POSTER CONTEST & SESSION
Stem Cells: Immune Tolerance and Immunotherapy
14th Annual Wisconsin Stem Cell Symposium
April 17, 2019 – Madison, WI

NOTE: Each submitter's name is in bold and italicized.

POSTER CONTEST FINALISTS

(1) Microfluidics and oncoimmunology: new *in vitro* tools to study solid tumor immunotherapy

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Immunotherapies against solid tumors face daunting challenges compared with hematological cancers. In solid tumors, immune cells and therapeutic antibodies need to extravasate from the vasculature; find the tumor; and migrate through a dense tumor mass of cells where nutrients are depleted, and waste products accumulate. All these factors pose significant obstacles for solid tumor immunotherapy, commonly leading to immune exhaustion and compromising the immune response. Thus, finding effective immunotherapies against solid tumors require *in vitro* models that accurately mimic the solid tumor microenvironment. In this work, we present microfluidic models for solid tumor immunotherapy and show how they mimic the tumor architecture in an unprecedented way compared with other traditional *in vitro* models based on Petri dishes. Breast cancer cells were cultured as a dense mass and embedded in a 3D collagen hydrogel into a microfluidic device. Endothelial cells were cultured in lateral flanking lumens to mimic the blood vessels, allowing the perfusion of therapeutic antibodies or effector immune cells (e.g., natural killer cells). Antibody and immune cell extravasation, diffusion, migration and tumor clearance were evaluated in the model. Natural killer cells were able to detect the presence of the tumor several hundreds of microns away, exhibiting a directional migration towards the tumor. Once inside the organoid, real-time microscopy revealed natural killer cells were able to destroy tumor cells at the tumor periphery and, more importantly, also at the innermost layers. Finally, the combination of antibodies and genetically-engineered natural killer cells led to an enhanced cytotoxicity, showing the potential of the model to evaluate new immunotherapy combinations.

(2) Title- Interferon-γ inhibits neo-adipogenesis from adipose resident Mesenchymal Stromal Cells via SMAD3-TGFβ pathway

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Mesenchymal stromal cells (MSC) are tissue resident multipotent progenitors that differentiate into specific cell types in response to various stimulatory conditions. Adipose tissue resident MSC differentiate into mature adipocytes to store excess nutrients under conditions of prolonged metabolic stimulation. This de novo adipogenesis, rather than adipose hypertrophy where preformed adipocytes increase in size to accommodate nutrient overload, plays positive roles in controlling nutrient homeostasis by promoting nutrient absorption and preventing ectopic lipid deposition. Inflammation under chronic metabolic stress inhibits de novo adipogenesis, primarily by the release of pro-inflammatory factors from adipose depot resident immune-effector cells and adipocytes themselves. Interferon-γ (IFN-γ) is a key mediator of the metabolic inflammation circuit and heavily contributes to metabolic dysfunction. Although the effects of IFN-γ exposure in pre-adipocyte and mature adipocyte has been investigated, its effect on adipose MSC remains largely unknown.

We observed that IFNγ potently inhibits differentiation of primary human visceral adipose MSC (hVA-MSC) as well as bone marrow derived MSC (hBM-MSC) into mature adipocytes. IFN-γ prevents the critical buildup of two key adipogenic transcription factors, namely C/EBPα and PPARγ. Although significant activation of the JAK-STAT pathway was observed in hVA-MSC upon IFN-γ treatment, several JAK/STAT inhibitors completely failed to rescue IFN-γ's effect, indicating that JAK-STAT pathway is not a suitable target for rescuing adipogenesis under metabolically stressful conditions. In search for other molecular targets, we found that IFN-γ significantly upregulates Smad2/3 signaling in MSC by specifically increasing Smad3 phosphorylation as well as total Smad3 protein levels; Smad3 in turn prevents adipogenic differentiation of MSC. Co-treatment with Galunisertib, a small molecule inhibitor of TGFβR1 kinase activity was sufficient to rescue the adipogenesis blockade caused by IFN-γ.

These results point toward potentially beneficial metabolic effects of rescuing IFN-γ blocked adipogenesis by employing Galunisertib, especially in insulin resistant obese individuals, to improve metabolic homeostasis and decrease ectopic fat buildup under chronic metabolic stress.

(3) Human monocytes educated with exosomes from TLR4 primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery

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Total body irradiation is often used as a conditioning regimen for bone marrow transplants but can cause life threatening damage to host tissues especially the bone marrow. Developing a cellular therapy that can protect the bone marrow from acute radiation syndrome and stimulate hematopoiesis is a priority for patients exposed to therapeutic or even accidental radiation

injury. In this study, exosomes derived from MSCs stimulated with the TLR4 agonist lipopolysaccharide (LPS) were used to alternatively activate human monocytes, termed LPS EEMos, as a potential novel radioprotective cellular therapy. LPS EEMos expressed higher levels of PD-L1 ($p<0.0001$), and lower levels of CD16 ($p<0.01$), CD86 ($p<0.01$), and CD206 ($p<0.0001$) by flow cytometry compared to monocytes educated with exosomes from unstimulated MSCs (EEMos). Using qPCR, increased gene expression in LPS EEMos of IL-10 ($p<0.05$), IDO ($p<0.001$), FGF2 ($p<0.05$), IL-15 ($p<0.05$), and IL-6 ($p<0.0001$) were detected compared to EEMos. Using a xenogeneic radiation injury model, infusion of human LPS EEMos 4 hours after lethal radiation led to reduced clinical scores and an increased survival at 40 days postinfusionas compared to infusions of PBS, EEMos, and monocytes alone, all of which led to worse clinical scores and 0% survival with uniform death by 20 days ($p<0.05$). Complete blood cell counts in LPS EEMo recipients showed leukocyte, erythrocyte and platelet counts equivalent to non-irradiated mice, demonstrating complete restoration of hematopoiesis. Infusion of LPS EEMos may be a useful strategy to protect the bone marrow from acute radiation syndrome by expression of anti-inflammatory molecules and cytokines that promote hematopoiesis/engraftment.

