a BMP inhibitor at day 3 of hPSC differentiation induced neural olfactory placodal cells and prevented non-neural olfactory and lens placodal cells at day 24. Secondly, a WNT inhibitor treated at day 3 promoted olfactory placodal cells while preventing lens placodal cells at day 24. Finally, addition of TGFα at day 9 enhanced olfactory placode at day 24. Future experiments will involve the combination of these factors and assess the functionality of hPSC-derived olfactory placodal cells by producing olfactory sensory neurons. These results will positively impact the field because they will lead to new therapies for diseases of the olfactory tissue and promotion of sensory nerve regeneration.

Funding Source
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Keywords: olfactory; hPSCs; differentiation

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PROTEIN-TYROSINE PHOSPHATASE 9 (PTPN9) NEGATIVELY REGULATE THE PARACRINE FUNCTION OF BONE-MARROW DERIVED PROANGIOGENIC CELLS (PACS): IMPACT IN OXYGEN-INDUCED RETINOPATHY (OIR)

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Insufficient post-ischemic revascularization is an initial key step in the pathogenesis of OIR. In the past decade, studies have demonstrated the potential of angio-therapy using different population of bone-marrow (BM) derived stem cells and particularly, early outgrowth EPC, also called PACs. During revascularization, BM-PACs are mobilized to ischemic area to integrate local microvessels and secrete large amount of growth factors promoting angiogenesis. However, the paracrine activity of PACs during oxygen-induced retinopathy (OIR) and the associated mechanism remain to be explored. Because PTPN9 is reported to be a negative regulator of stem cell differentiation and angiogenesis signaling, we proceeded to study its function on PACs activity in OIR. Using a rat model of OIR, we found that lower levels of mobilization factor SDF-1 and decreased PAC (markers CD34,

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CD117, CD133) are associated with a greater retinal avascular area, which in turn correlated positively with PTPN9 expression in retina as well as in isolated BM-PAC. In addition, fewer PACs (CD34+cells) co-localized with a lower number of choriocapillaris observed in OIR (compared to controls). Next, we characterized ex-vivo the effects of hyperoxia on BM-PAC-generated conditioned media (CM), and explored the role of PTPN9 in this process (by knocking down its expression). PACs subjected to hyperoxia displayed increased PTPN9 expression which corresponded to impaired ability of PAC-CM to promote angiogenesis as attested by lower choroidal vascular sprouting and tubulogenesis of endothelial cells compared to CM from normoxic-exposed PACs. Suppression of PTPN9 (using siRNA) normalized PACs secretome during hyperoxia, and restored their CM ability in angiogenesis; accordingly, endothelial and microglial cells treated with siPTPN9-treated PAC-CM generated more VEGF, SDF-1, and VEGFR2 phosphorylation (active form). Collectively, these results suggest that hyperoxia alters the paracrine proangiogenic activity of BM-PACs by inducing PTPN9 which can contribute to impaired post-ischemic revascularization in OIR. Targeting PTPN9 can improve PAC efficacy, and represents a novel angio-therapeutic strategy for ischemic retinopathies.

Keywords: bone-marrow derived proangiogenic cells; oxygen-induced retinopathy; PROTEIN-TYROSINE PHOSPHATASE 9 (PTPN9)

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LABELING OF MOUSE DERIVED MESENCHYMAL STEM CELLS USING PAMAM DENDRIMER NANOMOLECULES AND ITS EFFECT ON INCREASING THE LEVELS OF ENDOGENOUS BDNF IN VITRO AS A POTENTIAL THERAPY FOR HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disease caused by the death of medium spiny neurons in the striata of the brain. Mesenchymal stem cells (MSCs) are multipotent cells that have the potential therapeutic effects for HD, by releasing trophic factors such as brain-derived trophic factor (BDNF) that is essential for the survivability of neurons. In addition, MSCs also have anti-inflammatory properties. Our previous studies have shown that MSCs by themselves (derived from various sources) and/or when over-expressing BDNF reduce the motor and cognitive defects in HD mice and rats. Moreover, these MSCs also slow down the rate of neurodegeneration because BDNF helps spare vulnerable neurons from dying or becoming completely dysfunctional. We also showed that the therapeutic effects of BM-MSCs depend on numbers of passages prior to transplantation: higher-passaged MSCs (P40 to P50), releasing BDNF, delayed the onset of deficits in HD mice. However, one of the major pitfalls is how the cells are labeled prior to transplantation. One of the current labeling method involves using Hoechst stain, which is known to have adverse effects, such as toxicity and leakiness, causing labeling of the host cells of the brain. To overcome the adverse effects, we developed a new strategy to label the MSCs using fluorescently tagged G4 PAMAM dendrimer nanomolecules with cystamine core (S=S), which are less toxic to cells, and can safely label the MSCs in vitro prior to transplantation. Following their uptake by the cells, we showed that the BM-MSCs retained their stemness property in vitro. Following transplantations of dendrimer labeled MSCs into healthy C57BL/6J mice, we tracked the MSCs in the brain using IVIS. Previous studies have shown that cystamine and cysteamine drugs increases BDNF levels in HD brain. Therefore, it is important to study BDNF levels with our dendrimers. In the present study, our results showed that the dendrimers by themselves increased the amount of BDNF released by the MSCs compared to control cells. Therefore, our results indicate that our dendrimers: (1) could serve as an alternate method to label and track the best suitable MSCs for treating HD; (2) help increase the endogenous BDNF levels; and (3) could be used as a potential therapy for HD following transplantations into the HD brain.

