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**WSCS13**

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# POSTER ABSTRACTS

**Submission ID: 23203**

**Submission Title: Neo organo histo genesis of Fallopian tube: A Desired Metaplasia of adult autogenous mesoderm stem cells.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Organs and tissues seriously damaged by trauma or diseases or become nonfunctional need replacement for life to continue. Transplantation is with known complications and non availability of donor organs. Growing tissues and organs in vivo exists in nature in lower life forms. But it is rare or absent in mammals and man. If tissues and organs are regenerated in the body using adult tissue cells it will be ideal modality and obviate all the problems faced in transplant surgery. Diseases of Fallopian tube some times result in block in transfer of ovum from ovary to uterus or sperms from uterus to ampulla of fallopian tube for fertilisation. Repair of tube mostly is difficult and unsuccessful, therefore regeneration is attempted in mammals, dog and later standardised in near human model monkeys before it is attempted in Human beings.

**Background**

A single celled fertilised ovum is capable to form whole body having different tissues and organs having different structure and different function. Repairing cells in tissues and organs are capable for repair of damaged or diseased cells and also replace cells lost during normal wear and tear of body during life. Authors realised as surgeon that in chronic peritonitis patients metaplastic tissue formation has relation with cells of germ layer of germ disc of developing embryo. Peritoneum is developed from Mesoderm and various metaplastic tissues in chronic peritonitis are limited to tissue formations developed from germ layer mesoderm. In developing embryo proper and normal tissues are formed only at a site in embryo. For that particular cells of germ layer of germ disc are migrated to the particular cells during foetal growth. Not only this no one has ever observed full and normal growth of brain in pelvis or kidneys in skull. If such cells are trapped in these areas these cells only form incomplete or abnormal tissues in those regions and are termed teratomas or Metaplasia due to abnormal formation of tissues at that site, due to exposure to abnormal environment and function.

With this background of knowledge, the research attempted to study effect of guided site, environment and functional need that is, if tissue stem cells of a germ layer are colonised with tissues and tissue systems developed from same germ layer.

**Hypothesis**

Nature is committed & capable of miraculous changes to meet the goals of life. If a fertilised ovum is transplanted in uterus of, even a female of post menopausal age the whole body of that female is transformed to benefit the fertilised ovum to grow to full term & get breast milk to feed the offspring incapable to live independently after extra uterine life. Considering this capacity of nature the present research is attempted. Embryogenesis of Fallopian tube is from cells of coelomic epithelium. This duct forms Fallopian duct and Uterus. In other words the coelomic epithelial cells form Uterus and Fallopian tube. This

epithelium is termed as peritoneum of adult developed body. These cells retain the capacity to form all tissues formed from mesoderm layer of germ disc of embryo. Based on this, after experimentation on various tissues and organs in experimental animals a hypothesis was proposed which states that, "In post embryonic body if stem cells of Autologous tissue derived from a germ layer of germ disc of developing embryo are colonised with the tissues and tissue system which are also derived from the same germ layer, neoregeneration of tissues and organs is possible by desired Metaplasia, provided that both colonised and recipient tissues have developed as physical neighbours in the developing embryo. The essential part is the donor and host tissues must have developed in neighbourhood to each other in developing embryo and belong to same germ layer. (Widely published in peer reviewed scientific journals).

Based on this the in vivo regeneration of Fallopian tube planned and presented with photographic evidence in the poster.

## **Research**

Regeneration of Fallopian tube was carried out in seven female mongrel dogs (5 to 7 year old between 6 to 10 Kg body weight). The abdomen opened through infra umbilical midline incision. The Fallopian tube segment was excised and preserved, and a tube constructed from selected peritoneum was anastomosed in place over a stent. One end of stent was kept up to fimbrial end of Fallopian tube while the other end brought through uterus in vagina where it was secured to the wall of vagina, for a period of three months a time needed for foetal viability in growing embryo. The stent was kept in situ for three months till the colonised peritoneal tube retrieved for gross and histological examination. The peritoneal tube anastomosed with spatulated ends of fallopian tube. This is to avoid stricture formation at the anastomotic site. After three months of post operative period the Grafted peritoneal tube was subjected to gross and histological examination and compared with normal preserved Fallopian tube. The photographic evidence is presented in poster

## **Observations**

On reexploration of operated site showed no collection of any inflammatory exudate or blood serum etc. in the pelvic cavity. There were a few flimsy adhesions at the anastomotic site and over the grafted membrane, easily separable with blunt dissection only. No sharp dissection needed to separate the anastomosed peritoneal membrane. The gross appearance showed thickening of thin peritoneal membrane. The consistency found to be comparable to normal Fallopian tube. Pale whitish colour of membrane became the same colour as the rest of the fallopian tube. The anastomosed segment was excised along with part of the healthy fallopian tube. There was no stricture or constriction on anastomotic line at both the ends. Histologically membrane showed all the layers of Fallopian tube comparable to the normal one in high and low power microscope in H & E stain. The smooth muscle layer in MTS stain was found to be comparable to normal Fallopian tube and could be differentiated from fibrous tissue which stained green.

The photographic evidence of gross and microscopic examination H E stain and MTS stain is presented in the poster.

**Submission ID: 23380**

**Submission Title: The Effect of Biotech Industry, Government, and Public Opinion on Embryonic Stem Cell Policies in Europe**

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**Topic**

Ethics, Law and Society

**Problem**

The nature of the problem in this research is what factor (s) affects embryonic stem cell policy in European Countries. Typically countries have adopted either a permissive or a restrictive policy toward using embryos or embryonic stem cells in research. Identification of factors may help explain and predict science policy in the future. Factors selected for 28 countries are type of government (unicameral vs bicameral), public opinion poll, and the number of biotechnology companies in each country

**Background**

Nearly half of the countries in the European Union have a unicameral legislature. One of the great proponents of a one chamber legislature was Jeremy Bentham (Rockow, 1928). Bentham thought a second chamber or bicameral legislature would encourage special interests and lead to mischievous activities. In addition, having two chambers in government would require double the amount of work and may be prohibitive in passing laws. Opposing views are represented by James Madison and Lord Viscount Bryce who thought a bicameral government would provide a check on legislation and therefore supported a two chamber system (Ali, 1995). If in fact Madison and Bryce are correct a bicameral system may have a prohibitive effect on passing legislation creating an environment of either a bill not be presented or getting lost in committee. In the realm of embryonic stem cell research policy this may lead to a country having no law on the issue or in the case of one chamber laws that are passed very expeditiously. Embryonic stem cell research is a category of biotechnology. If a country has an aggregation of biotechnology companies such a presence may have an impact on policy especially when located in close proximity to research institutes and universities. This factor will be analyzed along with unicameralism in affecting embryonic stem cell research policy. The significance of this study is passing laws that represent the people's views in type of government and if a stronghold presence of biotechnology sectors is influencing lawmakers toward supporting embryonic stem cell research.

**Hypothesis**

Countries with a unicameral government will have permissive policies on embryonic stem cell research compared to those with bicameral governments. A large number of biotechnology firms is associated with permissive policies on embryonic stem cell research.

## Research

A logistical regression design is used to determine if the number of biotechnology firms, public opinion (from a Eurobarometer 2010 poll) and type of government has a significant effect on embryonic stem cell policy in 28 countries in Europe at the .05 level. The dependent variable is policy and is coded 0 for restrictive policy and 1 for permissive policy. Restrictive is defined as forbidding embryonic stem cell research or only allowing importation of embryonic stem cells. Permissive is defined as allowing research on left over embryos from in vitro fertilization procedures and/or allowing therapeutic cloning. Country is the unit of measure. Independent variables are type of government, 0 for unicameral and 1 for bicameral, public opinion (interval/ratio variable) and number of biotechnology firms (interval/ratio) in the country. SPSS is the software used for binary regression.

## Observations

Fifteen countries have a unicameral government and of those 73 percent have a permissive policy on embryonic stem cell research. The number of biotechnology countries ranged from 2 to 2098 with Sweden having the greatest number. While Sweden has the largest number of biotech firms and is characterized as permissive, other countries like Germany with 763 firms has a restrictive policy. Using binary regression the poll variable resulted in a P value of  $<.05$  while government and biotech variables yielded P values  $>.05$ . A unicameral government and large number of biotech firms are not significantly associated with permissive policies in embryonic stem cell research in 28 countries. The significance of the Eurobarometer poll indicates national approval is reflective of ESC policy.

**Submission ID: 23399**

**Submission Title: Study on cancer stem cell marker**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Does cancer stem cell marker plays a role in mediating tumorigenic ability and stemness property?

**Background**

CD90 is a glycoposphatidylinositol (GPI)-anchored glycoprotein, which is highly expressed in stem cells. CD90 is related to immunological function, such as T cell activation. Besides, CD90 involves in nonimmunological functions, including cell adhesion, migration and cell death. The role of CD90 in malignant tumor has been studied. The published data show that CD90 is a tumor suppressor gene in ovarian cancer and nasopharyngeal carcinoma. However, several studies indicate that CD90 is a potential marker for liver cancer stem cells (CSCs). The published data show that CD90 is a tumor suppressor gene in ovarian cancer and nasopharyngeal carcinoma. However, several studies indicate that CD90 is a potential marker for liver cancer stem cells (CSCs).It suggests that CD90 leads to different function in different tumors.Several cell surface markers have been used to identify liver cancer stem cells including CD90, EpCAM, CD133, CD44, and CD13. Among these markers, CD90 is particularly interesting since overexpression of CD90 inhibited the growth of another type of tumor.

**Hypothesis**

Cancer stem cell marker is not just a marker, which plays some roles in mediating stemness ability and cancer progression.

**Research**

In order to elucidate the role of CD90 in liver cancer progression, the HepG2 and Hep3B stable transfectants expressing exogenous CD90 were established and the tumorigenic ability was analyzed *in vivo* and *in vitro*. We further analyzed the stemness properties of the transfectants.

**Observations**

We further analyzed the stemness properties of the transfectants and found that CD90 increased the stemness property.We analyzed the cross-talk interaction between these CSC markers and found that there was a cause-effect relationship. We further identified the signaling transduction pathway in mediating the processes of CD90-induced tumorigenic ability. Our findings provide a concept to target stemness-associated signaling axis as a strategy to treat cancer.



**Submission ID: 24177**

**Submission Title: LifeMap Discovery™: The Stem Cells, Embryonic Development and Regenerative Medicine research portal**

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**Topic**

Industry Infrastructure

**Problem**

The LifeMap Discovery™ database (<http://discovery.lifemapsc.com>) is designed to provide the research community with viable, scalable, and easy to use data portal describing embryonic development, along with vast information about stem and progenitor cells, and cell therapy applications for regenerative medicine.

Understanding how cells differentiate during embryonic development is invaluable for the derivation of functional cells from stem cells *in vitro*. The database has therefore been created to integrate data from the *in vivo* and the *in vitro*, including gene expression and signaling information which, in developing cells, is essential for stem cells identification. Further, this information can be used to develop novel differentiation protocols and therapeutic products.

The database is divided into the following parts:

1. **Embryonic development** - cell lineages and anatomical compartments developed in the mammalian body, supplemented with gene expression, signaling, *in situ* hybridizations and high-throughput experiments, related diseases, images and relevant references.
2. **Stem cell differentiation**- cultured cells and differentiation protocols, supplemented with gene expression, high throughput experiments, growth conditions and functional assays.
  1. **Regenerative medicine** – Cell therapy applications in different phases of the development from research through marketing. The information includes; the relevant stem/ progenitor cells, used as the "Therapeutic cell" for the application, mode and regimen of cell delivery, mechanism of action, treated disease, related animal models, pre-clinical and clinical information, publications and links.

These different parts are interconnected by computational and hand curated methods. The value provided by LifeMap Discovery originates from the combined power of this data, which enables identifying, predicting and indicating possible differentiation paths and future regenerative medicine applications. LifeMap Discovery integrates symbiotically with the more elemental database GeneCards where rich information is available at the gene level, and MalaCards, that provides human disease information.

## Background

n/a

## Hypothesis

n/a

## Research

n/a

## Observations

n/a

**Submission ID: 25794**

**Submission Title: Elucidating the Mechanism of Monoclonal Antibody-Induced Human Pluripotent Stem Cell Death**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Various studies have demonstrated the great potential of human pluripotent stem cells (hPSC) for tissue engineering, regenerative medicine and drug testing. However, the risk of in vivo teratoma formation by residual undifferentiated hPSC is still a major safety concern.

**Background**

Previously, our group has demonstrated the ability to prevent teratoma formation using a cytotoxic IgM antibody, mAb84, which binds to and kills undifferentiated hPSC. However, penetration of the pentameric mAb84 into cell aggregates to kill residual hPSC may be impeded because of its size (MW~950kDa vs IgG ~150kDa). Intriguingly, we have successfully generated another cytotoxic antibody, TAG-A1 (A1).

**Hypothesis**

In this study, we benchmarked the characteristics of A1 against mAb84. Additionally, the death mechanism induced by A1 was investigated.

**Research**

Firstly, the binding and killing of A1 on hPSC were characterized by flow cytometry. A1 target antigens on hPSC were identified by immunoprecipitation-mass spectrometry. Subsequently, western blot and sugar inhibition assay were performed to find out the target glycan type and its epitopes. Caspase assay and TUNEL assay were used to determine the mode of A1-induced cell death. Morphological changes of A1-treated hPSC were investigated by Scanning Electron Microscope and Transmission Electron Microscope.

**Observations**

We found that A1 kills hPSC as efficiently as mAb84 in a time and dosage-dependent manner. Most of the killing occurs within the first 5 minutes of incubation. Unlike mAb84 that targets podocalyxin (PODXL) on PSC, A1 binds to multiple proteins on PSC via O-linked glycan epitopes. Its target antigens can be divided into four protein groups: cell adhesion proteins, cytoskeleton-associated proteins, ATPase proteins and transporter proteins.

Mechanistically, A1 killing was not a consequence of apoptosis i.e. absence of caspase activity and DNA fragmentation. Instead, significant changes in both intracellular and extracellular morphology were observed. Under Scanning Electron Microscope, the formation of cell aggregations, loss of microvilli, formation of membrane pores as well as cell swelling followed by cell shrinkage were hallmarks of A1-induced killing. Under Transmission Electron Microscope, we observed varying degrees of cytoplasm lysis accompanied by cellular volume alteration, nucleus dilation, and mitochondria enrichment as well as peripheral relocalization. All these observations suggest that A1 induces hPSC death via an oncotic-like pathway. We also found that A1 cytotoxicity on hPSC can be partially reduced by inhibitors of actin polymerization. Moving forward, our plan is to elucidate the links between these observations and consequently propose a model for A1-induced hPSC death.

**Submission ID: 27113**

**Submission Title: Stop the disposal of cord blood stem cells- Creating awareness through social networks**

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**Topic**

Patient Advocacy and Communications

**Problem**

The sources of stem cells are varied. These can be classified as

- a). Organ specific, which are invasive, few in numbers and economically not affordable.
- b). Stem cells available from products identified as biomedical waste during various medical procedures like labour (umbilical cord, cord blood, placenta etc), circumcision (foreskin), menstrual discharge etc. Though all are economically affordable, the abundant source of stem cells are available in umbilical cord blood and placenta.
- c). Induced stem cells and other forms of reprogrammed cells - currently they are not available in adequate quantities for research and therapy.

The hinge in acquiring the required stem cells lies in donor's acceptance which is highly influenced by ethics, practicing religions, customs and largely not informed. The best way to achieve and ensure the availability of stem cells to biomedical research and therapy are by educating individuals via social networking sites, media & internet etc; on the therapeutic potentials of stem cells in medicine and research.

**Background**

The potentials of stem cells are well known to scientific community today, still millions of affordable and reliable sources of stem cells from cord blood, lining, placenta are disposed as biological waste in both developing and developed countries. While considering the ethics of geographical boundaries and individual's decision on umbilical cord, it is noteworthy that majority of the parents or individuals concerned with umbilical cord etc are not aware of its biological & clinical importance, at least to the lesser extent; its use in biomedical research. Therefore it becomes necessary for both public and private sector to invest on creating awareness among individuals on umbilical source of stem cells for various therapeutic, if not least for research use. This can be achieved at large by writeups and other forms of educative advertisements in social networking sites, internet sites, blogs (best example of success is from Paul Knoepfler receiving GPI's National Advocacy Award for this year 2013). The current scenario in developing countries on the role of stem cell therapies are encouraging, as the creamy layer of educated advocate, while to an extent that economically strong avail this facility. Most ( almost 95%) of people either are not aware of stem cell therapies/ research or sources of stem cells or not up to the reach of stem cell therapies.

## Hypothesis

Do social networking sites have the power to create awareness to stop the disposal of cord blood and cord products stem cells as Biomedical waste?. Does it persuade public/private or state/industrial partnerships to successfully ascribe the availability of economically affordable stem cells to therapy and research?.

## Research

The importance of stem cell therapies can be harnessed via supporting stem cell research in biomedical/translational research and advocating stem cell therapies through various government agencies for social educations and at large internet and social networking sites. While locating the economically affordable source of stem cells, the umbilical cord blood, cord lining, placenta etc come first. Therefore the importance of centralized public cord products storage banks at state levels would be sufficient to support ongoing research in various fields of translational, industrial and basic research. Considering the economic burden on the public cord banks mostly run as state owned, private or non-profit organizations, the use of stem cells for industrial research has been appealing thus paving a strong path for beneficial public/private partnerships (PPP). There may be  $n$  number of ethical, logical, religious, cultural, economic reasons and bunch of scientific objections on cord blood stem cells , which may be based on current real time observations, however it would be like an early abortion to alienate and dematerialize the concept of cord blood banks either public or private.

## Observations

Increased awareness in expected parents to store the umbilical cord blood or donate for research has been well documented in various developed and developing countries. Such success has been possible only via education and advertisements through various social networking sites and media . Recently there has been new conceptual ideas for public umbilical cord banks with special reference to utilize the private owned autologous cord blood units on the requirement. Securing and saving more units of cord blood (initially) would be beneficial in coming days to fill up the gap for combined allogenic matched donor units in various therapies. Thus education through social networking sites will be one of the best ways to create awareness to stop the disposal of easily available stem cells and to save it for future use through public or private cord blood banks. Further this will enable both public and private banks along with state or industrial funding to establish huge cord blood banks as a source for stem cells for therapies and research. Huge storage facilities of stem cells would be adequate to cover the mosaic of HLA signatures with regional specificities, which answers a big question among the cord blood banking advocates. Utilizing the child's cord blood and cord products under public donor setup might look as huge investment and may not even sound ethical or can be considered as interference of individual's right, but still a humanitarian need gets fulfilled and a real public/private endeavor would be achieved.

Let me finish like this: " If at all beyond all ethics lies humanitarian need, then need fulfills the requirement of ethics".

**Submission ID: 27354**

**Submission Title: IMMUNOHISTOCHEMICAL EVALUATION OF ODONTOGENIC DIFFERENTIATION POTENTIAL OF HUMAN BUCCAL TISSUE FIBROBLASTS IN VITRO**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Pulpitis and periapical diseases that are types of chronic, bacterial and infective diseases are often found in oral cavity. Currently, the root canal therapy is the most effective and thorough treatment for pulpitis and periapical disease. Despite reported clinical success, endodontically treated teeth become devitalized and brittle, susceptible to postoperative fracture and other complications, including reinfections due to coronal leakage or microleakage. Removal of substantial amount of tooth constituents including enamel and dentin, during endodontic treatment, potentially leads to post treatment tooth fracture and trauma, therefore, endodontical manipulation is not considered as a first line treatment of a teeth. The complications of current endodontic treatment are inevitable because of pulp devitalization or the loss of the tooth's innate homeostasis and defense mechanisms. One novel approach to restore tooth structure is based on biology: regenerative endodontic procedures by application of tissue engineering. Tissue engineering practices are needed to implement the regenerative approaches in the field of endodontic; so that differentiating the cells from different tissues to maintain tooth vitality, will provide a significant advantage to increase the success of endodontic treatments.

**Background**

Tooth regeneration using tissue engineering concepts is a promising biological approach that aims to regenerate natural tooth-like mineral tissues in terms of structure and function. Tooth development is regulated by the interactions between oral epithelium and cranial neural crest-derived ectomesenchyme. In papers studying these interactions at a level of differentiation of cells from non-dental tissues to embryonic dental epithelium and mesenchyme, it has been demonstrated that odontogenic differentiation signals are capable to differentiate the cells obtained from various non-dental tissues such as embryonic cells, nerve cells and bone marrow cells into the dental epithelium. Differentiation potential of dental stem cells is largely investigated in current studies of dentistry and these researches yield promising results. In addition to the studies

showing that cell populations in the mesenchyme can be used as a graft material in the regeneration of various tissues in adults, it is also stated that there are cells functioning as reserve in such tissues as muscle, connective tissue, bone marrow and these cells can be used as source in tissue regeneration. Although odontogenic differentiation potential of cells from dental pulp, periodontal connective tissue and deciduous teeth has been shown in several studies, these cells are less applicable in clinical practice. Experimental studies to investigate the availability of cells from different regions will provide significant results. However, studies of differentiating cells from different tissues into dental cells are few, and there is no study investigating whether stromal cells in adult buccal submucosa function as dental reserve cells in literature.

## **Hypothesis**

Due to the ease of tissue sampling at any age, buccal submucosa can be regarded as a suitable tissue, in terms of proliferation characteristics and efficiency, as a cell source, instead of dental pulp and periodontal connective tissue. Furthermore, the fact that any damage to occur in the region will heal in a short time, depending on the high proliferation and regeneration ability of fibroblasts in connective tissue under the buccal epithelium supports the idea of using buccal submucosa as source. In this study, we investigated whether buccal submucosa fibroblasts grown in culture have odontogenic differentiation capability, and can be regarded as a reproducible source for in vitro regeneration studies.

## **Research**

A buccal mucosa sample was obtained from three volunteers at a standard age range, during root canal therapy with informed consent. The study was conducted in accordance with the Ethics Committee of the Istanbul Medical Faculty, Istanbul, Turkey. The buccal mucosa samples were cut into pieces with a scalpel and were transferred into a 10mm Petri dish containing maintenance media to allow cell attachment and seeding. Upon confluency, the cells were detached and transferred into 75cm<sup>2</sup> culture flasks. For differentiation experiments buccal mucosa fibroblast (BMF) of passage 4 were used. The cells were seeded onto a 12-well culture plate at a density of  $1 \times 10^5$  cells/cm<sup>2</sup>. The osteo/odontogenic differentiation was carried out in DMEM/F12 supplemented with 10% FBS, 50  $\mu$ L-ascorbic acid 2-phosphate, 10 mM of  $\beta$ -glycerophosphate, 100 nM of dexamethasone for 7, 14 and 21 days. The control cells were cultured in maintenance media. Expression of odontogenic differentiation markers Runx-2, Dentin Matrix Protein 1 (DMP1), Dentin Specific Sialoprotein (DSPP), collagen type I and osteocalcin were evaluated by immunohistochemical staining with anti-Runx2, anti-DSPP, anti-DMP1, anti-osteocalcin, anti-collagen type 1 antibodies. Also mineralization was assessed histochemically with Von Kossa staining. Staining pattern and mineralized nodules were visualized and photographed by digital camera attached to a light microscope. Results were compared with control cells cultured in maintenance medium.

## **Observations**

Prominent nuclear immunostaining with anti-Runx2 were observed in differentiation groups beginning from day 3 to day 7, and staining pattern was observed to be decreased in day 14, and disappeared until day 21. Cells grown in ordinary maintenance medium were not stained with anti-Runx2 antibody on all time endpoints. Any staining was observed for DSPP, in cells grown in differentiation medium on day 7. Faint cytoplasmic staining was seen in day 14 and homogenous and prominent staining pattern were detected on day 21. Cells grown in maintenance medium were not stained with anti-DSPP antibody on any of the time endpoints. Immunostaining with anti-DMP-1 antibody were resulted positive reaction in cell nuclei grown in differentiation medium beginning from day 14 and this staining were increased gradually until the end of the study on day 21. Cells grown in maintenance medium were not stained with anti-DMP-1 antibody on any of the time endpoints. Immunostaining with anti-osteocalcin antibody were resulted positive reaction in cytoplasm of cells grown in differentiation medium beginning from day 14 and become prominent on day 21. Cells grown in maintenance medium were not stained with anti-osteocalcin antibody on any of the time endpoints. Any staining was observed for collagen type 1 on day 7, in cells grown in both differentiation and maintenance media. Immunostaining with anti-collagen antibody were resulted

positive reaction in cytoplasm and intercellular space of cells cultured in differentiation medium beginning from day 14 and become prominent on day 21.

The results obtained from the current study indicate that buccal submucosa cells have the potential to change. Definitive conclusion about the differentiation pattern of these cells would be possible by studies performed with further methods such as PCR, northern blot, micro array, which can be important in terms of supporting these results. Considering that intermediate markers in osteo/odontogenic differentiation have yet to be fully enlightened, it is not a far prospect to use buccal submucosa cells as a source for stem cells.

**Submission ID: 27431**

**Submission Title: Induced Pluripotent Stem Cell Transplantation in the Treatment of Chronic Myocardial Ischemia**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Discovering a new way for induced pluripotent stem cell (iPSC) based therapy in cardiovascular disease. We found that iPSC cells can be allogeneically transplanted into immunologically competent host without creating notable graft against host disease (GvHD).

**Background**

In order to create a large animal model to treat cardiovascular diseases using iPSCs, we generated iPSCs from pig myocardial fibroblasts, which presumably have a higher tendency to differentiate into cardiac lineages due to epigenetic memory. This study was designed to test iPSC effects in vivo and compare to MSCs'.

**Hypothesis**

iPS cells will have more ability to differentiate into cardiomyocyte lineage while transplanted into the ischemic myocardia environment.

**Research**

P3 myocardial fibroblasts were used for reprogramming using the lentiviral vector containing 4 human factors: OCT4, SOX2, KLF4, and cMYC. The iPSC Colonies at P12-17 were allogeneically transplanted into chronically ischemic myocardium of eight swine by direct intramyocardial injection. Cohorts of two animals were sacrificed at 2, 4, 6 and 8 wks after injection.

**Observations**

No signs of graft versus host disease were found at any time points. At 2 wks, clusters of SSEA-4 positive iPSCs were detected in the injected area. Four to 8 wks later, these cells started to proliferate into small spheres surrounded by thin capsules. The cells inside these masses demonstrated a homogeneous phenotype with no sign of differentiation into any specific lineage. In MSCs injected animals, cell clusters were also clearly found in the injected area. However in contradistinction, there was no sign of cell proliferation, no capsules around the MSC clusters, and the number of MSC decreased gradually in the period of 8wks post-injection. Increased smooth muscle actin or vWF positive cells were found inside and around the iPSC clusters, compared with non-injected areas. By RT-PCR, the levels of VEGF, FGF, and ANRT expression were significantly higher in the iPSC treated myocardium compared to untreated areas. These results suggest that injected iPSCs might have contributed to the formation of new blood vessels to a level that is comparable with those injected with MSCs.

Allogeneic transplantation of iPSCs and MSCs in a large animal model can be used to study stem cell behavior in vivo. Despite an ischemic environment pig iPSCs continue to proliferate in vivo after injection. However the proliferation ability of the iPSCs was limited within the immunocompetent hosts. Injected MSCs survived in the ischemic environment but showed no signs of proliferation. Both iPSCs and MSCs demonstrated paracrine proangiogenic effects.

**Submission ID: 27786**

**Submission Title: Retinal organotypic culture conditioned media induces neural marker expression in human derived stem cells from dental pulp**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

This work aimed to explore the interaction of dental pulp stem cells with the retinal non regenerative environment.

**Background**

Dental pulp stem cells arise from the neural crest during development and migrate to form several craniofacial structures and tissues including the dental pulp, which is composed of differentiated and undifferentiated cells that maintain their multipotentiality through adulthood and are known to be capable of differentiating in vitro to mesenchymal and neuronal lineages. Their valuable relation with the CNS is well established since they promote survival of dopaminergic embryonic neurons, trigeminal ganglion neurons and spinal cord motoneurons in different injury models through the following mechanisms: neurotrophic support, apoptosis inhibition, axon growth inhibitors signaling inhibition and replacement of damaged cells through oligodendrocyte differentiation. Besides their ability to differentiate into functionally active neurons in vivo on a chick embryo model as well as in the adult mice hippocampus led us to think it would be an asset -due to their availability and source- to evaluate their regenerative potential within another site of the CNS: the retina. The mammal retina is a non regenerative tissue, responsible of visual transduction and perception. It is integrated of two nuclear layers and three synaptic layers that contain several neuronal (ganglion, amacrine, horizontal, bipolar and photoreceptor cells) and glial (Muller cells, astroglia and microglia) elements.

**Hypothesis**

Ectomesenchymal stem cells within the dental pulp are capable of differentiating into retinal neurons when cultured with retinal organotypic conditioned media.

**Research**

After culturing dental pulp stem cells with retinal organotypic culture conditioned media of control and MNU injured retina, bright field microscopy, immunocytochemistry and RT-PCR were performed in order to assess neural markers expression.

## Observations

Dental pulp stem cells (DPSCs) cultured with control retina conditioned media from day 14 ex vivo showed morphological changes that resembled neuronal morphologies after treatment with conditioned media, developing cytoplasmic projections and stellate somas different from the characteristic fibroblast like morphology of control DPSCs.

When cultured with MNU injured retina conditioned media from day 2 and 5, DPSCs developed round or elongated somas. Besides the morphological changes, DPSCs were immunoreactive to polysialic acid neuronal cell adhesion molecule (PSA-NCAM) when cultured with both control and MNU injured retina media. However they remained immunoreactive to glial fibrillary acidic protein (GFAP), a typical marker of DPSCs.

The cells were also capable of expressing Rhodopsin, a photoreceptor cells marker, but only after treatment with MNU injured retina conditioned media.

Furthermore, dental pulp stem cells expressed the neural markers OTX2, SOX2, ASCL1 and POU4F1 (retinal ganglion cells marker) when cultured with retinal organotypic culture conditioned media.

**Submission ID: 27883**

**Submission Title: TGF- $\beta$ 3 gene knockdown enhances stem cell properties in adipose derived stromal cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Notwithstanding many ongoing clinical trials of stem cell therapy targeting a variety of diseases are testing transfusion of stem cells originated from adult tissues such as adipose tissue, significant improvement after stem cell transplant has yet to be seen that likely because the need to raise the stem cell number as well as to improve the quality of transplantable cells is not met. Whereas both the number and quality of stem cells decline with aging, adipose derived stromal cells (ADSCs) have been considered as the most abundant mesenchymal stem cell-like cells of the elderly. A strategy to maximize the stemness of ADSCs will, therefore, enhance their therapeutic prospective and promote the realization of stem cell therapy.

**Background**

TGF- $\beta$  superfamily members such as transforming growth factor beta (TGF- $\beta$ s), bone morphogenic proteins (BMPs) regulate a wide array of cellular processes including proliferation, differentiation, immune regulation. Through cross-talk between their respective pathways, TGF- $\beta$ s and BMPs can modulate the activity of each other. Most of our knowledge was acquired from the studies on TGF- $\beta$ 1 and that other TGF- $\beta$ s may display distinct, even opposing, functions has been shown elsewhere. Unlike TGF- $\beta$ 1, the role of TGF- $\beta$ 3 in many biological processes has not been extensively explored.

**Hypothesis**

We proposed that knockdown of TGF- $\beta$ 3 expression may enhance the stem cell features and increase their immune-modulating capacity in ADSCs.

**Research**

ADSCs isolated from wildtype and TGF- $\beta$ 3(+/-) mice carrying only one intact TGF- $\beta$ 3 allele were cultured. These ADSCs were subjected to adipogenic or osteogenic induction to compare their differentiation potential. To test the immune-modulating function, serum-free condition medium that was used to culture ADSCs for 24 h were collected, followed by concentrating step, and included in the stress challenge assays involving treating lung epithelial cells or fibroblasts with cigarette smoke extract (CSE). The cell death and the cytokines induced by CSE in these cells was used as the indication of cell injury, and the

protection offered by ADSC condition medium was used to measure immune suppression capacity and pro-survival benefit of ADSCs. We also performed gene array analyses to study the involved pathways affected by TGF- $\beta$ 3 knockdown in ADSCs.

## **Observations**

Compared to wildtype, TGF- $\beta$ 3(+/-) ADSCs displayed significantly higher differentiation potential toward to both adipogenic and osteogenic lineage. The conditioned medium that was used to culture TGF- $\beta$ 3(+/-) ADSCs provided better protection against CSE-induced injury in lung epithelial cells and fibroblasts as indicated by an increased cell survival and by reduction of inflammatory cytokines in cells treated by TGF- $\beta$ 3(+/-) condition medium. Microarray data revealed interesting findings that losing one functional TGF- $\beta$ 3 allele in ADSCs not only reduced the level of TGF- $\beta$ 3 expression but also significantly decreased the expression of other TGF- $\beta$  family members such as Bmp4. These results were confirmed further by experiments using ADSCs with TGF- $\beta$ 3 or BMP4 expression knockdown by siRNA transfections. Taken together, our study indicate that reduced TGF- $\beta$ 3 or BMP4 expression in ADSCs can simultaneously increase their differentiation potential toward multiple lineages and enhance their immune modulating capacity, both likely contributing to clinical success of stem cell therapy.