(4) Modeling the blood-nerve barrier using human pluripotent stem cell–derived Schwann–like cells and endothelial cells

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The blood-nerve barrier (BNB) is formed by peripheral nerve endoneurial microvessels and maintains the environment necessary for proper neuronal function by regulating ionic, molecular, and cellular transport. BNB dysfunction accompanies peripheral nerve disorders including chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barré syndrome. While functional and molecular features of BNB-forming endothelial cells such as low permeability to small and large molecule tracers, formation of tight junctions, and expression of nutrient and efflux transporters have recently been characterized, little is known about the cellular and molecular mechanisms that regulate the development of these properties. We hypothesized that Schwann cells, glia of the peripheral nervous system that ensheath axons and lie in close proximity to microvessels, could induce BNB properties. Since a renewable source of human Schwann cells is lacking, we differentiated Schwann–like cells from human pluripotent stem cells (hPSCs) through a neural crest intermediate. hPSC–derived Schwann–like cells adopt classic Schwann cell morphology, express glial markers including GFAP and S100β, and express Schwann cell–associated transcription factors (Sox10, EGR2, POU3F1) and myelin proteins (PMP22, MBP, MPZ). Coculture of these Schwann–like cells with hPSC–derived endothelial cells yielded improved endothelial barrier properties including fivefold–increased transendothelial electrical resistance, improved tight junction morphology, and a decreased rate of transcellular dextran transport, suggesting a possible role for Schwann cell–derived factors in BNB development. This *in vitro* model may offer scalability and fidelity benefits over currently employed BNB endothelial cell lines, and may be further employed to study molecular mechanisms of BNB development and disease.

(5) *In vitro* protection of SC-islet cells from NK cell cytotoxicity

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Type 1 diabetes (T1D) results from the autoimmune destruction of the insulin-producing pancreatic beta-cells. Allogeneic stem cell-derived beta cells (SC-beta cells) are an attractive source of replacement beta cells as an "off-the-shelf" therapy. However, immunosuppression is required to permit graft survival in the absence of an encapsulation device. Thus, the genetic engineering of allogeneic SC-beta cells to evade immune destruction is of interest. Natural biological phenomena such as tumor development and immune-tolerance at the fetal-maternal interface are both characterized by reduced classical MHC class I expression, and consequently have developed strategies such as increasing the expression of non-classical MHC to evade NK cell cytotoxicity due to the "missing-self" response. Thus, non-classical MHC class I molecules such as HLA-E, HLA-G and HLA-F are of interest for alloprotection of cell replacement therapies in a classical MHC-deficient context. The aim of this study was to assess the protective effect of the HLA-E long-chain fusion in SC-beta cells in a classical MHC-deficient context. Beta 2 microglobulin (B2M)-deficient human embryonic stem cells (hESCs) were genetically-modified to co-express Firefly luciferase (Luc2) and the HLA-E long-chain fusion at the AAVS1 safe-harbor locus. HLA-E expressing $B2M^{-/-}$ hESCs demonstrated a ~30-fold increase in HLA-E expression at the plasma membrane in comparison to parental controls. Differentiation into SC-beta cells resulted in ~25-40% $C\text{-peptide}^{+}/GLP2^{-}$ (SC-beta cells), however, HLA-E was not detected by FACS in differentiated cells. Stage-specific reporter analysis and bulk RNA-seq showed a relationship between a decrease in luciferase activity and an increase in DNMT1 expression during stage 2 and 3 of differentiation, which was corroborated by increased CAG promoter methylation. Despite this, HLA-E expressing SC-beta cells demonstrated protection against *in vitro* NK cell cytotoxicity, suggesting a low level of HLA-E expression is sufficient to protect against NK cell cytotoxicity.

(6) Engineering 3-D Neural Organoid Morphology using Alginate Hydrogels

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Human pluripotent stem cells (hPSCs) provide unlimited potential for engineering a wide variety of in vitro tissue models to investigate human physiology and disease. Much progress has been achieved in using embryoid body culture approaches to generate 3D mimetics of brain, kidney, liver and prostate tissues, a.k.a. organoids. However, the morphogenesis that occurs within these organoid cultures is uncontrolled and spontaneous, thereby producing tissues with highly variable cytoarchitectures and morphologies. In the body, the brain and spinal cord develop not from a spheroid but from the neural tube. It is formed via primary and secondary neurulation to give rise to a tube of polarized neural stem cells. Here, we have developed a versatile method for controlling the microscale morphology of neural organoids derived from hPSCs, which resembles the in vivo neural tube. We demonstrate reproducible engineering of 3-D, cylindrical neural organoids using 250∝m diameter fishing line and alginate hydrogels. The fishing line is used to cast internal channels inside of alginate hydrogels, which are then injected with embryonic stem cells. The cells are differentiated *in situ* using chemically defined media and

organize into a polarized tube of neural stem cells apically expressing N-Cadherin and basally expressing laminin. These neural tube organoids approximate the morphology of the germinal neural tube in the developing human embryo. We hypothesize that this enhanced control of organoid morphology will yield a more biomimetic organoid morphogenesis process and thereby more physiologically relevant 3-D tissue models.

(7) Hox5 genes establish and maintain the lung elastin network

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Alveoli are the terminal airways of the lung and these structures are critical for efficient gas exchange throughout life. Little is understood regarding the molecular mechanisms that regulate both alveolar development and maintenance, but mesodermally-derived lung fibroblasts are critical drivers of alveologenesis. These fibroblasts have primary responsibility for secreting the elastin required for subdividing the saccular airspace into mature alveoli. Our laboratory has previously demonstrated that all three Hox5 genes (Hoxa5, Hoxb5 and Hoxc5) are exclusively expressed in the lung mesenchyme, and loss of all three Hox5 genes from embryonic stages leads to severe developmental lung defects and perinatal death. Intriguingly, expression levels of all three Hox5 genes peak during the postnatal stages to higher levels than those observed in at any embryonic stage. We have recently demonstrated that postnatal mesenchymal deletion of Hox5 in the lung, using a newly generated Hoxa5 conditional allele, results in severe alveolar simplification. The establishment of the ECM/elastin network required for alveologenesis is disrupted in the Hox5 conditional mutant, characterized by the absence of the thick elastin cables that subdivide the nascent alveoli into smaller units. Immunofluorescence and western blot analyses demonstrate that both the basement membrane and ECM components are established normally in Hox5 conditional mutants. However, mutant fibroblasts exhibit significant adhesion defects in culture, and preliminary data show loss of Integrina5 expression in Hox5 conditional mutant fibroblasts. Further, additional preliminary data from mice in which Hoxa5 has been deleted at adult stages also show a dramatic disruption in elastin, demonstrating a continued role for Hox5 genes in the maintenance of the elastin network of the adult lung. Collectively, our data indicate that Hox5 genes regulate function of mesenchymal fibroblasts ultimately controlling lung matrix formation and maintenance critical for proper alveolar structure.