Funding Source
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Keywords: Mesenchymal stem cells; Dendrimers; Huntington's disease

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ELECTROPHYSIOLOGICAL PHENOTYPE CHARACTERIZATION OF MULTIPLE HUMAN IPSC-DERIVED NEURON LINES BY MEANS OF HIGH-RESOLUTION MICROELECTRODE ARRAYS

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High-resolution-microelectrode-array (MEA) technology enables to study neuronal dynamics at different scales, ranging from axonal physiology to network connectivity. We have used this MEA technology (26'400 electrodes at 17.5 µm pitch) to characterize and compare the electrical phenotypes of 6 commercially available human neurons (dopaminergic neurons, dopaminergic neurons A53T α-synuclein, glutamatergic neurons, motor neurons, motor neurons SOD1-G93A, motor neurons TDP43-Q331K, Fujifilm Cellular Dynamics International). All neuronal lines were co-cultured with human astrocytes (Fujifilm Cellular Dynamics International). Neurons' electrical activity was recorded at DIV7, DIV14 and DIV21. Al neuronal lines showed robust synchronized/oscillating busting activity at DIV 14 and 21. We extracted the following metrics, in order to characterize neuron's electrical activity: Mean Firing Rate (MFR, Hz), Mean Spike Amplitude (MSA, µV), Percentage of Active Electrodes (PAE), Burst Peak Amplitude (BP, Hz) and Interburst Interval (IBI, s). Using high-resolution MEA technology, we measured the following median coefficient of variation (CV) for the each metric, across developmental points: MFR = 0.2, MSA = 0.10, PAE = 0.24, BP = 0.27 and IBI = 0.13. Furthermore, we quantified MEA metric CVs for low-resolution MEAs recordings (16 electrodes at a pitch of 300 µm pitch): MFR = 0.64, MSA = 0.28, PAE = 0.5, BP = 0.42 and IBI = 0.18. Therefore, high-resolution MEA technology enables to phenotype the electrical activity of multiple neuron lines, with lower sample-to-sample variability as compared to state-of-the art low-resolution MEA technology. Furthermore, we isolated single-neuron electrical activity and quantified (1) network functional connectivity and (2) subcellular electrical features (e.g. axon propagation velocity along axons). We found differences between wildtype and disease lines in network connectivity between the different lines tested, and most importantly, between wild-type lines and their isogenic disease model counterpart. High-resolution MEA systems enable to access novel electrophysiological parameters of iPSC-derived neurons, which can be potentially used as biomarkers for phenotype screening and drug testing.

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Keywords: h-iPSC-derived neurons; Neurodegenerative Disease Modeling; High-Resolution Microelectrode Arrays

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GENERATION OF MICROGLIA FROM HUMAN PLURIPOTENT STEM CELLS FOR NEURODEGENERATIVE DISEASE MODELING

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Microglia are critical modulators of neurodegenerative disease. As microglia are of mesodermal origin, current human pluripotent stem cells (hPSC)-derived neuroectodermal differentiation models do not give rise to this critical cell type. We attempted to standardize microglia differentiation by developing microglia differentiation and maturation media, which efficiently differentiate hPSC into functional microglia based on the publications of Abud et al. (2017) and McQuade et al. (2018). Human pluripotent stem cells maintained in mTeSR™1 were differentiated into CD43-expressing hematopoietic progenitors using the STEMdiff™ Hematopoietic Kit for 12 days. The resulting hematopoietic cells were maintained using the microglia differentiation media and supplements for 25 days and followed by the maturation media and supplements for 4-10 days. At the end of maturation stage, the cells were characterized by expression of CD11b and CD45 through flow cytometry, and for microglia-specific gene and protein expression by qPCR. Functional characterization by phagocytosis assays were performed with pHrodo™ Red Zymosan Bioparticles™. Finally, we tested several microglia densities in co-culture with cerebral organoids produced using the STEMdiff™ Cerebral Organoid Kit. Flow cytometric analysis reveals that 96.3% ± 0.6% of the resulting population co-express CD11b and CD45 (mean ± SEM, n=7; 3 ESC and 4 iPSC lines). They also express the microglia-specific markers

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TMEM119, P2RY12, TREM2, and IBA1- by qPCR. The microglia are functional, with the ability to phagocytose pHrodo™ Red Zymosan Bioparticles™ beads. Adding between 250-500,000 microglia was optimal for integration into 40 day old cerebral organoids. These data demonstrate robust differentiation of functional microglia across multiple cell lines using the optimized microglia culture system. Using these microglia in co-culture with cerebral organoids will provide a useful tool for modeling neuroinflammation in disease.
Keywords: Microglia; Stemcell; Differentiation

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CELL FUSION: DEVELOPING A NEW WAY TO TREAT DISEASE WITH CELLS

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The formation of polyploid cells via cell fusion is an essential process that occurs in several aspects of mammalian life including fertilization, placenta formation, and tissue development. The tumorigenicity and immunogenicity of pluripotent stem cells are major barriers that impede their clinical application in cell-based therapies. We have developed a culture platform that allows us to fuse cells engineered to overcome these barriers into new therapeutic cells. The cell fusion platform uses a designer mouse embryonic stem cell (mESC) line, termed Cdk1_Bridge, previously engineered such that expression of Cdk1, and cell survival are absolutely dependent on doxycycline. We hypothesized that wildtype mESCs are capable of fusing with Cdk1_ Bridge mESCs and that resulting cells will express wildtype Cdk1 and therefore, will survive and proliferate independent of doxycycline. Fused cells were selected for by adding puromycin and removing doxycycline from culture media. Mixing of both mESC lines followed by selection resulted in independent fusion events that effectively lead to tetraploid cell colonies. Fused mESCs express pluripotency markers at levels similar to diploid mESCs and differentiate into all three germ layers in vivo. Future directions are focused on studying the inheritance of genetic modifications and parental properties in fused cells. Cell fusion could be used to generate new tetraploid cells with therapeutic value.
Funding Source
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Keywords: Cell fusion; Cell therapy; Immune evasion

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SUSTAINED DELIVERY OF THERMO-STABILIZED CHONDROITINASE ABC AS A THERAPEUTIC STRATEGY AFTER STROKE