**Submission ID: 27931**

## **Submission Title: Growth of Human Adipose-Derived Mesenchymal Stem Cells in Three Dimensional Culture**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Mesenchymal stem cells (MSCs) have anti-inflammatory and tissue repair properties that suggest these cells will play an important role in future regenerative therapies. Supplying effective doses of MSCs requires efficient generation of a large numbers of these cells, yet traditional cell culture techniques (using traditional cell culture plasticware) are costly, require substantial space, and are labor intensive. Alternative culture methodologies using bioreactors have shown promise to meet this clinical need, but require optimization to translate these technologies to prepare cells for clinical use.

### **Background**

Human MSCs are multipotent cells capable of differentiating into osteoblasts, adipocytes, and chondroblasts, and are actively being explored for the treatment of multiple diseases. Conventional cell culture uses two-dimensional plasticware and requires enzymatic manipulation to remove or passage cells, ultimately limiting the number of cells that can be practically prepared for the clinic. As the number of cells required for treatment grows, and trial enrollment increases, three dimensional culture techniques, using bioreactors, may be more practical and may better reflect the cell growth in vivo.

### **Hypothesis**

We hypothesized that two dimensional tissue culture methods for human, adipose-derived MSCs could be successfully adapted to expand and recover MSCs in three dimensional systems using microcarriers to provide a growth platform. Specifically, we hypothesized that the growth in three dimensions would yield a comparable number of cells and similar growth rates to traditional cell culture.

### **Research**

Small scale studies using microscopy, multi-well plates, microcarriers (Plastic Plus Microcarriers (Product Code PP102-1521) from Solohill; Ann Arbor, MI), and spinner flasks were conducted to optimize a protocol for cellular attachment, growth, and dissociation of MSCs. Cellular growth, morphology, and viability of MSCs were compared in three growth conditions (traditional plastic culture flasks, spinner flasks, and a bioreactor (Mobius CellReady, Millipore, Bedford, MA)) over eight days. Cell counts, photomicrographs, and metabolic profiles were obtained on the samples to follow cellular growth and

performance.

### **Observations**

Human MSCs were successfully adapted to two different three dimensional culture systems and grown, harvested, and passaged by a procedure developed for this application. We found that: 1) suspension cultures maintained viable cells throughout the duration of the study; 2) cellular morphology in suspension cultures differed from that in two dimensional culture; 3) traditional cell culture can successfully be adapted to three dimensional culture with comparable yields. Further refinement to three dimensional culture methods of MSCs is necessary, as the significant reduction in time and labor utilizing such methods favors this technique for further clinical stem cell development.

**Submission ID: 27936**

**Submission Title: Variable effects of tumor secreted factors on human mesenchymal stem cell**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Mesenchymal Stem cells have a presumed role in tumorigenesis, the goal is to asses that role and identify the mechanisms in which it is carried.

**Background**

Recent years have witnessed huge interest in studying the tumor microenvironment, given its apparent role in driving tumor progression and metastasis. Of particular interest, mesenchymal stem cells (MSCs) have been the focus of many research groups as the exact role of MSCs in driving cancer progression remains controversial. Herein, we investigated the effects of tumor secreted factors from a panel of human cancer cell lines (breast (MCF7 and MDA-MB-231); prostate (PC-3); lung (NCI-H522); and head & neck (FaDu)) on MSCs.

**Hypothesis**

MSCs have an active role in tumorigenesis by an inflamatory mechanism, proved by alterations in morphology, gene expression, and migration studies.

**Research**

Morphological changes were asseses using fluorescent microscopy. Changes in gene expression were assessed using Agilent microarray and qRT-PCR. Cell migration was assessed using transwell migration system.

**Observations**

Morphologically, MSCs exposed to secreted factor from FaDu, MDA-MB-231, PC-3, and NCI-H522, but not from MCF7, exhibited a spindle-shaped morphology, and the cells were more elongated with bipolar processes, compared to control MSCs which were larger and more flattened with multiple processes. Integrated analysis of gene expression and bioinformatics

revealed a proinflammatory response of MSCs when exposed to conditioned media (CM) from all cell lines, but not MCF7. Nonetheless, MSCs exhibited significant tropism toward secreted factors from the aforementioned tumor cell lines.

**Submission ID: 28685**

**Submission Title: Tumour-Derived Interleukin-1 Beta Induces Pro-inflammatory Response in Human Mesenchymal Stem Cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Studying cancer tumors microenvironment may reveal a novel role in driving cancer progression and metastasis. The biological interaction between stromal (mesenchymal) stem cells (MSCs) and cancer cells remains incompletely understood. Herein, we investigated the effects of tumor cells' secreted factors as represented by a panel of human cancer cell lines (breast (MCF7 and MDA-MB-231); prostate (PC-3); lung (NCI-H522); colon (HT-29) and head & neck (FaDu)) on the biological characteristics of MSCs.

**Background**

Over the past several years, significant amount of research has emerged documenting a role for MSCs in promoting epithelial-to-mesenchymal transition (ETM), and accelerating tumor growth and metastasis. In addition, MSCs are being introduced into therapy for a number of clinical indications and there is a concern of possible promoting effects on tumour growth by MSCs. On the other hand, several other studies reported that MSCs exert tumor suppressive effects. Therefore, understanding the crosstalk between MSCs and tumor growth is very crucial for the safe utilization of MSCs in regenerative medicine.

## **Hypothesis**

Given this complex interplay between MSCs and tumor cells, the goal of this study was to assess the cellular and molecular changes in MSCs in response to secreted factors present in conditioned media (CM) from a panel of human tumor cell lines covering a spectrum of human cancers (Breast, Prostate, Lung, colon, and head and neck).

## **Research**

Morphological changes were assessed using fluorescence microscopy. Changes in gene expression were assessed using Agilent microarray and qRT-PCR. GeneSpring X and DAVID tools were used for bioinformatic and signaling pathway analyses. Cell migration was assessed using transwell migration system. SB-431542, PF-573228, and PD98059 were used to inhibit TGFb, FAK, and MAPKK pathways, respectively. IL1b was measured using ELISA.

## **Observations**

MSCs exposed to secreted factors present in conditioned media (CM) from FaDu, MDA-MB-231, PC-3, and NCI-H522, but not from MCF7 and HT-29, developed an elongated, spindle-shaped morphology with bipolar processes. In association with phenotypic changes, genome-wide gene expression and bioinformatics analysis revealed an enhanced pro-inflammatory response of those MSCs. Pharmacological inhibitions of FAK and MAPKK severely impaired the pro-inflammatory response of MSCs to tumor CM (~80-99%, and 55-88% inhibition, respectively), while inhibition of the TGFb pathway was found to promote the pro-inflammatory response (~3-fold increase). In addition, bioinformatics and pathway analysis of gene expression data from tumor cell lines combined with experimental validation revealed tumor-derived IL1b as one mediator of the pro-inflammatory phenotype observed in MSCs exposed to tumor CM.

MSCs exhibited significant tropism toward secreted factors from the aforementioned tumor cell lines, while both normal and MSCs exposed to tumor conditioned media were capable of attracting human peripheral blood mononuclear cells (PBMCs).

**Conclusions:** Our data revealed tumor-derived IL1b as one mediator of the pro-inflammatory response in MSCs exposed to tumor CM, which was found to be positively regulated by FAK and MAPK signaling, and negatively regulated by TGFb signaling. Thus, our data support a model where MSCs could promote cancer progression through becoming pro-inflammatory cells within the cancer stroma.

**Acknowledgment:** This work was supported by grant 11-MED1942-02 from the National Plan for Sciences and Technology, King Saud University

**Submission ID: 28975**

**Submission Title: Human Embryonic Stem Cell Research Activity in California, 2010-2012**

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**Role:** Author

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**Topic**

Ethics, Law and Society

**Problem**

The California Department of Public Health (CDPH), Human Stem Cell Research (HSCR) Program has the legislative mandate to develop statewide guidelines for research involving human embryonic stem cells, with the exception of research fully funded by the California Institute for Regenerative Medicine (CIRM). Thereby, the HSCR Program has authority in the oversight of stem cell research that receives funding from other sources. The challenge of the HSCR Program was to ensure the reporting of human stem cell research activity in California that is not fully funded by CIRM.

**Background**

In July 2007, the first version of the CDPH Guidelines for Human Stem Cell Research were finalized and published. These guidelines were developed in collaboration with CIRM to ensure alignment and are revised accordingly. The CDPH guidelines require that all research projects involving the derivation or use of human embryonic stem cells (hESCs) be reviewed and approved by a Stem Cell Research Oversight (SCRO) Committee prior to being undertaken and on an annual basis thereafter. The SCRO Committees are required to apply the CDPH guidelines and report to the HSCR Program on the status and disposition of the hESC projects they oversee.

**Hypothesis**

The requirement of SCRO Committees to annually report the status and disposition of each project they review and oversee will allow the HSCR Program to monitor human embryonic stem cell research in California that is not fully funded by CIRM.

## Research

To ensure effective oversight of hESC research in California, the HSCR Program developed and implemented a system for ongoing data collection, monitoring and analysis. The HSCR Program developed a standard reporting form for SCRO Committees. These reports are collected and maintained in a database for analysis. All reports were reviewed to identify trends related to hESC research for reporting years 1 through 5. This abstract presents data from reporting years 4 and 5, which covers July 1, 2010 to June 30, 2012.

## Observations

The HSCR Program began collecting its first SCRO Committee reporting forms on August 1, 2008. All subsequent reporting forms, which cover a reporting period of July 1 through June 30, were collected on an annual basis thereafter. In the two year period, 36 SCRO Committees provided information on 623 hESC research projects. These reporting institutions comprised of universities, private companies, medical centers and research institutes.

The SCRO Committees are required to report on the status and disposition of each project. Nearly all research projects were still in progress at the end of reporting years 4 and 5, 96% (n=294) and 97% (n=306), respectively. During the two reporting years, 4 projects were completed and 17 were terminated early or closed. A project that was terminated early or closed does not imply improper research conduct or noncompliance. It could indicate, for example, that the project did not receive renewal funding or the researcher did not complete the project.

The SCRO Committees report on the type of each research project and can select these non-mutually exclusive project types: use of hESC *in vitro*, use of hESC *in vivo*, creation/derivation of hESCs or cell lines, use of human oocytes or embryos, somatic cell nuclear transfer (SCNT), parthenogenesis and clinical trial. For both reporting years, two types of research were most common: use of hESC *in vitro* and *in vivo*. Nearly all of the research projects in years 4 and 5 involve research of hESC *in vitro* (93% and 95%, respectively) and almost half of the projects involve research of hESC *in vivo* (48% and 52%). A total of 61 projects in the two reporting years were involved in deriving new hESC lines. Less common were projects that involved the use of human embryos, human oocytes, SCNT and parthenogenesis.

The research project titles were used to determine more specific information regarding the focus of the research projects. Diseases specifically studied were Alzheimer's, amyotrophic lateral sclerosis (ALS), cancer, HIV, Huntington's disease, Parkinson's disease and stroke. Other research included those related to blood, cartilage/bone, heart, pancreas, retina and spine/nerve. Some projects also investigated the mechanisms of stem cell biology, such as modeling of diseases, self-renewal and differentiation.

One of the key elements of hESC research oversight includes monitoring whether ethical problems arise during a research project and responding to these issues. SCRO Committees are required to report on any unanticipated problems, unforeseen issues, or serious instances of investigator noncompliance. In the two year period, SCRO Committees did not report any issues or problems.

The collection of mandated reporting from all SCRO Committees that have reviewed research projects involving the derivation or use of hESCs (excluding projects fully funded by CIRM) is an essential component of the effective monitoring of stem cell research. While the SCRO Committee reports received may not be representative of all hESC projects in California, they do provide an overview of the diversity of hESC research types and activities occurring within the state.



**Submission ID: 29681**

**Submission Title: Stimulation of Adult Stem Cell Derived Cardiospheres via Pulsed Infrared Radiation Leads to Contractility Mediated by Mitochondrial Calcium Cycling**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

*In vitro* adult stem cell derived cardiomyocyte studies, have been limited due to the immaturity of the stem cell derived cardiomyocytes. These cells express proteins and genes representative of cardiomyocytes, but do not undergo spontaneous contractile motion, which is the ultimate functional role of *in vivo* cardiomyocytes.

**Background**

Cellular cardiomyoplasty has been shown to help improve cardiac functional parameters post infarct including increased ejection fraction and decreased scar size. In order to gain a better understanding of the *in vivo* differentiation mechanisms, *in vitro* studies of adult stem cell derived cardiomyocytes (ASC-CM) are necessary. Recent work has shown that pulsed infrared radiation (IR,  $\lambda=1862\text{nm}$ ) can be used to pace neonatal cardiomyocytes undergoing spontaneous contractions. The present study investigates the response of adult stem cell-derived cardiospheres (CS) to IR stimulation.

**Hypothesis**

We hypothesize that pulsed infrared radiation may be able to initiate the contraction of stem cell derived cardiospheres.

**Research**

Adult stem cells were isolated from periodontal ligament derived from wisdom teeth explants. Differentiation was induced by culturing cells on collagen I coated silicone membranes. When cells reached 40% confluence, complete culture media was replaced with a growth factor cocktail. Growth factor conditioned media is replaced every other day for two weeks. After two weeks of culture, cells are lifted from the silicone membrane using Trypsin/EDTA. ASC-CM are then resuspended at a concentration of 500,000 cells/mL. With this suspension, ASC-CM are cultured according the hanging drop aggregation assay, to induce CS formation. After one day CS's are macroscopically visible and moved to gelatin coated six well plates to promote adherence for stimulation studies. CS are stimulated using a Lockheed Martin Capella IR source ( $\lambda=1863\text{nm}$ ) coupled to a 400  $\mu\text{m}$  optical fiber. Frequency was varied from 0.5 to 4 Hz during the experiments. To quantify the results of stimulation, cells

were loaded with the intracellular calcium indicator Fluo-4 AM. Image sequences during IR stimulation were collected using a fluorescent microscope. Average fluorescence values of cells in each frame were computed using region of interest (ROI) analysis (ImageJ). These intensity values were imported in Matlab (MathWorks, Natick, MA, USA) to plot the normalized fluorescence variations.

## **Observations**

The aggregates and single cells underwent contractile motion in concert with the applied IR pulses. The fluorescence imaging showed pulse-by-pulse changes in the cells. In order to determine the mechanism of the induced  $\text{Ca}^{2+}$  release, pharmacological studies were performed. A pharmacological array including ryanodine (20  $\mu\text{M}$ ), a blocker of the ryanodine receptor channel in the sarcoplasmic reticulum and CGP-37157 (20  $\mu\text{M}$ ), an inhibitor of the mitochondrial sodium calcium exchanger (mNCX) was used. Upon application of CGP-37157 the observed  $\text{Ca}^{2+}$  was dramatically reduced, and rate of contraction was significantly altered. Ryanodine did not alter the IR-evoked responses. In addition to IR, we are exploring electrical stimuli for inducing contraction of stem cell derived cardiomyocytes.

**Submission ID: 29924**

**Submission Title: Novel 3-Dimensional Hydrogel Constructs with Patterned and Functionalized Nanofiber Scaffolding for Enhanced Neurite Outgrowth and Directional Control**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Damage to neural tissue is one of the leading causes of death and permanent disability in the world and presents one of the greatest challenges in current medical care. Stroke, traumatic brain injury, spinal cord injury, and neurodegenerative diseases all cause a tremendous amount of long-term suffering, yet medical interventions that can directly repair and restore neural tissue structure and function remain extremely limited.

**Background**

Neural tissue engineering holds incredible potential to restore functional capabilities to damaged neural tissue in a wide range of diseases and conditions. Reconstruction of functional neural tissue is best achieved in 3-dimensional culture where the innate structure-function relationship found in neuronal tissue can be achieved. Ideal engineering of neural tissue will enable cellular and subcellular control over network formation, neuroanatomical structure, synaptogenesis, and cell differentiation. This work sought to investigate novel approaches for achieving these aims.

**Hypothesis**

It was hypothesized that the combination of patterned and functionalized fibers within 3-dimensional hydrogel cultures could help guide neural development and control neurite extension, thereby providing detailed control of neural regeneration and replicating innate neuroanatomical structures of the brain and spinal cord.

**Research**

3-dimensional hydrogels were constructed with a layer of patterned nanofibers to support neuronal cell cultures. Electrospinning techniques were used to create nanofibers, which were composed of poly-caprolactone (PCL) or PCL with a

biofunctional coating. A method of creating aligned nanofibers was developed. Fiber characteristics were analyzed using environmental scanning electron microscopy. A diffusion model was created to evaluate viable diffusion of oxygen and nutrients into the 3-dimensional hydrogel construct, and a method of analyzing neurite morphology with reference to aligned fibers was developed. Microscopic images were captured at high-resolution in single and multi-focal planes with GFP-expressing neuronal cells in a fluorescent channel and the nanofiber scaffolding in a separate channel. Neurite tracking of nanofibers and neurite morphology, including neurite segment lengths, angles, and tracking distances were then analyzed in detail.

## **Observations**

It was found that biofunctionalized nanofibers in 3-dimensional hydrogels enabled significant alignment of neurites with fibers, significantly increased the distance over which neurites could extend, and resulted in significant neurite tracking of nanofibers. This work demonstrates the ability to create unique 3-dimensional neural tissue constructs using a combined system of hydrogel and micro-patterned nanofiber scaffolding. Furthermore, it is shown that patterned and biofunctionalized nanofiber scaffolds can control direction and enhance length of neurite outgrowth. This approach thus offers several advancements in the development of implantable neural tissue constructs, including enhanced control of neural development and reproduction of neuroanatomical pathways. These unique tissue constructs also have the advantages of being scalable in complexity, biocompatible, and gel-stabilized for direct implantation as neural tissue grafts. This work therefore holds important potential as a novel therapeutic approach for many diseases of neural tissue, including spinal cord injury, traumatic brain injury, neurodegenerative diseases, and stroke.

**Submission ID: 29925**

**Submission Title: Introduction of stem cells derived from placenta as alternative cell source for umbilical cord blood transplantation**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

to overcome cell dose limitation of UCB transplantation, hematopoietic and mesenchymal stem cells of placenta can be isolated and transplanted with cord blood cells

**Background**

to overcome cell dose limitation of UCB transplantation, hematopoietic and mesenchymal stem cells of placenta can be isolated and transplanted with cord blood cells. in this manner , the problem of cell dose limitation will be solved due to increasing the number of primary hematopoietic stem cells and homing of cells through mesenchymal stem cells

**Hypothesis**

hematopoietic and mesenchymal stem cells of placenta can be isolated and transplanted with cord blood cells, to increase the number of primary hematopoietic stem cells and homing of cells through mesenchymal stem cells

**Research**

The placenta tissue was treated with collagenase and after omitting red blood cells with lysis buffer, the remaining cells were studied for presenting of hematopoietic and mesenchymal stem cells.

**Observations**

The result showed that  $6.02 \pm 0.6$  and  $3.48 \pm 1$  percent of isolated cells was CD34+ and CD34+, CD38- respectively. These cells generated all hematopoietic lineages and adherent stromal cells with property of mesenchymal stem cells were obtained from culture of placenta cells.

**Submission ID: 30367**

**Submission Title: Effects of environmental carcinogen, benzo(a)pyrene, on canine adipose-derived mesenchymal stem cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The environmental carcinogens have been shown to affect the human mesenchymal stem cells (MSC), but their complete mechanisms are still not known. In this study, we evaluated the effects of the environmental carcinogen, benzo(a)pyrene (BaP), using canine adipose-derived mesenchymal stem cells (K9ADMSC) as model for human ADMSC.

**Background**

Breast cancer is the most common cancer in women and will affect one in eight women in their lifetime. It is the second major cause of female patient mortality in the western world. One of the risk factors for the breast cancers are endogenous estrogens and the environmental carcinogens such as, bisphenol-A (BPA) and polycyclic aromatic hydrocarbons like benzo(a)pyrene (BaP). The dogs with naturally-occurring tumors offer a unique opportunity to study human cancers given the spontaneous development of the disease, their similarities in physiological development, and exposure to environmental carcinogens. The dogs offer an opportunity to study human cancers, obesity and weight loss given the spontaneous development of these diseases. Breast cancers have been shown to be affected by their microenvironment composed of surrounding stroma, which include MSCs, fibroblasts, endothelial cells, and fat cells. MSC are multipotent cells that can differentiate into a variety of cell types, including: osteoblasts, chondrocytes, and adipocytes. The purpose of this study was to isolate and characterize adipose-derived mesenchymal stem cells (K9ADMSC) from female dogs and evaluate the effects of the environmental carcinogens, BaP and BPA on the proliferation and differentiation of K9ADMSC after exposure to BaP and BPA.

**Hypothesis**

In this study, we evaluated the effects of two carcinogens, BaP and BPA on K9ADMSC to assess the effects of these carcinogens on cell proliferation and adipogenic differentiation of K9ADMSC through aryl hydrocarbon receptor (AhR) signaling pathway.

## Research

The K9ADMSC were isolated from unutilized abdominal fat tissues after spaying of female dogs. The K9ADMSC were characterized by expression of MSC markers (CD90), adherence to surface, and their ability to differentiate into adipocytes, chondrocytes and osteocytes. Further the effects of the two carcinogens were studied on the K9ADMSC. Interestingly, BaP and BPA did not affect proliferation of K9ADMCS after short-time (48 h) neither after long-time (2 weeks) exposures. However; BaP inhibited adipogenesis of K9ADMSC in a dose-dependent manner; but on the other hand, BPA did not affected adipogenesis of K9ADMSC using OilRed O staining. The AhR is known target of BaP. BaP down-regulated AhR expression in K9ADMSC; however increased its translocation from cytoplasm to nucleus in K9ADMSC during adipogenesis. CYP1A1 is a member of the cytochrome P450 superfamily, which catalyzes many drugs and its expression is induced by polycyclic aromatic hydrocarbons. CYP1A1 cytoplasmic expression was increased by BaP in K9ADMSC-derived adipocytes as compared to BaP-treated undifferentiated ADMSC. We also confirmed that peroxisome proliferator-activated receptors-gamma (PPAR $\gamma$ ), an important regulator of adipogenesis, was inhibited by BaP treatment. This confirms negative regulation of adipogenesis by BaP.

## Observations

The K9ADMSC were affected by environmental carcinogen BaP, similarly as was shown in human ADMSC. Our data demonstrated that BaP prevented adipogenic differentiation of K9ADMSCs in dose-dependent manner. AhR was down-regulated by BaP; however AhR activity was increased through increased CYP1A1 expression during adipogenesis of K9ADMSC. In conclusion, our data suggests that ADMSC as part of stroma are susceptible to environmental carcinogens and play an important role during breast carcinogenesis.

**Submission ID: 30423**

**Submission Title: ChIP-Seq on 10,000 Cells Using Diagenode's MicroChIP and MicroPlex Library Preparation Protocols**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-Seq) has become the gold standard for whole-genome mapping of protein-DNA interactions. However, conventional ChIP and library preparation protocols associated with current high-throughput sequencing platforms require large amounts of starting material (at least hundreds of thousands of cells per immunoprecipitation) making it difficult for stem cell researchers to apply this technology when working with various stem cell populations.

**Background**

To overcome this barrier, Diagenode has developed a small-scale ChIP-Seq protocol compatible with 10,000 cells of starting material.

**Hypothesis**

Several experimental parameters have been optimized in the True MicroChIP protocol to enable successful low cell number ChIP-Seq such as; low concentration chromatin shearing, antibody titration, use of different carriers and wash stringencies. The MicroPlex Library Preparation protocol requires only picogram amounts of immunoprecipitated DNA inputs for sequencing library preparation and it is compatible with Illumina® platforms and barcodes.

**Research**

We will present new ChIP-seq tools for genome-wide analysis using the True MicroChIP protocol followed by MicroPlex Library Preparation protocols. The performance of this optimized method was evaluated for read mapping, sensitivity and specificity at a range of starting cell numbers covering three orders of magnitude of 1 million cells per IP down to 10,000 cells per IP.

### **Observations**

The combination of the True MicroChIP and MicroPlex Library protocols will now enable stem cell researchers to perform epigenomic experiments who were previously unable to do so due to limited amounts of starting material.

**Submission ID: 30593**

**Submission Title: IL-6 secretion from mesenchymal stem cells supports proliferation of injured neuronal cells in a stroke model**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Mesenchymal Stem Cells (MSCs) are multipotent progenitor cells that have emerged as a promising treatment for inflammatory diseases and acute injury.

**Background**

MSCs display protective, regenerative, and anti-inflammatory properties, and are able to exert their effects in a paracrine manner. These properties make MSCs a promising treatment for stroke, as they can act from afar and implantation into the injured site is not necessary for MSCs to provide beneficial effects.

**Hypothesis**

To investigate the potential for MSC use in stroke treatment, we used the oxygen-glucose deprivation (OGD) stress cell culture model to determine if MSCs could rescue injured neuronal cells, which model cells in the stroke affected brain. Additionally, we sought to elucidate the mechanisms involved.

**Research**

In our studies, neuronal cells were subjected to OGD stress and subsequently co-cultured with MSCs in a non-contact system. A cytokine assay was performed to determine the factors responsible for the protective or regenerative effects of the MSCs.

**Observations**

Here we show that MSCs were able to rescue neuronal cells as illustrated by increased proliferative activity in the rescue group compared to the stress only group. The cytokine assay showed that OGD stress led to an increased in inflammatory cytokines, such as TNF- $\alpha$ , from the injured cells, and that MSC co-culture with these cells attenuated the release of these factors. Also, MSCs were shown to innately secrete high levels of the neuroprotective cytokine IL-6 and angiogenic factor VEGF; interestingly, MSCs secreted a higher amount of IL-6 and VEGF when co-cultured with injured neuronal cells. When IL-6 signaling was blocked, there was a decrease in proliferative activity in the rescue group. This suggests that IL-6 is contributing to MSCs neuroprotection or regeneration of the injured neuronal cells. Further studies are needed to determine whether IL-6 acts in a protective or regenerative manner, as well as to identify additional factors that contribute to MSCs protective and regenerative properties. As these factors are identified, new potential indications for MSCs in medicine may be revealed.

**Submission ID: 30864**

**Submission Title: Human Umbilical Vein Endothelial Cell (HUVEC) as a New Source of Induced Pluripotent Stem Cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

In the last two decades, reprogramming mechanisms have been studied in animal cloning experiments, which in 1997 resulted in successful reprogramming of adult fully differentiated sheep cell nuclei transferred to the ooplasm resulting in the Dolly (Wilmut, Schnieke et al. 1997). Yamanaka found that with the insertion of 4 genes OCT 3/4, Sox 2 Klf4 and c-Myc it was possible to reprogram differentiated cells to pluripotent cells, which were termed *Induced Pluripotent Stem* (iPS) cells. Thomson's group demonstrated that the reprogramming to pluripotent state could be done with human cells and without the use of the oncogene c-Myc (genes OCT 3/4, Sox 2, Nanog and Lin 28), the result confirmed by Nakagawa, Koyanagi et al. (2008).

**Background**

Then, it has been reported that only Oct 4 and Sox 2 were indispensable for reprogramming to occur (Huangfu, Osafune et al. 2008). So far successful reprogramming has been done with many cell types, such as mouse embryonic and adult fibroblast, human fetal and dermal fibroblasts, progenitor neural cells, mouse hepatocytes, etc, all of them being important both for research and regenerative purposes. Meanwhile, the need for new cell type reprogramming is of importance. Umbilical cord is attractive in this respect as a source of a large number of not only hematopoietic cells but also the endothelial, HUVEC (Human Umbilical Vein Endothelial Cells) cells that to this moment were not reprogrammed. In our study we performed expression of Oct 4, Sox2, Nanog and Lin 28 genes in HUVEC and investigated their ability of inducing the expression of pluripotency markers to eventually obtain IPS cells. The primary culture of HUVEC was obtained from fresh umbilical cords collected in PBS. The culture was infected for twenty-four hours with four lentiviral vectors containing the genes of interest. On the 14<sup>th</sup> day, three colonies were cloned mechanically (like iPS (HUVEC) 1-3). The colonies were selected based on their morphological similarity to stem cells (compact colonies, high nucleus to cytoplasm ratio, prominent nucleoli. The colonies were expanded and sub- cultured for three weeks in order to perform a detailed analysis. Three colonies were transferred to plates with BD Matrigel™ and mTeSR™1, for their propagation; later on, at the 4<sup>th</sup> passage it was verified in an individual way the presence of AP. By means of inmuno-fluorescence assay we verified the presence of pluripotency markers OCT 4, Nanog, SSEA-1, SSEA-4, Tra-1-60, Tra-1-81 and found that the colonies were positive with the respect of all these five markers while the parent

**Hypothesis**

HUVECs were negative. The expression of the genes (OCT 4, Nanog, Sox 2, Lin 28) was analyzed by RT-PCR. The cells expressed them at similar levels. If the colonies were maintained without desegregation of the cells they formed embryonic bodies.

**Research**

The iPS-like (HUVEC) clones were passaged at the same ratio (1:4) every 4 to 5 days and found after 8 passages the cells to present the stem cell morphology and maintaining an undifferentiated state. The iPS-like HUVEC analyzed in the present work displayed some of the characteristics of Stem Cells. The assays of teratoma formation and *in vitro* differentiation are to be done for the complete proof of the reprogramming of these cells (HUVEC) to iPS.

**Observations**

The currently observed advances of IPS field make them candidate sources of cellular material for basic studies of reprogramming mechanism and their potential of differentiation to other cell types.

**Submission ID: 31261**

**Submission Title: Graphene-Regulated Cardiomyogenic Differentiation Process of Mesenchymal Stem Cells by Enhancing the Expression of Extracellular Matrix Proteins and Cell Signaling Molecules**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The therapeutic efficacy of the stem cell treatment toward myocardial infarction is quite limited as mesenchymal stem cells (MSCs) hardly differentiate into cardiomyocytes in vivo. In addition, There is not yet a plausible method to differentiate MSCs into cardiomyogenic lineage in vitro without having risk of interfering with normal cell activity by using exogenous chemical inducers.

**Background**

It is reported that MSC differentiation could be modulated through cellular interactions with culture substrates in vitro. However, only a few studies have studied the effect of cell culture substrates on cardiomyogenic differentiation of MSCs. Therefore, we have utilized graphene, which has drawn attention as a platform for cell culture due to its unique physical, chemical, and mechanical properties and its effects on stem cell lineage specifications, as cell culture substrate to induce cardiomyogenic differentiation of MSCs.

**Hypothesis**

Graphene would promote cardiomyogenic differentiation of MSCs through the regulation of extracellular matrix and signaling molecule expression levels.

**Research**

The biocompatibility of graphene was examined through live/dead assay, cell counting kit-8, polymerase chain reaction (PCR),

cell counting, and proliferating cell nuclear antigen immunostaining. Then, the expression of cardiomyogenic genes of MSCs were analyzed by PCR. The regulation of gene expression of ECM proteins and activation of molecules involved in cardiomyogenic differentiation-related signal transduction pathways in MSCs cultured on graphene were analyzed through PCR and western blotting.

### **Observations**

Compared with MSC culture on coverslips, the MSC culture on graphene upregulated the gene expressions of cardiomyogenic differentiation-related ECM proteins (Col I, Col III, Col IV, fibronectin, and laminin). In addition, cardiomyogenic differentiation-related signal transduction was promoted in MSCs cultured on graphene compared with MSCs cultured on coverslips. As a result, graphene promoted the cardiomyogenic differentiation process of MSCs without the use of any exogenous chemical inducers.

**Submission ID: 31631**

**Submission Title: The Medical Student Stem Cell Club: A Community Initiative Enhancing Stem Cell Donor Recruitment**

**Author(s)**

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**Topic**

Patient Advocacy and Communications

**Problem**

The global stem cell and marrow network is a database used to identify potential stem cell donors for patients in need of hematopoietic stem cell transplants. In Canada, individuals age 17-35 can register to be potential stem cell donors online or at stem cell drives, where they consent to join the Canadian stem cell donor database: OneMatch Stem Cell and Marrow Network and swab their cheeks to provide a tissue sample. It is challenging to secure a genetic match for a stem cell transplant; currently, over 1000 Canadians cannot find a match.

**Background**

Patients are more likely to match to a donor in their own ethnic group, and younger donors lead to better outcomes. Additionally, males are preferred donors, as female donors increase the risk of recipients developing chronic graft-versus-host disease. However, males under the age of 35 only represent 12% of the current Canadian donor-database (5% ethnic males).