(8) Non-destructive high-content analysis of heterogeneity in CAR T cell immunotherapies

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Genetically engineered human T cells expressing cancer-specific chimeric antigen receptors (CARs) are an exciting new therapeutic for relapsed and refractory cancers. However, in spite of notable successes in combating leukemia, CAR T cells show limited efficacy against solid tumors. We hypothesize that this difficulty stems in part from heterogeneous cell populations, with variable CAR expression after viral transduction. To mitigate this issue, we are using CRISPR/Cas9 to precisely insert the CAR transgene at defined loci. Additionally, we are developing Optical Metabolic Imaging (OMI) to probe T cell behavior and differentiation in a

non-destructive, label-free manner. OMI assesses the fluorescence intensity and lifetime of the metabolic coenzymes NAD(P)H and FAD, providing information about their relative proportions and co-enzyme binding states, and therefore, the redox state of the cell. We have demonstrated that OMI robustly identifies activated from quiescent T cells by metabolic state, and further distinguishes CD8+ naïve vs. memory T cells. Cell heterogeneity can be resolved to a high degree by metabolic fingerprint; thus, this technology constitutes a powerful tool for monitoring immune cell function during the manufacturing process.

(9) Mitochondrial deficits contribute to impaired dendritic maturation in FXS human and mouse neurons

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Fragile X syndrome results from a loss of the RNA-binding protein fragile X mental retardation protein (FMRP). How FMRP regulates neuronal development and function remains unclear. During embryonic development, mitochondria are important for neural progenitor proliferation and neuronal survival. Alterations in mitochondrial morphology and function directly impact morphological development of neurons. Here, we report that FMRP has a critical role in dendritic maturation of adult new neurons, neonatal hippocampal neurons, and human neurons developed in transplanted mouse brains. We discover that FMRP-deficient immature neurons exhibited altered expression of mitochondrial genes, fragmented mitochondria, impaired mitochondrial function, and increased oxidative stress. Enhancing mitochondria fusion by either a chemical activator or exogenous expression of mitochondrial fusion genes rescued both mitochondrial morphology and dendritic maturation deficits of FMRP-deficient neurons. We discovered that FMRP deficient neurons had reduced HTT levels and acute knockdown of HTT recapitulates both mitochondrial fusion and neuronal maturation deficits seen in Fmr1 KO neurons. We used guide RNAs to target modified CRISPR/Cas9 (dCas9VP64-SAM) to selectively activate the endogenous Htt gene in neurons and show that increased Htt transcription rescued both mitochondrial fusion and dendritic maturation deficits of Fmr1 KO neurons. Finally, we show that mice with HTT knockdown in the hippocampus exhibit several behavioral deficits similar to Fmr1 mutant mice and treatment with a mitochondrial fusion compound rescued behavioral deficits of both Fmr1 KO mice and mice with hippocampal knockdown of HTT. Our data demonstrate that mitochondrial dysfunction contributes to the impaired maturation of FMRP-deficient developing neurons and present a crosstalk between FMRP and HTT in pathogenesis of human diseases.

(10) Pancreatic ECM co-culture enhances stem cell-derived beta cell differentiation

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Introduction: Stem cell-derived beta cells (SCBCs) could provide an abundant supply of insulin-producing cells for diabetic patients. Despite progress in SCBC differentiation, fully mature phenotypes have not been consistently achieved. Our lab has produced a novel human pancreas decellularization protocol, generating pancreas-specific extracellular matrix (ECM) which provides chemical signals to developing cells. We hypothesize that co-culture of hP-ECM hydrogel (hP-HG) with SCBCs will enhance beta cell maturation, reflected in increased beta cell-specific gene expression and function of the cells.

Methods: SCBCs were embedded into 10 uL hP-HG droplets on day 11 of differentiation and cultured through the remainder of the 28-day differentiation protocol. In parallel, cells were cultured in suspension without added ECM. On day 28, cells were collected for comparative analysis. Immunofluorescent staining was performed and quantified to assess expression of mature beta cell markers: Pdx1/Nkx6.1 co-expression, Insulin and Urocortin-3 (Ucn3). A panel of 17 key islet endocrine genes was used to assess gene expression through QPCR, comparing control cells to hydrogel treatment. All groups were normalized to undifferentiated stem cells and compared to isolated human islets.

Results: Insulin, somatostatin, Ucn3, glucose transporter-2, NeuroD1 and IAPP gene expression was significantly upregulated in SCBCs cultured with hP-HG compared to suspension. Cell clusters co-cultured with hP-HG contained 34.9% Ins+ area, while suspension cultures contained only 16.2%. Co-expression of the essential beta cell transcription factors Pdx1 and Nkx6.1 was found in 72.1% of hP-HG cultured cells, while only in 43.5% of suspension cultured cells.

Conclusions: These results suggest pancreatic ECM may improve the efficiency of differentiation toward a more mature beta cell fate. This is reflected in a higher percentage of Pdx1/Nkx6.1 co-positive cells, as well as improved gene expression of a variety of endocrine genes. Future studies will test beta cell survival and function following hP-HG co-culture.

GENERAL POSTER SESSION

(11) iNKT cells interact with monocytes to regulate the inflammatory activation of human umbilical cord blood T cells after hematopoietic transplantation

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iNKT cells have been shown to promote immunological tolerance following solid organ transplantation, but their effects on hematopoietic transplantation have not yet been well characterized. We have observed that co-transplantation of allogeneic human iNKT cells is associated with diminished expansion and inflammatory activation of human umbilical cord T cells in an in vivo hematopoietic engraftment model. This study aims to determine the mechanisms by which iNKT cells limit cord T cell response. We first established an in vitro system to induce either TCR-(anti-CD3/CD28) or cytokine-(IL-7) driven activation of isolated $CD4^+$ cord T cells. Cord T cells that expanded in response to IL-7 maintained CD45RA expression and efficiently produced TNFa but were deficient in IFN-g production. In contrast, TCR-driven expansion resulted in loss of CD45RA expression, and in acquisition of the ability to produce IFN-g in addition to TNFa. Remarkably, the presence of autologous monocytes during IL-7 driven expansion was sufficient to replicate the effects of TCR-driven expansion (loss of CD45RA and acquisition of IFN-g production). However, in the presence of monocytes along with a small fraction of iNKT cells the proliferative response of the cord T cells was reduced, CD45RA expression was maintained, and IFN-g production was deficient. Moreover, even TNFa production appeared to be suppressed. Since cord T cells cultured with iNKT cells alone did not result in similar limitation of cord T cell responses, we conclude that the interaction of iNKT cell with monocytes leads to the induction of a potent tolerogenic pathway. We are currently using

this in vitro model system to dissect the mechanisms behind the tolerogenic effects of the iNKT-monocyte axis, which will then allow us to test the impact of specific pathways in vivo following hematopoietic transplantation. These studies will thus provide important new information on how iNKT cells impact outcomes of hematopoietic transplantation.