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Central nervous system (CNS) injuries, such as stroke, are characterized by the formation of a proteoglycan-rich glial scar, which limits the regenerative capacity of the CNS. Stem cell transplantation has been proposed to replace lost neurons and promote functional repair. Yet, their survival is reported to be poor, limited by the inflammatory microenvironment and loss of brain tissue plasticity. The degradation of the glial scar may provide a means to improve local plasticity and promote transplanted cell survival. Chondroitinase ABC (ChABC) is a bacterial lyase, able to degrade chondroitin sulfate proteoglycans in the glial scar and facilitate CNS repair. ChABC has generated great interest as a therapeutic protein following CNS injuries, yet its inherent instability, characterized by rapid protein aggregation and subsequent loss of activity (typically within several hours at physiological temperature), limits its use. Nonetheless, prolonged, local delivery is needed to obtain a significant improvement. Here we report the sustained delivery of a thermo-stabilized ChABC to serve as a therapeutic strategy. We have previously reported an affinity-controlled delivery strategy for ChABC in which the enzyme was expressed as a fusion protein with a Src homology 3 (SH3) domain and encapsulated in an injectable cross-linked methylcellulose hydrogel containing SH3 binding peptides. Here, redesign of the enzyme by introduction of a single point mutation (asparagine to glycine at position 1000), and modification of the protein with the attachment of 20 kDa polyethylene glycol (PEG) chains, resulted in a thermo-stabilized mutant ChABC, which demonstrated higher melting temperature, increased half-life at 37°C and increased activity for the substrate chondroitin sulfate A. The mutation did not alter affinity-based release of ChABC from the hydrogel. Using an endothelin-1-induced stroke injury model in rats, SH3-ChABC was delivered directly to the

brain cortex using a minimally invasive, sustained release strategy. Importantly, the stabilized ChABC reduced proteoglycans levels in the stroke penumbra up to 28 days post-injury, demonstrating prolonged in vivo bioactivity. Ongoing studies are examining the efficacy of ChABC delivery after stroke injury in rats.

Funding Source
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Keywords: Chondroitinase ABC; Stroke; Sustained delivery

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PLURIPOTENT CHARACTERISTICS OF HUMAN MESENCHYMAL STROMAL CELLS FROM PERINATAL MEMBRANES: EPIGENETIC, PHENOTYPIC AND DIFFERENTIATIVE ANALYSIS

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Human mesenchymal stromal cells isolated from amniochorionic membrane (hFM-MSCs) are not tumorigenic and have a low immunogenicity thus representing potential candidates for regenerative medicine; nevertheless, their real position in the stemness hierarchy and their differentiative potential are still unclear. The aim of this study was to characterize the stemness of hFM-MSCs by outlining their epigenetic, genetic and biological characteristics in comparison with human induced pluripotent stem cells (hiPSCs) The methylation status and the protein expression of pluripotency transcription factors (NANOG, OCT4, SOX2) and c-KIT were analyzed by pyrosequencing and flow cytometry analyses. Moreover, to clarify their stemness properties, the differentiation potential toward the ectodermal lineage (cholinergic and dopaminergic neurons) was evaluated. The methylation profile for the pluripotency markers differed between hFM-MSCs and hiPSCs for SOX2 (33.7±13.3 vs 0.5±0.7, $p<.01$) and c-KIT (20.2±3.4 vs 4.5±2.1, $p<.01$), but was comparable for NANOG and OCT4. The protein expression reflected this methylation pattern, since the cytometric analysis evidenced a similar % of the Nanog+ cells, whereas Sox2+, c-Kit+ and Oct4+ cells were significantly less represented in hFM-MSCs than in hiPSCs (29.0±1.4 vs 42.5± 3,5; 0.4±0.5 vs 13.7±0.4; 18.3±5.7 vs 78.5±10.6 %, respectively). We then verified whether hFM-MSCs could be induced to overcome their mesenchymal fate and differentiate into neurons in vitro. hFM-MSCs underwent two different differentiation protocols for cholinergic and dopaminergic neuronal cells. After the treatment, up to 70% of the cells expressed markers of cholinergic (Neurofilament L, HB9 and acetylcholine transporter) or dopaminergic (Tyrosine hydroxylase, LMX1b and dopamine transporter) neurons and were able to respond to KCl stimulation with an intracellular calcium increase. In conclusion, although originating from extraembryonic mesoderm, the hFM-MSCs display some epigenetic traits of hiPSCs, express pluripotency markers and are not restricted to mesenchymal fates

Keywords: mesenchymal stromal cells; Epigenetic analisys; Neuronal differentiation

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RECOMBINANT HUMAN GROWTH FACTORS AS AN EFFECTIVE ALTERNATIVE TO PLATELET-RICH PLASMA (PRP) FOR TISSUE REPAIR

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Platelet Rich Plasma (PRP) is a blood cell derived product obtained by centrifugation, which contains a high concentration of growth factors (GFs) in a small volume of plasma: Platelet-derived Growth Factors (PDGF), Transforming Growth Factors (TGF-β 1, 3), Fibroblast Growth Factors (FGF), Vascular Endothelial Growth Factors (VEGF), Bone Morphogenetic Proteins (BMPs) and others. By applying PRP at the site of injury, a robust healing response is achieved. It is important to maintain adequate levels of GFs in all the different stages of tissue repair. The preparation of PRP is a non-standard procedure and variable results are being observed in the literature, due to the amount of growth factors and/or methods of preparation. Besides that, usually more than one PRP application is required involving a new blood collection for each application in order to accelerate tissue regeneration. The early preparation of PRP with possibility of storage for use in future applications during treatment could be an important solution to avoid the discomfort of patients; however, the problem of the absence of a gold protocol stays. Recombinant GFs were produced in a safe and reproductive heterologous system using mammalian cells

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(293, CHO) to obtain overproducing cell clones, to guarantee their quality and post-translational modifications, which are important for their production or stability. The GFs were purified using heparin affinity chromatography and tested using a specific in vitro biological activity assays for each GF. The rhPDGF-BB, rhVEGF, rhTGF-ß application and human PRP were tested in a model of dorsal wound healing in rats or mice (normal and diabetic animals induced with streptozotocin). rhBMPs (BMP2, BMP4, BMP7) were applied subcutaneously to induce ectopic bone formation in nude rats. The results showed comparative efficacies in the treatments of wounds with rhPDGF-BB, rhVEGF, rhTGF-ß in comparison with PRP application, without significant differences, indicating that the use of recombinant GFs are so effective as the application of PRP for the tissue regeneration process, as observed in the new tissue with collagen deposition and granulation tissue formation. This work shows the possibility of substitute the PRP application using a safe, effective and reproductive source of GFs using a mammalian cell platform.