The UBC Medicine Stem Cell Club was founded two years ago, to increase membership in the stem cell donor database and address the need for young, ethnically-diverse male registrants. Our club is community partnered with OneMatch: we are certified to run drives independently on their behalf.

## **Hypothesis**

Our club aims to sign up over 1500 registrants to effect multiple donor-patient matches annually, by targeting the most-needed demographics with the highest chances of matching to patients in need: ethnically-diverse males under the age of 35.

## **Research**

Our club has spearheaded several stem cell drive campaigns, including the 2012/2013 “Will You Marrow Me?” and the 2012 star-wars themed “May The Swab Be With You”, at 6 university campuses. Funding was obtained primarily from community initiative grants. Prior to the drives, all volunteers completed training emphasizing our target demographic: young, ethnically-diverse males. In 2013, drives were piloted at a university residence and in a rural setting (Inuvik, Northwest Territories). Data was compiled from post-event reports. Stem cell drive planning and coordination is ongoing.

## **Observations**

To date, we have coordinated 16 stem cell drives, and recruited 1989 potential stem-cell donors. We have run 11 campus drives, 2 community drives, 1 classroom drive, 1 rural drive, and 1 university residence drive. In winter 2012, we signed up 646 registrants; in 2012-2013 we signed up 1024; and from Sept 2013-publication, we signed up an additional 319. Collectively, our campus drives in Greater Vancouver, Victoria, and Kelowna have signed up 1048, 434, and 87 registrants respectively, and our community drives (at a shopping mall and a community centre) recruited 334 registrants. The rural stem cell drive pilot signed up 44 individuals, including 26 Aboriginal males. Our UBC residence pilot drive signed up 41 individuals (34 males). Since November 2012, 75.9% of the 1060 registrants recruited at our university drives have been male, and 100% have been under 35. Overall, the vast majority of our registrants are 18-25 years old. These results demonstrate that our initiative is feasible and will help to improve membership of young, ethnically-diverse males on the Canadian stem cell donor database.

**Submission ID: 31854**

**Submission Title: Surrogate progenitors for cardiogenesis: direct reprogramming of somatic cells to cross lineage restriction bypassing pluripotency**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Ischemic heart disease is a major cause of mortality and morbidity worldwide. The development of novel curative intervention for myocardial repair and regeneration remains an area of intense research for the treatment of infarcted myocardium. A number of cell types are currently being studied for the regeneration of the ischemic heart.

**Background**

Among these Induced pluripotent stem cells (iPSC) are a promising cell type as they can be derived from autologous source and have an excellent differentiation potential. However teratogenicity of pluripotent stem cells has marred their progress from bench-to bedside. In this context, the approach of direct reprogramming of somatic cells into cardiac (CPC) and vascular progenitor cells (VPC) for *de novo* cardiogenesis may be a safer alternative for stem cell therapy.

**Hypothesis**

We hypothesized that cardiomyoblasts (CMy) would be a suitable cell type for direct reprogramming into multiple progenitors for *de novo* cardiac regeneration and repair.

**Research**

CMy from young male,  $\alpha$ -MHC-cre/*loxP* transgenic mice were reprogrammed by short term retroviral transduction with Yamanaka factors followed by sequential treatment with JAK-1 inhibitor and cardiac and endothelial specific cytokines. Eleven days after transduction with stemness genes and 6 days after VEGF treatment, CMy were reprogrammed to VPC and expressed endothelial markers Flk1, Flt1, vWFactor-VIII and eNOS, and expressed pro-angiogenic factors including VEGF and Ang-1 as confirmed by immunocytochemistry and RT-PCR. On the contrary, cells treated with cardiac cytokines showed spontaneous beating and expressed cardiac markers Gata4, Mef2c, Nkx2.5, myosin heavy chain and troponin-I. Electron microscopy showed typical striated myofibrillar structures in spontaneously beating CPC. Electrophysiological studies showed cell membrane potential similar to atrial and ventricular cardiomyocytes. For *in vivo* studies,  $1 \times 10^5$  male CMy or CPC were injected intramyocardially using DMEM without cells in control animals. Masson's trichrome staining (n=4 animals/ group) showed significantly attenuated infarct size as compared to CMy and DMEM treated controls at 4 weeks after transplantation.

Immunohistology of the hearts for cardiac specific structural proteins showed extensive myogenic differentiation of CPC into neofibers which re-populated the infarct and peri-infarct regions.

### **Observations**

Directly reprogrammed CPC differentiated into morphofunctionally competent cardiomyocytes which successfully repopulated the infarcted heart

without teratogenicity and reduced the infarct size expansion. These data confirm the safety and superiority of direct reprogramming method to generate a continuous source of CPC/ VPC for *de novo* cardiogenesis.

**Submission ID: 31874**

**Submission Title: Pomegranate and the Breast Cancer Stem Cells - a detailed study about their link and a novel nutraceutical therapy**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

We are in need of a therapy/treatment to selectively kill cancer stem cells at the original tumor site and in distant metastases with no toxic effects on healthy cells, including normal stem cells.

**Background**

Breast Cancer Stem Cell is a single, genetically abnormal cell. As this one culprit cell divides, it eventually becomes a tumor and develops a blood supply to nourish its continued growth. The target should be the cancer stem cells and not any normal cells. At some point, the cancer stem cells may break off from the primary mass and move through the blood supply or nearby lymph system to other parts of the body and this process is called metastasis. The most common sign of breast cancer is a new lump or mass. Most often when untreated a breast cancer stem cells trigger the cancer growth to spread to underarm lymph nodes even before the original tumor in the breast tissue is large enough to be detected. We are in need of a therapy/treatment to selectively kill cancer stem cells at the original tumor site and in distant metastases with no toxic effects on healthy cells, including normal stem cells. Here comes the role of pomegranate which has a long history of use as a food and medicine in Asia and South America. According to the WHO, this medicinal plant is the best source to obtain variety of drugs. The plant part or the compounds derived from the plants are now established recipe of nutraceuticals. There are many evidences that the pomegranate has good anti-cancer properties against prostate, bowel and liver cancer. But there are no studies so far looking at the use of pomegranate in humans. The objective of this paper is to provide a detailed report about why pomegranate in human breast cancer, scientific research proof for the medicinal quality of pomegranate, Sensitivity of Pomegranate and its hypersensitive reactions, its interactions with drugs and a novel nutraceutical therapy using pomegranate to selectively kill breast cancer stem cells.

**Hypothesis**

Pomegranate and human breast cancer cells - their link

**Research**

Breast Cancer Stem Cell is a single, genetically abnormal cell. As this one culprit cell divides, it eventually becomes a tumor and develops a blood supply to nourish its continued growth. The target should be the cancer stem cells and not any normal cells. At some point, the cancer stem cells may break off from the primary mass and move through the blood supply or nearby

lymph system to other parts of the body and this process is called metastasis. The most common sign of breast cancer is a new lump or mass. Most often when untreated a breast cancer stem cells trigger the cancer growth to spread to underarm lymph nodes even before the original tumor in the breast tissue is large enough to be detected. We are in need of a therapy/treatment to selectively kill cancer stem cells at the original tumor site and in distant metastases with no toxic effects on healthy cells, including normal stem cells. Cancer stem cells are critical to a cancer's ability to recur following conventional chemotherapies and radiation therapy because they can quickly multiply and establish new tumors that are often therapy resistant. Here comes the role of pomegranate which has a long history of use as a food and medicine in Asia and South America. According to the WHO, this medicinal plant is the best source to obtain variety of drugs. About 80% of individuals from developed countries use them in traditional medicine. The plant part or the compounds derived from the plants are now established recipe of both pharmaceuticals and nutraceuticals. There are many evidences that the pomegranate has good anti-cancer properties against prostate, bowel and liver cancer. But there are no studies so far looking at the use of pomegranate in humans. The objective of this paper is to provide a detailed report about why pomegranate in human breast cancer, scientific research proof for the medicinal quality of pomegranate, Sensitivity of Pomegranate and its hypersensitive reactions, its interactions with drugs and a novel nutraceutical therapy using pomegranate.

### **Observations**

The objective of this paper is to provide a detailed report about why pomegranate in human breast cancer, scientific research proof for the medicinal quality of pomegranate, Sensitivity of Pomegranate and its hypersensitive reactions, its interactions with drugs and a novel nutraceutical therapy using pomegranate

**Submission ID: 31878**

**Submission Title: Knockdown of p53 impairs self-renewal of embryonic stem cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The role of p53 in embryonic stem cells (ESCs) is controversial, and its function under basal culture conditions is not completely understood. Therefore, in the current study, we examined whether the p53 is functional or unfunctional under unstressful conditions in mouse ESCs.

**Background**

ESCs have unlimited ability for self-renewal and a great potential to differentiate into all cell types. The importance of p53 signaling pathway in stem cell biology has recently received great interest. Mouse ESCs express high levels of p53 protein in the cytoplasm under basal conditions, and in response to DNA damage, p53 is accumulated in the nucleus leading to ES cell differentiation by directly inhibiting Nanog expression. Although earlier studies claimed that the p53-mediated response is inactive in ESCs due to the cytoplasmic sequestration of p53, a recent report showed an antidifferentiation function of p53 in mouse ESCs through directly regulating the Wnt signaling pathway. Also, our recent results obtained by using a p53 chemical inhibitor showed that under unstressful conditions, p53 expression might be involved in self-renewal of mouse ESCs.

**Hypothesis**

We postulate that under unstressful conditions, p53 in mouse ESCs is required to maintain the stem cell characteristics.

**Research**

The role of p53 was examined in undifferentiated mouse ESCs under un-stressful conditions using a small interfering RNA (siRNA)-based technique. To specifically knockdown the p53 gene in mouse ESCs, two independent siRNAs, which target different regions of the p53 mRNA were used to specifically knockdown p53 expression in ESCs. The effect of p53 knockdown on self-renewal and pluripotency of ESCs were examined using different techniques, including Western blotting, RT-PCR, immunofluorescence, alkaline phosphatase (AP) activity assay, apoptosis assay and bromodeoxyuridine (BrdU) incorporation.

**Observations**

Knockdown of p53 resulted in inhibition of ESC proliferation, as indicated by a reduction in the cell number, and a significant reduction in DNA synthesis. Also, deficiency of p53 led to downregulation in the expression of p21 and cyclin D1. Concurrently with p53 reduction, we found a dramatic reduction in the mRNA and protein levels of Nanog at 24 h post-transfection. Furthermore, Oct4 was slightly downregulated at 48 h post-transfection. Conversely, after treatment of ESCs with DNA-damaging agents, the reduction in Nanog expression was more pronounced in control siRNA-treated cells than those of p53 siRNA-treated cells, suggesting that in the absence of p53, DNA damage has no direct effect on Nanog expression. These findings indicate that p53 pathway in ESCs is functional under unstressful conditions, and it plays different roles in regulating both self-renewal and differentiation through its effects on Nanog expression.

**Submission ID: 31918**

## **Submission Title: Heparin-Based Hydrogel as a Selective Matrix Platform for Human Mesenchymal Stem Cell Culture**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Efficient matrix platform that can provide a good expansion as well as guided differentiation of human mesenchymal stem cell (hMSC) is necessary to get enough cell numbers for further application of hMSCs.

### **Background**

A conventional microenvironment for hMSC culture comprises tissue culture polystyrene plate (TCP) surfaces, but the culture of hMSCs on TCP normally leads to a limited proliferative capacity, incomplete pluripotency and uncontrollable differentiation of the cells due to non-specific and non-biological interactions associated with the TCP.

### **Hypothesis**

Heparin-based hydrogels could be a good culture matrix for hMSC culture via direct interaction with heparin moieties as well as tunability of mechanical properties.

### **Research**

We prepared heparin-based hydrogel with a wide range of elasticity ranging from 400 to 43,300 Pa after swelling by thiol-ene crosslinking reaction between thiolated heparin and acrylated PEG. Then, we analyzed culture of hMSCs (human adipose derived stem cells and human mesenchymal stem cells) on heparin-based hydrogels with various mechanical properties including cell adhesion, proliferation, maintenance of pluripotency as well as adipogenic differentiation under the appropriate differentiation environment, and compared with other surfaces and hydrogels. To see the enhanced effect of heparin-based hydrogel mediated by the serum proteins contained in the culture media, hMSC culture was compared both using serum-containing and serum-free media.

### **Observations**

Heparin-based hydrogels without additional modification provided an excellent surface for adhesion and proliferation of hADSCs, which were further tunable by both the amount of heparin (in a positive way) and the elasticity of hydrogel (in a negative way). The optimized heparin-based hydrogel could selectively modulate the adhesion of hADSCs and human bone marrow stem cells (but not all kinds of cells), and resulted in a significant increase in cell proliferation compared to TCP. Furthermore, in terms of the maintenance of pluripotency and specific differentiation, heparin-based hydrogel was much superior to TCP. The selective binding and proliferation of human mesenchymal stem cells on heparin-based hydrogel over

other hydrogels were largely mediated by integrin b1 and selectin, and these superior characteristics were observed regardless of the presence of serum proteins in the culture medium.

Reference: Kim M, Kim YH, Tae G. *Acta Biomater.* 2013,9(8):7833-44.

**Submission ID: 31933**

**Submission Title: Development of a protein marker panel for characterization of human induced pluripotent stem cells (hiPSCs) using global quantitative proteome analysis**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The technology for generation of human induced pluripotent stem cells (hiPSCs) is already progressed to the clinical trials stage of more than 20 iPSC-derived biological products ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). Clinical translation would benefit from in depth understanding of the molecular mechanisms underlying the self-renewal, expansion and differentiation of hiPSCs.

**Background**

While many studies have compared gene expression profiles of hiPSCs and human embryonic stem cells (hESCs), very few have dealt with their proteomes.

**Hypothesis**

Our goal is to build a protein marker database for characterization of hiPSC, as a cell therapy product precursor, using qualitative and quantitative proteomics.

**Research**

We performed global proteome characterization of hiPSCs of different somatic origin: fibroblasts and CD34<sup>+</sup> cells circulating in peripheral blood; and compared them with the H9 hESC line, as well as with the original donors' primary cells. The uniqueness of the study is in parallel analysis of proteomes of hiPSC lines from different sources and of different somatic origin using the same sample preparation approach and a combination of two LC/MS/MS techniques: electrospray ionization (ESI)-MS<sup>e</sup> and MALDI-TOF/TOF. Label-free quantification of proteins was performed by ESI-MS<sup>e</sup> using normalization against internal reference standards.

## Observations

A total of 800 to 1,700 proteins were confidently quantified in different cell lines. Quantitative comparison between 5 cell lines (H9, two iPSC and donors' PBMC and fibroblasts) revealed about 300 proteins overexpressed in three pluripotent cell lines compare to primary cells. Classification of overexpressed proteins by PANTHER and functional networks mapping by Ingenuity Pathway Analysis revealed that the differences mainly affected: RNA Post-Transcriptional Modification, RNA Trafficking; Molecular Transport; Protein synthesis; Ribosomal complex; Cell Morphology; Cell cycle; DNA Replication, Recombination, and Repair. The expression level of 25 previously reported pluripotency markers in hiPSC/hESC was shown to be up-regulated (>1.5 fold) by ESI-MS<sup>e</sup> quantification compare to primary cells, and 12 of them were confirmed by Western-blot analysis. Fifteen (15) novel candidate marker proteins with the highest fold-change difference between hiPSCs/hESCs and somatic cells were observed and confirmed by Western-blot analysis. Qualitative analysis of hiPSCs/hESC proteomes by MALDI-TOF/TOF revealed additional 150-1,000 proteins in both biological replicates; from them 30 proteins were common between hESC and both hiPSC cell lines and 5 were confirmed to be overexpressed in hESC and hiPSC by Western-blot analysis. This resulted in the development of a protein marker panel of 27 proteins (including novel and some previously described markers) that was validated in 9 more hiPSC lines derived in different laboratories by different reprogramming techniques and of different somatic origin by Western-blot analysis. The development and further validation of this panel should facilitate the standardization of hiPSC quality control and their potential application.

**Submission ID: 32127**

**Submission Title: Co-localized transplantation of heterospheroids of islet cells and mesenchymal stem cells for effective angiogenesis and anti-apoptosis**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

There are efficiency concerns that are attributed to poor engraftment of transplanted islets. Hypoxic condition and delayed vasculogenesis induce necrosis and apoptosis of the transplanted islets.

**Background**

Islet transplantation is a promising method for the treatment of type 1 diabetes. However, the specialized vasculature of islets is disrupted as extracellular matrix and vessels are lost, which inhibits the survival of core cells of islets. Transplanted islets suffer from a hypoxic environment and may lose their viability and function during the early stage of transplantation until vascular network formation occurs in approximately 14 days after the transplantation. In result, it compromises cell viability and function.

**Hypothesis**

Transplantation of heterospheroids (HSs) consisting of dissociated islet cells(ICs) and mesenchymal stem cells (MSCs) will improve co-localization of islets and MSCs after transplantation

**Research**

For the viability test, Live/Dead assay and qRT-PCR was performed, and angiogenic factor release was observed with dot blots and ELISA. HSs were transplanted into kidney and transplanted intraprtally into the liver for the obsevation of angiogenesis and co-localization.

## **Observations**

The HSs cultured under hypoxic condition system exhibited a significant increase in anti-apoptotic gene expression in ICs. hMSCs in the HSs secreted angiogenic and anti-apoptotic proteins. With the HS system, we were able to successfully co-localize ICs and hMSCs in the liver after transplantation of HSs via portal vein, whereas the transplantation of islets and the dissociated hMSCs did not result in co-localization. HS transplantation resulted in an increase in angiogenesis at the transplantation area and a decrease in the apoptosis of transplanted ICs after transplantation into the kidney subcapsule compared to transplantation of islet cell clusters.

**Submission ID: 32132**

**Submission Title: Gelatin nanofibrous scaffold to maintain human ES/iPS cells in a feeder-free culture condition for long-term period**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human pluripotent stem cells (hPSCs), i.e., embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively) hold a great potential for basic research as well as industrial and clinical applications. Their current advanced culture substrates are still problematic due to the use of recombinant proteins and synthetic polymers, which might cause big lot variation, labor intensive processes and cost issues. Yet, these two-dimensional (2D) substrates are not applicable for large expansion of hPSCs. Thus, there is a clear need to establish a novel cell culture substrate to maintain stemness of hPSCs and expand them with high quality.

**Background**

In this decade, there are tremendous efforts to establish a cellular scaffold for expanding hPSCs under feeder-/serum-free conditions due to the limitations of using mouse embryonic fibroblasts and Matrigel as a feeder layer, which might cause xenogenic contamination to human cells. New substrates addressing this question have been reported in the literatures; however, these substrates for hPSC cultures still include recombinant proteins (Rodin, S. et al., *Nat. Biotechnol.* (2010); Miyazaki, T. et al., *Biochem. Biophys. Res. Commun.* (2008); Miyazaki T., et al, *Nature Commun.* (2012)) and synthetic polymers (Villa-Diaz, L.G. et al., *Nat. Biotechnol.* (2010); Zhang R. et al., *Nature Commun.* (2013)), which may cause large lot-to-lot variations, require labor intensive processing and are associated with cost issues. Thus, *it clearly necessitates a new generation of hPSC culture substrates that meet today's requirements.*

**Hypothesis**

Recently, nanofiber technology has attracted the focus of many researchers at universities and in industry due to their advantageous properties over common 2D substrate/material, such as (i) high-surface area to enhance interaction with the

other molecules/cells, (ii) flexible structure simplifying handling and manipulation, and (iii) porousness to filter, detect and/or capture targeted molecules/cells. A number of companies have already commercialized products, such as air and liquid filters, membrane fuel cells, dye-sensitized solar cells, sport apparel and rain gears.

## Research

To address these issues, we used nanofiber technology to develop a new cellular scaffold for robust long-term hPSC self-renewal. Recent studies have found that nanoengineered substrates are advantageous for cell culture because, at the subcellular level, their three-dimensional (3D) topological features facilitate intracellular signaling pathways for adhesion and proliferation. The electrospinning technique is a straightforward method to fabricate nanofibers by applying a high voltage between a syringe/needle setup containing a precursor solution and a grounded collector. The technique allows (i) the use of a variety of polymers and biomolecules as materials, (ii) the generation of large quantities of nanofibers, and (iii) the production of a quality-controlled substrate.

## Observations

We were able to develop gelatin nanofibers suitable of long-term self-renewal of hPSCs. Gelatin is an approved material for use in cosmetics and medicine, can be formed into nanofibrous structures and used as a cellular scaffold for hPSC CULTURE. Maintaining hPSCs on optimized nanofiber substrates is comparable to using Matrigel in mTeSR-1 (Ludwig, T. E., et al., *Nat. Meth.* (2006)), commonly used as a defined medium (at least 3 months). hPSCs cultured on gelatin nanofibers successfully maintained their pluripotency and normal karyotype after long-term culture. For hPSC passaging with our culture method, enzymatic dissociation processes (i.e., trypsin, dispase, accutase and collagenase IV) are not required. In addition, chemically defined cell dissociation buffer based on EDTA is suitable for this method. Therefore, we are able to avoid the xenogenic contamination due to the use of these enzymes, reduce cell damage during the procedure and simplify the passaging procedure in a robust fashion.

**Submission ID: 32176**

**Submission Title: TaqMan® hPSC Scorecard™ Assay: A TaqMan® Panel and Analysis Software for the Characterization of Induced Pluripotent Stem Cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The numerous human iPSC clones being generated from diverse patient sources have created a challenge for comprehensive characterization that cannot be addressed by current methods. Current methods rely on a combination of *in vitro* and *in vivo* cellular methods to confirm pluripotency and tri-lineage differentiation potential and are not amenable to high-throughput environments. Molecular analysis solves this characterization bottleneck by providing a quantitative, accurate, and efficient alternative to the current characterization methods.

**Background**

Rapid advancements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the creation of patient-derived stem cells which are valuable tools in drug discovery and future cell therapies. In order to confirm and standardize the quality of iPSCs, thorough characterization has become a necessity. Current methods used to identify viable iPSCs rely on a combination of *in vitro* and *in vivo* cellular methods to confirm pluripotency and tri-lineage differentiation potential.

**Hypothesis**

A focused gene expression array can be used to accurately confirm gene signatures in the undifferentiated state but also confirm functional pluripotency based on tri-lineage gene expression pattern of differentiating cells.

**Research**

Several platforms are available for gene expression analysis and vary in content and complexity. To determine the optimal method and minimal set of genes required for definitive characterization of pluripotency, we have utilized high and medium density arrays along with low density TaqMan<sup>®</sup> qPCR arrays to compare expression patterns of partially reprogrammed clones and fully reprogrammed human iPSCs to parental fibroblasts and control human embryonic stem cells.

Human Embryonic stem cell (ESC) and iPSC clones confirmed to have normal karyotype were characterized using standard cellular methods and detailed transcriptome analysis was performed using whole genome microarrays. A subset of these characterized lines was further analyzed by RNA-seq to generate a focused sequencing panel of 560 genes. Analysis of 3 pluripotent lines and their spontaneously differentiated progeny showed distinct clustering of expression patterns between these two main groups. A further reduced 94 gene TaqMan<sup>®</sup> qPCR array was used with reference standard data to compare expression from several ESC and iPSC clones. Over 20 ESC and iPSC clones and cells differentiated via spontaneous or directed differentiation were analyzed using this TaqMan<sup>®</sup> panel, providing confirmation via cellular methods that these samples were correctly scored for pluripotency or appropriate lineages.

## **Observations**

The results indicate that a focused set of genes in a low density array can recapitulate the information obtained with medium and high density arrays with distinct clustering of samples based on their pluripotency. As a result, a 94-gene TaqMan<sup>®</sup> hPSC Scorecard<sup>™</sup> panel and analysis software was developed to provide scientists with a more efficient and universal method to characterize their human iPSCs. This method provides a sensitive, fast and accurate measure to determine pluripotency and differentiation into the three germ layers: endoderm, ectoderm and mesoderm, thus confirming functional pluripotency of pluripotent lines.

**Submission ID: 32263**

**Submission Title: PCR Assays for Comprehensive Characterization of Pluripotent Stem Cells**

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**Topic**

Industry Infrastructure

**Problem**

Induced pluripotent stem cells (iPSC) offer an ideal platform to generate patient-derived cell models to dissect basic biology and for therapeutic use either in drug screening or cell replacement. As iPSCs are generated from various genetic backgrounds using different derivation and culture conditions, it is essential to carryout comprehensive characterization to ensure their identity, functionality, and genetic stability. Traditional methods for characterization of iPSCs and ES cells necessitate a combination of in vitro and in vivo cellular analysis. These methods are laborious with subjective measures and are not amenable to high throughput analysis. Molecular analysis platforms offer an appealing alternative for rapid generation of quantitative data to confirm the quality of the generated clones based on genomic and epigenomic expression patterns.

**Background**

The ability to generate footprint-free iPSCs using platforms such as the Cytotune® iPS Sendai Reprogramming Kit has created patient specific models for pathway and disease studies, providing traction for the translation of disease research. The bottleneck in this process has now shifted from efficient methods of generation, to a lack of throughput tools for characterization that are easily incorporated into the iPSC workflow. Molecular analysis platforms offer a quantitative, accurate, and fast alternative to current methods, stated earlier, and have recently been utilized to qualify pluripotent stem cells. Furthermore, several platforms are available for gene expression analysis varying in content and complexity.

**Hypothesis**

The focus of this work is to identify a focused set of genetic and epigenetic markers that can be analyzed on an easy, simple to use platform to predict the quality and functional outcome of iPSCs. TaqMan® Gene Expression Assays has been extensively used for dissecting molecular signatures of several cells including stem cells and their intermediates. Utilizing medium and low density [TaqMan® gene expression](#) and [MicroRNA Cards](#), expression profiling data was generated for pair-wise comparison to identify differences or similarities between undifferentiated and differentiated sample sets. Identification of a differential

set of transcriptome and miRNA targets enables design and development of a validated focused array for rapid characterization and functional confirmation of pluripotent stem cells.

## **Research**

Control H9 ESC and iPSC generated from human fibroblasts using CytoTune®, were cultured under varying conditions either in their undifferentiated state or after 1-2 weeks of undirected differentiation. Cells characterized using traditional cellular methods were further subjected to detailed transcriptome and miRNA expression analysis using TaqMan® Arrays. Resulting data was analyzed to identify consistently up-regulated and down-regulated genes and miRNA between the two cell states. In addition, PCR based method for methylation pattern of these cells were also examined.

## **Observations**

High density and medium density arrays demonstrate hierarchical clustering of samples consistent with cell state. A subset of 93 genes, comprised from both pluripotent and differentiation genes, was used in a low density focused TaqMan® hPSC ScoreCard™ Panel. The resulting expression profile recapitulated the pattern observed with higher density panels with distinct clustering of undifferentiated and differentiated cells. This panel in combination with a validated reference standard generated from several functionally confirmed pluripotent lines, was used for rapid confirmation of pluripotency and trilineage differentiation of pluripotent lines. Further, microRNA analysis identified 10 up-regulated and 7 down-regulated miRNAs between undifferentiated and differentiated cells. These 17 differentially expressed miRNAs also correlated to the differentially expressed genes, suggesting them for potential use as identifying markers of cell state. Methylation patterns further provide an additional measure of identifying and distinguishing cell states. Overall, comprehensive PCR assays can be utilized for identification and confirmation of cell state and differences in profile evaluated for further impact on functionality and long-term stability.

**Submission ID: 32274**

**Submission Title: The qPCR panel for evaluation of iPS/ES cells gene expression**

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**Co-Author(s)**

**Topic**

Basic Research, Medicine, and Health

**Problem**

Development of Core Technologies for Industrial Applications of Human Stem Cell is very important. In recent years, great progress has been made worldwide in developing drug discovery screening and cell therapy applications for hPSCs (human pluripotent stem cells, such as hES cells). However, large scale PSC culture methods vary widely across the globe, and are in need of standardization.

**Background**

Since 2011, Takara Bio has participated in the NEDO project; “Fundamental Technology Development for Promoting the Industrial Application of Human pluripotent Stem Cells “.

**Hypothesis**

We play the roles for development of profiling and evaluation reagents for quality control of hPSCs as Development of quality evaluation and control for hPSC lines.

**Research**

Then, using the ES cell materials supplied by our NEDO project, we have developed a set of real-time RT-PCR primers for the analysis of the expression of genes associated with ES cell pluripotency. This set can be used to analyze the gene expression in differentiated and undifferentiated ES cells.

**Observations**

Each array contains 96 primer pairs representing 88 pathway-related genes and 8 housekeeping genes. When comparing an unknown sample to a control sample, gene expression differences can be expressed using the relative quantification method. In addition, expression levels of multiple genes can be screened simultaneously.

**Submission ID: 32275**

**Submission Title: Modulation of T Cells by Mesenchymal Stem Cells via Control of CD25**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Recently, Mesenchymal Stem Cells (MSCs) have been identified as playing an important role in regulating inflammation, and have become an attractive tool in clinical settings for treating inflammatory diseases. However, the mechanisms by which they achieve this regulatory role are not fully understood. We endeavored to identify and characterize these mechanisms so that MSC biology can be better understood, and so that they can be more effectively used in clinical settings.

**Background**

Mesenchymal Stem Cells (MSCs) are tissue resident, multipotent stem cells capable of self renewal and differentiation into a number of cell types including adipocytes, chondrocytes, and osteoblasts, and have been noted for their immunosuppressive characteristics. We have previously identified that while cytokine stimulated mouse MSCs primarily owe their immunosuppressive capabilities to the expression of iNOS, human MSCs achieve this through the expression of indoleamine 2,3 dioxygenase (IDO). IDO is thought to exert its suppressive effects via tryptophan depletion, but recent evidence suggests that IDO derived metabolites of the kynurenine pathway may exert direct effects on lymphocytes.

**Hypothesis**

We hypothesise that MSCs exert their immunosuppressive effects, at least in part, by releasing IDO metabolites into the environment in which they have attracted local lymphocytes. These IDO metabolites can then directly inhibit proliferation of lymphocytes, or indirectly suppress lymphocytes by enhancing regulatory T cell (Treg) differentiation.

**Research**

We have measured the activity of lymphocytes, especially T cells, in response to both MSC coculture and treatment with various metabolites in the kynurenine pathway, the first step of which is catalyzed by IDO. We have used proliferation, cytokine secretion, surface marker expression, and mRNA transcription as readouts of lymphocyte activity following anti-CD3 activation of T cells. Additionally, we have analyzed the proportion of Tregs in the lymphocyte population after culture in these conditions.

**Observations**

Human MSCs that have been activated with cytokines reduce CD25 expression on T cells and potently inhibit lymphocyte proliferation induced by anti-CD3 activation in an IDO dependent manner, as evidenced by the reversal of these observations with the addition of the IDO specific inhibitor 1-MT. L-kynurenine, the product of IDO catalysis, similarly reduces CD25 expression and proliferation of T cells in a dose dependant manner. While we did not observe an increase in the proportion of

Tregs with L-kynurenine treatment alone, L-kynurenine combined with TGF $\beta$ 1 induced a much higher proportion of Tregs compared to TGF $\beta$ 1 treatment alone. L-kynurenine imposed a bimodal modulation of CD25 expression on T cells, with low doses increasing CD25 expression preferentially in Tregs compared to Teff, and high doses suppressing CD25 expression in both groups; this suggests that IDO expressing MSCs inhibit T effector proliferation by inhibiting IL-2 signaling via reduction of surface expressed CD25, while enhancing the Treg population via enhancement of surface expressed CD25, which may synergize with TGF $\beta$ 1 signaling to enhance the Treg population.

**Submission ID: 32297**

**Submission Title: VeraVec endothelial cells recreate the vascular stem cell niche in vitro for unprecedented expansion of stem and progenitor cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The scarcity of stem cells from various tissues has created a bottle neck for basic research, transplantation, and regenerative medicine at large. Current methodologies for the expansion of stem cells in vitro invariably reduce the capacity for engraftment and multi lineage commitment of the expanded stem cells. The ability to recapitulate the stem cell niche in vitro represents an ideal solution to this shortcoming in stem cell expansion. However, this has eluded scientists as the creation of such a niche often requires irreducibly complex media additives, such as serum.

**Background**

The recent appreciation of the association of stem cells with the endothelial cells forming the vascular stem cell niche has only recently been understood. Angiocrine factors, the growth factors supplied by endothelial cells, are indispensable for the maintenance and expansion of stem cells in vivo and numerous models of development and recovery from injury. Historically, this observation has not been transferable to in vitro cultures due to the numerous additives needed to cultivate endothelial cells which are toxic to both the stem cells and endothelial cells.

**Hypothesis**

VeraVec endothelial cells, created with the stabilizing effects of the E4ORF1 gene, are capable of adapting endothelial cells from both mouse and human to tissue culture conditions without the need for such toxic and deleterious media additives. VeraVec endothelial cells are phenotypically stable for numerous passaging until their eventual senescence and are capable of recreating the vascular stem cell niche in vitro with defined media conditions.