(12) Coculture of cardiac cells with hPSC-derived cardiac progenitors to enhance cardiomyocyte maturation

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Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) hold a great potential for cardiac repair and drug screening. Though we now can generate essentially pure populations of hPSC-derived CMs, these CMs are immature or embryonic-like, causing arrhythmias when implanted into non-human primates. This immaturity is characterized by a difference in cardiac protein expression, electrical and mechanical functionality, and morphology in comparison to adult CMs. Recent research has shown some benefits of coculturing endothelial cells (ECs) with the hPSC-derived CMs to increase the CM maturation. Epicardial cells (EpiCs) also impact heart development, directly interacting with the CM to regulate proliferation and other phenotypes. Through coculturing hPSC-derived cardiac progenitor cells (CPCs) or beating hPSC-derived CMs with hPSC-derived ECs, we demonstrate that the addition of the EC interactions at an earlier point in the CM development has a greater potential for maturation than when coculture is initiated with fully differentiated CMs. Specifically the percent of CMs expressing cTnI and MLC2v were only significantly increased by the introduction of ECs at the cardiac progenitor stage. hPSC-derived CPCs cultured with EpiCs increased the percent of CMs expressing MLC2v yet no change was seen in cTnI. Alternatively, CPCs with EpiC-derived cells only induced cTnI expression and not MLC2v. This research demonstrates the importance in mimicking heart development by the inclusion of other cardiac cell types in the culture of CPCs to yield the most adult-like cell type. In the future we will work to identify the methods of interactions between the CPCs and the ECs, EpiCs, and EpiC-derived cells to determine the important signals required for maturation.

(13) Molecular and functional characterization of hPSC-derived cardiac stromal cells

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Coronary heart disease results in the death of cardiomyocytes, the muscle cells of the heart, and loss in contractile function which could be restored with a cellular therapy**.** Sourcing of cardiomyocytes is challenging, because there are not enough donors to fulfill the demand for heart transplantation, and there is no source for the non-proliferative human adult cardiomyocytes. Human pluripotent stem cells (hPSCs) readily provide a proliferative cell source that can be differentiated into cardiomyocytes and cocultured with other cell types of the heart.

While our group has developed a protocol to generate many of the cell types in the heart from hPSCs, hPSC-cardiomyocytes are still immature compared to adult cardiomyocytes. To mimic the heart *in vitro*, researchers are attempting to mature cardiomyocytes by exposing the cells to electrical stimulation, engineered substrates, or biological cues. However, cardiomyocytes are not the only cell type present in the heart niche, and interactions with other cell types are crucial for development. Epicardial-derived stromal cells are of particular interest, because they

are crucial for normal cardiac muscle regeneration in zebrafish and play a role in heart fibrosis, remodeling, and repair in mammals. In this work, we have optimized differentiation of hPSC-epicardial cells into stromal cells looking for robust marker expression and proliferation. We characterized the resulting population molecularly comparing with primary dermal fibroblasts, smooth muscle cells, epicardial cells, and stem cells. Finally, we have begun functional characterization including the ability to deposit extracellular matrix, contractility, and capability to induce hPSC-cardiomyocyte maturation.

(14) Small molecule mimicry of shear stress izmpacts on BMECs

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Diseases of the central nervous system (CNS), such as brain cancers and neurodegenerative diseases currently effect over 60 million people worldwide, but currently 98% of small molecule and 100% of large molecule therapeutics fail to penetrate the blood-brain barrier (BBB) in clinically relevant doses. The BBB is comprised of brain microvascular endothelial cells (BMECs), a specialized type of endothelial cells. The human pluripotent stem cell (hPSC) derived BMECs, developed in our labs, provide a scalable and human model of the BBB which possess high barrier tight junction resistance compared to other models, but still less than *in vivo* measurements. Most current hPSC-BMEC models of the BBB are operated under static conditions, however *in vivo* BMECs are exposed to the flow of blood. Therefore, our lab has begun investigating the role of shear on hPSC-BMEC model fidelity and to investigate the signaling pathways involved in hPSC-BMEC shear stress response. We have identified transcriptional upregulation of p21 and TGFβ1 signaling pathways in hPSC-BMECs under shear stress. Application of agonists of these signaling pathways had minor effects on hPSC-BMEC barrier quality, contrary to expectations. Further experiments being conducted are using gene set enrichment analysis of transcriptional data to identify upregulated pathways with the ultimate goal of creating a static culture model that can replicate improvements seen under shear stress without the need for microfluidic devices.

(15) Controlled study measuring the impact of a new software platform on reproducing a stem cell protocol

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Many stem cell scientists have experienced significant challenges reproducing protocols they have not previously run. Much of this difficulty arises from incomplete documentation of highly complex stem cell protocols and their associated material recipes in typical scientific publications and other records.

In this poster we report on a controlled laboratory study (NIH STTR Grant 1R41GM125489-01) measuring the impact of a new software platform on how successfully stem cell scientists could reproduce a protocol they had not seen previously. A simple protocol for differentiation of neuroepithelial cells from IMR-90 cells was used (Lippmann et al, *Stem Cells* 2014). The scientists were divided into two matched groups based on workplace and experience. The Control group was given the original and ancillary references together with vendor instructions, and was asked to write a culture plan, execute the protocol in the lab and document their work in a paper lab notebook sufficiently that a co-worker could reproduce or troubleshoot it.

The Treatment group was trained for 3 hours on the software, then given the protocol using only the software on a tablet device, which was used in place of a paper notebook to document their work. The software used in the study was designed for stem cell research and captures critical details such as cell line lineages, vessels & wells, materials used, specific actions taken, and analytical data collected, which are linked together in a highly semantic, non-relational data structure.

The completeness and accuracy of the experimental documentation was scored against a standard, and the success of each scientist in completing the protocol was judged by daily observation of their work and by analyzing the resulting cells.