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Keywords: Wounds Healing; Bone Morphogenetic Protein; Platelet-Derived Growth Factor

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TCD4+ ACTIVATION PROFILE IN SICKLE CELL DISEASE PATIENTS WITH OSTEONECROSIS AND HMSC MEDIATED IMMUNOSUPRESSION IN VITRO

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Sickle cell disease (SCD) has characteristic painful vaso-occlusive episodes, which triggers inflammatory processes. Osteonecrosis (ON) is a chronic severe complication in sickle cell disease. Mesenchymal stromal cells (MSC) therapy has been the best alternative in treating initial stage ON due to their regenerative properties. Cytokine and T helper cells (TCD4+) play an important role in ON pathophysiology. Here we assessed whether SCD/ON and nSCD/ON patients bone marrow derived MSCs suppress TCD4+ cells proliferation and their supernatant cytokine profile in co-culture. Prior to co-culture experiments we assessed the TCD4+ intracellular cytokine profile, which showed statistical significance when comparing Th1, Th2 and Th17 profile of nSCD to SCD/ON when activated or not. MSC immunopotential towards activated (anti-CD3/CD28 beads) and not activated TCD4+ cells showed suppression values did not vary between nSCD/ON and SCD/ON MSC, meaning SCD/ON MSC display intact function and imunosupression potential. Co-culture supernatant showed statistical significance in IL-2 ($p<0,02$), IL-4 ($p<0,02$), IL-10 ($p<0.0001$), IFN-γ ($p<0,02$) and IL-17A ($p<0,04$) secretion comparing nSCD and SCD/ON PBMC. These results show that an acute expression of pro-inflammatory cytokines and a strong polarized behavior of TCD4+ cells may display an important role in SCD osteonecrosis pathophysiology and MSC might be a viable suppressor for the resolution of this inflammatory profile as seen in co-culture supernatant.

Funding Source
FAPESB, CAPES, CNPq
Keywords: T helper cells; Human mesenchymal stromal cells; Osteonecrosis

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TREATMENT OF DIABETIC ULCERS WITH ACTIVATED ADULT STEM/PRECURSOR CELLS

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Non-healing wounds affect more than 6 million people in the US and result in an estimated cost of 25 billion dollars annually. Approximately 84,000 Americans with diabetes will have a limb amputated due to non-healing wounds per year. Current wound care has had limited success and is particularly expensive for the treatment of the non-healing wounds in diabetic patients or patients subject to venous stasis ulcers. Stem cells play an essential role in tissue renewal and skin regeneration after injury. Studies show that repair of skin wounds depends on the activation of a pool of epidermal stem/precursor cells defined with markers such as CD34, LGR5, residing in the basal keratinocyte layer and hair follicle (HF) bulge. They retain homeostasis of the local skin and play an essential role in wound healing. Some studies observed that bone marrow-derived CD34+ cells improve blood flow and wound healing after injection into the ischemic limbs of diabetic mice. However, it is still unclear whether non-epidermal stem cells can be used for treatment of chronic skin wounds. We have discovered a unique population of CD34+ precursor cells (BDPCs) from peripheral blood of adult mice without mobilization or stressful stimulation. These BDPCs are distinct from any known stem cells in terms of size, cellular components, surface marker profile, and natural state in mouse blood. We have established methodologies to expand the BDPCs in vitro and further successfully induced their trans-differentiation into epithelial-like cells (eBDPCs). eBDPCs showed a superior therapeutic effect in the treatment of full thickness skin wound in diabetic mice. The closure of the wounds treated with eBDPCs occurred on day 16 compared with saline treated control wounds ($p<0.001$). The advantages of eBDPCs, such as easy access, rapid expansion and tissue specific differentiation hold great potential for eBDPCs to serve as novel and effective cell-based therapies for diabetic wound ulcers.

Keywords: diabetic ulcers; stem cell therapy; CD34 positive cells

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THE GENERATION OF PHENOTYPE-STABLE TYPE II ALVEOLAR EPITHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The alveolar epithelium is primarily comprised of two types of cells: type I alveolar epithelial cells (AECI), which are responsible for gas exchange; and type II alveolar epithelial cells (AECII), which secrete surfactant and are considered alveolar stem cells. AECIIs hence are required for lung regeneration. The isolation of primary AECIIs for cell therapy is complicated with technical challenges including slow turnover and inability to maintain AECII phenotype ex vivo. AECIIs have been differentiated from induced pluripotent stem cells (iPSCs) by using specific morphogens. However, several technical gaps in methodology have yet to be resolved including: 1) generation of a pure AECII culture; 2) amplification of differentiated cells in long-term feeder-free cultures; 3) storage of cells for effective recovery; and 4) generation of functional AECIIs. We hereby sought to develop an AECII differentiation methodology to address these limitations. Two previously published differentiation protocols from the Snoeck laboratory at Columbia University were combined to generate a new adapted method that maximizes AECII production. iPSCs were cultured and differentiated according to this new protocol. At the end of differentiation, cultures were further purified for the AECII phenotype with magnetic-assisted cell sorting (MACS) for EpCAM expression. iPSCs were successfully differentiated into AECII, which was confirmed by expression of Pro-Surfactant Protein C and EpCAM. In addition to phenotypic validation, we detected phosphatidylcholine in the culture media, suggesting functional secretion of surfactant from the generated AECII. MACS enabled generation of highly homogenous AECII cultures, which were maintained long term for 180 days. Taken together, our results point to the feasibility of differentiating AECIIs from iPSCs for cell therapy.

Funding Source
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Keywords: iPSCs; AECII; cell therapy

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IL-10 IN HUMAN UMBILICAL CORD MESENCHYMAL STROMAL/STEM CELLS IMPROVES THE CELL EFFICACY IN PNEUMONIA ANIMAL MODEL