**Research**

Endothelial cells from both mouse and human were characterized via transcriptional profiling from both in vivo and in vitro sources. This data set revealed the complexity and depth of the angiocrine factor expression in each tissue responsible for the creation of the vascular stem cell niche and also the stability of the VeraVec endothelial cells in vitro. The endothelial cells and VeraVec endothelial cells were revealed to be dynamic sources of growth factors, many of which had never before been attributed to endothelial cells but known to be critical for stem cell expansion.

**Observations**

The co-culture of stem cells, with hematopoietic stem cells used as an example, with VeraVec endothelial cells resulted in a profound 1200 expansion within 12 days. Importantly, the expanded stem cell population maintained multi lineage engraftment capacity while simultaneously having enhanced engraftment. Transplantation studies revealed that the long

term engrafting stem cells were both serially transplantable and at a higher frequency when compared to primary stem cell populations. Collectively, these observations underscore the capacity to use the VeraVec endothelial cells to recreate the vascular stem cell niche in vitro for the expansion of stem cells for both research and clinical applications.

**Submission ID: 32353**

**Submission Title: Stem Cell Clinics And The Internet**

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**Topic**

Patient Advocacy and Communications

**Problem**

Stem cell therapies which are yet to be proven efficacious in appropriately designed clinical trials are easily available through clinics represented online. Online clinics describe a vast array of treatments with a diverse range of indications making them relevant for a substantial portion of the global population. The clinics and therapies offered are described in a very favorable manner. Despite demonstrating a lack of appropriate accreditation, clinics report major clinical improvements and the curative potential of treatment. The provision of stem cell therapies in such an unregulated online environment is jeopardising the development of this nascent branch of medicine and offers a substantial risk to both the health of patients availing of these treatments and to the credibility of long term research in this domain. The aim of this study is to objectively analyse stem cell clinics represented online and within that analysis to ascertain the nature and description of the stem cell therapies offered, to determine how the clinics are portrayed, to examine the methods employed to attract patients and also to explore the accreditations offered by online stem cell clinics.

**Background**

59% of the total American population use the internet as a source of health information [1]. With the advent of smart phones and tablets, the internet will undoubtedly gain an even greater influence within the field of patient information and education. Online stem cell clinics are employing the internet to directly communicate with patients and to advertise largely unproven stem cell therapies. These clinics are readily accessible online and operate globally. Many have already harnessed the powerful resource that is the internet, with potentially detrimental effects on the development of stem cell therapies. While many clinics have been the subject of intense scrutiny in the past, they continue to function despite a lack of accreditation [2]. Today, with unprecedented use of the internet, the need for appropriate regulation of online stem cell clinics is greater than ever. The significance of this cannot be understated as the spectre of negative publicity generated by an unapproved clinic could dampen the esteem with which stem cell therapy is held. The major concern is that such an event could diminish both public and government support, and ultimately funding for genuine research in this area of immense medical potential.

[1] Fox, S., Duggan, M., *Health Online 2013*. Pew Internet & American Life Project, January 15, 2013, available: <http://www.pewinternet.org/Reports/2013/Health-online.aspx> accessed on 11/05/13

[2] Kiatpongsan, S., Sipp, D., (2009) *Medicine. Monitoring and regulating offshore stem cell clinics*. *Science*, 323 (5921):1564-5. Doi:10.1126/science.1168451

## **Hypothesis**

Stem cell therapy represents a realm of novel therapeutic possibilities for both patients and clinicians. The substantial demand for stem cell therapy is irrefutable. However, many of these therapies are only taking elementary steps towards the successful completion of approved clinical translational pathways. This represents a fundamental problem as patients are employing the services of online stem cell providers, despite the experimental nature of treatments and the lack of accreditation and outcome data displayed by clinics, thereby overcoming the delay imposed by the evolutionary process of clinical pathways. Thus, the underlying hypothesis of this study is that there is a substantial online demand from patients for stem cell therapy, despite poor certification and international authorization.

## **Research**

This research is based on an analysis of stem cell clinics as represented online. A web-based search utilising five search terms as follows was employed: stem cell clinic, stem cell cure, stem cell therapy, stem cell treatment and stem cell centre. The first twenty pages of each search result were screened. This strategy yielded 1091 web pages and the homepage of each site was assessed as to whether or not it was a stem cell clinic that administered stem cells to treat human disease. 224 of the 1091 pages represented stem cell clinics. A number of the web pages representing online clinics appeared on multiple occasions within the search process and were analyzed once only. Web pages representing the same clinic despite a different URL were excluded. In addition, web pages promoting clinics led by the same physician and clinics operating under the same umbrella network were also excluded. After the exclusion criteria were enforced 68 sites remained. Over 340 variables were then employed to critique the websites. This analysis ranged from the description of stem cell therapies offered, to the portrayal of the clinics and doctors involved as well as the marketing devices used to attract potential patients. A literature review pertaining to stem cell therapy was also included as part of the research process with particular emphasis on the online representation of stem cell therapy.

## **Observations**

### Stem Cell Clinics and the Internet

The essential element of online stem cell clinics appears to be the creation of user friendly sites, which are easy to navigate and which provide information in the vernacular of the target audience. Over 50% of the clinics employed social media outlets such as Twitter, Facebook, YouTube and Skype to further enhance and augment the accessibility of their service. Online advertisement was utilised by 9% of the analysed clinics. A further 88% invited patient contact via the means of online comment boxes. In many instances patients are prompted to initiate contact by the promise of further information following such communication.

## Clinic Descriptions

It was observed that many web pages representing stem cell clinics contain much complimentary text. The terms “experienced”, “renowned” and “acclaimed” are frequently used to describe both the doctors and the clinics involved. Doctors are named in 62% of cases, while 80% of doctors are classed as “specialists”. The terms “modern” “advanced” and “state of the art” are commonly referenced to portray the clinics. 34% of sites mention the number of patients they have treated while one quarter of clinics provide outcome data and have patented their therapies. 29% of clinics demonstrate an internationally recognised accreditation with 65% of these accreditations laboratory related and not pertinent to the clinical applications themselves.

## Therapy Descriptions

The stem cell therapies were described as safe and effective. 90% of clinics advocate the safety of treatment, while 15% state that there is “no risk” involved. 88% of clinics claim treatment effectiveness, with 16% describing the curative potential of therapy. 9% refer to specific research publications to support their outcomes.

Information on precise treatment protocols was deficient. Over 40% of sites did not specify the number of treatments required, duration of procedure or therapeutic course. One treatment lasting 1-3 hours as an outpatient was the most common regimen of those mentioned.

Of the sites analysed 53% request access to patients medical records and 12% recommend patients discuss the proposed therapy with their general practitioner. Almost one quarter of sites reference contraindications to treatment with 41% of sites mentioning follow up patient care.

Treatments were often presented as a medical tourism package, where patients combine a treatment with various additional services such as flights, accommodation, adjunctive therapies and even guided tours of the local surrounds. This practice highlighted the geographic spread of online stem cell clinics with 22 countries spanning 5 continents represented. The USA had the highest density of clinics, with American clinics accounting for one quarter of clinics studied. Asia also contributed a large portion of the online clinics. Costs of treatments were mentioned by 35% of sites with costs ranging from \$5,000 to \$50,000.

## Indications

Over 390 conditions spanning a broad spectrum of disease categories are indicated by the online clinics. The top five indications for stem cell therapy encountered were; Multiple Sclerosis, Anti-Aging, Parkinson’s Disease, Stroke and Spinal Cord Injury with neurologic and musculoskeletal conditions particularly to the fore.

## Stem Cell Type

Adult, autologous stem cells were the most commonly utilized stem cell with 82% of clinics employing them for their therapies. These were frequently sourced from bone marrow and adipose tissue. Additional sources of stem cells included peripheral blood, umbilical cords, and blood/marrow donors with 10% of clinics using fetal stem cells. Stem cells were administered intravenously in 60% of cases with 11 means of administration encountered in total.

## Conclusion

It is imperative that the scientific community is made aware of the threat to stem cell therapy posed by unregulated online clinics. These clinics are harnessing the internet to attract a wide range of patients suffering from a diverse catalogue of

conditions to therapies that are described in an attractive manner. A concerted effort from scientists, researchers, doctors, advocacy groups and governments alike is required to rapidly address the existing legislative deficiencies to prevent these clinics from offering clinically unproven treatments to vulnerable patients. It remains beyond doubt that enhanced regulation would benefit vulnerable patients and also to protect the unquestionable, immense potential of stem cells as a therapy for human beings. Stem cell therapy is only in its infancy and needs to be regulated and monitored adequately today so that it can change the lives of patients tomorrow.

**Submission ID: 32361**

**Submission Title: Establishing the piggyBac-iPSC based stem cell therapy for Sanfilippo syndrome in MPSIIIA mouse model**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Sanfilippo Syndrome is a lysosomal storage disease (LSD) which affects majorly on nerve system. Currently, there is no effective treatment for this type of disease. In this study, we applied a MPSIIIA mutant mouse as a disease model for Sanfilippo syndrome and used piggyBac-iPSC for studying the possibility of treatment.

**Background**

Human brain is a vital organ highly protected in the [cranium](#) and suspended in [cerebrospinal fluid](#). Yet, it is still vulnerable to many types of damages and diseases. Current medical strategies including enzyme replacement treatment (ERT) and gene therapy do not effectively treat those diseases due to the complicated nature of brain anomalies and pathologies. Thus, devising a novel therapeutic strategy urgently needs a well characterized disease model with a quantifiable measurement for evaluating therapeutic efficacy.

**Hypothesis**

We hypothesized that applying iPSC technology to acquire stemness from a differentiated cell will allow us to repair the mutant sulfamidase and establish the dishy model of disease.

**Research**

The iPSC technology holds great promises for regenerative medicine. In this study, we explored the feasibility of iPSC-based therapy in treating the neural disorder of Sanfilippo syndrome A (MPS IIIA). To tackle this problem, we have successfully established the *piggyBac*-iPS based MPS IIIA genetic disease mouse model system by integrating the *piggyBac* genetic engineering platform with iPSC technology in the natural existing MPS IIIA mouse model.

**Observations**

The system consists of a *piggyBac*-based regulatable vector system expressing Yamanaks factors (referred hereafter as

*piggyBac*-TEO-iPSC inducers) and a triple transgenic mouse line (MOS) with following features: rtTA (a tetracycline regulator) and GFP knocked-in to the endogenous ROSA26 and OCT4 loci, respectively, in the MPS IIIA mutant background. By introducing *piggyBac*-TEO-iPSC inducers into the embryonic fibroblasts derived from MOS mice in the presence of Doxycycline, MPS IIIA defected mouse iPSCs will be generated and further engineered to expressing the wild type human MPS IIIA. The genetic corrected MPS IIIA iPSCs will be differentiated into neuronal stem cells and transplanted to brains of MOS mice (MPS IIIA defected mice). Utilizing the Moris water maze, open-field locomotor activity, and Gait analyses, we have established a highly sensitive behavior assessment platform for evaluating the therapeutic efficacy of *piggyBac*-iPSC based gene/stem cell therapy in the triple transgenic MPS IIIA disease model.

**Submission ID: 32366**

**Submission Title: Rapid Generation of Mature Hepatocyte-Like Cells from Mesenchymal Stem Cells by a Novel Bio-Microfluidic Device**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Hepatic different platforms take about one month to complete the hepatic differentiation process, thus it is important to improve the efficiency of the platform. The traditional static culture method does not allow growth factors in the medium to be maintained at an optimal level and cellular waste to be removed efficiently.

**Background**

Mesenchymal stem cells (MSCs) are multipotent cells capable of being induced and differentiated into a variety of cell types and have great potential in clinical cell therapy. However, the optimal condition for cell differentiation is not well established.

**Hypothesis**

Using novel microfluidic system improves efficiency of hepatic differentiation from mesenchymal stem cells

**Research**

In the study, we have developed a microfluidic device that provides a stable uniform flow for cells grown in device chamber and allows us to observe changes in cell morphology continuously under microscopy. We compared the difference between mesenchymal stem cells (MSCs) grown on microfluidic device and in static culture after 3 weeks of hepatic differentiation medium induction.

**Observations**

We observed cuboidal morphology characteristic of hepatocytes and expression of hepatocyte marker genes on MSCs grown with both culturing methods. MSCs grown on a microfluidic device showed greater expression of hepatocyte marker genes compared to MSCs grown in static culture. Moreover, the functional analysis performed at 21 days of differentiation using the microfluidic device system demonstrated that urea production with a fold change above 2 were statistically differently produced on microfluidic device cultures compared to static plate culture. In summary, our novel microfluidic device may induce highly efficient hepatic differentiation of MSCs and such device may be useful in the industrial scale up of cell production.



**Submission ID: 32423**

**Submission Title: The Potential Therapeutic Role of Cardiovascular Progenitor Cells (PC) Derived from Induced Pluripotent Stem Cells (iPSC) after Myocardial Infarction**

**Author(s)**

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**Co-Author(s)**

**Topic**

Basic Research, Medicine, and Health

**Problem**

There is a long standing debate as to whether post-myocardial infarction (MI) restoration of cardiac function is due to non-cell-based therapy mediated by paracrine factors secreted by progenitor cells or due to cell-based therapy.

**Background**

In support of this paracrine hypothesis, many studies have observed that progenitor cells secrete cytokines, chemokines, and growth factors that could potentially repair injured cardiac tissue. This paracrine hypothesis could potentially be a foundation for a non-cell-based alternative to progenitor cell therapy for cardiovascular disease. Non-cell-based therapies diverge from cell-based therapies as they are generally easier to produce and are safer as they are nonviable. However, increasing evidence supports the use of iPSC cell based therapy. In order to clearly distinguish the nature of therapeutic effects (non-cell-based vs. cell-based), an experiment was designed in which progenitor cells derived from iPSC were genetically modified to express the herpes simplex virus thymidine kinase (TK) "suicide" gene.

**Hypothesis**

Assess the potential role of (1) PC differentiated directly into cardiomyocytes (CM) and endothelial cells (EC) to the site of injury, or (2) paracrine factors released from PC on heart post myocardial infarction.

**Research**

These concepts were evaluated using iPSC-derived PC genetically modified to express the herpes simplex virus thymidine kinase (TK) under the control of cardiomyocyte (NCX1) or endothelial cell (VE-cadherin) specific promoters. PC expressing the TK permitted ablation at the first week (WK1) or the third week (WK3) by injection of ganciclovir (GCV). If GCV applied at the WK1, but not at WK3, altered cardiac function, we would conclude that myocardial contractile recovery depends on CM and EC derived from iPSC. If the beneficial effects on cardiac function persisted after GCV was given at the WK3, we would surmise that the PC effect was via by a paracrine action.

**Observations**

The levels of IGF-1 $\alpha$  and VEGF released from ischemic tissues were significantly higher in the cell patch group. Heart function, infarction size, and vessel density were also significantly improved after cell patch treatment. However, this beneficial effect on cardiac function was completely abolished in the group given GCV at WK1, and partially abolished in the group given GCV at WK3 compared to the untreated cell patch group.

**Conclusions:** The specific gene suicide approach allowed us to determine that the early salutary effects of cardiovascular cells derived from iPSC on angiogenesis and consequent improvements in LV function were largely due to paracrine effects, while the long-term, potentially important, therapeutic effects were largely due to the new EC and CM populations derived from iPSC.

**Submission ID: 32437**

**Submission Title: Immunomodulatory effects of stem cells from the bone marrow combination therapy with BMP-2 and on canine osteosarcoma**

**Author(s)**

**Rose Eli Grassi. Rici**

Dr.

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The main cause of death in osteosarcoma is assigned to the progression of metastatic disease. Canine Osteosarcoma is highly comparative with human osteosarcoma in their morphology, thus being a model for human study. This tumor is considered locally invasive and potentially metastatic, with early death of the patient. These patients mortality is not associated with inadequate surgical therapy of primary tumor, but with failure in treating metastasis (Dernell et al., 2007). However, metastatic disease remains the leading cause of death in 80% of cases in which chemotherapy was established. Therefore, new chemotherapeutic agents and new molecular immunomodulatory therapies are subject of intense research in the scientific community, in order to prolong the neoplastic remission, and thereby extend the animals' survival period (Dernell et al., 2007).

Cells - mesenchymal stem cells (MSCs) – are able to modulate the function of other cells through cell-cell contact and through the release of a broad spectrum of bioactive factors - cytokines and chemokines (Fu et al., 2009; Meirelles et al., 2008). This interaction is essential for the promotion of an immunosuppressive or pro-inflammatory profile, dependent on factors from soluble factors from other immunoregulatory cells (Dazzi et al., 2012). An understanding of these MSC populations may provide a new therapeutic strategy in modulating the immune response against tumors.

## **Background**

Osteosarcoma is a highly metastatic primary bone cancer observed in humans and animals. Studies show that tumors use mechanisms that suppress the immune system to prevent host immunity (Liyanage et al. 2,002; Pardoll, 2003). These mechanisms include components of the host's immune system, regulatory T cells CD4<sup>+</sup> CD25<sup>+</sup>, myeloid suppressor cells and natural killer T cells (NKT) (Botti et al. 2,004). The infiltration of CD3<sup>+</sup> lymphocytes correlates with malignancy and is associated with metastases at diagnosis (Trieb et al. 1998). The association of CD4<sup>+</sup> and CD25<sup>+</sup> cells are capable of maintaining the tumor environment and reduce its immunogenicity thus allowing its progressive growth by blocking the cytotoxic action of the CD8<sup>+</sup> cell (Anthony et al. 2,005; Kojima et al. 2005).

Activation of macrophages is mainly characterized by the secretion of IL-1, IL-6 and TNF- $\alpha$  in addition to the secretion of reactive oxygen and nitrogen and increased phagocytic ability (Shurin, 2005). In this study, the tumor was implanted in Balb-nude mice and treated with the combination of canine bone marrow stem cells and morphogenetic protein (BMP-2) and analyzed using markers of the immune system and proinflammatory by flow cytometry.

## **Hypothesis**

Mesenchymal stem cells from canine bone marrow, associated with morphogenetic protein, modulate the immune system of mice with canine osteosarcoma.

## **Research**

Osteosarcoma cell lines (OST) (Ethical Committee Protocol number 1654/2009) and undifferentiated mesenchymal stem cells from the bone marrow of canine fetuses (MSC) (Ethical Committee Protocol number 931/2006) and undifferentiated mesenchymal stem cells from the bone marrow of canine fetuses (MSC) were obtained from the cell bank of the Stem Cells Laboratory of the Anatomy of Domestic and Wild Animals Division, School of Veterinary Medicine and Animal Science, University of São Paulo and from the Laboratory of Biochemistry and Biophysics of Butantan Institute, São Paulo, Brazil.

The osteosarcoma and mesenchymal bone marrow cell lines from canine fetuses were rapidly thawed in a water bath at 37°C. The pellet was centrifuged to remove the culture medium. Two washes were subsequently performed in PBS (phosphate buffer solution) in a centrifuge at 24°C and 1000 rpm for five

minutes. Cells were maintained in 25-cm<sup>2</sup> culture flasks using DMEM-H (LGC) cell culture media supplemented with 10% fetal bovine serum (VITROCELL, Campinas, SP), 1% antibiotics penicillin and streptomycin (GIBCO) and 1% pyruvic acid (GIBCO) at pH=7.4 in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Cells were grown in a monolayer adherent to the culture dish surface. Following 72 hours of adherence and confluence, OST and MSC cells were trypsinized, centrifuged and resuspended in PBS for application in the experimental animals. Trypan blue dye and a Neubauer chamber were used to count the cells and assess their viability, and a 1×10<sup>6</sup> concentration was prepared for the treatment. MSC cells were split into two groups one of which was treated with a 5 nM rhBMP2.

The “in vivo” research was approved by the Ethical Committee in the Use of Animals (Protocol number 1654/2009) of School of Veterinary Medicine and Animal Science, University of São Paulo (Protocol n. 2997/2013). 13 female BALB/c<sup>nu/nu</sup> mice, with an approximate weight of 22 g each and age of 6–8 weeks, received 5 x 10<sup>6</sup> transduced canine OST cells, applied dorsally under sterile conditions subcutaneously. After 4 weeks applying the OST cells, mice were randomly separated into three groups (group I – Control, group II for treatment with MSC and group III for MSC combined with rhBMP-2 (B355510U6- Sigma, Gillingham, UK). Weekly, MSC and MSC combined with rhBMP-2 were applied intraperitoneally (4 cycles of treatments). Animals were kept in micro-isolator cages in ventilated racks located at the Butantan Institute Animal House and euthanized after 4 weeks of treatment in a CO<sub>2</sub> chamber with 30% oxygen; a small quantity of CO<sub>2</sub> was injected for 30s to reduce the anxiety that results from the discomfort of hypoxia.

The lymph nodes were macerated and filtered through a 30-µm membrane to prepare a cell suspension. Cells were resuspended in FACS (fluorescence-activated cell sorting) buffer, and the concentration was adjusted to 10<sup>6</sup> cells/mL. For intracytoplasmic and nuclear markers, cells were permeabilized with 5 µl 0.1% Triton X-100 for 30 minutes prior to incubation with specific primary antibodies in a concentration of 1:100 for, CD4 (cluster of differentiation 4) (ab25475), CD8 (cluster of differentiation 8) (ab22378), IL-1 (interleukin-1) (ab7632), IL-8 (interleukin-8) (ab89336), IL-6 (interleukin-6) (ab6672), CD3 (cluster of differentiation 3) (ab113628), CD25 (cluster of differentiation 25). Tubes were centrifuged and the supernatant discarded; the pellet was resuspended in 100 µL, and 1 µL of secondary antibody was added (ALEXA fluor 488 Molecular-Probe, mouse anti-goat IgG-FITC). The analysis was performed using a flow cytometer (FACSCalibur, BD), and the expression of the markers was determined by comparison with the control isotype.

## Observations

Immunophenotyping of T lymphocytes subpopulations from lymph nodes showed a decrease in the expression of all markers in treated groups. Especially for CD4, the expression was significant in Group II ( $p < 0.001$ ) and group III ( $p < 0.05$ ). While CD25 expression was significant for Group II ( $p < 0.05$ ) and III ( $p < 0.001$ ), CD3 was significant for Group II ( $p < 0.05$ ) and III ( $p < 0.001$ ). The expression of CD8 in Group III had a significant increase ( $p < 0.01$ ). For CD4 lymphocyte markers, CD3 and CD25 showed an modular effect with decreased expression of CD4<sup>+</sup> and CD25<sup>+</sup> subpopulations, in both treated groups, and increased subpopulation of CD8<sup>+</sup> cytotoxic T lymphocytes.

The modulating effect of this treatment was observed for the increase in lymphocyte subpopulations that showed double CD4<sup>+</sup> and CD25<sup>+</sup> staining. An increase in the mean percentage of infiltrating CD4<sup>+</sup> T lymphocytes was noted in patients with lymph node metastasis, but not CD8<sup>+</sup> T cells. The relationship between the tumor stage and the T lymphocyte infiltration has been shown in individuals with prostate, gallbladder, kidney, colorectal and breast cancer (Guise, Chirgwin, 2003). The infiltration of CD3<sup>+</sup> lymphocytes is correlated with malignancy and the occurrence of metastasis upon diagnosis. Phenotypic analysis indicated that these infiltrating lymphocytes in osteosarcoma were 95% CD3<sup>+</sup> and 68% CD8<sup>+</sup> (Holzer et al., 2012).

In the treated group there was a reduction in the expression of IL-1, IL-6 and IL-8 when compared to the control group. A significant reduction of IL-1 ( $p < 0.05$ ) in group II and III was observed. The expression of IL-6 was significantly reduced in Group II and III ( $p < 0.05$ ) and the expression of IL-8 was significantly reduced in group III ( $p < 0.01$ ) in group II ( $p < 0.05$ ). Our results indicate a decreased expression of the IL-1, IL-6 and IL-8 receptors in the MSC/rhBMP-2-treated groups, suggesting an inhibition of the local inflammatory response. According to Wierenga et al. (2002), JAK protein degradation can interfere with the IL-6 signaling. Treatment with 4 doses of MSC/rhBMP-2 (Group III) showed a significant decrease in expression of IL-1, IL-8 and IL-6 receptors. Several studies have shown that cytokines can modulate tumor development (Weifeng et al., 2012).

The combination of CTM/rhBMP-2 showed an efficient role in modulating tumor immune response when evaluated in treatment of canine osteosarcoma in mice, CD3, CD4 and CD25, IL-1, IL-6. Thus, the CTM/rhBMP-2 combination therapy was able to generate a cellular immune response with CD8<sup>+</sup> T cells and suppress the inhibitory effects of CD25<sup>+</sup> regulatory T cells, suggesting that it may be used as a tool for osteosarcoma treatment.

**Submission ID: 32462**

**Submission Title: Inhibition of PDGFR $\alpha$ + Sca-1+ Mesenchymal Progenitor Proliferation and Differentiation By Nilotinib Leads to Improved Cardiac Function Following Ischemic Injury**

**Author(s)**

**Ben Paylor**

PhD Candidate / Blogger

University of British Columbia / Signals Blog

**Co-Author(s)**

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[Fabio Rossi, MD PhD](#) - Principal Investigator, University of British Columbia

**Topic**

Basic Research, Medicine, and Health

**Problem**

Fibrosis is defined by excess deposition of extracellular matrix components (ECM) including collagen, and is associated with a wide variety of pathological states in numerous different tissues. The functional implications of pathological cardiac fibrosis are well documented, but a thorough understanding of the cellular and molecular mechanisms underlying its development are still lacking.

**Background**

Classical dogma in the heart states that in situations of injury, “cardiac fibroblasts” differentiate into mature “myofibroblasts” that are the principal cell population involved in deposition of collagen. A lack of robust *in vivo* markers for both of these populations, as well as thorough understanding of heterogeneity within the loose definitions used to identify them has hampered efforts to develop of therapies targeting cardiac fibrosis. Recent data from our lab has demonstrated that multiple tissues, including the heart, harbour a tissue-resident population of mesenchymal progenitor cells (MPCs) that are involved in the development of fibrosis.

**Hypothesis**

**Pharmacological modulation of cardiac-resident mesenchymal progenitor cell (Lin<sup>-</sup>:PDGFR $\alpha$ <sup>+</sup>:Sca1<sup>+</sup>) proliferation and differentiation following cardiac injury represents a potential therapeutic strategy to improve cardiac function**

**Research**

We isolated cardiac Sca1+ PDGFRa+ (S+P+) and Sca1- PDGFRa+ (P+) cells and following *in vitro* clonal expansion demonstrated their multipotency towards mesenchymal lineages (cultured in osteo- and adipogenic conditions). RNA from freshly sorted S+P+ and P+ was shown (via qPCR) to contain the predominant expression of ECM-associated genes (Col1a1, CTGF, TGFB1) when compared to other cardiac cell populations (S-P-, CD31+ & CD45+). Utilizing two models of cardiac injury (isoproterenol & experimental MI through ligation of the left-anterior descending artery) we observed significant expansion of S+P+ cells at D3 after injury followed by expansion of P+ cells at D7 and D14 after injury. We also observed that proliferation of both populations (assessed by EdU incorporation) co-localized with collagen deposition and CD68+ macrophage infiltration in the myocardium. In both injury models collagen expression was found by qPCR to be upregulated primarily in S+P+ cells at D3 after injury but by D7 expression was predominantly found in P+ cells. Treatment with daily I.P. injections of nilotinib (20mg/kg/day) led to a reduction in PDGFRa+ cells quantity, and also resulted in an increased S+P+ and decreased P+ fractions at D7 after injury as well as reduced collagen expression in P+ cells and reduced collagen deposition in both injury models. Further, daily administration of nilotinib for 28-days following LAD ligation lead to improved cardiac function (EF - 29% in Treated vs 19% Untreated). We conclude that nilotinib has anti-fibrotic effects following cardiac injury that result primarily through its effect on PDGFRa+ mesenchymal progenitor differentiation.

### Observations

We provided in vitro and in vivo evidence that Nilotinib blocks both the proliferation of PDGFRa+ Sca1+ mesenchymal progenitors in the heart as well as their differentiation into more mature PDGFRa+ Sca1- progeny. Utilizing two models of cardiac injury, herein we demonstrate that treatment with nilotinib leads to reduced collagen and improved cardiac function, highlighting it as a potential therapeutic strategy for treatment of cardiovascular disease.

Reviewer	Comments	Total	Reco
Alan Jakimo	In what species has this experiment been carried out? If in humans, were there applicable IRB requirements? If in non-human laboratory animals, were there applicable animal welfare requirements?		Accept
Bernard Siegel			Accept
<b>AVERAGE</b>			

**Submission ID: 32558**

**Submission Title: LESSONS FROM THE TWO-YEAR PRIVATE DENTAL STEM CELL BANKING EXPERIENCE IN MEXICO**

**Author(s)**

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**Topic**

Industry Infrastructure

**Problem**

Stem cell banking is an activity in the border of the science and busyness in which so far the commercial part prevailed over the scientific merits. This is due to the demand for the depositing of their stem cells stimulated by the propaganda of banks on the marvelous possibilities of the treatment of any disease by means of these cells in near future when the science and technology develops effective cell therapy methods. The most expanded are banks of the umbilical cord blood mononuclear cells containing small quantities of mesenchymal cells and hematopoietic stem/progenitors. The mononuclear fractions are cryoconserved without expansion and insurance that will be viable upon thawing after many years. Transplantation of these cells is practiced in which the payment is not justified by the effects.

**Background**

In the 2000 Gronthous and collaborators discovered dental stem cells which have several biomedical and technological advantages over all previously described adult stem cells. Several publications analyzed the importance of these advantages for public and private banking. The biomedical advantages are that these cells originate from the neuroectoderm (neural crest) actively migrating populations characterized by multi-potentiality. The technological advantages rely on their excellent expansion potentials to provide in 2-3 passages the amount sufficient for their therapeutic use

**Hypothesis**

Mesenchymal cells from dental pulp are next generation of the stem cell banking issues and will gradually substitute the cord blood banks for their features to expand in vitro preserving the stem cell potentials

## Research

In 2011 a company INDEBIOC was founded in Mexico by university scientists engaged in stem cell research and odontologists with the aim to create a type of banking resembling more to the bone marrow than cord blood private banking style. We will present summarized information on the economic and technological challenges/solutions and demonstrate the feasibility of the private stem cell banking without any false promises. To our understanding this is the first lesson from a short-term but successful dental pulp stem cell banking practice.

## Observations

despite these novel possibilities that open dental cells for the regenerative medicine the first private banks offer the same established style of the banking – just crioconservation. In

Reviewer	Comments	Total	Reco
Alan Jakimo	Assuming this speaks to banking of human cells, what type of informed consent procedures and related ethical procedures are being followed? The Observations are incomplete and contain typographical errors.		Maybe
Bernard Siegel			Maybe
<b>AVERAGE</b>			

**Submission ID: 32588**

**Submission Title: Comparative analysis of human adipose-derived stem cells selected through the low-affinity nerve growth factor receptor and through plastic adherence**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human adipose-derived stem cells selected by plastic adherence (PA-ADSC) have been extensively characterized and they comprise in early passages, a heterogeneous cell population. For clinical applications, a homogeneous, fresh, and fully characterized ADSC population is desirable. Immunomagnetic selection of ADSC using the low-affinity nerve growth factor receptor (CD271) yields a homogeneous but smaller cell population. However, the role of CD271 surface receptor in mesenchymal stem cells is unclear and little is known about the similarity between CD271<sup>+</sup>ADSC and PA-ADSC regarding immunophenotype, genotype, and duplication time. This study's aim is to determine similarities/differences in the incidence of surface markers, expression of stem-cell related genes and duplication time between CD271<sup>+</sup>ADSC and PA-ADSC.

**Background**

CD271 is a cell surface marker which has been used for enriching mesenchymal stem cells from human bone marrow aspirates and lipoaspirates. CD271<sup>+</sup> cells immuno-magnetically selected from ADSC have been demonstrated to have a higher clonogenic and differentiation potential compared to PA-ADSC.

**Hypothesis**

The fact of possessing or not the CD271 cell surface receptor is possibly linked to a cellular process and/or state. We believe

that CD271<sup>+</sup>ADSC are a more primitive subpopulation with differences in the expression of stem cell-related genes and proliferation ability.

## Research

Mononuclear cells were harvested using a well-established protocol from the lower backs of 5 female donors (35-50 years old) and purified using magnetic beads. The positive and negative fractions were cultured for 1 week and used for subsequent experiments. Flow cytometry was performed to determine the incidence of CD34, CD45, CD73, CD90, and CD105 surface receptors. RNA was extracted for performing RT-PCR for stem cell-related transcription factors Sox2, Notch1, Rex1, Oct4, Nanog, Nestin, and reference gene GAPDH. Duplication times were obtained through a cell-titer assay.