In the study, the software group showed significantly improved reproducibility and efficiency of both lab work and documentation. Of the 13 Control group participants, only 9 successfully passaged their IMR90-4 cells, with four subjects having no cell attachment. Of these nine, rosette morphology on Day 6 post-seeding was observed for only 5 participants due to incorrect seeding density. The 15 Treatment group participants were all successful at passaging their cells and obtaining rosette morphology. The average time for both lab work and documentation was 33% less for the Treatment group than the Control group.

(16) Mesenchymal Stromal Cells mediate intestinal homeostasis through the synergistic action of CCL2 and CXCL12 induced M2 macrophage polarization

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The key parameter to establish MSC based therapeutic approach is to understand the ways to enhance its immunomodulatory capacity. In this regard, it is critical to glean insight into the MSCmacrophage interaction, since macrophage is the primary regulator of the body immunity. Previous study demonstrated, that, the macrophage activation towards its immunosuppressive M2 state, is promoted by MSC. However, the impact of MSC secretory factors in this class switching phenomenon, has never been adequately addressed. Here we depleted macrophage and its M2 signature cytokine, IL10, in colitis mice, to establish their critical role in bone marrow derived MSC (BMSC) mediated intestinal immune regulation. Our study showed, for the first time, that the MSC secreted chemokines CCL2 and CXCL12 synergistically operate, to induce M2 polarization of macrophage. Additionally, IFN-gamma, which enhances CCL2 production from MSC, was observed to generate more therapeutically potent MSC. Therefore, our study indicates, enhancement of CCL2 production by MSCs could be a new therapeutic modality for clinical inflammatory diseases.

Key words
Bone marrow derived MSC (BMSC), Peritoneal macrophage (PMf), chemokine ligand 2 (CCL2), chemokine ligand 2 (CCL2), C-X-C motif chemokine 12 (CXCL12).

(17) Hematopoietic stem cell differentiation but not self-renewal is suppressed in response to GVHD induced cytokines in a humanized mouse model of HSCT

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Graft-vs-host disease (GVHD) is a major complication that occurs after hematopoietic stem cell transplantations (HSCT) and leads to cytopenia in the patient. Despite four decades of HSCT clinical practice and accompanying clinical trials, there is little evidence to explain how these two phenomenon are connected. Using clinically relevant human HSCT grafts as a source of human cells and the NBSGW immunodeficient mouse model developed at UW-Madison, we are able to recapitulate clinical HSCT outcomes and their biological mechanisms. Using this method, we have been able to show a significant suppression of hematopoietic output in the bone marrow of these mice 12 weeks post engraftment when T cells are included in the graft. Furthermore, we have shown that T cell activation and TNF production in particular is responsible for suppressing hematopoietic output in our mouse model. This biological phenomenon exists in all three clinically relevant HSCT graft sources; bone marrow, GCSF mobilized blood and cord blood. Despite the lack of hematopoiesis in the presence of T cell activation, the HSCs persist in the bone marrow of these mice and future experiments will determine if they are still functional. Overall, this study examines a clinically relevant complication of HSCT that previously had no known biological mechanism. Armed with this knowledge, clinicians may now be able to prevent HSCT patients experiencing GVHD from having cytopenia that will protect them from additional pathogenic infections.

(18) Regional patterning of hPSC-derived posterior central nervous system tissue

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In vitro modeling of the human central nervous system (CNS) is complicated by the diversity of constituent cell phenotypes, which arise from neural stem cells (NSCs) that are regionally patterned along both the rostrocaudal (R/C) and dorsoventral (D/V) axes during early development of the neural tube. Recapitulating this patterning with chemically defined, scalable protocols using human pluripotent stem cells (hPSCs) is necessary to optimally model CNS disorders, screen potential drug therapies, and develop cell replacement strategies for regeneration. We have previously reported methods to regionally pattern hPSCs to neural stem cells (NSCs) expressing discrete R/C Hox profiles that span the hindbrain through spinal cord and subsequently direct them to a ventral fate by modulating Shh signaling. Here we focus on developing protocols to differentiate these regionally defined NSCs to dorsal interneuron (dIN) fates, thereby generating the first regional spectrum of human somatosensory spinal populations. Because progenitor domains in the dorsal spinal cord are generated by both TGF-β dependent (Class A dINs) and TGF-β independent (Class B dINs) mechanisms, we sought to develop separate differentiation protocols to obtain populations from both classes. qRT-PCR demonstrates a shift in expression from intermediate to dorsally expressed transcription factors in response to exposure to single agonists and/or antagonists of Wnt/β-catenin and BMP signaling. By combining the most efficacious factors to optimize for Class A or Class B progenitors, we observed a 10-1000 fold decrease or increase in transcriptional markers for intermediate fates in Class A-optimized or Class B-optimized cultures respectively. Importantly, we demonstrate the differentiation of mature dorsal horn (dhIN) a valuable resource for gaining

insights into regional differences in somatosensory network development, modeling chronic pain neuropathies, and conducting neurotoxicity and drug screening.

(19) Developing endothelial cell vascular networks in beta cell co-cultures.

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Background: Diabetes is a disease that affects millions around the world, causing major late-stage health complications and being a leading cause of death. Despite the promise of pancreatic cell replacement therapies, stem cell-derived beta cell (SCBC) transplantation has not yet reached routine clinical application due to the lack of mature function and survival of cells *in vivo*, although clinical trials are underway. Insufficient vascularization, lack of necessary growth factors, and incomplete matrix environments have been identified as issues that affect the viability of transplanted cells. Our lab is the first to successfully apply decellularization techniques to cadaveric human pancreata to produce human pancreatic extracellular matrix hydrogels (hP-HG) that mimic normal human pancreatic matrix microenvironments. However, issues with insufficient vascularization remained unresolved. We hypothesize that endothelial cells (ECs) embedded in hP-HG *in vitro* will form vascular networks that can support the engraftment and maturation of SCBCs following transplant.
Methods: To ascertain optimal techniques of prevascularizing hP-HG gels *in vitro,* ECs were embedded in 5 uL hP-HG droplets and cultured in various media to assess 3D vascular formation over 3 days. AngioTool was used to quantify vascular formation on 2D maximum-intensity confocal images of gels.
Results: ECs cultured in hP-HG with EC medium formed 3D vasculature covering 29% of the area of a 2D maximum-intensity confocal image. When cultured with beta cell media, the percentage was reduced (14.2% in CMRL, 23.7% in INS1E media), but when cultured with a hybrid medium combining the EC and INS1E media, the percentage was greater at 31.7%.
Conclusions: We demonstrate that ECs are able to form vascular networks in hP-HG, which could be used to further optimize SCBC cell replacement techniques involving *in vitro* co-cultures of ECs and SCBCs in hP-HG. This could one day be used in clinical settings as an optimized therapy for diabetes.