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Mesenchymal Stromal/Stem Cells (MSCs) demonstrate therapeutic potential for acute respiratory distress syndrome (ARDS) in preclinical models, and are in early phase clinical testing. ARDS is characterized by dysregulated inflammation and excessive release of pro-inflammatory cytokines. IL-10 plays a key role in the regulation of the host inflammatory response and treatment with MSCs carrying IL-10 improved acid-primed lung injury in mice. We hypothesized that overexpressing IL-10 in human umbilical cord (UC)-MSCs would further increase their efficacy in a rat ARDS model. IL-10 overexpression in UC-MSCs was established through adenoviral transduction. ARDS was induced by intra-tracheal E. coli instillation (8x10^9 CFU/kg), and regular or IL-10 UC-MSCs (obtained from Tissue Regeneration Therapeutics, Toronto) were given to the rats by tail vein 1h after ARDS induction (10 mill/kg) while control group received vehicle. Survival, pulmonary function and morphology, inflammation and bacterial clearance were assessed 48h later. The effect of MSCs on macrophage (Mϕ) phagocytosis was tested in vitro using FITC labeled Zymosan. IL-10 UC-MSCs were more effective than regular UC-MSCs in reducing lung bacterial burden in ARDS rats. Lungs were less injured and alveolar airspace increased in rats treated with IL-10 compared to regular UC-MSCs. Lung inflammatory cell infiltration was lower in both IL-10 and regular UC-MSCs treated groups compared with vehicle treated animals. However, IL-10 UC-MSCs decreased percentage of neutrophils while macrophage differential count was increased in IL-10 UC-MSCs-treated group only (30.4+/-8.3%) compared to both vehicle (20.1+/-10.7%) and regular UC-MSCs-treated group (24.3+/-10 %). Our in vitro data have shown that both regular and IL-10 UC-MSCs increase ability of Mϕ to phagocytose while phagocytic index is further increased by IL-10 UC-MSCs. IL-10 overexpressing UC-MSCs were more effective than naïve UC-MSCs in our rodent E. coli pneumonia model. This enhanced effect of IL-10 UC-MSCs may be mediated via further improvement in macrophage function. Therapeutic use of these cells might be beneficial for subset of ARDS patients, especially for ARDS caused by bacterial and viral pneumonia.

Funding Source
Canadian Institutes for Health Research (CIHR) and by the Keenan Research Centre for Biomedical Sciences at St. Michael's Hospital.
Keywords: Mesenchymal Stromal/Stem Cells (MSCs); Interleukin 10 (IL-10); acute respiratory distress syndrome (ARDS)

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GENERATING PVALB-FAST SPIKING INTERNEURONS FROM HUMAN STEM CELLS

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GABAergic interneurons are the primary source of inhibition in the cerebral cortex and play key roles in nearly every aspect of brain function by balancing and synchronizing excitation in cortical circuits. Interneurons comprise ~20% of cortical neurons and are divided into subgroups and subtypes based on their neurochemical markers, connectivity and physiological properties. Abnormal development and function of cortical interneuron subgroups has been implicated in the pathobiology of major neuropsychiatric disorders, including schizophrenia, autism, and epilepsy. Advances in the derivation of cortical interneurons (CIns) from human pluripotent stem cells (hPSCs) demonstrate their utility in studies of neuronal developmental genetics, function, and disease. Unfortunately, there has been limited success at generating highly enriched preparations of parvalbumin-expressing fast-spiking (PV-FS) subgroup from hPSCs, even though this is the most plentiful subgroup of cortical interneuron and their dysfunction is frequently associated with neuropsychiatric disease. In mice, the PV-FS CIns are specified in the medial ganglionic eminence (MGE) at lower levels of Shh signaling than somatostatin (Sst) interneurons. In addition, in vivo fate mapping and other studies suggest that the PV-FS CIns derive from divisions in the subventricular zone of the MGE, whereas the Sst CIns derive from ventricular zone divisions. These results have led to the generation of effective protocols for the enrichment of PV-FS CIns from mouse embryonic stem cells. Our goal is to "humanize" these protocols, using a hESC line modified to express fluorescent citrine when MGE-derived CIns become postmitotic (Lhx6-Citrine). The initial goal is to modify our previous CIn protocol to generate more MGE SVZ-like divisions, defined by expression of Nkx2.1 and CCND2 (cyclin D2) in both dissociated

cultures and neurospheres. Based on our previous work in mESCs and based on that of Freda Miller's lab in mice, SVZ divisions can be stimulated in neural progenitors by inhibition of protein kinase c zeta. Generating PV-FS interneurons from human PSCs and applying them to both in vitro and in vivo assays will ultimately lay crucial groundwork for their use in the study of disease related processes.
Keywords: interneurons; schizophrenia; autism

709

SPATIALLY CONTROLLED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Spatial determination of cell fate during early development is critical in organogenesis. The patterned specification of different cell types occurs not only during early development but also during later stages of development to form complex tissue structures. Recapitulating this spatial patterning in human pluripotent stem cells might provide a more physiologically relevant stem cell niche to produce high-quality stem cell-derived cell types for translational research. Since the regional cell fate determinations are induced by elaborate signals from the cellular microenvironment in vivo, mimicking these patterning in an in vitro system has been challenging, especially due to difficulties in localizing signals, mostly morphogens, in a specific region of the in vitro culture system. Most of the previous studies to spatially control cell fate are based on specially designed apparatus such as microfluidic devices, bioprinters, or engineered substrates. In this study, however, we show that the macroscopic localized differentiation of human embryonic stem cells (hESCs) into mesoderm and endoderm can be achieved by simply placing protein-containing solutions on a part of a tissue culture plate before cell seeding. Possibly due to the hydrophobic interaction between proteins and the substrate, morphogens such as BMP4, Activin A, and Wnt3a remained deposited after the washing step and induced patterned differentiation. Circle-, star-, and heart-shaped mesoderm and endoderm were differentiated from hESCs by deposited BMP4 and Activin A, respectively. The mesodermal cells further differentiated into cardiomyocytes with the retained patterns. Furthermore, localized differentiation of epicardial cells from hESC-derived cardiac progenitor cells was induced by deposited Wnt3a and BMP4. This simple strategy to achieve patterned stem cell differentiation will provide a platform to study intercellular communications as well as signaling cues during development in a spatial context. Moreover, this study will contribute to constructing spatially ordered tissue structures in an in vitro system for various therapeutic purposes.
Funding Source
This material is based upon work supported by the National Science Foundation under Grant No. EEC-1648035
Keywords: Human pluripotent stem cells; Spatially-controlled differentiation; Cardiomyocytes and epicardial cells

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DEVELOPING NOVEL TREATMENTS FOR SETBP1-MEDIATED NEURODEVELOPMENTAL DISORDERS