## Observations

CD271<sup>+</sup>ADSC and PA-ADSC demonstrated similar immunophenotypes: CD34<sup>+</sup>/ CD45<sup>-</sup>/ CD73<sup>+</sup>/ CD90<sup>+</sup>/and CD105<sup>+</sup>. Multipotency capacity was tested by determining the relative gene expression of stem cell-related transcription factors. PA-ADSC had no expression of transcription factor Sox2 while a clear band appeared on an agarose gel for CD271<sup>+</sup>ADSC. Furthermore, Oct4 and Nanog showed a smaller relative gene expression for PA-ADSC as compared to CD271<sup>+</sup>ADSC. Mean duplication time was significantly longer for CD271<sup>+</sup>ADSC than for PA-ADSC indicating that this antigen probably addresses a population of resting primitive mesenchymal stem cells. In conclusion, our findings demonstrate differences between CD271-ADSC and PA-ADSC in genotype and proliferation ability.

Reviewer	Comments	Total	Reco
Alan Jakimo			Accept
Bernard Siegel			Accept
<b>AVERAGE</b>			

**Submission ID: 32897**

**Submission Title: Deletion of *Asxl1* Results in Myelodysplasia and Severe Developmental Defects in Vivo**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Acute Myeloid Leukemia (AML) is the most common acute leukemia diagnosed in adults and is responsible for approximately 9,000 deaths/year in the United States. AML is a part of a wider family of myeloid malignancies that include myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and chronic myelomonocytic leukemia (CMML). Currently, there are no targeted therapies for most of these diseases making the study of the molecular mechanisms of their induction and progression of biologic and clinical significance.

**Background**

Candidate gene and genome-wide discovery studies have identified a set of novel disease alleles in patients with myeloid malignancies, including myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and myeloproliferative neoplasms (MPN). This includes somatic mutations in genes with a known, or putative role in the epigenetic regulation of gene expression. Somatic *Addition of Sex Combs Like 1 (ASXL1)* mutations occur in 10-30% of patients with myeloid malignancies, most commonly in myelodysplastic syndromes (MDS), and are associated with adverse outcome.

## Hypothesis

We set out to investigate the role of *Asx1* in normal hematopoiesis and myeloid disease.

## Research

We generated the *Asx1* knockout mouse model and deleted *Asx1* constitutively or specifically in hematopoietic compartments in vivo.

## Observations

Here we show that constitutive loss of *Asx1* results in developmental abnormalities including anophthalmia, microcephaly, cleft palates, and mandibular malformations. By contrast, hematopoietic-specific deletion of *Asx1* resulted in progressive, multilineage cytopenias and dysplasia in the context of increased numbers of hematopoietic stem/progenitor cells (HSPC's), characteristic features of human MDS. Serial transplantation of *Asx1*-null hematopoietic cells resulted in a lethal myeloid disorder at a shorter latency than primary *Asx1* knockout mice. *Asx1* deletion reduced hematopoietic stem-cell self-renewal, which was restored by concomitant deletion of *Tet2*, a gene commonly co-mutated with *ASXL1* in MDS patients. Moreover, compound *Asx1/Tet2* deletion resulted in an MDS phenotype with hastened death compared to single-gene knockout mice. *Asx1* loss resulted in a global reduction of H3K27 trimethylation and dysregulated expression of known regulators of hematopoiesis. RNA-seq/ChIP-seq analyses of *Asx1* in hematopoietic cells identified a subset of differentially expressed genes as direct targets of *Asx1*. These findings underscore the importance of *Asx1* in Polycomb-group function, development, and hematopoiesis.

Reviewer	Comments	Total	Reco
Alan Jakimo			Accept
Bernard Siegel			Accept
<b>AVERAGE</b>			

**Submission ID: 32913**

**Submission Title: Etoposide pre-treatment reduces teratoma formation and enhances the safety of induced pluripotent stem cell-based therapy for the heart**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The advent of induced pluripotent stem cell (iPSC) technology created new opportunities for transplant-based therapeutic strategies and disease modeling. While experimental evidence increasingly supports its reparative benefit for heart disease and neurodegenerative disorders, iPSC-based clinical translation is currently hindered by the risk of dysregulated cell growth known as tumorigenicity.

**Background**

It is known that pluripotent stem cells derived by three-factor (Sox2, Klf, Oct4) and four-factor (Sox2, Klf, Oct4, c-Myc) reprogramming results in teratogenic growth. Studies suggest that the therapeutic application of differentiated stem cells carries the potential to form teratomas, which are composed of the three embryonic germ layers, due to residual undifferentiated cells in the transplanted population. Hence, the removal of residual undifferentiated stem cells from the differentiated cell population has been considered a critical requirement for iPSC-based treatment to progress toward pragmatic clinical application.

**Hypothesis**

One viable strategy to prevent tumor formation is the use of pharmacological agents to selectively target and purge the teratoma-forming cells prior to clinical utilization. Our study aimed to demonstrate the effect of etoposide treatment in decreasing teratoma formation by eliminating the pluripotent cells in the early cardiac progenitor population.

### Research

Immunodeficient murine hearts were infarcted and received implantation of the therapeutic cardiac progenitor population. *In vivo* bioluminescence imaging (BLI) measured the luciferase-labeled iPSCs and cardiac progenitors in the infarcted myocardium at four weeks post-implantation. The total percentage, area and location of mass formation following the pharmacological treatment of etoposide at 0.01  $\mu$ M concentration was measured by echocardiography and gross autopsy.

### Observations

Etoposide-treated cell implantation produced none or reduced teratogenicity in the intra-cardiac and extra-cardiac/chest cavity compared to the control untreated group. *In vivo* bioluminescence imaging confirmed the localization and engraftment of transplanted cells in the myocardium four weeks post-injection. Comparatively, the cell population without treatment demonstrated a greater incidence and size of teratoma formation. Hence, pretreatment with genotoxic etoposide could significantly lower the threat of teratogenicity, allowing the ability to harness the clinical-grade application of iPSC-derived therapeutic agents.

Reviewer	Comments	Total	Reco
Alan Jakimo			Accept
Bernard Siegel			Accept
<b>AVERAGE</b>			

**Submission ID: 32938**

**Submission Title: DEVELOPING SYNTHETIC POLYMERIC SURFACES FOR GROWING HUMAN MESENCHYMAL STEM CELLS IN A XENO-FREE AND SERUM-FREE MEDIUM**

**Author(s)**

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**Topic**

Industry Infrastructure

**Problem**

In this study, we seek to replace ECM proteins with synthetic polymers which are cheaper, from pure sources and better suited for industrial scale cell production in the serum-free culture.

**Background**

Pre-clinical and clinical studies indicate that mesenchymal stem cells (MSCs), functioning particularly in angiogenicity and immune modulation, have potential in treating multiple diseases. MSCs are widely cultured in serum-containing media which provides nutrients, oncotic pressure, growth signals and extracellular matrix proteins that adhere to the culture dish to allow initial cell attachment. Recently MSCs are increasingly cultured in serum-free media which offer better culture consistency. ECM proteins are routinely blended in the serum-free media or coated on Petri dishes before cell culture. However, ECM protein is expensive, extracted from tissues or blood, and often contains unwanted impurities. Therefore, we seek to replace ECM proteins with synthetic polymers in the serum-free culture.

## **Hypothesis**

We hypothesize that the synthetic polymers mimic the function of ECM proteins which enhances cell attachment during the serum-free culture.

## **Research**

More than 1500 polymers were initially screened for MSC attachment and growth using Ilika's polymer microarrays in combination with ITRI-SFM, which is a xeno-free, serum free and chemically defined medium developed by ITRI. CellStart™ from Invitrogen was used as a positive control. Based on the results of the polymer microarrays, more than 80 polymers were selected for the next screening phase on cover glasses. About two dozen polymer compositions were further selected and tested for coating on polystyrene surface using 24-well plates. From these results, three polymers which were the best performing in terms of cell growth were finally selected for coating on 6-well plates. One polymer was further selected for coating on 10 cm plates to demonstrate the coating ability on the larger surface area.

## **Observations**

Using the culture system with ITRI-SFM and selected synthetic polymers on the polystyrene surface, MSCs were able to grow and maintain their stem cell properties after serial passages of culture. Cells were able to maintain characteristic MSC phenotypes of surface markers (CD73+, CD90+, CD105+, CD34-, and CD45-) and capabilities of tri-lineage differentiation. These results demonstrate that the synthetic polymeric surface developed here is suitable for GMP-compliant expansion under xeno-free and serum-free condition for MSC-based therapy.

**Submission ID: 32949**

**Submission Title: Human mesenchymal stem cells cultured with a xeno-free industrial pharmaceutical grade supplement for cell culture (SCC) preserve their capacity for differentiation**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human mesenchymal stem cells (hMSCs) have a great potential for several cellular therapies but, in most cases, the cells are still expanded using media supplemented with animal products, such as Fetal Bovine Serum (FBS) which is a concern for future clinical applications.

**Background**

Despite the many disadvantages related to FBS usage, such as the high variability between batches and the effect on the immune response, the lack of GMP, pharmaceutical grade, xeno-free alternatives to FBS means that many laboratories are performing cell culture research by using supplements of animal origin which could be a concern when transferring the research from the lab bench to the clinical setting.

**Hypothesis**

The pharmaceutical grade, GMP xeno-free supplement for cell culture (SCC) under development at Grifols avoids the use of FBS and other non-xeno-free supplements. hMSCs cultured with SCC maintain their characteristics and preserve their potential for differentiation into different cell types

## **Research**

Grifols' SCC is obtained from industrial cold ethanol human plasma fractionation and it is manufactured under GMP rules. Plasma is collected from healthy donors at FDA-licensed plasmapheresis centers. Every plasma pool contains donation from over 1000 different donors and every donation is tested for viral markers and all plasma is tested, using nucleic acid techniques, for the presence of transfusion transmissible viral agents (HIV, HAV, HBV, HCV and B19 virus). SCC has a specific viral inactivation step (gamma irradiation) besides the production process purification steps with pathogen removal capacity. hMSCs (commercial origin) from bone marrow cultured with SCC, after differentiation induction with commercial media, as previously has been shown, were trans-differentiated into neurons and, now, these cells have shown their capacity to differentiate into adipocytes and osteoblasts. Differentiation was confirmed using the Nilss staining for neurons and the Oil Red O staining for adipocytes. The detection of calcium deposits with the Alizarin Red S staining and the alkaline phosphatase activity was performed for confirming the differentiation into osteoblasts.

## **Observations**

Commercial origin hMSCs from bone marrow cultured with Grifols' SCC preserve their capacity to differentiate into neurons, adipocytes and osteoblasts. Grifols' SCC could be a suitable substitute of FBS and other animal origin supplement because of its advantages such as the high consistence between batches due to the high number of donations from different donors who contribute in a plasma pool, the human origin of SCC, and the plasma-derived product safety regarding the screening of donor and donations and, also the incorporation of a specific viral inactivation step to the production process. SCC not only support the growth of undifferentiated hMSCs with full capacity for differentiation, but also the embryonic stem cells, induced pluripotent stem cells and several continuous cell lines. These results indicate that SCC could be a good candidate for cell supplementation in advanced therapies.

**Submission ID: 33035**

**Submission Title: Treatment of Cerebellar Ataxia with Mesenchymal Stem Cells: A Phase I/II Trial**

**Author(s)**

**Bing-Wen Soong, M.D., Ph.D., FAAN, CPI, Professor, US Board Certified Neurologist**

Treatment of Cerebellar Ataxia with Mesenchymal Stem Cells: A Phase I/II Trial

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Spinocerebellar ataxias (SCA) are rare, debilitating neurodegenerative diseases which cause progressive difficulty with coordination and gait which progressively interferes with performing normal daily functions. SCA is categorized as a “rare disease” by the Office of Rare Diseases Research (ORDR) at the National Institutes of Health (NIH) with an estimated prevalence of 2-7 per 100,000 (Schols L, 2004) (Whaley RN, 2011).

A progressive, serious neurodegenerative disease, there are more than 30 subtypes of SCA that involve the cerebellum (in particular Purkinje cells), brainstem, and other non-spinocerebellar tissues. Each subtype of SCA is caused by a mutation of a different gene. Although different gene mutations are identified in different subclasses of SCAs, all the polyQ SCA patients clinically present limb and gait ataxia because the same ataxia interactome is shared among these different sub-groups of patients.

SCA patients typically first lose their ability to work, followed by the loss of their ability to walk, and then suffer complications from being bed-ridden over an extensive period of time. SCA patients typically die from respiratory failure, aspiration pneumonia or severe infection within 20 years of onset.

There are no approved, disease altering, medical treatments for SCA (spinocerebellar ataxia) patients. This poster presents a Phase I/II clinical trial for StemChymal, a potential cell therapy candidate, to assess safety and early evidence of efficacy in SCA3 patients.

**Background**

PolyQ SCAs are caused by an extensive CAG sequence which encodes for expanded polyQ residues within the mutated protein. The severity of the disease being correlated with the length of CAG repeat and the level of oxidative stress.

SCA2 and SCA3 are two common subclass types with large numbers of CAG repeats in mutant ATXN2 and ATXN3 genes, resulting in the expansion of polyQ residues in ataxin-2 and ataxin-3 proteins. Physiologically, ataxin-2 has been found to be a component of translating polysomes, cytoplasmic stress granules, P-bodies, and the miRNA pathway; polyQ in ataxin-2 may have an imbalance of RNA metabolism and regulation of translation in target cells. Ataxin-3, which is associated with transcriptional repression and protein homeostasis, promotes the interaction between Bxl-XL and Bax and directly regulates

the ubiquitin-proteasome system (Evert BO, 2006) (Reina CP, 2010) (Burnett B, 2010) (Zhou L, 2013). From the cellular point of view, oxidative stress is a co-factor of ataxin-2 and worsens the severity of SCA2, and, in addition, up-regulation of superoxide dismutase-2 in response to oxidative stress is impaired in cells from SCA3 patients. Therefore, polyQ in ataxin-2 causes dysfunction of SCA, resulting in increased cellular stress. Extensive polyQ in the affected cells, including Purkinje neurons, leads to cell dysfunction and ultimately triggers cell apoptosis. Loss of Purkinje cells leads to the symptoms and disease outcomes of SCA.

## **Hypothesis**

Mechanisms of action for potential therapeutic benefit using mesenchymal stem cells have been proposed to include; immunologic, paracrine, endocrine and direct cellular effects (Stem Cell Research & Therapy 2011, 2:23 (May 11), Carrion, Figueroa). In the lab, our research has achieved pre-clinical evidence that suggests adipose tissue-derived mesenchymal stem cell transplantation ameliorates motor function deterioration of spinocerebellar ataxia in SCA2 transgenic mice by rescuing cerebellar Purkinje cells (Journal of Biomedical Science 2011, 18:54; Chang, et al). In addition, MSC for the treatment of neurodegenerative disease has been assessed previously (RegenMed. 2010 November ; 5(6): 933–946; MSC for the treatment of neurodegenerative disease; Joyce, Annett, et al; UC Davis, CA, USA).

The infusion of MSC into SCA patients may be safe and may also show evidence of ameliorating motor function deterioration, by preventing or arresting continued loss of Purkinje cells to premature apoptosis caused by oxidative stress from excessive PolyQ expression?

This ongoing Phase I/II clinical trial has been designed and conducted to investigate the safety, and early signs of efficacy, of the infusion of allogeneic adipose-derived mesenchymal stem cells (Stemchymal™) in patients with cerebellar ataxias.

## **Research**

This Stemchymal Phase I/II clinical trial was designed and approved by the IRB of Taipei Veterans General Hospital and Taiwan Food and Drug Administration in April, 2012. Two healthy donors, 6 patients with spinocerebellar ataxia type 3 (SCA3) and one patient with multiple system atrophy-cerebellar type were recruited.

The Primary outcome measures for Safety are evaluated according to vital signs, clinical lab tests and adverse events (AEs). The Secondary outcome measures for early evidence of efficacy include: changes in the “scale for the assessment and rating of ataxia (SARA)” score, changes in sensory organization test (SOT) score, changes in adaptation test (ADT) scores and changes in electronystagmogram (ENG).

The trial design includes: Visit 1; screening (inclusion / exclusion), Visit 2; baseline+ Stemchymal IV infusion (day 0): \*7 x10<sup>7</sup>cells/infusion, Visits 3, 4, 5, 6, 7, 8 → Follow-up for safety measures and signs of efficacy measures: chemistry, imaging: PET, MRI, functional: SOT, AT, ENG, SARA, language and swallowing.

The progress of the trial has been regularly and closely monitored by the Data and Safety Monitoring Board to ensure the safety of the subjects. The subjects have been monitored closely on their scheduled follow-ups and the first recipient has been followed for 10 months.

## **Observations**

This is a phase I/II clinical trial aiming to evaluate the safety and efficacy of IV infused Stemchymal (allogeneic AT-derived MSCs) for the treatment of cerebellar ataxia.

The trial was approved by the IRB of Taipei Veterans General Hospital and Taiwan Food and Drug Administration in April, 2012. Two healthy donors, 6 patients with spinocerebellar ataxia type 3 (SCA3) and one patient with multiple system atrophy-cerebellar type were recruited.

Subjects were given one intravenous infusion of allogeneic adipose MSCs and followed for one year\*. The progress of the trial has been regularly and closely monitored by the Data and Safety Monitoring Board to ensure the safety of the subjects.

The subjects have been monitored closely on their scheduled follow-ups and the first recipient has been followed for 10 months. So far, no MSCs-related adverse event is observed and some evidence of efficacy has been discerned from the preliminary data. The Phase I/II interim safety and early efficacy data, to date, supports the feasibility of using allogeneic Stemchymal™ Cell Therapy in the treatment of SCA3 patients. Longer term follow-up and larger, well-controlled clinical trials will be required to get to a definitive conclusion for Stemchymal treatment of SCA.

Positive results in SCA patients, could support potential for further development of Stemchymal™ cell therapy for multi-type SCA and other neurodegenerative diseases (e.g. HD, ALS, MS).

**Submission ID: 33050**

**Submission Title: Recombinant Adeno-Associated Viral Vectors for Widespread Regulatable Therapeutic Gene Delivery to the CNS**

Benjamin E. Deverman, Bryan Simpson, Piers Pravdo and Paul Patterson

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We previously demonstrated that delivery of the cytokine leukemia inhibitory factor (LIF) to the brain by recombinant adenovirus enhances oligodendrocyte progenitor cell proliferation and remyelination in a mouse model of multiple sclerosis (MS). To extend these findings, we are developing clinically relevant delivery vehicles capable of providing long-term, controllable expression of LIF, or other transgenes, that is widespread within, yet restricted to, the CNS. Recombinant adeno-associated virus 9 (rAAV9) is an attractive candidate for such a vector as it can deliver genes to neurons and glia throughout the CNS when injected intravenously (IV). For this systemic rAA9-based approach to succeed, however, significant improvements in CNS transduction efficiency and selectivity are needed, as is a better means of modulating or turning off transgene expression post-delivery. We are making these enhancements by (i) developing rAAV genomes that use gene regulatory elements (promoter/enhancer elements and miRNA binding sites) to restrict expression to CNS astrocytes, (ii) optimizing dox-inducible regulatory elements to provide control over transgene expression post-delivery and (iii) using directed evolution/in vivo screening to develop AAV9-based capsids that more efficiently transduce CNS astrocytes. These optimized vectors will be used as LIF delivery vehicles in animal models of MS, and should also be applicable to the treatment of other CNS neurodegenerative and developmental disorders.

**Submission ID: 33060**

**Submission Title: mRNA-Engineered Mesenchymal Stem Cells for Targeted Delivery of Interleukin-10 to Sites of Inflammation**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

While biologics have had a profound impact on rheumatology, oncology, cardiology, dermatology, gastroenterology, and neurology, there have been significant challenges given the often multiple administrations required, poor stability, and the release kinetics are often difficult to control. Aside from antibody-based therapeutics, protein therapies are not targeted and several biological agents fail to advance given the complexity involved in their synthesis, purification, or delivery.

**Background**

Using cells to deliver protein therapeutics is a viable approach, yet current strategies entail viral modifications that complicate the regulatory process and introduce potential safety concerns. Non-viral cell based therapy has shown significant potential to address several medical problems through transient expression of biological agents. Cells such as mesenchymal stromal cells (MSCs) are rich stores of immunomodulatory, angiogenic, and trophic factors that exert therapeutic effects in multiple disease models. MSCs represent a potentially ideal cell type to deliver biological agents, given their safety profile in thousands of human patients, their ease of isolation, culture expansion, and immune-evasive phenotype that permits off-the-shelf allogeneic therapy.

**Hypothesis**

We hypothesized that the targeted delivery of potent therapeutic factors (e.g., immunomodulatory molecules) via MSCs to diseased sites will improve the current therapeutic use of protein biologics.

**Research**

In this study, we used mRNA transfection to generate MSCs that simultaneously express functional rolling machinery (P-selectin glycoprotein ligand-1 (PSGL-1) and Sialyl-Lewisx (SLeX)) to rapidly target inflamed tissues following systemic infusion, and that express the potent immunosuppressive cytokine interleukin-10 (IL-10), that is not inherently expressed by human MSCs. Triple transfected PSGL-1/SLeX/IL-10 MSCs transiently increased levels of IL-10 in the inflamed ear and showed a superior anti-inflammatory effect in-vivo, significantly reducing local inflammation following systemic administration.

**Observations**

Overall, this study demonstrates that mRNA-engineered MSCs can be harnessed via a “hit-and-run” action for the targeted delivery of potent immunomodulatory factors to treat distant sites of inflammation. These results also highlight mRNA transfection as a promising platform to improve cell-based therapy via simultaneous control over multiple cell properties following transplantation, and to non-virally target biologics to disease sites.

**Submission ID: 33071**

## **Submission Title: Sample Microtopography Promotes Mechanomodulation of Histone H3 and Efficiency of Cell Reprogramming**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Differentiated cells can transform to become induced pluripotent stem cells through cell reprogramming. With cell reprogramming efficiency being less than 1%, biophysical factors are examined for cell reprogramming throughput. In addition, chemical factors and biological techniques have been studied extensively, but studies on the effect of mechanical factors on efficiency of cell reprogramming is still not sufficient.

### **Background**

Cell reprogramming requires 4 key transcription factors for a differentiated cell to become pluripotent. Some of these Yamanaka factors (namely OCT4, SOX2, and KLF42) are directly affected by the epigenetic framework of the cell. Histones and its molecular modifications, which regulate the histones, greatly influence DNA activity as they determine gene regulation such as which genes can be expressed or repressed and transcription kinetics; therefore, transcriptional activity can be determined by looking at the epigenetic posttranslational modifications. For instance, acetylation of histones affects the binding chemistry between the histone and the DNA backbone; the more acetylated the histones, the more neutralized the histone N-tails are, making them less attracted to the negatively charged phosphate groups of the DNA. HDACs (Histone Deacetylases) take off the acetyl groups, making histones wrap around the chromatin more tightly and thus making the cell transcriptionally silent.

### **Hypothesis**

By changing the microenvironment of the cell in a mechanistic way, cell topography will change. Through these topological changes in the cell, the cell will respond by increasing transcriptional activity as the nucleus also changes shape. Cell reprogramming is enhanced when cells are more transcriptionally active, making the DNA and RNA become more open to proteomic changes.

### **Research**

Summary of PDMS membrane fabrication: SU-8 Photoresist was coated onto Piranha chemical solution treated (mixture of H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub>) silicon wafer. After UV treatment and several bakes, PDMS mixed with curing solution at a 10:1 ratio that was de-gassed is poured onto center of silicon wafer and evenly coated by a spinner. After an hour of baking, PDMS is sonicated with ethanol and then plasma treated to change hydrophobicity.

Outline of Cell Culture Time Line: Fibroblasts were isolated from mouse ears, and passaged twice, each time allowing proliferation for 3 days. After cryogenic preservation, cells were thawed and seeded onto plates and allowed to grow for 2 days. They were then collected and seeded cells onto PDMS membranes. Cells were kept in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Mouse Embryonic Fibroblast media contained 10% FBS (fetal bovine serum) and 1% Penicillin/Streptomycin.

Immunostaining of Phalloidin and DAPI for cells seeded on different width microgroove membranes. Directionality/cell orientation and nuclear elongation are affected by the microgrooves. We observe a decrease in nuclear shape index as the width of microgrooves decreases. Through more immunostaining, we imaged colonies formed on microgroove and flat membranes. Quantification of Nanog colonies was also done on membranes of variable groove width.

Western Blots display the expression levels of various proteins in cells cultured on flat membranes versus grooved membranes. Quantification of Western blots normalized to Actin. Changes in signal/intensity correlate with level of protein expression. Higher signals relative to other bands means more of those specific proteins in that cell sample in comparison to other samples.

### **Observations**

Cell reprogramming efficiency can be increased by topography: 10 micrometer microgroove PDMS membranes produced the highest number of iPSC colonies. The cell's biophysical microenvironment affects the mechanomodulation of epigenetics: AcH3, WDR5, and H3K4me3 is higher on the groove than on the flat membranes and the opposite trend is seen for HDAC2. HDAC2 is the most affected in comparison to other HDACs.

**Submission ID: 33121**

**Submission Title: Morphology-based and non-invasive prediction of differentiation potential of mesenchymal stem cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Morphology of cells had long been used as an important indicator of cell quality. However, such experience-based morphological importance has not been well quantitatively examined in the aspect of automation for industrialization.

**Background**

For the cell therapy, such non-invasive technique to evaluate and assess the quality of cells is strongly required for its industrialization with the growth of stem cell research and its achievements. Human bone marrow-derived stem cells (hBMSCs) had been widely studied and applied to clinical cell therapies with their multipotency. However, it is also known that such stem cells require highly skilled cell culture, together with costly consumables, to carefully maintain their undifferentiated status, and to differentiate into the objective type of cells.

**Hypothesis**

Technological advances in optical systems and image-processing technologies have changed the status of image-based data from an art, available only to experts, to a technique that can be used to generate unbiased data. Many high-content image-analysis methodologies, based on imaging and image-processing technologies, are contributing to advances in drug discoveries. However, non-labeled cell-morphology analysis continues to be rare, and reports published to date have not yet demonstrated the practical usefulness of image analysis in the production of cells for regenerative medicine.

**Research**

In our research, we introduced bioinformatics machine learning strategy to build a prediction model, which links “the cell morphology information” and “the experimentally determined differentiation results” of hBMSCs. By modeling information from the time-lapse phase contrast images of more than 2,000 images of the continuously passaged hBMSC (over 8 passages), the experimentally defined staining results of differentiations could be predicted from extremely early period of culture.

## **Observations**

For construction of prediction model, we examined to use distributional information as input data, which show the heterogeneity in culture cells. Also 69 gene profile was determined to investigate the genetic mechanism which allowed our concept of morphological prediction of cellular quality collapse.

**Submission ID: 33160**

**Submission Title: Functional validation of a novel gene conferring selective advantage to human pluripotent stem cells (hPSCs) during propagation.**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human pluripotent stem cells (hPSCs) have generated a lot of interest in the scientific community based on their potential applications in regenerative medicine. However, numerous research groups have continued to report a propensity for genomic alterations during hPSC culture that poses concerns for basic research and clinical applications.

**Background**

Work from our laboratory and others has demonstrated that amplification of chromosomal regions is correlated with increased gene expression. To date, the phenotypic association of common genomic alterations remains unclear and is a cause for concern during clinical use. In our study, we focus on a common genomic aberration and a list of candidate genes with increased gene expression to hypothesize a gene that may confer selective advantage when overexpressed.

**Hypothesis**

We hypothesize a particular gene to confer selective advantage to transduced hPSCs when overexpressed.

**Research**

Combining karyotype and microarray data we identify a candidate gene for functional analysis. Using lentiviral transduction we overexpress our candidate gene in a manner similar to that observed in amplified genomic regions that cause gene overexpression.

**Observations**

Our transduced candidate gene overexpressing hPSC line exhibited culture dominance in co-cultures of modified overexpression lines with non-overexpression lines. Furthermore, during low density seeding, we demonstrate increased clonality of our overexpression line against matched controls. A striking observation is that we could reduce this selective advantage by varying the hPSC culture conditions. This work is unique in demonstrating (1) a novel gene that confers selective

advantage to hPSCs when overexpressed and may help explain a common trisomy dominance, (2) provide a selection model for studying culture conditions that reduce the appearance of genomically altered hPSCs, (3) and, aid in elucidation of a mechanism that may act as a molecular switch during culture adaptation.

**Submission ID: 33220**

**Submission Title: Developing an easy-to-understand booklet for everyone to discuss ethical issues in stem cell research**

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**Topic**

Ethics, Law and Society

**Problem**

Currently, two major issues lie in stem cell research in Japan. First, since the existing Japanese guidelines have not met the needs of the latest stem cell research, the guidelines impede taking the research to the next level. Second, misleading information released by the media is a routine problem for stem cell research stakeholders. Typical examples are that “ES cells are created by destroying fertilized eggs intended to become babies” and “ES cells are no longer necessary because iPS cells have been discovered.” Moreover, patients who believe the hype, consider treatments using stem cells as established treatments and receive non-evidence based therapy unknowingly. Some of them even end up suffering from unexpected side-effects. Thus, the current situation has the potential to undermine the relations of mutual trust between researchers and the general public about stem cell research.

**Background**

-The complexity of ES/iPS cell research makes it difficult to understand for non-experts.

-Most published books written for non-experts focus commonly on general information, such as, *what are stem cells* or *what are their benefits to our society*. It is also crucial for the general public to know *what we can and cannot do with stem cells, the arguments against their use, and how we should address these issues*. However, existing books contain too many technical terms that non-experts are unfamiliar with.

-The myth that “ES cells are no longer necessary since iPS cells have been discovered” is still dominating public opinion in Japan, while researchers have come to the consensus that both ES and iPS cells are indispensable in stem cell research. It is extremely difficult for front-line researchers to have their voices heard when creating guidelines and forming national policy.

**Hypothesis**

If we provide correct easy-to-understand information to the general public, we'll be able to contribute to developing a sound environment for stem cell research and protect patients from potential risks.

## Research

The working group has interviewed about 30 stem cell researchers. The interview questions varied from *how researchers view the current situation and consider the issues surrounding them to what researchers wish the general public to understand.*

What makes this book unique is showing the various aspects of these arguments and issues, by taking in front line researchers' comments.

Also, we avoided the lecture-like approach of scientists educating the general public, in favor of a more participatory approach. With everyone's involvement, we can help deepen the public understanding of issues surrounding stem cell research.

Contents are as follows:

### 1. Topics for non-experts

- 1) How do researchers study?
- 2) What are the controversial points? How do researchers address these problems?
- 3) Why do we need stem cell research?
- 4) What stem cells can and cannot do?
- 5) What are researchers' responsibilities?
- 6) Regenerative medicine is not an almighty treatment. There are untreatable cases with regenerative medicine.
- 7) What are the risks?

### 2. Basic scientific knowledge

- 1) What are stem cells?
- 2) What is regenerative medicine?
- 3) What is the process before treatment will be allowable?

### 3. How to judge the limit of advanced stem cell research

- 1) Medical principles
- 2) Ethical, Legal and Social Implications (ELSI)
- 3) Consider Japanese cultural background, such as, Buddhism and how Japanese view nature and death

### 4. How to behave when facing underdeveloped guidelines

1) Create a group of stem cell experts and abide by the principles

2) Make a mental note of prioritizing quality in research

### **Observations**

1. Considering the booklet is 80 pages long, illustrations are included on every page to make the booklet reader friendly.
2. Our target audience is high school students or above who have knowledge of basic science.
3. The contents consist of a dialog between a law student, as a non-expert, and her uncle, as a stem cell researcher. Following their dialogs, readers can think about the issues on their own.

### **Conclusion:**

1. Through casual conversation, it is easy to understand the contents.
2. We haven't distributed the booklet to the general populace yet. Evaluation will be made after more people read the booklet.

**Submission ID: 33256**

**Submission Title: Biological Characteristics and Effect of Human Umbilical Cord Mesenchymal Stem Cells (hUC-MSCs) Grafting with Blood Plasma on Bone Regeneration**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Delay or nonunion of fractures occur in approximately 5-10% of the 5.6 million patients who sustain a fracture annually. Nonetheless, the poor patient outcomes associated with fracture nonunion point to a critical need for more efficacious strategies for bone regeneration. Furthermore, the precise regulated mechanism of the differentiation of osteocytes remains unknown.

**Background**

These clinical scenarios underscore the need to develop novel strategies to enhance bone regeneration in the treatment of musculoskeletal disorders. Mesenchymal Stem Cells (MSCs) can be isolated from bone marrow and adipose tissues in adult stages and from placenta, umbilical cord blood and connective tissue (Wharton's Jelly) of human umbilical cord (UC), and MSCs can be induced in vitro and in vivo to differentiate into various mesenchymal tissues such as bone, cartilage, muscle, tendon, adipose tissue, and hematopoiesis-supporting stroma. Osteo-differentiation of MSCs is a complex, tightly regulated multi-stage process that is critical for proper bone formation and is influenced by a variety of endogenous and environmental factors and multiple signaling pathways.