(20) Cell-intrinsic control of cardiomyocyte differentiation from human pluripotent stem cells

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A myocardial infraction, also called a heart attack, can lead to a significant damage to the part of the heart muscle thereby severely restrict the heart's contractile function. The death of the heart muscle cells, also termed cardiomyocytes, is the main cause of this dysfunction, but the adult cardiomyocytes have very limited capacity for self-renewal hindering the restoration of their functional activity. Human pluripotent stem cell (hPSC)-derived cardiomyocytes have emerged as a promising source to replace the damaged heart muscle for the restoration of the heart function. This hPSC-based cardiac cell therapy have shown some promising results in non-human primate models but there is still room for improvement including scalable production of hPSC-derived cardiomyocytes. Most strategies for the biomanufacturing of the hPSC-derived

cardiomyocytes rely on the use of three-dimensional (3D) bioreactors. In the case of the 3D culture system it is important to uniformly stimulate each cell with differentiation induction

factors for the directed cardiomyocyte differentiation. However, most of the efficient cardiomyocyte differentiation protocols are based-on the use of cell-extrinsic inductive factors such as proteins and small molecules and the effects of these cell-extrinsic factors highly depend on the dynamic conditions of the 3D culture system such as mass transport and fluid shear stresses which can possibly cause heterogeneous responses of cells to inductive factors during cardiac differentiation. In this study, we suggest utilizing a genetically edited hPSC line where a beta-catenin short hairpin RNA is incorporated into as a cell-intrinsic inductive signal to regulate the Wnt signaling pathway which has been shown as a key pathway to derive cardiomyocytes from hPSCs. The use of the cell-intrinsic factor might increase the robustness of the 3D-based cardiomyocyte differentiation, thus contribute to the biomanufacturing of the hPSC-derived cardiomyocytes.

(21) Mesenchymal stem cell migration during bone marrow establishment

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The bone marrow stroma is home to a heterogeneous population of cells including hematopoietic stem cells and their niche, comprised of specialized stromal cells, referred to as mesenchymal stem cells (MSCs). The success of clinical bone marrow ablation for HSC replacement therapies is dependent on MSCs recolonizing the bone marrow space in a mechanism thought to mimic development, making this a fundamental process in translational and developmental biology; however, how MSC populations are established in the bone marrow is poorly understood. The current paradigm for MSC migration invokes the use of invading vasculature as a conduit into this compartment. Our lab has recently shown that Hox-expressing cells in the bone marrow are exclusively identified as progenitor-enriched skeletal MSCs. Evidence from our laboratory, using our Hoxa11eGFP allele as a marker for MSCs, reveals inconsistencies between vascular migration and MSC presence in the bone marrow. Hoxa11+ MSCs enter the bone marrow space through the mid-diaphysis beginning ~E15.5, spatially independent of discontinuous endothelial cells, which do not coalesce into vessels until birth (P0). In contrast, MSCs are not diffuse throughout the bone marrow until P3 and do not initially show any obvious association with vasculature, as they localize to distal cortical surfaces of the bone. Current results suggest that the proximodistal growth of bone growth plates may be responsible for shifting MSCs throughout the length of the bone marrow. Alternatively, MSCs could enter through 'paths' carved by osteoclastic resorption of cartilage matrix. These preliminary experiments have paved the way for further investigation of the migratory dynamics of an embryonically-established, self-renewing Hox-expressing MSC population into the bone marrow and the establishment of the HSC niche. The emerging knowledge of region-specific stem cell populations is critical in improving stem cell therapies in which bone marrow transplant rejection is common due to improper bone marrow reestablishment.

(22) Distinct Lineage-Derived Chondrocytes From Human Peripheral Blood-Induced Pluripotent Stem Cells For Articular Cartilage Regeneration

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Mesenchymal and neural crest lineage cells exist robustly during the early embryonic development. While mesoderm-derived mesenchymal stem cells (MSCs) have been widely studied as a promising cell source for cartilage regeneration, increasing attention has been given to neural crest stem cells (NCSCs) in the field of regenerative medicine because of their potential to differentiate into various tissue types. Here, we performed a comparison study to evaluate properties of mesoderm- and NCSC-derived chondrocyte pellets from human peripheral mononuclear blood cell-derived induced pluripotent stem cells (iPSCs) and the capability of both cells for hyaline cartilage regeneration. Multiple iPSC lines were induced with each into both mesodermal and NCSC lineages and further into chondrocytes. RNA-sequencing analysis was performed to compare the global transcriptome of distinct lineage-derived chondrocytes in reference to human primary chondrocytes. In the in vivo study, two lineage-derived chondrocyte pellets were implanted subcutaneously in mice for assessment of ectopic cartilage formation and in rat joints for evaluation of defect repair. Our results showed that NCSC-derived chondrocytes more resembled primary chondrocytes of articular cartilage than mesoderm-derived ones. In addition, unlike mesoderm-derived chondrocytes, NCSC-derived ones did not show hypertrophic characteristics, which are critical to the repair of articular cartilage. Taken together, our study showed distinct differences in transcriptome profiles, matrix production, and the repair capacity between mesoderm- and NCSC-derived chondrocytes. The collected information not only helps us gain better insight into how pluripotent stem cells undergo chondrogenesis following different developmental programs but also suggests that NCSC-derived chondrocytes may be a potent cell type for articular cartilage repair

(23) UM171 expands myeloid progenitors and lymphoid progenitors with NK potential from human pluripotent stem cells

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Scaling up blood cell production from hPSCs is critical to advancing hPSC technologies for blood transfusion, immunotherapy, and transplantation. We explored the potential of the HSC agonist and pyrimido-indole derivative UM171, to expand hematopoietic progenitors (HPs) that were derived from hPSCs, in chemically defined conditions. We revealed that culture of hPSC-HPs in HSC expansion conditions (SFEM with added TPO, SCF, Flt3L, IL3, and IL6) in the presence of

UM171 predominantly expanded HPs with a unique $CD34^{+}CD41^{lo}CD45^{+}$ phenotype and myeloid, particularly G-CFC clonogenicity, and that this expansion was mediated by increased proliferation and G0/G1 to S phase progression, and decreased apoptosis. Further extending these studies, both DMSO and UM171 expanded HPs could be effectively differentiated into functional neutrophils.