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Missense mutations in a 4-aminoacid hotspot found in SETBP1 (SET binding protein 1) cause Schinzel-Giedion syndrome, an autosomal dominant gain-of-function (GOF) disease characterized by severe mental retardation, bone and renal abnormalities and neurological degeneration. Conversely, loss-of-function (LOF) mutations cause a disease characterized by mild mental retardation, autism and expressive language impairment. We made forebrain neurons from 2 patients with SETBP1 GOF and 2 patients with SETBP1 LOF and their sex-matched controls through the use of induced pluripotent stem cells. We have also generated isogenic heterozygous KOs of SETBP1 and isogenic mutants by introducing a GOF mutation into control cells. We demonstrate the mechanism of disease for both pathologies is equal and opposite, whereby the expression of SETBP1 in GOF cells is elevated and in LOF cells is decreased. We have developed a series of Antisense Oligonucleotides (ASOs) which we can conclusively demonstrate reduces SETBP1 in GOF patients and we demonstrate small molecules which can affect critical downstream targets of SETBP1. We demonstrate that SETBP1 downregulation can be modulated in a dose-dependent manner and that a single treatment with ASOs can exert its effect for up to four days. These data are the first models of SETBP1 neurodevelopmental syndromes and provide realistic therapeutic avenues for their treatment.
Funding Source
Funding provided by the National Council of Science and Technology of Mexico.
Keywords: Neurodevelopment; Treatment; Antisense oligonucleotides

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RECOMBINANT HUMAN PEPTIDE GROWTH FACTORS (RHPDGF-BB AND RHVEGF165) AND / OR MESENCHYMAL STEM CELL DERIVED EXOSOMES APPLIED IN A NUDE ANIMAL HEALING MODEL FOR TISSUE REPAIR

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The regenerative medicine aims treatments to accelerate the different phases of tissue repair: blood clotting and inflammation, cell proliferation, tissue remodeling. By manipulating the growth factors (GF) composition, it is possible to accelerate or modify the process of regeneration and remodeling of damaged tissues. In this way, the application of Mesenchymal Stem Cells (MSCs) or their microvesicles/exosomes could contribute to the correct repair in a timely manner. This study aimed to establish a model of dorsal wound healing model in nude rat to test the in vivo biological activity of different molecules such as platelet-derived recombinant human peptide growth factor BB (rhPDGF-BB) and vascular endothelial growth factor 165 (rhVEGF165) produced in mammalian cells, and adipose mesenchymal stem cells (AD-MSCs) whether or not treated with these factors and their derived exosomes to evaluate their therapeutic effect on tissue repair. AD-MSCs were isolated and characterized, cultured and the conditioned media were collected under conditions without BFS for isolation of exosomes by concentration and ultracentrifugation. Samples were quantified resulting in higher yield in exosomes of cells treated for 24 hours using 10ng/mL of each GF or their combination. Animal healing model was established using 3µg of rhPDGF-BB and/or rhVEGF165 applied into each wound on dorsal region from nude rats. The GF were obtained from purified media collected from mammalian cell clones using affinity chromatography with heparin, quantified by ELISA, with in vitro biological activity tested by incorporation of H3-thymidine into DNA. The results showed comparable efficiencies for both treatments, with an accelerated healing rate compared to untreated injury or vehicle-treated injury alone. This work is expected to contribute to an improved protocol for tissue healing using exosomes from growth factor-treated MSCs or in the future to use combinations of these exosomes and growth factors for cell-free therapy. We aiming to develop and offer an improved and alternative therapeutic approach to wound healing for patients and animals with complex chronic ulcers and other skin lesions that have been treated are difficult to treat and/or associated with high treatment costs.

Funding Source
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Keywords: wound healing; mesenchymal stem cells; exosome

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THE ROLE OF RNA EXOSOME IN PLURIPOTENCY

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The role of different RNA binding proteins in cell fate transitions is not clear. Here we study the part of the RNA degradation component, EXOSC2 in pluripotency. We have previously shown that EXOSC2 is targeted and is upregulated by the pluripotency factor ZSCAN10 during reprogramming. We have also shown that one of the functions of exosome in ESC cells is to regulate the redox levels by regulating the levels of GPX2 mRNA, an enzyme involved in glutathione metabolism. In this study, we downregulate EXOSC2 in embryonic stem cells, and we unravel a connection between EXOSC2 regulation and cell plasticity. Our data show that downregulation of EXOSC2 in ESC blocks differentiation without affecting the self-renewal capacity of mouse ESC cells. Our data also reveal novel targets of cytoplasmic RNA exosome function. The importance of characterizing the function of RNA exosome in pluripotency is critical for basic stem biology, regenerative medicine, and cancer.

Keywords: RNA exosome; embryonic stem cells; pluripotency

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MAGNETIC IRON OXIDE NANOPARTICLE LABELING OF PHOTORECEPTOR PRECURSORS FOR MAGNETIC RESONANCE IMAGING

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In vivo cell tracking is a powerful tool for the optimization and monitoring of cell therapy. Magnetic resonance imaging (MRI) can be used to visualize transplanted cells labeled with superparamagnetic iron oxide nanoparticles (SPIONs) also the surrounding tissues. However, the applicability of these techniques in vivo in the retina has not been investigated. The goal of this study was to evaluate the feasibility of SPION labeling and MRI tracking of photoreceptor precursors transplanted into the subretinal space. Photoreceptor precursors derived from human embryonic stem cells were labeled with SPIONs using the FeraTrack MRI contrast agent kit (Miltenyi Biotec Inc., Auburn, CA, USA). The proliferation, viability, and differentiation capacity of SPION-labeled photoreceptor precursors were assessed in vitro and were found to be unaffected by SPION labeling. Royal College of Surgeons rats were classified into four experimental groups as follows: those injected with culture medium, unlabeled photoreceptor precursors, SPION-containing medium, and SPION-labeled photoreceptor precursors. All rats underwent subretinal injection by a transscleral approach and were examined by 9.4T MRI with T2*-weighted sequences (T2*WI) from 1 day to 12 weeks after transplantation. Hypointense signals corresponding to the transplanted SPION-labeled photoreceptor precursors and SPION-containing medium were clearly visible at the injection site at 1 day after transplantation. In contrast, no hypointense signal was observed in rats injected with culture medium or unlabeled photoreceptor precursors. The hypointense signal of the SPION-labeled photoreceptor precursors decreased but remained visible over the entire follow-up period. At 12 weeks after transplantation, histological analysis showed that transplanted SPION-labeled photoreceptor precursors were viable, and their distribution corresponded to the hypointense signal observed on T2*WI. However, the hypointense signal of the SPION-containing medium markedly decreased over time until it was undetectable at 12 weeks after transplantation. This study demonstrated that the SPION labeling and MRI tracking of photoreceptor precursors transplanted into the subretinal space is feasible and can be utilized in cell therapy for degenerative retinal diseases.