**Hypothesis**

The primary aim of this study was to examine the biological characteristics of hUC-MSCs on the course of impaired bone healing in vivo. The second aim was to observe the precise regulated mechanism of the osteo-differentiation from hUC-MSCs.

## **Research**

SD rats (142) were randomly divided into four groups: fracture group (positive control); nonunion group (negative control); hUC-MSCs grafting with blood plasma group; and hUC-MSCs grafting with saline group. Rats were administered tetracycline (30 mg/kg) and calcein blue (5 mg/kg) 8 days before killing. The animals were killed under deep anesthesia at 4 and 8 weeks post fracture for radiological evaluation and histological/immunohistological studies.

## **Observations**

The hUC-MSCs grafting with blood plasma group was similar to fracture group: the fracture line blurred in 4 weeks and disappeared in 8 weeks postoperatively. Histological/immunohistological studies showed that hUC-MSCs were of low immunogenicity which merged in rat bone tissue, differentiated into osteogenic lineages, and completed the healing of nonunion. After stem cell transplantation, regardless of whether plasma or saline was used, new multi-center bone formation was observed; fracture site density was better in stem cell grafting with blood plasma group.

**Submission ID: 33264**

**Submission Title: Nutrient and Metabolite Documentation for Standardization of Mesenchymal Stem Cell (MSC) Cultures Using the CEDEX Bio**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Standardization of human MSC culture development is limited by lack of cost effective methods for measuring nutrients and metabolites. While large scale MSC culture is standardized by expensive auto-analyzers, cost-effective measurements for smaller scale cultures are not available for MSC culture research, development, and production.

**Background**

MSC's respond to changes in culture conditions and media components by changes in morphologic markers, genomic expression and proteomic products. Affordable documentation of nutrients and metabolites is required to standardize MSC culture to control these variables.

**Hypothesis**

This study tested if the Roche CEDEX Bio (CDB) can function as an auto-analyzer for measuring important nutrients and metabolites for MSC culture standardization.

**Research**

This study utilized ATCC sourced human dermal fibroblast cell lines (HDF's): (CCL-110 & SCRC-1041) to measure outcomes for MDFc19, a MSC conditioned media as active component for skin care products. Six culture media were used for each HDF sample for culture days 0, 1, 4, 7 for CDB analyses: sodium, potassium, glucose, glutamine, lactate, ammonia, and lactic dehydrogenase (LDH). MEDIA KEY: a) Neg Cont1 - CMRL+HuABS, b) Neg Cont 2 - SCM9+HuABS, c) Basic Medium - CMRL+FBS, d) Production Medium - SCM9+FBS, e) Conditioned Medium - MDFC19, f) Pos Cont - ATCC optimized medium. HDF assay outcomes were a) % confluency, b) total Type 1 collagen production, and c) number of HDF's filling the Gap Assay in 24 hours.

**Observations**

Confluent growth of both types of HDF's over 7 days was highest with MDFC19 conditioned media. Total Collagen and Gap Assay results below:

<b>COLLAGEN LEVELS</b> (ng/ml)	CMRL+HuABS	SCM9+HuABS	CMRL+FBS	SCM9+FBS	MDFc19	ATCC Control	Culture Days
CCL-110	100	100	6,704	12,609	15,242	17,415	9
SCRC-1041	100	100	8,427	15,740	21,375	20,773	11
<b>GAP ASSAY</b> (# Cells / 24 Hrs)							
CCL-110	-	-	35	137	240	180	1
SCRC-1041	-	-	62	134	186	210	1

Since this HDF model shows that MDFc19 optimally increases cell confluency, increases collagen production, and stimulates more HDFs to enter the GAP, we utilize these potency assays in part as release criteria for MDFc19 lot distribution. But these assays do not provide metabolic activities of these cells.

The CDB determined there was no major difference in SODIUM or POTASSIUM levels in the 6 media over 7 days, as expected. The GLUCOSE values varied depending upon the number of cells initially plated. The results in the tables started with cells plated at 40% confluence with GLUCOSE levels falling to 0.0 during culture that did not occur when starting with 1% confluence. **CCL-110 HDF** results.

CULTURE MEDIA	GLUCOSE (mmol/L)			LACTATE (mmol/L)			LDH (U/L)			GLUTAMINE (mmol/L)			AMMONIA (mmol/L)		
	Day 1	4	7 ^	1	4	7 ^	1	4	7 ^	1	4	7 ^	1	4	7
a)CMRL+HuABS	4.32	5.60	4.34	0.86	0.53	0.39	6.50	3.07	5.23	0.17	0.26	0.23	0.13	0.16	0.18
b)SCM9+HuABS	5.35	5.60	3.44	0.87	0.45	0.38	0.63	0.60	0.27	8.98	9.06	5.34	5.47	5.19	4.72
c)CMRL+FBS	4.46	5.61	3.17	2.91	6.15	6.85	14.00	7.93	12.37	0.22	0.23	0.18	0.22	0.39	0.46
d)SCM9+FBS	3.96	0.78	0.00	3.61	9.88	11.86	9.60	3.07	10.07	7.32	6.66	5.73	4.70	5.37	6.08
e)MDFc19	3.26	1.24	0.00	4.39	10.33	10.19	3.40	0.27	2.70	12.24	13.70	10.39	2.93	3.22	4.34
f)ATCC Control	3.37	0.83	0.00	3.63	11.33	10.73	0.00	0.00	0.00	1.18	1.06	0.80	0.48	0.79	1.23

LACTATE levels correlate with GLUCOSE levels with little metabolism in HuABS controls and highest in 3 test media. GLUTAMINE levels vary in the different media with AMMONIA levels mimicing the GLUTAMINE levels. LDH levels are lowest in the MDFc19 test media suggesting optimal cell survival. CEDEX Bio results add nutrient and metabolite details that are essential to standardizing MSC involved cultures and their use.

**Submission ID: 33281**

**Submission Title: ESTABLISHING A COLONY ASSAY WITHOUT CONDITIONED MEDIA AND SERUM TO ASSESS ACTIVITIES OF PROGENITOR CELLS FROM ADULT MURINE PANCREAS IN VITRO**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Adult pancreatic stem and progenitor cells are a potential source of insulin-producing beta cells for cell replacement therapy of type 1 diabetes. However, it proved difficult to identify these progenitor cells and analyze their properties, in part due to a lack of an analytical tool.

**Background**

We recently developed a colony assay that allows quantitative and functional analysis of adult murine pancreatic progenitor-like cells in culture. Dissociated single cells from adult pancreas are plated into a semisolid medium containing methylcellulose (a viscous material), Matrigel (for extracellular matrix components), conditioned media, fetal calf serum and growth factors (nicotinamide, exendin 4, activin B and vascular endothelial growth factor). The semisolid medium restricts cell movement yet permits a cell to self-renew, proliferate, differentiate and form a colony of cells. Using this assay, we found that adult murine pancreatic progenitor-like cells are able to give rise to cystic colonies containing ductal-, acinar- and endocrine-like cells. The single cell that initiates and forms a colony is therefore termed a pancreatic colony-forming unit (PCFU).

**Hypothesis**

The undefined components in the conditioned media and serum may complicate the examination of molecular mechanisms that govern the self-renewal and differentiation of these progenitor-like cells. We therefore set out to establish a better-defined, serum-free culture condition for our colony assay.

**Research**

We replaced the conditioned media and fetal calf serum with epidermal growth factor (EGF), R-Spondin1, Noggin and Serum Replacement (Invitrogen) in our otherwise unchanged assay and analyzed for colony formation. To find which media components were essential we omitted single factors of the new culture conditions. Finally, we assessed the long-term expansion of the PCFUs using serial replating experiments.

## **Observations**

We found that our better-defined, serum free culture conditions allowed colony formation and that these colonies also expressed ductal, acinar and endocrine marker genes. Omission of EGF or nicotinamide abrogated colony formation, suggesting the survival and/or proliferation of the PCFUs are dependent on these factors. Media containing all factors tested favored the formation of ductal- and endocrine-like cells over acinar-like cells and supported the long-term expansion of PCFUs better than media containing only the essential factors EGF and nicotinamide.

In conclusion, this better-defined culture condition is an improvement of our existing pancreatic colony assay and will allow for studying mechanisms of self-renewal and differentiation of adult pancreatic progenitor-like cells towards endocrine beta cells in future studies.

**Submission ID: 33335**

**Submission Title: Characterization of in vivo tumorigenicity test using severe immunodeficient NOG mice for quality assessment of human cell-processed therapeutic products**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Contamination of tumorigenic cells in human cell-processed therapeutic products (hCTPs) is one of the major concerns in manufacturing and quality control of hCTPs. However, no quantitative method for detecting the unwanted contamination has been currently standardized.

**Background**

NOD/Shi-scid IL2Rg<sup>null</sup> (NOG) mice have shown highly engraftment potential, as compared with other well-known immunodeficient strains, e.g. nude mice, which are recommended as a rodent xenotransplantation model to assess tumorigenicity of mammalian cells as substrates for the manufacture of biological medicinal products in WHO Technical Report Series, No. 878..

**Hypothesis**

We hypothesized that tumorigenicity test using NOG mice as xenogenic hosts could be a sensitive and quantitative method to detect a small amount of tumorigenic cells in hCTPs.

**Research**

In the present study, we examined tumor formation after subcutaneous injection of HeLa cells in NOG (T, B, and NK cell-defective) and nude (T cell-defective) mice, to compare the sensitivity in detecting tumorigenic cells between the two strains.

Additionally, to establish a sensitive and quantitative assay for detection of tumorigenic cells in normal human cells, we evaluated the sensitivity of the NOG mouse test to HeLa cells that are spiked into human mesenchymal stem cells (hMSCs).

### **Observations**

The sensitivity to tumorigenic cells was defined as the reciprocal number of 50% tumor-producing dose (TPD<sub>50</sub>), which represents the number of cells that form tumors in 50% of the animals at a time point. Sixteen weeks after the inoculation, the TPD<sub>50</sub> value of HeLa cells was stable at  $1.3 \times 10^4$  and  $4.0 \times 10^5$  cells in NOG and nude mice, respectively, indicating 25-fold higher sensitivity of NOG mice, compared to that of nude mice. Matrigel, a basement membrane-like extracellular matrix extract used for suspending HeLa cells, decreased the TPD<sub>50</sub> value of HeLa cells in NOG mice to 80 cells, leading to 5,000-fold higher sensitivity, compared with that of nude mice. The TPD<sub>50</sub> values of HeLa cells suspended in Matrigel with  $1 \times 10^6$  or  $1 \times 10^7$  hMSCs were approximately 100, regardless of the number of hMSCs, sixteen weeks after the inoculation.

NOG mice in combination with Matrigel demonstrated superior efficiency in engraftment of HeLa cells, compared with nude mice that are recommended in WHO TRS 878. They also showed an ability to detect as little as 100 (0.001%) HeLa cells present in  $1 \times 10^7$  hMSCs at a probability of 50%. These results suggest that the *in vivo* tumorigenicity test using NOG mice with Matrigel is a highly sensitive and quantitative method to detect a trace amount of tumorigenic cell contamination in normal human cells, which can be useful in quality assessment of hCTPs.

**Submission ID: 33384**

**Submission Title: miRNA profiling as a quality signature for cellular therapies**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Stem cells derived from embryonic or adult tissue and from reprogrammed somatic (iPS) cells have significant promise for regenerative medicine. However, despite similarities in developmental potential, several groups have found fundamental differences between stem cell lines that could impact on the potency and/or safety of the resultant cell populations, but which were not predicted using current monitoring procedures based on flow cytometry and analysis of panels of mRNAs.

**Background**

There is a requirement for reliable tools to monitor cell populations during the processes of stem cell line development, directed differentiation and scale-up to safe, therapeutically-useful cell populations.

**Hypothesis**

MicroRNA (miRNA) profiling is proving highly informative for cell characterisation, and cell therapy developers are now exploring using miRNA profiling for product characterisation. Alterations in miRNA expression in cell populations provide a sensitive indicator of deviations in cell phenotype, which may crucially impact on the functional capacity/potency of cell populations.

**Research**

Sistemic Ltd. has developed a novel, reliable, generic monitoring tool (SistemQC™) that provides both a robust indication of cell identity, homogeneity and potency, as well as providing insights into the underlying changes in gene expression associated with observed biological phenotypes.

## **Observations**

SistemQC™ utilises a combination of microRNA expression profiling and customised, multi-layered data analysis to provide a simple, robust and cost-effective tool to monitor the maintenance of pluripotency in stem cell lines across passage, the staging of directed differentiation from embryonic, iPS or direct reprogramming strategies, and post scale-up, an assessment of functional attributes and safety profile of the cells. In this poster data will be presented to illustrate the application of SistemQC™ in donor selection, bioprocessing monitoring and potency testing.

Part of the data presented here has previously been presented at an international conference

**Submission ID: 33387**

**Submission Title: Simple, easy, and effective vascular and tissue regenerative therapy for diabetic patients by quality and quantity culture of peripheral blood mononuclear cells.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The quality and quantity of endothelial progenitor cells (EPC), i.e., CD34+ cells is known to be impaired in diabetic patients, thereby raising declined tissue repair in autologous EPC therapy. Since number and function of EPC is low in diabetic patients, large number of peripheral blood or bone marrow EPCs are needed to be isolated by apheresis or bone marrow aspiration. Therefore diabetic patients undergo present autologous EPC therapy by invasive isolation technique with transplantation of low number of dysfunctional EPCs. Less invasive and application of more functional EPCs for diabetic patients is needed for practical and effective tissue regeneration therapy.

**Background**

In 1997, Asahara first reported that a subset of circulating CD34+ cells can function as adult progenitor cells of endothelial lineage (Asahara T, Science. 1997). These endothelial progenitor cells (EPCs) could be isolated from peripheral blood and were found to incorporate into sites of physiological and pathological neovascularization in vivo. (Takahashi T, Nature Med 1999). Recent, studies have also shed light on the relationship between EPCs and diabetes. Tepper reported that ischemia, a well-recognized entity of diabetic wounds and related complications, is a major stimulus and regulator of EPC contribution (Tepper, Blood 2005). EPC transplantation has shown promise in both pre-clinical and clinical trials as a strategy for therapeutic vasculogenesis. Since many diabetic patients suffer from ischemic complications and non healing wounds these patients should benefit from therapeutic vascularization. However, impairment in diabetic EPCs may lead to less clinical benefit in this patient population. Accordingly, researchers have found only limited clinical benefit of diabetic EPCs (versus normals) to cure wound healing (Stepanovic, Awad et al. 2003). Other obstacles to this therapeutic modality have been inefficient isolation techniques and limited numbers of available cells. We have recently described an ex-vivo serum free expansion culture system, using murine bone marrow EPCs. Preliminary data shows an enhanced ability of bone marrow EPCs to differentiate

into vasculature after culturing the cells in an serum free ex vivo expansion system. For future clinical application of serum free ex vivo expansion system and to establish a simple and effective vascular and regenerative cell therapy for diabetic patients, we have recently disclosed the newly developed a serum free ex vivo expansion system called Hybrid Quantity and Quality Control Culture System (HyQQc) to potentiate the vasculogenic property of EPCs for tissue repair from small amount of peripheral blood.

## Hypothesis

Non-healing wound and ulcers are a major cause of morbidity and mortality in diabetic patients. It is well known that diabetics exhibit impaired neovascularization and thus are at risk for the formation of diabetic ulcers and non-healing wounds. Despite our understanding of this critical relationship, there currently is no effective pro-angiogenic therapy for the treatment of diabetic wounds. Recently, there have been advances in the field of vascular biology that offer new insights into mechanisms of neovascularization, and stem cell therapy has been proposed as a possible technique for augmenting diabetic revascularization. Unfortunately, stem cell therapy may confer less benefit to diabetic patients, as recent literature indicates that diabetic progenitor cells are dysfunctional. The objective of this study is to locate the dysfunction in the diabetic Endothelial Progenitor Cells (EPCs) and to test the efficacy of Hybrid QQc using peripheral blood mononuclear cells of diabetic patients for wound healing.

## Research

Mononuclear cells were isolated from 50 ml of peripheral blood in diabetic patients (n=40) and healthy volunteers (n=40). Then underwent HyQQc for 7 days using STEMSPAN serum free medium with VEGF, Flt-3 ligand, TPO, IL-6 and SCF. The vascular regeneration capability of HyQQ cells pre- or post QQc was evaluated with EPC colony forming assay (EPC-CFA), FACS, EPC culture assay, and qRT-PCR. In order to determine the efficacy of HyQQc cells in therapeutic vasculogenesis in wound healing,  $1 \times 10^4$  cells of pre and post HyQQc cells were injected in a BALBC nude wound healing model. The wound healing model is made with a silicon O-ring stent which prevents wound contractions and allow us to investigate wound healing due to granulation and re-epithelialization. Morphometric analysis of % wound closure and angio-vasculogenesis by co-staining of the antibodies for CD31 and/or human specific mitochondria antigen (HMA), was assessed for wound healing.

## Observations

Results: Diabetic patients demonstrated significantly lower number of total colonies pre HyQQc compared to healthy volunteers (total EPC-CFU No./1000 cells:  $1100.76 \pm 479.55$  vs  $151.92 \pm 317.92$   $p < 0.001$ ). However in post HyQQc, EPC-CFA disclosed the predominant generation of functional total EPC-CFU in post HyQQc cells vs pre HyQQc cells ( $151.92 \pm 317.92$  vs  $729.04 \pm 1241.78$ ,  $p < 0.01$ ) with higher RNA transcripts of VEGF, Ang1, Ang2, MMP9. FACS analysis demonstrated significantly higher percentage of CD34+ cells post HyQQc in diabetic patients ( $3.44 \pm 3.57\%$ .) Transplantation of post diabetic HyQQc cells consecutively unveiled the greater closure compared with that of pre QQc diabetic HyQQc Tx (% wound closure post- vs pre-QQc Tx at day 14:  $84.2 \pm 3.2$  vs  $66.9 \pm 2.5$ ,  $p < 0.05$ , n=10). Further, post-diabetic HyQQc cells promoted wound vascularity compared to pre- diabetic QQc HyQQc cells at day 14 (post- vs pre- QQc Tx for CD31+ cells/ hpf:  $321.8 \pm 162.9$  vs.  $145.7 \pm 69.9$ ;  $p < 0.05$ , n=10). Co-staining for HMA and CD31 revealed vasculogenesis of Tx cells. In conclusion, HyQQc system of autologous peripheral blood mononuclear cell provides the methodological clue to overcome the insufficient efficacy of naïve mononuclear cell therapy for wound healing in diabetic patients. From our data, we assume that 150cc of peripheral blood will be necessary to replace the existing EPC therapy for diabetic patients for wound healing. With this new technology, we

will be able to establish outpatient based simple, safe and effective vascular and regenerative therapy. We are now preparing clinical trial for non-healing diabetic wound patients using autologous peripheral blood HyQQc cells to further confirm its efficacy in future clinical application.

**Submission ID: 33518**

**Submission Title: Engineering local microenvironments reactivates dormant JAK-STAT signaling by tuning local gp130 and BMP ligands, leading to the reversion of epiblast stem cells to naïve mouse pluripotent stem cells.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Directing cell fate decisions from pluripotent stem cells (PSCs) requires the addition of cytokines in precise concentrations and for defined durations. While this is sufficient to direct the desired cell fate changes, the frequency of conversion remains inconsistent as the responsiveness to given cytokines is heterogeneous across populations of cells. This observation underscores the importance of colony-level heterogeneity in cells when instructing cell fate decisions. Specifically, the precise role of the cellular microenvironment (i.e. niche) in driving responsiveness to stimuli remains elusive. Here, we use conversion between two distinct PSC populations, Epiblast stem cells (EpiSCs) and mouse embryonic stem cells (mESCs), as a model for cell fate decisions to dissect the role of the stem cell niche.

**Background**

mESCs and EpiSCs represent pluripotent cell populations from the early pre-implantation inner cell mass and the later post-implantation epiblast, respectively. Importantly, self-renewal of the respective PSC populations is governed by distinct signaling pathways; mESCs by leukemia inhibitory factor (LIF), bone morphogenic protein 4 (BMP4), and Wnt, and EpiSCs by Activin A and basic fibroblast growth factor (bFGF).

**Hypothesis**

We hypothesized that transitions between the respective pluripotent states can occur by controlling extracellular signaling and that any challenges to responsiveness could be overcome by engineering the local cellular microenvironment.

**Research**

To explore this hypothesis, we used microcontact printing to control colony size and separation of EpiSCs. Additionally, we

developed an assay to measure responsiveness to LIF, taking advantage of single-cell level data as measured by a high content screening platform (Cellomics™). The final functional output of modulation of cytokine responsiveness was performed using a reversion assay whereby EpiSCs were seeded in stringent mESC media (2i media) to both drive and stabilize revertant cells.

## Observations

Consistent with the response to maintain pluripotency, we demonstrate that reversion of EpiSCs to mESCs can occur at a low frequency ( $0.0052\% \pm 0.0036\%$ ) in the presence of LIF, despite EpiSCs being refractory to LIF signaling. This reversion occurs due to reacquisition of LIF responsiveness in regions of high local cell density. We next, by micropatterning ( $\mu$ P), engineered the microenvironment to increase local cell density across the entire population. This led to both a robust increase in LIF responsiveness and a 9-fold increase ( $0.047\% \pm 0.018\%$ ) in reversion frequency, thus demonstrating the utility of tuning local cell density. We further demonstrate that LIF and BMP4 signaling are central to  $\mu$ P-mediated reacquisition of LIF responsiveness and in the subsequent reversion of EpiSCs. Addition of LIF and BMP to non-patterned EpiSC culture increases LIF responsiveness and drives reversion of EpiSCs  $\sim$ 5-fold over matched controls ( $0.22\% \pm 0.03\%$  vs.  $0.04\% \pm 0.02\%$ ). We identify LIF receptor (LIF-R) as a novel transcriptional target for STAT3 and Smad1, the respective downstream transcription factors of LIF and BMP. This complex is bridged by p300 and together regulates transcription of LIF-R. Silencing of p300 leads to the loss of LIF responsiveness and the concomitant decrease in reversion frequency. Taken together, we have identified, for the first time, the role of the microenvironment in driving responsiveness to an exogenous signal. As response to exogenous stimuli drives cell fate decisions, we propose that precise engineering of the stem cell niche can ultimately mitigate the variability observed in the generation of cell types useful for cell-based therapeutics. Specifically, we propose that niche engineering will have broad benefits for controlled generation of cells and tissues from PSCs.

**Submission ID: 33564**

**Submission Title: Antiproliferative effect regardless of the route of p53 in stem cell therapy with yolk sac for the treatment of equine squamous cell carcinoma equinus**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The occurrence of eye diseases in equine veterinary clinic ranges from 3% to 27% (SOMMER, 1984; SZEMES & GERHARDS, 2000). Among these diseases, the most diagnosed is the uvea, with a prevalence of up to approximately 50%, and corneal, with a prevalence of up to 28%. Diseases of the eyelid, conjunctiva and/or sclera, lacrimal system, vitreous humor and lens are less frequent in equines (SOMMER, 1984; SPIESS, 1997). One of the most frequent diseases in horses' populations is the equine recurrent uveitis (ERU), with a prevalence of 8% to 25% in the United States (GILGER et al., 2001). The occurrence of this disease in surveys of clinical cases ranges from about 1.4% to 50% (SOMMER, 1984; DWYER et al., 1995; SPEIRS, 1999; SZEMES & GERHARDS, 2000; MOSUNIC et al., 2004). Squamous cell carcinoma (SCC) is a common neoplasm in horses, present mainly in the third eyelid, where the occurrence is associated to excessive exposure to sunlight (MOSUNIC et al., 2004). This malignancy is also associated with age and in castrated males and it is possible that the concentration of androgens, estrogens or both are associated with the development of horses ocular structures (IWABE et al., 2009; MOSUNIC et al 2004). The lack of treatment for this type of carcinoma cause an invasion of the surrounding soft tissues such as bone, orbit, sinuses and brain. When the tumor invades the limbo it progresses rapidly causing visual impairment and destruction of the eyeball (BROOKS, 2002; BROOKS, 2005). The SCC is a malignant and invasive tumor, which can cause metastasis. The third eyelid is involved in 30% of cases and is the primary site with secondary involvement of the conjunctiva, lacrimal canal, eyelid and cornea in 50% to 60% of cases (DUGAN et al 1991; BROOKS, 2002; BROOKS, 2005).

**Background**

Changes in DNA are generally responsible for the onset of cancer and occur in oncogenesis and tumor suppressor genes, which are involved with proteins that regulate cellular cycle and cell growth and differentiation (JORDE et al., 2000; KOCH, 1998). Tumor suppression genes contribute to carcinogenesis when they are inactivated by mutations, therefore, when the

functional suppressor genes stop acting and there is an inappropriate growth inhibition, resulting in uncontrolled cell proliferation (RIVOIRE et al., 2006). The identification of tumor markers is a valuable method for analyzing the disease behavior and determine its prognosis, being little known in veterinary medicine. Cell therapy provides new expectations for the treatment of malignant tumors in horses. This new therapeutic approach with undifferentiated stem cells could lead to new cancer treatments in horses and domestic animals affected with this tumor, which so far have no meaningful answers and major side effects, in addition to recurrences or chemoresistance.

## Hypothesis

Antiproliferative and immunomodulatory cell therapy with stem cells undifferentiated from the yolk sac.

## Research

Samples of squamous cell carcinoma (SCC) in horses were obtained in the Veterinary Hospital of the School of Veterinary Medicine and Animal Science of the University of São Paulo, which were surgically removed in the animal's ocular region. The tumor tissue was cut into small pieces and placed in 25cm<sup>2</sup> culture bottles, kept in DMEM culture medium supplemented with 10% FBS, pH 7.4, in a humidified incubator at 37 °C with 5% CO<sub>2</sub>.

After growth and cell expansion, cells were trypsinized and inactivated with fetal bovine serum for 20 minutes. Then, the material was centrifuged at 1500rpm for 10 minutes and resuspended in FACS Flow. Markers used for expression analysis by flow cytometry were CD 90, STRO-1, OCT3/4, Nanog, Ki-67, PCNA, VEGF, Bax, Bad, Bcl-2, and TNF $\alpha$ . SCC cells were divided into trans-wells plates (Costar) and treated separately with SVE stem cells at concentrations 10<sup>4</sup>, 10<sup>5</sup> ; 10<sup>6</sup>, 10<sup>7</sup>. They were then kept in a incubator at 37 °C for 24, 48 and 72h of treatment.

Cells that actively migrated into the lower chamber were collected and the expression of markers involved in cell death by apoptosis and the proliferative response were determined by flow cytometry. Cells obtained from the treated plates were trypsinized and treated with inactivated FBS, centrifuged at 1500rpm for 10 minutes. The supernatant was discarded and the pellet, resuspended in 5 ml PBS for washing. After the second centrifugation, the supernatant was again discarded and the FACS flow was added. Cells were suspended and incubated with antibodies CD34, CD44, IL-6, Ki-67, VEGF, p53, p27, p21, Bad, Bcl-2, active Caspase-3 and incubated for 15 minutes at 4°C. The expression of nuclear and cytoplasmic antibodies were previously permeabilized with 10 $\mu$ l of Triton X-100 (0.1%) for 30 minutes before the addition of antibodies specific primers. The study was supported by FAPESP (2011/18765-8)

## Observations

SCC showed cells adhered to the surface of the culture bottle, with growth in monolayer with a fusiform appearance (*fibroblast like*), visualized by inverted microscope and confocal laser. Carcinoma tumor cells had significant expression of the antiapoptotic protein Bcl-2, relative to antiapoptotic markers BAD, BAX, cytochrome c, involved in cell death pathways. The expression of markers *stem-cell cancer*-type, such as NANOG, CD90, OCT3/4, STRO-1 and CD44, were determined. Markers of

TNF-dependent pathways – DR4, VEGF-R1 and CD44 – showed no significant differences. They had differential markers expression of the origin and precursors of tumor cells, similar to human squamous cell carcinoma.

The *in vitro* treatment with increasing doses of stem cells from the yolk sac showed dose-dependent effects in the suppression of the proliferative response of tumor cells. There is a significant reduction in the expression of receptors and precursors involved in neoangiogenesis (CD34 and VEGF-R1). Cell therapy with undifferentiated stem cells from the yolk sac induced, in greater treatment doses, the inhibition of expression of CD44 and IL-6 receptors involved in cell adhesion and migration. The antiproliferative effect is modulated by stem cell dose dependent; however, the reduction in proliferative capacity is independent of the p53 pathway and its regulators p21 and p27, which were unchanged at different cell concentrations used in the *in vitro* treatment.

**Submission ID: 33645**

**Submission Title: Generation of Human iPS Cells by a Synthetic Self-Replicative RNA**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The generation of iPS cells in the absence of integrative DNA vectors remains problematic.

**Background**

The generation of human induced Pluripotent Stem (iPS) cells holds great promise for development of regenerative medicine therapies to treat a wide range of human diseases.

**Hypothesis**

Here we report a simple, highly reproducible RNA-based iPS generation approach that utilizes a single, synthetic self-replicating VEE-RF RNA replicon that expresses four reprogramming factors, *OCT4*, *KLF4*, *SOX2* with *c-MYC* or *GLIS1* at consistent high levels prior to regulated RNA degradation.

**Research**

Transfection of the VEE-RF RNA replicon into newborn or adult human fibroblasts resulted in the efficient generation of iPS cells with all the hallmarks of embryonic stem cells, including cell surface markers, global gene expression profiles and *in vivo* pluripotency to differentiate into all three germ layers. Importantly, the RNA replicon had been lost in all iPSC colonies (>60) examined from multiple starting cells and using different combinations of expressed pluripotent factors after removal of B18R, which is required for expression of VEE-RF RNA to suppress the interferon responses induced by RNA replicon.

**Observations**

Of note, our system certainly reproduced the retrovirus-iPSC generation with no virus production, no additional reprogramming factors and no enhancers for reprogramming. The VEE-RF RNA-based approach has broad applicability for the generation of iPS cells for ultimate use in human stem cell therapies in regenerative medicine.

**Submission ID: 33677**

**Submission Title: Smek1/2 regulate cortical neurogenesis through the translocation of the cleaved Ryk receptor**

**Author(s)**

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**Role:** Author

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**Topic**

Basic Research, Medicine, and Health

**Problem**

It has been well established that Wnt signaling pathways play critical roles in neurogenesis in many species. We previously reported that the intracellular domain (ICD) of Ryk, a Wnt receptor, translocates into the nucleus and regulates GABAergic neuronal differentiation. However, it remains to be elucidated how Ryk ICD moves to the nucleus and regulate neuronal differentiation.

**Background**

The canonical Wnt/b-catenin pathway was shown to influence the self-renewal capacity/ differentiation ability in mouse neural progenitors (NPCs).

In our previous study, we have shown that the receptor tyrosine kinase (Ryk) plays an important role in cell fate-determination, neurite outgrowth, and cortical neurogenesis. Although we have shown that Ryk can be cleaved to allow the release of the intracellular domain from the membrane, the molecular mechanism of its downstream signaling remains unknown.

**Hypothesis**

Since there is no nuclear localization signal(NLS) on the Ryk protein, we hypothesized that Ryk ICD interacting with other co-regulator which would translocate it into cell nucleus and further activate downstream target genes. In this study, our goal is discovering the interacting partner and elucidate its molecular mechanism.

**Research**

Mass spectrometry analysis of the ICD pull-down cell lysate was performed for indentifying ICD-interacting protein. Smek1 and Smek2 were indentified as the candidates. The nuclear translocation event was observed by in vitro overexpression assay and the Smek1;Smek2 double mutant mice were generated for neurogenesis analysis.

**Observations**

In our study, we show that the Smeks interact with the Ryk-ICD and bring it into the nucleus. The nuclear translocation of the Ryk-ICD leads to GABAergic-specific transcription cascades and influences neurogenesis.

We showed that Smek proteins directly bind to the promoter of GABAergic neuron specifiers, Dlx1 and Dlx2, and control their transcriptions. Smek deficient transgenic mice show the same neurogenesis defect as we observed in the Ryk deficient mice.

In summary, together with the translocation event we observed in the mouse neural stem cells, we have refined the model of Wnt-Ryk signaling in the embryonic telencephalic neurogenesis.

**Submission ID: 33688**

**Submission Title: Cell Therapy Products: Stakeholders and Integrated, Collaborative Development Toward Commercialization**

**Author(s)**

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**Co-Author(s)**

**Topic**

Industry Infrastructure

**Problem**

Cell therapies are derived from various tissue types, and autologous and allogeneic sources. The problem is that development of cell therapy products is a complex and expensive undertaking. It requires a strong foundation of scientific and clinical knowledge, regulatory acumen, innovative capabilities and multi-disciplinary expertise for development of a commercial product.