In lymphoid cultures on OP9-DLL4, in the presence of SCF, Flt3L, and IL7, UM171 selectively expanded $CD34^{+}CD45^{+}$ progenitors that expressed the lymphoid progenitor markers, CD7 and CD45RA, and that possessed NK potential, resulting in an increase of up to 10-fold in NK cell output, with subsequent NK differentiation. We further showed that while fractions of cells with $CD41a^{lo}$ and $CD41^{hi}$ phenotypes were also detected in lymphoid cultures, $CD41a^{-}$ HPs had the most robust NK differentiation potential, and the highest frequency of $CD7^{+}$ cells. Our results thus indicate that lymphoid differentiation with UM171 primarily promotes amplification of a $CD41a^{-}$ $CD7^{+}$ lymphoid progenitor population.

Our studies should improve our understanding of the effect of UM171 on *de novo* generated HPs, and facilitate development of protocols for robust granulocyte and lymphoid cell production from hPSCs, for transfusion and adoptive immunotherapies.

(24) Weaponizing tumor infiltrating lymphocytes for ovarian cancer immunotherapy

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High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy with ~23,000 new cases and ~15,000 deaths estimated in the United States in 2019. Often diagnosed at an advanced stage, HGSOC spreads past the primary site and features ascites in most patients. In spite of current therapy regimen of aggressive cytoreductive surgery followed by chemotherapy, the 5-year survival rate remains low (~29%) with high recurrence rate in majority of patients (70 to 95%). Recent advances in cancer immunotherapy research suggest that harnessing human immune system could be an effective approach to treat high-grade serous ovarian cancer (HGSOC). Presence of CD8+ Tumor infiltrating Lymphocytes (TILs) in ovarian cancer ascites is now well established and these are also prognostic for improved survival. However, their effectiveness is limited by tumor-mediated suppression of immune function. Therefore, it becomes critically important to develop new approaches that weaponize TILs for effective eradication of HGSOC. In this study, we report development of a new approach for ovarian cancer therapy by augmenting host TILs using our innovative fusokine (recombinant chimeric cytokine). We show that GIFT4, a recombinant GM-CSF-IL-4 fusion cytokine, augments the physiology of B-cells and NK-cells in HGSOC patient-derived TILs. Our data suggest that GIFT4-priming may confer potent anti-tumor properties on natural killer (NK) and CD8+ T-cells which could consequently be used for personalized immune cell therapy for treatment of HGSOC.

(25) Immune effects of ruxolitinib on gamma deelta T cells *in vitro*

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Gamma delta T cells are a rare subset of T cells with extraordinary properties including the capacity for tumor cell killing and are an attractive cell type for novel immunotherapy approaches. Ruxolitinib is a selective JAK inhibitor that is currently being used for some hematologic malignancies and being investigated in clinical trials for others in patients with poor outcomes. Our goal was to investigate the immune effects of ruxolitinib on gamma delta T cells for the potential implications for combined novel immunotherapies. First, we examined levels of intracellular IFNy and pSTAT3 to examine ruxolitinib's effect (1.25 uM, 2.5 uM, 5 uM, and 10 uM) on activated gamma delta T cells. IFNy decreased compared to untreated control once 2.5 uM or higher dose was reached after 48 hr. As expected, pSTAT3 decreased compared to untreated control, however, this decrease could be seen even at the lowest dose of 1.25 uM. Next, we wanted to examine ruxolitinib's effects (2.5 uM) on gamma delta T cells' ability to kill leukemia cell lines, K562 and Jurkat. There was no difference observed in cytotoxicity measured by LDH release in K562 and Jurkat with or without ruxolitinib at the following target effector ratios: 20:1, 10:1, and 5:1 using previously expanded and cryopreserved gamma delta T cells. Finally, we were interested in examining whether zoledronate alone could be used to expand gamma delta T cells as we were inhibiting JAK/STAT signaling of IL-2 with the addition of ruxolitinib (2.5 uM). Ruxolitinib completely blocked gamma delta T cell expansion (14 days) and in post expansion analysis showed toxicity to PBMCs exposed to ruxolitinib after the 14 day expansion period.

(26) Using human neural progenitor cell models to conduct large-scale drug dcreens for Fragile X Syndrome

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Human patient-derived induced pluripotent stem cells (hiPSCs) provide unique opportunities for disease modeling and drug development. However, adapting hiPSCs or their differentiated progenies to high throughput assays for phenotyping or drug screening has been challenging.

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and a major genetic cause of autism. FXS is caused by mutational trinucleotide expansion in the FMR1 gene leading to hypermethylation and gene silencing. One potential therapeutic strategy is to reactivate the silenced FMR1 gene, which has been attempted using both candidate chemicals and cell-based screening. However, molecules that effectively reactivate the silenced FMR1 gene are yet to be identified; therefore, a high throughput unbiased screen is needed.

Here we demonstrate the creation of a robust FMR1-Nluc reporter hiPSC line by knocking in a Nano luciferase (Nluc) gene into the endogenous human FMR1 gene using the CRISPR/Cas9 genome editing method. We confirmed that luciferase activities faithfully report FMR1 gene expression levels and showed that neural progenitor cells derived from this line could be optimized for high throughput screening. The FMR1-Nluc reporter line is a good resource for drug screening as well as for testing potential genetic reactivation strategies. Here we demonstrate the screening of over 320,000 novel compounds from the NIH's MLPCN compound library along with identification of potential gene reactivators. In addition, our data provide valuable information for the generation of knock-in human iPSC reporter lines for disease modeling, drug screening, and mechanistic studies.

(27) Development of iPSC-derived Natural Killer Cells for Brain Cancer Immunotherapy

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Diffuse Intrinsic Pontine Glioma (DIPG) is incurable brain cancer that arises in children. Drug development is especially difficult for brain tumors due to the difficulties of drugs penetrating the blood brain barrier (BBB). The ability of a large population of immune cells to pass through the BBB and be involved in the anticancer response could contribute to immunotherapy efficacy. Recently, NK cells were recognized as a superior cellular immunotherapy product (Li et al, 2018; Saetersmoen, et al, 2019). They recognize a variety of cancer cells irrelevantly of underling mutations and thus can be very effective at slowing down disease progression. The development of an effective "off the shelf" cellular immunotherapy with NK cells is crucial for brain tumors where operability is limited.