Funding Source
This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea (grant number: NRF-2015M3A9B4067004) and the Korea Health Technology R&D project (grant number: HI14C1277).
Keywords: superparamagnetic iron oxide nanoparticles; magnetic resonance imaging; degenerative retinal diseases

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ENSURING "REASONABLE ACCOMMODATION" REGARDING THE INFORMED CONSENT PROCESS WITH VISUALLY IMPAIRED PATIENTS

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Ophthalmology is one of the leading medical fields that is conducting clinical stem cell trials, particularly trials using pluripotent stem cells. Given the complex scientific information provided to participants, the informed consent (IC) process must be carefully considered in order to protect participants' rights. Especially for someone who is the patient with visually impaired, it is necessary to ensure "reasonable accommodation," which refers "to necessary and appropriate modifications and adjustments, which do not impose a disproportionate or undue burden that ensure the enjoyment or exercise of all human rights and fundamental freedoms by individuals with disabilities on an equal basis with others," as defined in Article 2 of the United Nations' Convention on the Rights of Persons with Disabilities (2006). There are several approaches to providing information without visual contents, such as using a text-to-speech interface. However, comprehending scientific and complex information may require considerable concentration on the part of the patient to understand via audio alone. Therefore, we need to know the process of how such information is understood by patients with a visual impairment. In this poster, we will discuss the appropriate manner in which to provide information to visually impaired patients using an example of audio material that we created. We conducted a literature survey and gathered knowledge about information support from a support center for the visually impaired. We then created audio material to support the IC process using an actual IC form from a stem cell clinical trial for corneal disease. We discussed the audio material with a person with a visual

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impairment to determine whether the material was useful in improving their understanding of the IC and revised the material based on their response. We will present our findings on the use of audio support materials for visually impaired patients and offer recommendations on providing information to prospective trial participants.

Funding Source
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Keywords: Information support for visually impaired; Informed consent; Research Ethics

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ESTABLISHMENT OF A XENO-FREE CULTURE OF RETINAL PIGMENT EPITHELIAL (RPE) CELLS TO PRODUCE PERSONALIZED IPS-DERIVED AND GENETICALLY MODIFIED RPE CELLS TO TREAT AGE-RELATED MACULAR DEGENERATION (AMD)

Kropp, Martina, Conti, Alain, Harmening, Nina, Bascuas, Thais, Thumann, Gabriele, Verlooven, Robin

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Treatment available for neovascular AMD consists of frequent, costly intravitreal injections of anti-angiogenic agents; there is no treatment for avascular AMD, though, AMD is the 1st cause of blindness in high income countries. Since AMD results in retinal cell death, replacement of degenerated cells may be a promising treatment; however, replacement by autologous cells is not optimal because cells may carry the original defect of the disease or may be harmed by pathologic conditions in the retina. We suggest replacement by iPS-derived RPE cells genetically modified to produce elevated levels of the neuroprotective factors pigment epithelium-derived factor (PEDF) and granulocyte macrophage-colony forming factor (GM-CSF) to reconstitute the retinal milieu and protect both remaining retinal cells and transplanted RPE cells. Transplanted subretinally, iPS-derived transfected RPE cells secrete PEDF and GM-CSF to recover the retinal anti-angiogenic and neuroprotective environment necessary to prevent further RPE and photoreceptor degeneration. Manufacture of such Advanced Therapy Medicinal Products requires GMP-grade production protocols standardized but flexible to manufacture personalized products for each patient. To generate individually optimized products and reduce risks associated with animal products in cell culture, we aim to develop a xeno-free culture using autologous platelet lysate (PL). Human ARPE-19 (n=15) and primary RPE (n=3) cells were supplemented with 0.5-10% pooled, xeno-free PL in DMEM/Ham's 12. ARPE-19 cells in 5% PL reached confluence at 4.2 d vs. 8.2 d for 10% FBS; primary RPE cells grew best with 1-2% PL. Transepithelial electrical resistance and viability are similar for PL- (0.5-10%) and FBS-cultured ARPE-19 cells: 23.6±3.7 Ω and 24.4±7.8 Ω at 14 d; 78.3±12.1% and 75.1±18.3% viable cells. RPE65 and Na+/K+ ATPase staining, PEDF secretion (1%: 422.±132.4 ng/ml; 5%: 892.2±283.7 ng/ml; 10%: 1670±184.8 ng/ml) and significant phagocytosis (ingested latex beads) confirmed cell function. We established a 0.5-10% PL-based xeno-free cell culture system ideal for the development of human iPS-derived RPE cells. Analysis of PL-based cultures from individual donors correlated to demographic data will establish a dosing regimen for the manufacture of personalized cell products.

Keywords: Neuroretinal degeneration; Gene therapy; Xeno-free cell culture

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QUALITY CONTROL AUTOLOGOUS IPS CELL THERAPY PRODUCT: IRPE (RETINAL PIGMENT EPITHELIUM) PATCH

Jha, Shekhar[1], Bharti, Kapil[2], Dejene, Roba[2], Highfill, Steven[1], Hotaling, Nathan[2], Hua, Fang[1], Sarkar, Sarmila[1], Sharma, Ruchi[2], Stroncek, David[1], Thakkar, Aditi[1], Zhang, Connie[2]

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With an unprecedented use of stem cell based therapies, it is necessary to design standards and criteria to confirm quality of the product before it is released for clinical applications. The need for such quality-control and safety standards has become ever more critical as manufacturing of cell therapy products is becoming a routine practice in regenerative medicine field. Here we describe the cGMP compliant manufacturing process of autologous induced pluripotent stem cell derived retinal pigment epithelium (iRPE) patch for the treatment of Age-related macular degeneration; focusing on the process flow, in-process quality checks and release assays. Combining these quality checks with our robust manufacturing process and, we have generated high purity functionally validated cGMP compliant cell therapy product from three patients in our IND-enabling studies for phase I/IIa clinical trial. This manufacturing process will form the basis for our phase I clinical trial to treat AMD patients with an autologous iRPE patch. In addition, it provides a streamlined path for manufacturing of cGMP complaint iPSC based autologous cell therapy products.