**Background**

INCELL and its staff have been building trust relationships through its collaborative business model, and a strong foundation of tools and capabilities relevant to cell therapies, for over 40 years. Stakeholders, collaborators, clients and consultants come from many sectors: patients and families, academic research and development; clinical research groups; military and government; industry and business; and education and training. INCELL infrastructure tools and capabilities include trained staff, facilities, equipment, cryostorage and innovative technologies. Also in place are quality systems, standard operating procedures (SOPs), Good Manufacturing Practices (GMP) and other "GXP" standards, support media, solutions, reagents, and biobanking that meet clinical standards. INCELL has been manufacturing products for over 20 years and is a registered HCT/P and GMP products manufacturer with FDA.

**Hypothesis**

A strong foundation of expertise, tools, and innovative infrastructure can help accelerate the development of new cell therapy products and services. These are strengthened through collaborations and key stakeholder relationships.

## **Research**

The development and manufacturing of autologous and allogeneic cell and tissue therapy products for INCELL and its customers is teamwork in action. Customized contract services, active collaborations and Quality by Design (QbD) have been integrated into projects and product development. INCELL has proprietary reagents, media and processing methods that are in FDA Master Files and are being used for bioprocessing, cryostorage or culture of cells or tissues intended for therapeutic or research use. INCELL's existing infrastructure, collection kits, and tools are accelerating laboratory product concepts to practical manufacturing, quality assurance (QA), stability and quality control (QC) testing, QbD and other needed product development and release capabilities. They are also helping to alleviate costly documentation and validation to meet GMP and other "GXP" standards, since they are being customized for new products, but built on an existing foundation. Preclinical testing, characterization and laboratory assays of freshly isolated, minimally manipulated, cryostored and/or culture-expanded cell populations can be derived from many tissue sources, each with unique, but some overlapping requirements. Methods and improvements have been defined for cell counting, and assays have been used for safety, identity, potency and bio-efficacy of the cells or tissues, or for combination products such as bioengineered scaffolds or cell tracking reagents to assess biodistribution post-transplantation.

## **Observations**

INCELL is developing and manufacturing autologous and allogeneic cell and tissue therapy products for itself, its collaborators and its contract customers. The intellectual property for these products is owned, respectively, by INCELL, INCELL plus the collaborator, or the client paying for the contract. Current products and projects target soft tissue and wound repair, arthritis, osteochondral diseases, cancer, pulmonary diseases, and neural defects or degeneration. These products and projects are at various stages of completion. Some will be tissue products, while others are being prepared for submission to the FDA as IND or IDE applications. Multi-faceted and practical considerations are important for regulatory compliance and testing of human cell products. There is value added when product developers and innovators work closely with key stakeholders to define approaches that will lead to safe and effective cell therapies that are profitable but can be delivered at a reasonable cost. Key stakeholders include those who are directly affected by disease or dysfunction; research scientists and clinical investigators who are seeking successful therapies; commercial and government research, development and manufacturing entities; tissue procurement organizations; healthcare entities seeking lower-priced, competitive products; product distributors and sellers; FDA and other national and international regulatory bodies; and the individual, private and public funders of the new therapies. Depending on the type of product, location and intended use, additional issues may need to be addressed. Some of these include: patient interactions, customer education, clinician training, tissue procurement, logistics, processing, manufacturing, shipping, packaging, product and labeling designs, cryopreservation options, stability assessments, and clinical trials design. Other aspects for consideration are chain-of-custody, patient privacy and other risk assessment considerations, such as safety, efficacy, time to market, costs, insurance, shelf life, quality, and the disease or injury to be treated. Strong expertise and infrastructure, combined with unique tools and an integrated strategy for teaming and stakeholder

participation, are the foundation for new stem cell therapies that are intended to address unmet medical needs of our world family.

**Submission ID: 33740**

**Submission Title: A global view of neurogenic deficits in Fragile X Syndrome.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Little is known about the molecular mechanisms that contribute to neurodevelopmental disorders such as autism, and currently there are few therapeutic options.

**Background**

Culture of disease-relevant cell types offers the opportunity to investigate aspects of human disease that are impossible to study in patients or mouse models. Differentiation of human pluripotent stem cells has been shown to recapitulate many aspects of development, including the molecular cues required for corticogenesis. Most studies and potential therapies for autism have focused on defects found in mature neurons. Recent studies, however, have observed an altered neural differentiation potential of neural precursor cells (NPCs) in Fragile X Syndrome (FXS), a leading genetic cause of autism. This suggests that the underlying molecular defects of FXS are already present in NPCs.

**Hypothesis**

A major goal of our work is to identify molecular targets that may lead to new therapeutic strategies for FXS by targeting the early stages of neural differentiation.

**Research**

We have generated iPSCs (induced pluripotent stem cells) from several patients clinically diagnosed with FXS. Our FXS-iPSC lines exhibit a reproducible and previously uncharacterized aberrant phenotype during early neurogenesis. We used global gene expression and epigenetic profiling combined with high-dimensional molecular interaction network topography to build a molecular model of FXS during neural differentiation.

**Observations**

Results of these studies will identify dysfunctional genes/pathways in FXS and identify molecular targets with therapeutic potential.

**Submission ID: 33752**

## **Submission Title: Simulated Microgravity Culture: a Novel Approach to Embryonic Stem Cell Culture**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Leukemia inhibitory factor (LIF) is an indispensable factor for maintaining mouse embryonic stem (ES) cells pluripotency. Feeder layer and serum are also needed to keep an undifferentiated state, however, such animal derived materials needs to be eliminated to apply for clinical use.

### **Background**

We have reported that simulated microgravity inhibited stem cells differentiation such as human mesenchymal stem cells, mouse hematopoietic stem cells, and mouse bone marrow stromal cells. ES cells have great ability of proliferation and differentiation, however, undergo unexpected spontaneous cell differentiation.

### **Hypothesis**

We hypothesis that simulated microgravity environment can provide us simple and clinically-significant cell culture methods for ES cell.

### **Research**

A 3D-clinostat, simulated microgravity machine is a multidirectional G-force generator. By controlled simultaneous rotation of two axes, a 3D-clinostat cancels the cumulative gravitational vector at the center of the device, producing an environment with an average of  $10^{-3}G$  over time. This is accomplished by the rotation of a chamber at the center of the device to disperse the gravitational vector uniformly within a spherical volume at a constant angular velocity.

In the present study, we demonstrated mouse embryonic stem (ES) cells derived from C57BL/6 mice (BRC6; RIKEN BRC CELL BANK, Tsukuba, Japan) were seeded in OptiCell™ (Thermo Fisher Scientific Nunc brand, Rochester, NY, USA), and cultured in normal 1G condition (group 1G) or  $10^{-3}$  G condition (group CL), using feeder-free and serum-free medium of ESF-C medium (Cell Science & Technology Institute Inc., Sendai, Japan) without LIF.

## Observations

Cells in group 1G showed morphologically differences in normal ES cells. On the other hands, cells in group CL formed many small spheres after 3 days of culture, and these spheres were bigger during culture period. RT-PCR and ALP staining indicated the spheres were undifferentiated mouse ES cells. Moreover, teratomas were generated by subcutaneously injecting group CL cells into C57BL/6 mice.

We succeeded in developing novel LIF-free culture methods of mouse ES cells in this study, which concurrently allows feeder-free, serum-free, coating-free, and trypsin-free culture technique. Simulated microgravity produced by a 3D-clinostat enables an easier and more effective tool for stem cell culture.

**Submission ID: 33754**

**Submission Title: Recombinant Human BMPs 2 and 4 expressed in Mammalian Cells aiming at Bone Tissue Engineering and Stem Cell Proliferation and Differentiation**

**Author(s)**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

BMPs are promising molecules in periodontal regeneration to treat physiopathological bone loss and non-union fractures and in oral surgery, and to accelerate and increase osseointegration.

**Background**

Bone Morphogenetic Proteins (BMPs) are multifunctional, secreted cytokines belonging to the TGF- $\beta$  superfamily. These proteins act as a disulfide-linked homodimer, being potent regulators of bone and cartilage formation and repair, cell proliferation in embryonic development and adult bone homeostasis. BMPs are dimeric molecules displaying sites for N- and O-glycosylation, which increases the stability and half-life of the protein in the body, in addition to determining the specificity of receptor coupling. BMP-2 induces cartilage and bone formation. BMP4 has also been shown to play a role in triggering osteoblastic differentiation of mesenchymal stem cells, through activation of osteoblastic related genes.

**Hypothesis**

To ensure proper glycosylation and conformational folding and to prevent immunogenicity, we elected a mammalian cell expression system to produce these BMPs aiming at bone regeneration, stem cell proliferation and differentiation and their application in human and veterinary cell therapy.

## Research

BMPs 2 and 4 cDNAs were amplified from an in-house constructed cDNA Bank and cloned into the pGEM<sup>®</sup>-T-Easy vector. *E. coli* transformants were screened by colony PCR. Upon DNA sequencing, the BMP 2 and 4 inserts were transferred to a lentiviral expression vector. HEK293 cells were co-transfected with a lentiviral plasmid containing both BMP 2 or 4 and eGFP cDNAs, by co-transfection, at a 40:1 ratio, with a Hygro<sup>r</sup> vector for clone selection. Cell clones were selected using 100 ug/mL hygromycin. Several cell clones were characterized and highest overproducing ones were selected for each protein. BMPs expression was analyzed by qRT-PCR, Western blotting, and *in vitro* biological activity by alkaline phosphatase activity in C2C12 cells during 7 days. Recombinant proteins were purified using heparin affinity chromatography.

## Observations

Upon cell cloning, most of the cells present in the selected clones were positive for GFP, indicating that a high transfection efficiency was achieved. BMPs 2 and 4 were continuously secreted to the medium even after 120h of serum starvation. Purification of rhBMP2 and 4 from the conditioned medium resulted in more than 90% purity. The rhBMPs 2 and 4 bound to the resin were eluted in 450mM NaCl buffer, with a single dimeric 30–37 kDa band being observed in the eluates. *In vitro* assays showed that the purified rhBMPs 2 and 4 displayed high osteogenic activity. The *in vivo* osteogenic bioactivity analysis of the purified proteins by ectopic bone formation using Rowett rats is underway. Glycosylation analysis using exoglycosidase digestion and structural analysis of the purified proteins is underway. The use of these biopharmaceuticals in bone Tissue Engineering is likely to allow accelerated recovery to both human patients and animals.

**Acknowledgments:** BNDES, CAPES, CNPq, FAPESP, FAPERJ, FINEP, MCTI and MS-DECIT.

**Submission ID: 33758**

**Submission Title: Monitoring Stem Cell Fate In Vivo using NIS Reporter Gene**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Mesenchymal stem cells (MSC) are endowed with immune suppressive properties and are capable of differentiating into osteocytes, chondrocytes and adipocytes. Currently, there are more than 200 clinical trials worldwide investigating the safety and efficacy of MSC for tissue repair or immune modulation in a variety of conditions, including joint and tendon repair or healing. However, it remains unknown for how long the cells survive post-transplantation, if they proliferate, engraft or die.

**Background**

The thyroidal sodium iodide symporter (NIS) is a non-immunogenic protein expressed on the surface of thyroid follicular cells that mediates the uptake of iodide. NIS expression can be monitored noninvasively, quantitatively and repeatedly by imaging the biodistribution of various radioactive isotopes (I-125, I-123, I-124, Tc-99m pertechnetate) using gamma camera, SPECT or PET imaging modalities in small and large animals.

**Hypothesis**

We hypothesize that the fate of MSC can be monitored in vivo using NIS and that cells implanted in the inflamed joints will have a longer residence time than in normal joints.

**Research**

In this study, we evaluated 1) the feasibility and sensitivity of NIS as a reporter gene for noninvasive imaging of MSC in vivo and 2) compare and contrast the in vivo fate (and durability) of MSC in normal and injured Achilles tendons in a rat model.

**Observations**

MSC from rat, human and canine were collected from adipose tissues or the bone marrow, expanded and transduced with lentiviral vectors encoding the human or species specific NIS genes. NIS function in transduced MSC was first validated in vitro; NIS expressing MSC (MSC\_NIS) from multiple species concentrated high levels of I-125 with no side effects. The sensitivity of cell detection was determined by transplanting a known number of MSC\_NIS subcutaneously into mice and the animals were imaged daily until the signals disappear. We can reliably detect  $2 \times 10^5$  MSC\_NIS in mice using the newly acquired U-SPECT II machine (MI-Labs, Mayo Small Animal Imaging Core). Canine MSC derived from the bone marrow were surprisingly robust;

viable cells were still detected (albeit lower numbers) at day 28 in the athymic mice. In contrast, NIS signals from adipose tissue derived rat or human MSC disappeared by day 7 post transplantation. We are currently comparing the long term fate MSC\_NIS in normal Achilles tendons or in rats with collagenase I induced Achilles tendonitis.

**Submission ID: 33759**

**Submission Title: Policy Uncertainty, Research Funding and the Conduct of Stem Cell Science**

**Author(s)**

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**Co-Author(s)**

**Topic**

Ethics, Law and Society

**Problem**

Emerging technologies, such as stem cell research and nanotechnology, offer substantial promise to improve our quality of life. Yet, at the same time, many emerging technologies raise challenging ethical questions. As a result of these and other tensions, the outcomes of policy debates surrounding emerging technologies often vary both across jurisdictions and time, yielding a heterogeneous and uncertain policy environment. In this project, I consider policy uncertainty surrounding pluripotent stem cell research and assess its impact on scientists in this promising but controversial field.

**Background**

N/A

**Hypothesis**

N/A

**Research**

Drawing on data from a recent survey of stem cell scientists in the United States, I argue that policy uncertainty surrounding federal funding policies for human embryonic stem cell research has negatively impacted the development of the broader field of stem cell science, affecting not just scientists working with human embryonic stem cells, but also those working with less controversial types of stem cells. My analysis also illustrates the importance of state policy in this field. In particular, scientists in states that have provided funding for stem cell research, including research using human embryonic stem cells, are substantially less likely than scientists in states without such funding to report impacts of policy uncertainty. This effect is present for the full sample of stem cell scientists, including both scientists working with human embryonic stem cells and other less contentious cell types, but is strongest for scientists working with human embryonic stem cells. State policies that legalize or otherwise support stem cell research, but do not provide funding have no discernible effect.

**Observations**

Together results highlight the negative consequences of the policy uncertainty on the development of stem cell research and emphasize the importance of developing and maintaining stable funding structures in ethically contentious areas of biomedical research.

**Submission ID: 33761**

**Submission Title: Ethical Controversy, Graduate Education and the Development of Scientific Careers**

**Author(s)****Co-Author(s)**

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**Topic**

Ethics, Law and Society

**Problem**

Scientific advances in diverse fields ranging from stem cell research to nanotechnology pose challenging ethical questions. At the same time efforts to strengthen and broaden the U.S. workforce in science, technology, engineering and mathematics are crucial to the long-term competitiveness of the national economy. This research examines the nexus of these two trends, assessing the extent to which and the manner by which ethical controversy affects the training of graduate students and their transition to the scientific workforce.

**Background**

N/A

**Hypothesis**

N/A

**Research**

Insight into both the challenges and the benefits of training in an ethically contentious field is gathered through interviews with early career scientists in stem cell science, health-related nanotechnology and synthetic biology. Participants in these wide-ranging interviews were asked to reflect on the ethics of their own research, their sense of the public perception of their research and the various impacts of working in areas that raised ethical concerns, for at least some people, on their research.

**Observations**

Thematic analysis of interview transcripts revealed mismatches between scientists' perceptions of the ethics of their work and their beliefs about public perceptions. In particular, in the context of stem cell science, scientists expressed worries about animal research or human biopsies (for induced pluripotent stem cell derivation) at about the same rate as they expressed concerns about the use of embryos in research, while discussion of public perceptions of the field focused on concerns about human embryos. Scientists in contentious fields reported a range of funding challenges, some of which they attributed to ethical controversy and the resulting policy environment surrounding their field, rather than broader funding trends. Scientists also reported changing research topics or avoiding certain topics altogether due to policy concerns and frequently noted that scientists working in ethically contentious fields must navigate a complex bureaucracy to complete their work. Scientists also noted what they perceived as greater need to engage in public outreach in ethically contentious fields. The research concludes by drawing on this improved understanding of the experience of scientists in fields that raise ethical controversy to suggest strategies to strengthen graduate education in stem cell science and similar fields.



**Submission ID: 33765**

**Submission Title: USE OF AUTOLOGOUS FAT GRAFTING FOR RECONSTRUCTION POST-MASTECTOMY AND BREAST CONSERVING SURGERY: A Systematic Review and Meta-Analysis**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Breast cancer is a highly prevalent disease. It is commonly treated by surgical removal of the affected tissue or whole breast. Following this, patients often require reconstruction of the breast. The use of autologous fat grafting (AFG) for reconstruction, where fat is removed from other areas and injected into the breast has been a topic of controversy. Questions remain as to the impact of placing adipose tissue into an area of previous malignant change.

**Background**

There is growing interest in the potential of autologous fat grafting (AFG) for breast reconstruction. However, concerns remains regarding it's effectiveness, safety and intereference with mammography. The possibility of local growth factors and adipose derived stem cells causing cancer recurrence is also a key concern.

**Hypothesis**

This systematic review aimed to look into the oncological, clinical, aesthetic, patient reported, process and radiological outcomes of autologous fat grafting to the breast.

**Research**

All studies investigating AFG for women undergoing reconstruction post mastectomy or breast conserving surgery for treatment of breast cancer were considered. We assessed six domains as above. Electronic databases were searched to June 2013; additional grey literature searches were also performed. Two independent reviewers assessed eligibility of articles for inclusion and performed data extraction.

**Observations**

31 studies were included in this review (3,521 patients). Current studies have a median follow up of 14.8 months, and a high degree of patient and surgeon satisfaction over an average of 1.9 sessions. Fat necrosis is the commonest complication in 4.4% and the majority were *Clavien-Dindo* Grade 1. Other harms include further radiological investigation (interval mammograms in 11.5%) and the need for biopsy (2.5%) to exclude malignancy. The weighted mean recurrence rate was 4.4% at a median of 18.3 months. Restricting to moderate quality studies focusing on in-situ disease, the recurrence rate was 9.4%, compared with 1.6% in matched controls ( $p=0.03$ ). There is a requirement to further investigate this relationship, possibly through high quality randomised controlled trials.

**Submission ID: 33779**

**Submission Title: Zinc finger protein 423 integrates signaling pathways by the niche and targets hedgehog signaling gene expression during adult neurogenesis.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

In mice lacking *Zfp423* (*Zfp423<sup>n12/n12</sup>*), adult neurogenic regions are malformed, including the ventricular (VZ) and subventricular (SVZ) zones lining the lateral ventricles and the subgranular zone of the dentate gyrus.

**Background**

Zinc finger protein 423 (*Zfp423*) is a 30 Kruppel like C<sub>2</sub>H<sub>2</sub>zinc finger transcription factor that binds to EBF and BMP-responsive SMAD.

**Hypothesis**

Therefore, we suggested that *Zfp423* could have an essential function in adult neural stem and progenitor cells.

**Research**

We found significant accumulation of Sox2+, Pdgfr $\alpha$ + and DCX+ cells lining the LV, especially at the dorsal lateral wall and ventral tip, and in the anterior LV. Increased DCX+ signal is also detected at the *Zfp423<sup>n12/n12</sup>* olfactory bulb. Adult neural stem cells (aNSCs) cultured from the VZ and SVZ of the *Zfp423<sup>n12/n12</sup>* showed accelerated growth under both renewal and differentiation conditions, increased number of DCX+ neuroblasts after RA differentiation, but decreased neurite formation after either BMP or RA differentiation. Whole genome expression analysis, supported by high-throughput microfluidic quantitative PCR confirmation, showed an altered timing of gene expression after RA or BMP differentiation, and implicates hedgehog signaling as the most significant *Zfp423* target under renewal and RA or BMP differentiation conditions. Surprisingly, lack of *Zfp423* inverted the relative expression levels of Gli2 and Gli3 transcription factors relative to wild type control cultures. Furthermore, *Zfp423* alters the expression of other essential genes, such as *Nrn1*, *Anxa3*, and *Zfp410*.

## Observations

In conclusion, RA, BMP and hedgehog signals from the niche require *Zfp423* to coordinate the appropriate early progenitor and stem cell response during neurogenesis.

**Submission ID: 33804**

## **Submission Title: Cellular Mobility Mapping for Non-invasive Prediction of Differentiation Quality of Stem Cells**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

For clinical stem cell therapy, the quality control of produced stem cells are essential for higher safety and therapeutic success. However, the current cell evaluation methods are mostly invasive, and therefore not compatible for the daily monitoring quality control of cells for therapy. Especially, for stem cells, which are sustained in vitro under long and laborious process of differentiation to satisfy the therapeutic condition, cells are commonly in the state of confluent stage. Although there had been some image analysis techniques to evaluate cellular morphology to evaluate cellular status, the techniques are still limited to sparsely grown cells, since confluent cells are nearly impossible to recognize individual cells. Therefore new technique for cell quality evaluation, specialized on cells in confluent stage, is strongly required.

### **Background**

From the lack of technological advance in the evaluation technologies for non-invasive cellular evaluation, few reports suggest the enabling technology to assess the cells in confluent status. The cells in confluent state is an essential criteria for differentiation, however increases the difficulty for image-processing technology to precisely recognize and evaluate cells. Therefore, we here report an novel approach to utilize mobility information of cells under confluent status to estimate their cellular quality, which we define as potential of differentiation.

### **Hypothesis**

Our hypothesis is that by combining several concepts of image processing and their informatic analysis, the disadvantages of image processing, time-point monitoring, and image analysis can be reinforced to visualize stable data for cellular evaluation.

### **Research**

In this work, we have analyzed the differentiation prediction of human mesenchymal stem cells, and iPS derived retina or retinal pigment epithelium cells by our newly constructed image processing and bioinformatic analysis scheme. Our data shows that non-stained native cells under confluent state can be successfully measured and modeled to predict the differentiation potential of their future results.

### **Observations**

Our analysis showed that mobility information can be an effective parameter for non-invasive evaluation of stem cells for

cellular production in the clinical facilities. The most important finding should be that the mobility information is very naive therefore should be accompanied by combinational strategy of analysis to provide stable evaluation data.

**Submission ID: 33806**

**Submission Title: The Center for Regenerative Medicine (CRM) Biotrust: Translating Regenerative Therapies at Mayo Clinic**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The availability of readily accessible biospecimens for translational studies is critical to advancing regenerative therapeutics in the clinical setting. Unfortunately, this process can be costly, labor intensive and lengthy.

**Background**

The Mayo Clinic Center for Regenerative Medicine (CRM) Biotrust has been established to facilitate the translation of regenerative medicine applications at Mayo Clinic. This involves infrastructure support for collection of patient samples, processing biospecimens for storage or biomaterial derivation, including induced pluripotent stem cells (iPS), and maintaining a biorespository of relevant and universally accessible materials. The goal of the CRM Biotrust is to generate, process, store and disseminate cost-effective, quality-assured biomaterials including iPS cells to advance regenerative medicine at Mayo Clinic.

**Hypothesis**

The biomaterials stored by the CRM Biotrust provide an infrastructure resource to advance research and clinical applications in regenerative medicine.

**Research**

The CRM Biotrust assists investigators in the development of IRB-approved protocols that allow for the contribution of samples to the Biotrust, coordinates the sharing of biomaterials between investigators, and facilitates commercialization opportunities for the stored products. The cryostored biomaterials include somatic cells such as skin fibroblasts, umbilical cord blood mononuclear cells, and iPS cells. The CRM Biotrust works collaboratively with investigators in developing directed differentiation protocols for iPS cells to produce cardiac, pancreatic, lung epithelial, neuronal and other cell types based on individual investigator requirements and Biotrust expertise. Cells collected and processed by, or submitted to the CRM

Biotrust by Mayo Clinic investigators as a form of redundant storage, formulate a biorepository that can advance the translation of novel, iPS or other cellular-based therapeutics for regenerative medicine applications in patients.

### **Observations**

The CRM Biotrust has derived and reprogrammed adult somatic cell types to a pluripotent state for differentiation into various cell types based on collaborative needs. To date, the CRM Biotrust has identified and obtained regenerative medicine-related biospecimens from over 250 patients for either immediate use by investigators, or archived for later accession based on need or reprogramming to iPS cell lines. The current iPS lines readily available from the biorepository includes over 100 iPS cell lines generated from patients with a variety of underlying disease states, including cardiac, lung, and neurodegenerative diseases. Our goal is to increase the collection to over 200 iPS cell lines by the end of 2014. These cryopreserved samples constitute a shared repository that forms a basis for both retrospective and prospective regenerative medicine studies.

**Submission ID: 33829**

## **Submission Title: Autologous Cell Therapy for Parkinson's Disease**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Currently no treatment exists to stop the progressive loss of dopaminergic neurons which occurs with Parkinson's disease (PD). Pharmacological treatments lose efficacy over time and require larger doses which result in increased side effects. Therefore there exists an unmet clinical need for long-term restoration of dopamine signaling for treatment of PD.

### **Background**

PD is a progressive, neurodegenerative disease which primarily attacks the dopaminergic neurons in the *substantia nigra*. Due to the specificity of the type of cells that are lost, PD is an attractive target for cell-based regenerative medicine therapy. Past trials with fetal-derived tissue demonstrated that it is possible to provide long-term motor symptom improvement through cell replacement and the scientific community learned several important lessons to guide development of future cell therapies for PD. Namely, careful patient selection and the use of high quality of cells will likely increase the probability of successful outcomes.

### **Hypothesis**

The hypothesis of this work is that authentic dopaminergic neurons can be produced from patient-derived induced pluripotent stem cells (iPSCs) for the purposes of treating motor-associated symptoms of PD.

### **Research**

We derived induced pluripotent stem cells (iPSCs) from dermal biopsies of 9 patients with PD under IRB approval. Patient stem cells were then differentiated into dopaminergic neurons using a floor plate progenitor method recently reported by Kriks *et al.* We characterized floor plate differentiation by gene expression and immunocytochemical staining. Cells were further differentiated into immature (day 25) and mature dopaminergic neurons (>50 days) and further characterized by genome-wide gene expression, immunocytochemical staining and electrophysiology.

### **Observations**

Patient iPSCs differentiated for 11 days robustly expressed floor plate markers FOXA2, LMX1A, OTX2 and expressed low levels of PAX6, a marker of non-midbrain neural progenitor cells. After 25 days of differentiation, cells expressed high levels of dopaminergic genes including tyrosine hydroxylase, EN1, PITX3, LMX1A and FOXA2. Additionally, markers of serotonergic

neurons and GABAergic neurons were expressed at low levels suggesting a high percentage of dopaminergic neurons in the cultures. We confirmed this finding through immunocytochemical detection of a high percentage of tyrosine hydroxylase positive (50-70%) cells and rare serotonergic and GABAergic (<5%) positive neurons. Overall, immature dopaminergic neurons differentiated from patient-derived iPSCs hold promise for future clinical investigation of an autologous cell therapy to treat PD and warrant further pre-clinical study for efficacy in treating animal models of PD.

**Submission ID: 33856**

**Submission Title: Intranasal delivery of therapeutic neural stem cells to target intracerebral glioma: Enzyme/prodrug CE/CPT-11 therapy**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Despite aggressive multimodal therapy and advances in imaging, surgical and radiation techniques, high-grade gliomas remain incurable, with survival often measured in months. Treatment failure is largely attributable to the diffuse and invasive nature of glioma cells, ineffective delivery of chemotherapeutic agents across the blood-brain barrier (BBB), and associated dose-limiting systemic toxicities.

**Background**

Neural stem cells (NSCs) display inherent tumor-tropic properties that can be exploited for targeted delivery of anti-cancer agents to invasive and metastatic tumors, and may offer an unprecedented advantage over conventional therapeutic approaches. NSCs can overcome the major obstacles limiting the efficacy of current treatments by their ability to cross the BBB, target therapeutic agents to primary and invasive tumor foci throughout the brain, and minimize toxicity to normal tissues. Used as a delivery vehicle, NSCs have been engineered to express a variety of anti-cancer agents, demonstrating >70% therapeutic efficacy in pre-clinical models of brain tumors. Cell based therapies for neurodegenerative diseases depend on efficient delivery of the therapeutic cells to the areas of damage. Administration of NSCs intracranially may cause damage to normal tissues and demonstrate poor engraftment into the brain. Alternatively, when stem cells are injected intravenously immunological reaction and other systemic complications may occur.

**Hypothesis**

Here we hypothesize that we can deliver localized chemotherapy using tumor-specific NSCs (HB1.F3.CDs) to human glioma xenografts in mouse models.

## **Research**

In this study, severely immunodeficient mice were implanted intracranially with U251 human glioma cells. NSCs were administered intranasally and biodistribution of the stem cells was determined via MRI and histological imaging.

## **Observations**

NSCs specifically localized in and around tumors, but not to non-tumor areas of the brain. Finally, NSCs expressing carboxylesterase, a CPT-11 activating enzyme, can inhibit tumor growth in animal models of orthotopic glioma. Intranasal delivery of the NSCs is a non-invasive method that allows stem cell treatments for non-resectable invasive tumors and may allow repeated rounds of treatments. This study has tremendous potential significance because the NSC-based delivery paradigm could improve survival and minimize the serious brain damage caused by current radiation and chemotherapy regimens, thus preserving intellectual function and improving the quality of survivorship of patients.

**Submission ID: 33924**

**Submission Title: Altered characteristics of human bone marrow stem cells under the hypoxic condition**

**Author(s)**

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Altered characteristics of human bone marrow stem cells under the hypoxic condition

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**Topic**

Basic Research, Medicine, and Health

**Problem**

An increasing number of evidence has demonstrated that human bone marrow stem cells are involved in the healing of damaged tissues in a direct and indirect manner. However, in order for hBMSCs to be applicable to a clinical setting as a promising therapeutics for the tissue regeneration, various factors affecting the activity of hBMSCs should be addressed.

**Background**

Since hypoxic microenvironment is a very specific characteristic of damaged sites, the altered characteristics of hBMSCs under the hypoxic condition should be understood. The purpose of the present study was to investigate the altered biologic activity of hBMSCs under the hypoxic condition using *in vitro* and *in vivo* assay models.

**Hypothesis**

hBMSCs show altered characteristics under the hypoxic condition.

## Research

In order to isolate human bone marrow stem cell, fresh bone marrow aspirate was obtained from the vertebral body during vertebral surgery with informed consent. A group of cells could be isolated using well established culture technique. To clarify the stem cell characteristics in the isolated cell population, characterization of cells was performed using well-documented *in vitro* and *in vivo* analysis models, including the colony-forming efficiency (CFE) assay, expression of cell-surface-markers, proliferation, migration, *in vitro* differentiations, and *in vivo* ectopic transplantation assay. We examined BSMC cultured under a hypoxic (1% O<sub>2</sub>) condition compared to a normoxic (21% O<sub>2</sub>) condition for control. In addition to the basic characteristics of cells under the hypoxic condition, collagen regeneration potency, a critical healing factor, was analyzed. Insoluble and soluble collagen and corresponding hydroxyproline synthesis was examined. Expression of the mRNA of collagen and Lox family gene was examined using real time PCR. *In vivo* collagen tissue regeneration was conducted by transplanting hBMSCs into the subcutaneous pockets of immunocompromised mice using hyaluronic acid as a cell carrier. Histologic and immunohistochemical analyses were conducted.

## Observations

hBMSCs population used in the present study fully presented the characteristics of mesenchymal stem cells in terms of adherent characteristics, immunophenotypes and *in vitro* and *in vivo* multipotency. Results revealed that the hypoxic condition reduced the proliferative potential and CFE of hBMSCs. The expression of CD 90 showed slightly decrease in the hypoxic condition while the expression of other positive surface markers showed similar patterns in both conditions. hBMSCs in the hypoxic condition showed significantly enhanced osteogenic differentiation potential but lower adipogenic differentiation than those in normoxia. Chondrogenic differentiation was increased under the hypoxic condition. When *in vivo* differentiation was evaluated using ectopic transplantation models, results showed that hBMSC provoked enhanced regenerative potential of bone and bone marrow-like tissue. *In vitro* synthesis of soluble and insoluble types of collagen was increased in the hypoxic condition. *In vivo* collagen tissue regeneration was also enhanced by hBMSCs cultured under the hypoxic condition. Increased expression of subtype of collagen and LOX family mRNA might contribute to such enhanced regeneration.

**Submission ID: 33982**

**Submission Title: Stem cell-based screening model for Botulinum neurotoxin inhibitors**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Botulinum neurotoxin (BoNT) is the most toxic protein known to man with a  $LD_{50} \sim 1$  ng/kg, where exposure to the toxin ultimately results in host paralysis and death. Although multiple *in vitro* screening campaigns have resulted in BoNT inhibitors with good *in vitro* potency, all leads to date have failed when advanced to *in vivo* testing due to poor pharmacologic properties such as low aqueous solubility, high cytotoxicity and low cell permeability. In addition, cellular models that rely on secondary cell lines have been shown to have poor predictive value in lead advancement. These limitations, therefore, dictate the need for new cellular models that faithfully recapitulate the phenotype of BoNT intoxication, are better predictors of *in vivo* efficacy, and are compatible with existing automation and high-throughput screening procedures.