We set out to manufacture an "off- the- shelf" iPSC derived NK cell immunotherapy product to efficiently combat DIPG. iPSC-NK cells were developed as described in (Galat et al, 2017) using an adherent system suitable for translation and scalable production.

In order to evaluate the killing efficiency of NK cells against the DIPG SF8628 cell line *in vitro*, we incubated the target cells with either PBMC-NK or iPSC-NK at ratios of 1:1, 1:5, and 1:10 for 4 hours. Killing activity was assessed using FACS analysis as well as an automated cell counting method. Both PBMC-NK and iPSC-NK were successful at killing the DIPG cells, with iPSC-NK performing at a higher efficiency of up to 80% at a 1:5 ratio.

This data suggests that our iPSC -derived NK cells can provide a potential cellular therapeutic tool to combat DIPG and other brain cancers.

(28) Ruxolitinib blocks IFNγ mediated activation of Mesenchymal Stromal Cells

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Ruxolitinib, an FDA approved JAK1/JAK2 inhibitor, is currently in use for myoproliferative disorders and has recently shown promising clinical effects in inflammatory disorders such as Graft versus Host Disease (GVHD). Although it is widely known that Ruxolitinib blocks JAK2 mediated activation of hematopoietic immune effector cells, its effect on nonhematopoietic immune modulating cell types such as Mesenchymal Stromal Cells (MSCs) is not known. MSCs are immune modulators responsive to IFNγ that signal through JAK2/STAT1. For this reason, we

investigated the effect of Ruxolitinib on the immunobiology of MSCs. MSCs were isolated from bone marrow aspirates of healthy human individuals. Culture adapted MSCs were found to express standard cell surface markers of MSCs including CD44, CD73 and CD105 and were negative for CD45. Proliferation of MSC as measured by MTT assay demonstrate that Ruxolitinib alone does not have negative proliferation effects on MSCs. However, the drug blocks IFNγ mediated cytostatic effects on MSCs. IFNγ activates STAT1 phosphorylation in MSCs. Western Blot analysis demonstrates that Ruxolitinib blocks this IFNγ mediated STAT1 phosphorylation in a dosage dependent manner. Next, we investigated the effector pathways downstream of STAT1 phosphorylation. Ruxolitinib effectively blocks MSC IFNγ mediated upregulation of Major Histocompatibility Complex (MHC) class II antigens as well as immunosuppressive enzyme Indoleamine 2,3-dioxygenase (IDO). Similarly, flow cytometry results show that Ruxolitinib attenuates the expression of immunosuppressive ligand Programmed-Death Ligand 1 (PDL1) in MSCs. MSC and Peripheral Blood Mononuclear Cell (PBMC) co-culture experiments demonstrate that Ruxolitinib partially blocks the immunosuppressive effect of MSCs on PBMC proliferation. Together, our results demonstrate that Ruxolitinib substantially modulates the immunobiology of MSCs by blocking both pro and anti-inflammatory pathways. These observations open an entirely new perspective on the mechanism of action of immunomodulatory drugs such as Ruxolitinib on nonhematopoietic stem cells.

(29) Developing methods for the scalable monitoring and maturation of induced pluripotent stem cell-derived cardiomyocytes

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Heart disease is the leading cause of death in the United States, killing hundreds of thousands of people and costing hundreds of billions of dollars annually. A promising regenerative therapy for reversing damage from heart disease is the implantation of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs); however, several hurdles currently prevent the adoption of said strategy.

One significant impediment to the clinical translation of iPSC-CMs is the time-intensive and low-throughput nature of traditional methods to assess and monitor these cells throughout differentiation. Thus, improved schemes must be developed for the robust, scalable, real-time, continuous monitoring of iPSC-CMs. Another major limitation to iPSC-CM translation is the functional and structural immaturity thereof as compared with adult *in vivo* cardiomyocytes. Such maturation mis-matches result in poor electromechanical coupling *in vivo*, which manifests as arrhythmias. Most efforts to date to improve iPSC-CM maturation utilize inherently low-scale methods which pose numerous limitations to scale-up.

In addressing these hurdles, I am implementing systems biology approaches to comprehensively characterize the temporal transitions as iPSCs differentiate into iPSC-CMs. Parallel *in vivo* analyses of mouse hearts and recent literature in human fetal hearts will provide comparative datasets to identify conserved and differential developmental trends. Subsequent analyses will identify predictive multivariate markers for real-time assessment of iPSC-CM quality throughout differentiation and elucidate novel pathways correlated with CM maturation thereby allowing for higher efficiency scaled-up iPSC-CM production for various uses including clinical intervention, disease modeling, drug screening, and developmental biology applications.

(30) Hox11-expressing stromal cells contribute to muscle growth, regeneration and repair

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Skeletal muscle is among the most regenerative adult tissues. Its remarkable regenerative capacity originates from a population of resident stem cells, termed satellite cells. While satellite cells are essential for muscle regeneration, surprisingly, their genetic ablation in adult mice does not accelerate sarcopenia. Thus, additional mechanisms or cell types might contribute to maintenance of muscle mass during muscle regeneration and repair. Satellite cells are marked by expression of Pax7, a transcription factor critical for muscle regeneration. However, a recent study provided the first evidence that a non-satellite (Pax7-negative), muscle interstitial population that lies outside the basal lamina and expresses Twist2 (Tw2) has myogenic potential at adult stages. The Hox transcription factors are also expressed in the interstitial muscle tissue, and the real-time reporter for Hoxa11, Hoxa11eGFP, reflects this expression which is exclusive of the muscle-derived satellite cells. We have also previously reported that loss of *Hox11* function at embryonic stages leads to mis-patterning of muscle. Here, we present novel results using a newly generated conditional *Hoxd11* allele. Using this in the background of *Hoxa11* null mutations, postnatal- and adult-initiated loss of *Hoxd11* function reveals dramatic defects in growth and homeostasis of the muscle (in the absence of injury). More unexpectedly, following the lineage of these *Hox*-expressing cells using a *Hoxa11-CreERT2* generated in the Wellik lab during postnatal and adult stages (but not embryonic stages), we observe strong lineage labeling from the *Hox11*-expressing stromal cells into muscle tissue. Our preliminary data supports a functional role for *Hox11* genes during postnatal muscle growth and adult homeostasis. Current work is examining function and lineage labeling in muscle regeneration and repair after injury as well.