Keywords: Quality Control; cGMP; RPE

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COMBINED CELL AND GENE THERAPY TOWARDS THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION AND DIABETIC RETINOPATHY

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Neovascularization, caused by an up-regulation of VEGF, is the common cause for vascular leakage, vessel dilatation, tortuosity, haemorrhage, cell death and ultimately blindness in age-related macular degeneration and diabetic retinopathy. Current anti-VEGF treatments (e.g. Avastin) can only temporarily improve visual function and therefore patients require monthly eye injections. In addition, the anti-VEGF therapies cause side effects such as stroke, gastrointestinal perforations and bleeding. To address these problems, we have generated a novel bi-functional VEGF trap (VEGF Sticky-trap) that is able to trap VEGF as well as bind ("stick") to the extracellular matrix (ECM). This consequently allows inhibition of neovascularization only at the site of administration without causing systemic side effects as we have demonstrated in a retinopathy mouse model. To avoid the invasiveness of current therapies we have generated retinal pigment epithelium cells that express VEGF Sticky-trap in an inducible manner. We have shown that VEGF Sticky-trap expressed by these cells in vitro is able to bind to ECM and trap soluble VEGF only upon doxycycline induction. In addition, we have shown that these cells incorporate into the subretinal space long-term (up to 10 month), express VEGF Sticky-trap that a) remains bound to eye ECM and b) inhibits laser-induced chorodial neovascularization. Further, injected cells prolong the degeneration of the outer nuclear layer in a retinal degeneration mouse model. Here we have shown that the combination of cell and novel anti-VEGF gene therapy can inhibit neovascularization in a controlled and long-term manner; potentially improve vision, while avoiding the risk of side effects.

Funding Source
McEwen Acceleration Award, The Foundation Fighting Blindness, CIHR foundation scheme, Canadian Research Chair and Medicine by Design (University of Toronto)
Keywords: Age-related Macular Degeneration; Diabetic Retinopathy; Cell and Gene Therapy

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A COMBINED CELL AND GENE THERAPY TO TREAT RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is an inherited retinal degenerative disease caused by any single mutation in over 70 identified genes. If left untreated, it can lead to primary rod and secondary cone photoreceptor degeneration, and ultimately blindness. Therapeutic biologics hold great promise in treating patients with RP. However, they exhibit short half-lives which would require frequent injections to the eye. On the other hand, cell replacement has been pursued but integration into the neural retina remains a major problem. Our lab has previously demonstrated the potential for combined cell and gene therapies to treat various diseases, including neovascular retinopathies and arthritis. Additionally, we have developed two platform technologies that mitigate the issues of allogeneic rejection (induced allogeneic cell tolerance, iACT) and teratoma development (Safe-Cell) in cell therapy. To this end, we have engineered novel mouse and human pluripotent stem cell (PSC) lines, exhibiting both iACT and Safe-Cell technologies, to secrete the 4IG domain of neogenin and soluble Cx3cl1 to address the short half-lives of such biologics. These therapeutic proteins have been shown to delay the progression of photoreceptor degeneration in mouse models of RP. To evaluate the therapeutic effectiveness of these 'designer' cells and expressed biologics, we will generate retinal organoids from PSCs derived from mouse models of RP (rd1 and rd10 mouse). Ultimately, upon grafting differentiated 'designer' eye-specific cells into mouse models of RP, we hypothesize that the combination of cell and gene therapy can significantly delay the progression of RP in a controlled and long-term manner. Overall, these therapeutic cells will not only provide greater insight into the pathology of RP, but may also have the potential to treat other neurodegenerative diseases.

Funding Source
CIHR Foundation Grant
Keywords: Retinitis Pigmentosa; Immune Evasion; Cell Therapy

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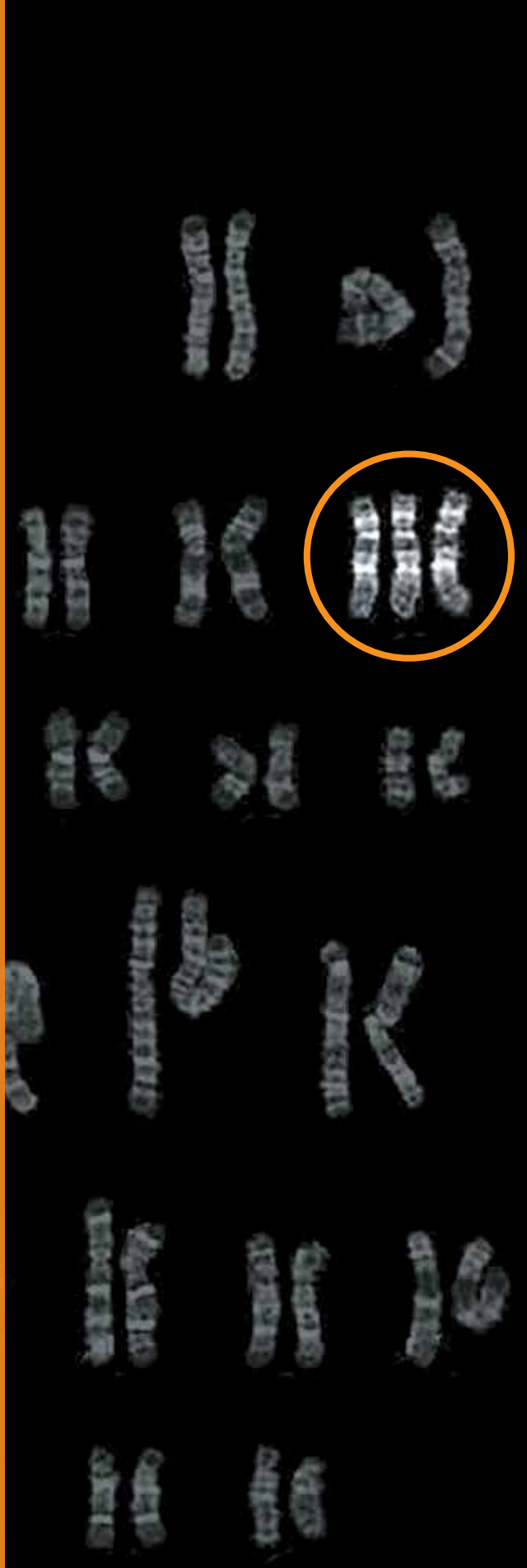

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