**Background**

Despite broad clinical utility, BoNT also can pose a safety threat if misused in the event of a bioterrorist attack. As a result, much effort has been put into the discovery and development of therapeutic strategies for the treatment of botulism. Traditionally, molecules with anti-BoNT activities have been identified through *in vitro* screening campaigns using fluorescent peptide substrates, or by using cellular models and low-throughput immunological detection of SNARE protein cleavage.

**Hypothesis**

We have taken advantage of human stem cell-derived motor neurons that have been demonstrated to exhibit high BoNT sensitivities and represent a feasible approach for expansion to cell numbers required for screening applications. We have proposed that a FRET-based reporter of BoNT activity allows for real time monitoring of the intoxication process.

**Research**

To achieve this goal, two different approaches have been used to engineer reporter expression in stem cells. In the first approach, cells are being prepared to constitutively express the BoNT reporter and allow for the direct comparison of BoNT sensitivity between differentiation stages. The second prong to our approach was to develop stem cell-derived motor neurons that express the BoNT reporter only in a specific differentiation stage. Stage-specific expression of the reporter protein allows for monitoring of intracellular BoNT activity in real time as well as enables the evaluation of the efficiency of the differentiation protocol and provides a means to purify cells of interest.

**Observations**

The FRET-based reporter of BoNT activity was successfully cloned and tested for its functionality and proper intracellular localization in a neuroblastoma cell line. In order to deliver the FRET-based BoNT reporter construct into stem cells, various delivery methods have been tested. Inefficient expression of the reporter that was observed with different delivery methods may result from gene silencing, incompatibility of the promoters used or cytotoxicity of the reporter in stem cells. Therefore, further optimizations will be required to obtain expression levels of the FRET BoNT reporter that will allow high-content imaging and will result in the first renewable BoNT intoxication cellular model system that is compatible with high-throughput screening applications for the identification of pharmacologically viable BoNT inhibitors.

**Submission ID: 34049**

**Submission Title: Immunogenic properties of autologous and allogeneic neural and cardiovascular progenitors derived from human pluripotent stem cells**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Currently there is a great deal of debate about the immunogenicity of early stage derivatives of pluripotent stem cells (PSCs), and previous work has been limited to mouse models. For human pluripotent stem cell derivatives that will be used for cell therapy, it is critical to assess their antigenicity and immunogenicity, particularly to determine whether it will be necessary to immune match hPSC derived tissues and cells to recipient.

**Background**

Human pluripotent stem cells (hPSCs) are a promising cell source for regenerative medicine because they can replicate indefinitely and generate any cell type in the body. The Major Histocompatibility Complex (MHC) enables immune cells to distinguish “self” from “non-self” and plays a critical role in graft rejection. It has been demonstrated that undifferentiated hPSCs do not express high levels of MHC, which may underlie their ability to evade immune detection even in an allogeneic setting (MHC mismatch between donor and recipient). However, if hPSC derivatives express MHC on their surfaces and are transplanted in an allogeneic setting, it is likely that the grafted cells will not survive due to immune cell mediated rejection.

**Hypothesis**

We expect that as hPSCs leave their pluripotent state and differentiate toward a specific cell fate, expression of MHC will increase, allowing the cells to become vulnerable to detection and elimination in an allogeneic setting. Conversely, hPSC derivatives transplanted into an autologous setting (MHC match between donor and recipient) will be accepted and the grafted cells will be allowed to survive.

**Research**

We have generated and characterized a group of human iPSC (hiPSC) lines with a variety of different MHC haplotypes. For each line, expression of MHC and other immune ligands was examined in undifferentiated cells and cells differentiated into neural, melanocytic and cardiovascular progenitors, using a combination of flow cytometry and gene expression analysis. To address the immune stimulating potential of the cell types, mixed lymphocyte reactions were carried out in both allogeneic and autologous settings. Responses were measured by cell proliferation and analyzed using flow cytometry.

## **Observations**

We have shown that selected ethnically diverse hiPSC lines expressed haplotypes consistent with their reported ancestry. As these hiPSC lines, as well as an embryonic stem cell line, were differentiated into selected cell types, expression of MHC did in fact increase. When these derivatives were mixed with autologous immune cells no rejection was observed. However when they were mixed with allogeneic immune cells a significant amount of immune cell proliferation was observed, which is associated with immune rejection of the cells. Furthermore, these hiPSC derivatives evoked similar immune responses as the parental fibroblasts in the mixed lymphocyte reactions.

**Submission ID: 34064**

## **Submission Title: Intramuscular Injection of Umbilical Cord Mesenchymal Stroma Cells Leads to Significant Improvements in Pediatric Dilated Cardiomyopathy**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Dilated cardiomyopathy (DCM) is a refractory fatal condition with no effective treatments other than heart transplantation.

### **Background**

Mesenchymal stroma cells (MSCs) can promote angiogenesis and heart function via paracrine mechanisms. Previously, intracoronary and intramyocardial administration of MSCs has shown beneficial effects on DCM.

### **Hypothesis**

**Intramuscular Injection of Umbilical Cord Mesenchymal Stroma Cells Leads to Significant Improvements in Pediatric Dilated Cardiomyopathy**

### **Research**

Three pediatric DCM patients with NYHA class III cardiac function and left ventricular ejection fraction (LVEF) at 30-40%, and one pediatric left ventricular non-compaction (LVNC) patient with NYHA class III-IV cardiac function and LVEF at 18%, in whom medication treatment failed to evoke improvements, were included in the current study. These patients were administered with allogenic umbilical cord MSCs (UCMSCs,  $1.2 \times 10^7$  cells) via intramuscular injection, and assessed for up to three years after the treatment.

### **Observations**

MSCs injection induced significant improvements in cardiac function and symptoms in all the patients. In the DCM patients, LVEF gradually elevated to the 50% or 60% level within 12 months, associated with improvements in other parameters and cardiac function. MSC injection also resulted in an elevation of LVEF to 38% in the LVNC patient within 18 months, associated with improvements in cardiac function.

**Submission ID: 34069**

**Submission Title: THE CELLULAR CARDIOMYPLASTY FOR MYOCARDIAL REGENERATION**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Heart is a terminally differentiated organ with limited capacity of regeneration. Heart failure is the leading cause of death in most countries. The present mode of treatment primarily involves medicinal therapy, interventional methods such as angioplasty or surgery. However, when a considerable portion of the heart muscle is significantly affected, the patient may require a Left ventricular assist device (LVAD) or heart transplant. This has disadvantages of surgical complications, the perils of immuno-suppression drugs, lifelong expenditure and paucity in donor graft availability. Such tribulation cues the necessity for other modalities of cell therapy.

**Background**

Cellular cardiomyoplasty for myocardial damage appears promising therapeutic option by stimulating neo-angiogenesis and regeneration. Various types of stem cells are experimented and used for clinical trial. Among them adult stem cells derived from peripheral blood, bonemarrow, cordblood, skeletal myoblast, cardiac stem cells are clinically applicable.

**Hypothesis**

Autologus Stem cells derived from pheripheral blood and bone marrow can promote cardiac regeneration by stimulating neoangiogenesis and myogenesis.

**Research**

The Granulocyte colony stimulating factor (GCSF) induced Peripheral blood derived CD34+ Endothelial progenitor cells (EPCs) and iliac crest bone marrow derived mononuclear cells (MNCs) were collected from 80 patients suffering from ischemic/dilated cardiomyopathy (mean age group: 42) (autologus EPCs: 26 cases, autologus MNCs: 53 cases, allogenic paternal EPCs: 1 case) with the approval from Clinical trial registry, Govt of India (Clinical trial number: CTRI/2009/091/000590). The EPCs were isolated from peripheral blood by cell aphaeresis. The bone marrow was processed by closed vial method using RBC sedimentation technique. The isolated MNCs were subjected to a battery of tests such as flowcytometry to analyze the presence of CD34+ & CD45+, total cell viability and cell count, microbial sterility check and endotoxin analysis. The route of cell delivery was trans-coronary for 56 patients and epicardial for 24 patients. The patients were injected with total cell yield approximately  $6 \times 10^8$  cells/ml of 99.5% viable cells.

**Observations**

The Ischemic cardiomyopathy patients (26/40) had shown significant improvement in Ejection fraction (65%), whereas dilated cardiomyopathy patients (9/24) had an improved cardiac output (37.5%) during a average follow up of 2.5 years. 12 patients in either group lost the follow up. Four patients (25%) had expired during the follow up due to other complications. No side effects and adverse complications. The autologous cellular cardiomyoplasty with EPCs and MNCs is more safe and effective therapy for Left ventricular dysfunction. However, many refinements are required to standardize the dosage and route of cell delivery for appropriate homing of stem cells.

**Submission ID: 34085**

## **Submission Title: T Cell Mediated Suppression of Neurotropic Coronavirus Replication in Neural Precursor Cells**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Transplantation of multipotent neural precursor cells (NPCs) is emerging as a feasible therapeutic strategy for the treatment of a variety of neurological disorders. However, despite the clinical and histological benefits of NPC transplantation in pre-clinical animal models of neurologic disease, there is limited evidence addressing the capacity of neural grafts to act as reservoirs for viral replication.

### **Background**

Recent studies have demonstrated both short and long-term clinical benefits following NPC engraftment within the context of rodent models of Alzheimer's disease, Parkinson's disease, Huntington's disease, and acute spinal cord injury. The apparent antigenicity of NPCs suggests successful engraftment may require the use of immunomodulatory agents and lifelong suppression of the immune system, as with solid organ transplants. An unintended consequence of immune suppression is the potential for latent viruses to become activated, or for uncontrolled viral replication to occur following opportunistic infection.

### **Hypothesis**

We propose that T cells play a critical role in controlling replication of a neurotropic virus in NPCs, which has important implications when considering immune modulation for NPC-based therapies for treatment of human neurologic diseases.

### **Research**

Cultured murine NPCs were infected with the neurotropic JHM strain of mouse hepatitis virus (JHMV), which induces acute encephalomyelitis and chronic demyelination when injected intracranially into immunocompetent mice. We evaluated the ability of JHMV to replicate and lyse infected NPCs *in vitro* and analysed the consequences of infection on expression of major histocompatibility molecules class I and II. Furthermore, NPCs were co-cultured with virus-specific CD8+ T cells to evaluate cytotoxic T lymphocyte-mediated killing of infected cells, and JHMV-infected NPCs were treated with supernatants from virus-specific CD4+ T cells to determine if pro-inflammatory cytokines play a role in suppression of virus replication.

### **Observations**

- Murine neural precursor cells are infected by JHMV and support a productive infection.
- NPCs are targeted for lysis by virus-specific CD8+ T cells.

- JHMV replication in NPCs is suppressed by CD4+ T cells through IFN- $\gamma$  secretion.
- IFN- $\gamma$  dampens JHMV receptor expression and JHMV protein production in NPCs.

**Submission ID: 34134**

**Submission Title: Transplantation of pluripotent stem cell derived human neural progenitor cells into a viral model of multiple sclerosis leads to stable clinical recovery**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Multiple Sclerosis (MS) is a chronic degenerative disease of the central nervous system characterized by autoimmune mediated demyelination that leads to scars called plaques and loss of function. Significant research hurdles remain before we will understand and eventually cure this disease.

**Background**

There are currently 2.5 million people worldwide including approximately 400,000 people in the United States who are thought to have multiple sclerosis. Currently it is the most common cause of non-traumatic disability in young adults in the United States and Europe. Although the etiology of this disease remains unknown, a number of factors including genetic background and latitude of origin have been implicated.

**Hypothesis**

Human neural precursor cells (hNPCs) transplanted into the spinal cords of mice subjected to a viral model of multiple sclerosis decrease inflammation and pathology by secreting factors that induce regulatory T-cells and promote oligodendrocyte maturation.

**Research**

Using mice infected with a neurotropic JHM variant of mouse hepatitis virus (JHMV) as a model of MS, we injected hNPCs derived from human pluripotent stem cells to explore a potential treatment option for the disease. We used clinical pathology scores to determine the efficacy of our treatment and immunohistochemistry to track changes in immune cell infiltration and myelination. Cells derived for transplantation as well as the spinal cords of both treated and untreated mice were examined using whole genome expression analysis. Genes of interest were identified and further investigated using *in vitro* techniques to assess their immunomodulatory and pro-glial potential.

**Observations**

Our results show that hNPC transplantation can mediate recovery in a mouse model of MS. This effect is consistent and long

lasting. Although the hNPCs are rapidly cleared, the mice showed a robust and stable recovery that included remyelination, a decrease in inflammation and a reduction of pathological symptoms. The lack of persistence of the transplanted hNPCs points to an indirect cause of the recovery. *In vitro* work with the hNPCs shows that they are immunomodulatory and proglial. They reduced T-cell proliferation in a dose-dependent manner and induced T regulatory cells. We also observed maturation of oligodendrocyte precursor cells when they were in the presence of the hNPCs. A set of genes that are upregulated in hNPCs and code for secreted proteins has been identified. Ongoing work will determine which of these proteins are necessary and sufficient to cause the observed outcome. Long term, we hope to develop novel stem cell-based therapies to help the recovery of MS patients.

**Submission ID: 34138**

**Submission Title: Precarious molecular balance determines cell fate**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Cell development is a precarious process where pluripotency and differentiated states are controlled through complex pathways which carefully control the tipping of the balance at each deciding point. Transcription factors play key roles in modulating these events.

**Background**

Transcription factor specificity has been studied by many groups using many methods. The method by which TFs combine at an atomic level to give specific readouts has been perplexing but is important for understanding early pathway development.

**Hypothesis**

We hypothesized that TFs combine using specific protein-protein interaction elements. It is the interaction interface which helps to install the high specificity seen for transcription factors.

**Research**

We used a variety of methods including bioinformatics, genomics, X-ray crystallography as well as cell biology to look at the problem from many angles. In vitro results were obtained using purified protein and oligonucleotides. Ex vivo results were generated using iPSC and modified novel TFs. Both mouse and human iPSC were used to make the results more general.

**Observations**

We were able to show that TFs indeed partner at specific interaction surfaces to install the high specificity which would be difficult if they only recognized the small 6 to 8bp elements on their own. We were able to modify this interface and create molecules with higher efficiency for iPSC production when substituting the original Yamanaka cocktail. In addition we were able to dissect the cis element recognition role of the HMG domain and the activation role of the C-terminal activation domain.

**Submission ID: 34171**

**Submission Title: Characterization of dopaminergic neurons differentiated from Parkinson's disease patient-derived induced pluripotent stem cells**

## Author(s)

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## Topic

Basic Research, Medicine, and Health

## Problem

Current treatments for Parkinson's disease temporarily allay the debilitating symptoms but do not reverse the loss of dopaminergic (DA) neurons in the substantia nigra, and eventually become ineffective. Alternative cell therapy-based treatments using patient specific induced pluripotent stem cell (iPSC)-derived DA neurons are a viable option targeting the cause of the problem.

## Background

Parkinson's disease is a debilitating degenerative disorder of the central nervous system resulting in severe motor deficits caused by the death of dopamine-generating cells in the region of the midbrain called the substantia nigra. Unfortunately, current treatments do not reverse the loss of DA neurons and eventually become ineffective at treating the symptoms. With the development of iPSC technology, it is now possible to generate pluripotent stem cells from the skin of a patient. These patient-derived iPSCs can then be differentiated into the DA neurons that are missing in the patient. Stem cell transplants for Parkinson's disease are currently being investigated and may hold the key to treatment of this intractable disease

## Hypothesis

DA neurons can be derived from patient iPSCs and *in vitro* analysis can be used as a tool to verify potential *in vivo* efficacy for cell therapy-based treatment of Parkinson's disease.

## Research

iPSCs were generated from the skin of nine Parkinson's disease patients. To begin to characterize the lines, embryoid bodies were generated. The embryoid bodies were characterized using immunocytochemistry to verify the presence of the three germ layers. In addition, DA neurons were derived from these iPSCs using a dual SMAD inhibition protocol developed in the Studer lab. RNA from patient derived DA neurons that had been differentiated for 90 days was isolated and analyzed using qPCR.

## Observations

Embryoid bodies formed from patient specific iPSCs were immunolabeled for mesoderm markers including Brachyury and Smooth Muscle Actin, as well as endoderm markers including Alpha Feto Protein and GATA4, and the ectoderm markers Nestin and Tuj1. The embryoid bodies were positive for each lineage, indicating that they are truly pluripotent. Genes indicative of neural lineage differentiation including PITX3, TH, DAT, SERT, GAD1, PAX6, GIRK2, FoxA2, LMX1A and VMAT were assayed via qPCR. These neural markers were expressed on mature, patient specific iPSC derived neurons that had been differentiated for 90 days. Though this work is ongoing, thus far it suggests that it will be possible to derive DA neurons from our patient specific iPSCs and *in vitro* analysis may indicate which lines will be successful *in vivo* following transplantation.



**Submission ID: 34178**

**Submission Title: Identification of possible Bipolar 1 Disorder related brain areas and lithium drug targets via human iPSC derived neurons**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

**Bipolar 1 Disorder (BD1)** is a psychiatric illness that affects 2.6% of the adult US population and is characterized by severe fluctuations between manic and depressive episodes. Despite the prevalence of BD1, science does not yet know what the molecular pathology of BD1 or which structures in the brain the pathology originates.

**Background**

One of the foremost therapeutics for BD1 is lithium, which interestingly is not efficacious for any other major psychiatric disorder. Lithium is a potent mood stabilizer but is known to cause serious side effects. The mechanism for how lithium works to treat bipolar is unknown.

**Hypothesis**

We hypothesize that studying the lithium response pathway in mixed neuronal cultures derived from human BD1 iPSCs would reveal novel insights into lithium's mechanism of action in BD1, possibly indicate regions of the human brain involved with BD1, and identify novel drug targets for future BD1 therapeutics.

**Research**

BD1 induced pluripotent stem cells were reprogrammed from verified BD1 human subjects, which were then differentiated into neurons. A 2D DIGE was performed to identify protein targets that are modulated by lithium in our BD1 iPSC derived neurons. Our list of modulated lithium targets were verified via western blotting. A search was performed with the Allen Brain Atlas to determine what structures of the brain contain high levels of our identified targets, which we cross referenced with brain areas postulated to be involved with BD1. Our 2D DIGE results were also analyzed by Ingenuity IPA to determine which canonical pathways were being modulated by lithium in our BD1 neurons.

**Observations**

Our 2D DIGE, which is sensitive to post-translational modifications, identified 15 proteins affected by lithium in BD1 neurons. The Allen Brain Atlas search performed with our list of lithium targeted proteins identified four brain areas that could possibly be significant in lithium's therapeutic pathway for BD1. Our Allen Brain Atlas search substantiated previous human fMRI studies' assertions of two specific brain areas being implicated in BD1, and identified two new brain areas that have yet to be systematically studied in BD1. Ingenuity IPA analysis of our target list provided a map of canonical pathways modulated by lithium in BD1 neurons. One of the most interesting pathways identified was axonal guidance signaling, as it was an effector of 75% of the pathways identified, and had a significant p-value. The Ingenuity IPA pathway results indicated one of our targeted proteins as the most significant for the effect of lithium on treating BD1, and we are now currently elucidating this protein's role in BD1, with the long term goal of finding a targeted therapeutic for the protein of interest that is efficacious and safe for bipolar.

**Submission ID: 34188**

**Submission Title: Functional Significance of Sialyltransferase ST6GAL1 in the Regulation of Pluripotency**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Recent studies show that post-translational modification of membrane bound glycoproteins may be a significant factor involved in the regulation of pluripotency. Further understanding of how these mechanisms operate in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) may facilitate the use of human pluripotent stem cells (hPSCs) for research and clinical applications.

**Background**

Our group previously observed that the *ST6GAL1* gene encoding a sialyltransferase that catalyzes the terminal addition of sialic acid to  $\beta$ -galactosides in  $\alpha$ -2,6 linkage was preferentially expressed in undifferentiated hPSCs compared to non-pluripotent cells.

**Hypothesis**

ST6GAL1 is functionally significant for the regulation of cellular pluripotency in hPSCs.

**Research**

In this study, we attempt to investigate the potential function of sialyltransferase ST6GAL1 in the regulation of cellular pluripotency. Using Western blotting, we have confirmed that several lines of undifferentiated hESCs and hiPSCs had enhanced expression of ST6GAL1 protein, compared to their differentiated derivatives or parental somatic cells used for reprogramming. In addition, SNA lectin which preferentially binds to sialic acid attached to terminal galactose in an  $\alpha$ -2,6 linkage showed stronger binding reactivity with glycoproteins extracted from hESCs and hiPSCs in the pluripotent state, suggesting that a group of glycoproteins was specifically sialylated in undifferentiated hPSCs due to the high expression of

ST6GAL1. To test the functional significance of ST6GAL1 in the maintenance and establishment of cellular pluripotency, we used shRNA-mediated gene knockdown to downregulate the expression of ST6GAL1 in undifferentiated hPSCs and somatic cells undergoing cellular reprogramming. We found that downregulation of ST6GAL1 decreased the level of POU5F1/OCT4 protein in WA09 hESCs. In addition, we found that the efficiency of retrovirus-mediated reprogramming of human dermal fibroblasts with POU5F1/OCT4, SOX2, KLF4 and MYC was also reduced when ST6GAL1 was knocked down as determined by alkaline phosphatase and NANOG expression in the reprogrammed cells.

## **Observations**

In summary, our data indicate that the sialyltransferase ST6GAL1 and its enzymatic products are highly expressed in undifferentiated hESCs and hiPSCs, and suggest that the protein glycosylation conducted by ST6GAL1 may be critically involved in the regulation of cellular pluripotency in human cells. We will further explore signaling networks affected by the downregulation of ST6GAL1 in hESCs and hiPSCs using global gene expression analysis and qRT-PCR techniques.

**Submission ID: 34191**

**Submission Title: Dissecting Molecular Heterogeneity in Pluripotent Stem Cells with Single-cell Resolution**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) hold great promise for the success of regenerative medicine. Although the expanding interest in studying hPSCs have greatly advanced our knowledge of cellular pluripotency and its potential utility, it is still unclear how these cells establish, maintain and regulate their pluripotency during reprogramming and differentiation.

**Background**

We have previously identified a pluripotency-associated lectin mark UEA-I that is suitable for isolating viable hPSCs and purifying differentiated derivatives. Using microfluidic dielectrophoresis arrays (DEParrays), we have observed substantial heterogeneity of UEA-I mediated staining in populations of undifferentiated hPSCs.

**Hypothesis**

The heterogeneity of UEA-I staining in undifferentiated hPSCs may indicate the molecular divergence and differentiation capacity associated with a population of hPSCs.

**Research**

To better dissect the biological significance of this heterogeneity with regard to the regulation of cellular pluripotency in a molecular context, single-cell samples representing the subpopulations of undifferentiated hPSCs that show high and low reactivity with UEA-I lectin was isolated using DEParrays and subjected to single-cell gene expression analysis using Fluidigm dynamic arrays for high-throughput quantitative RT-PCR.

**Observations**

We have established a working pipeline to simultaneously acquire gene expression profiles of 48 gene transcripts relevant to cellular pluripotency, neuronal differentiation, endoderm differentiation, and malignancy signaling in the single-cell hPSC samples. A similar protocol can be applied to analyzing gene expression in neuronal precursor cells differentiated from hPSCs at the single-cell level. In addition, the single hPSCs isolated using DEPArrays can survive, proliferate and maintain their pluripotency, indicated by colony reformation and the expression of pluripotency biomarkers in cells of the colonies. Our results suggested that the molecular heterogeneity may commonly exist in a population of hPSCs maintained in in vitro culture conditions. The analytical pipeline consists of DEPArray assays followed by single-cell gene expression profiling may represent an effective approach for systematically addressing the molecular complexity in a population of hPSCs and their differentiated derivatives.

**Submission ID: 34200**

## **Submission Title: Development of FGF/TGF $\beta$ -Independent Human ES/iPS Cell Culture System with Chemical Compounds**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

An optimal culture system for human pluripotent stem cells (hPSCs) should be fully defined and free of animal components. To date, most xeno-free culture systems require human feeder cells and/or complicated culture media that contain high dose of activators of the bFGF and TGF $\beta$  signaling pathways. However, these culture systems including recombinant FGF/TGF $\beta$  will be expensive for quality control in future applications. None provide of chemical compounds for replacement of bFGF/TGF $\beta$  ligands is impairing regenerative medicine using hPSCs.

### **Background**

Decision of self-renewal or differentiation in stem cells in vitro culture and in vivo is controlled by extrinsic factors such as signaling molecules. The extrinsic factors regulating human pluripotent stem cell (hPSC) self-renewal and early differentiation events seem to differ from mouse pluripotent stem cell and to date are incompletely understood, but activation of bFGF and TGF $\beta$ /activin/Nodal signaling forms the cornerstone of most systems for hPSC propagation. To date, we could not identify small molecule substitutes for bFGF and TGF $\beta$ . Therefore, we focused on other signals may be involved in hPSC self-renewal. The Wnt/ $\beta$ -catenin signaling pathway plays an important role in mouse ES cell self-renewal in LIF-independent culture but it is dispensable in LIF-dependent culture. In hPSCs, the role of Wnt/ $\beta$ -catenin signaling is still poorly understood and controversial because of the dichotomous behavior of Wnt/ $\beta$ -catenin signaling in proliferation and differentiation.

### **Hypothesis**

We hypothesized that Wnt signals can support hPSC self-renewal if we can identify molecular mechanisms in the dual role of Wnt signaling.

### **Research**

We screened chemical compounds can segregate the dual role of Wnt signaling in hPSCs. After identification of the chemical compounds, we investigated the target of the compounds, its effect of Wnt signaling pathway and molecular action of the pathway in hPSC self-renewal. Utilizing the compounds and other compounds can support the signaling pathways, we developed novel and simple defined hPSC culture system without supplement of FGF or TGF $\beta$  proteins.

### **Observations**

Building on our previous investigations of small molecules modulating Wnt/ $\beta$ -catenin signaling, we found a compound DYRK inhibitor ID-8 that could support Wnt-induced hPSC proliferation and survival without differentiation. Utilizing Wnt and ID-8,

we have developed a novel and simple defined xeno-free culture system that allows for long-term expansion of hPSCs without FGF or TGF $\beta$  activation. Cells in this culture system could self-renew even with FGFR or ALK inhibitors.

In further investigation, we found a chemical compound could replace Wnt and support hPSC self-renewal in combination of ID-8. These culture conditions do not include xenobiotic supplements, serum, serum replacement or albumin. These culture conditions do not include xenobiotic supplements, serum, serum replacement or albumin. Using this culture system, we have shown that several hPSC lines maintained pluripotency and a normal karyotype, and still retained the ability to differentiate into derivatives of all three germ layers. This Wnt-dependent and bFGF/TGF $\beta$ -independent hPSC renewal and the culture system would provide an ideal platform for complete replacement of growth factors with chemical compounds.

**Submission ID: 34207**

**Submission Title: Mesenchymal stem cells biodistribution and effects on neuronal survival and axon regeneration after optic nerve crush and cell therapy**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Diseases that affect the optic nerve, such as glaucoma and diabetic retinopathy, are common causes of blindness worldwide. Visual loss occurs because, in mammals, injury to the optic nerve, e.g. crush or transection, results in the progressive retrograde degeneration of axons and death of retinal ganglion cells, mainly by apoptosis.

**Background**

Strategies developed to enhance survival and regeneration of RGC include the inhibition of myelin-derived proteins and blockage of rho kinase, deletion of PTEN and/or SOCS-3, macrophage activation and delivery of oncomodulin, delivery and stimulation of CNTF, KLF family members regulation, cell therapy and combination of multiple approaches. Despite the remarkable progress in the understanding of mechanisms and pathways involved in neuronal survival and regeneration, at present there are no clinically and currently applicable therapies to sustain retinal ganglion cells survival and/or to promote long-distance axon regeneration.

**Hypothesis**

Bone marrow derived cells have been used in different animal models of neurologic diseases. In this study we investigate the therapeutic potential of mesenchymal stem cells injected into the vitreous body in a model of optic nerve injury.

**Research**

Adult (3-5 months old) Lister Hooded rats underwent unilateral optic nerve crush followed by MSC or vehicle injection into the vitreous body. MSC were labeled before injected with a fluorescent dye or with superparamagnetic iron oxide nanoparticles,

which allowed us to track the cells *in vivo* by magnetic resonance imaging. Sixteen and 28 days after injury, retinal ganglion cells survival was evaluated assessing the number of Tuj1- or Brn3a-positive cells in flat-mounted retinas, and optic nerve regeneration was investigated after anterograde labeling of the optic axons with cholera toxin B conjugated to Alexa 488.

### **Observations**

Transplanted MSC remain in the vitreous body and are found in the eye for several weeks. Cell therapy significantly increases the number of Tuj1- and Brn3a-positive cells in the retina and the number of axons distal to the crush site both at 16 and 28 days after optic nerve crush, although there is a decrease in RGC number overtime. In summary, MSC protects RGC and stimulates axon regeneration after optic nerve crush. The long permanence of the transplanted cells in the eye may account for the effect observed. However, further studies are needed in order to sustain the neuroprotective effect overtime.

**Submission ID: 34213**

**Submission Title: MicroRNA profiling of Mesenchymal Stem Cells (MSCs) provides a putative, general MSC signature and further discriminates cells derived from different tissues.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

MSCs are emerging as a clinically and ethically acceptable treatment for autoimmune and inflammatory diseases and for regenerative medicine. However, heterogeneity of MSCs, arising from the different tissue sources and culturing techniques used, can lead to substantial variations in the therapeutic effectiveness of MSCs. This heterogeneity provides a significant challenge to understand and preserve the favourable characteristics of these cells during their manufacture.

**Background**

Several studies have focused on specific diseases that can be treated with MSCs from various sources. However, no study has set out to define and optimise the mechanisms which specifically control the expansion and differentiation of MSCs into progeny with characterised functional phenotypes. Consistent cell production, expansion and differentiation protocols are needed to conduct the multi-centre clinical trials essential for the translation of current pre-clinical research and small-scale clinical studies. Therefore, there is a need for reliable assays to characterise MSC providing insight into their batch consistency and which are feasible ways to assess the potency of the final product at release.

**Hypothesis**

MicroRNA (miRNA) profiling is proving highly informative for cell characterisation. MiRNA expression profiling provides sensitive assays to analyse the heterogeneity of cell populations and detect contaminating cells. Alterations in miRNA expression also provide an indication of deviations in cell phenotype which may crucially impact on the potency of manufactured cell populations.

## **Research**

SistemQC™, utilises a combination of microRNA expression profiling and customised data analysis to provide a simple, robust and cost-effective assay to monitor the comparability of stem cell populations during culturing and expansion and post scale-up, provide an assessment of the identity and functional attributes/potency of the cells.

## **Observations**

MicroRNA profiles were derived for human monocytes, leucocytes and MSC populations. The MSC cells were from three different tissue sources – adipose tissue, bone marrow and cord blood. In total, 55 datasets were generated from the microarray analysis. High-level visualisation demonstrated clustering of the cells based on lineage. Further analysis of the MSC cells revealed a high degree of homogeneity in miRNA profiles (>40% identity) between all MSCs irrespective of tissue origin. However, miRNA differences were also identified which define the tissue of origin. These data support the use of microRNA profiles to define: (1). a microRNA signature which describes an MSC population irrespective of tissue of origin and with potential as a standardisation assay; (2) miRNA biomarkers that permit the identification of the tissue of origin of the MSCs.

**Submission ID: 34216**

**Submission Title: A qualitative study to identify an appropriate regulatory framework for human stem cell research in Malaysia.**

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**Topic**

Ethics, Law and Society

**Problem**

Malaysia aspires to advance its stem cell research and to ensure the advancement progresses in an ethical way. However, it is not evident that the current regulatory framework adopted is able to facilitate stem cell research and to ensure ethical compliance in the country.

**Background**

**Regulatory approach.** Studies have shown the existence of a regulatory patchwork in which policies on embryonic stem cell research vary from liberal/permissive to restrictive (Salter, 2007 ; Caufield et al., 2009). Some scholars have argued that these different approaches have different impacts on research development (Caufield et al., 2009). It has been claimed that liberal countries such as the UK and Singapore are perceived as “havens” for stem cell research attracting renowned scientists from all over the world (Levine, 2008 ; Arnold, 2006), in contrary, restrictive countries are facing a risk of a ‘brain drain’ (Wiedemann et al., 2004). Since Malaysia wants to advance its stem cell research, a major question is how should the regulatory framework be changed to facilitate stem cell research in Malaysia?

**Compliance measures.** Since stem cell research is surrounded by protracted debate on ethical issues, effective regulation is essential to tackle this situation. It is worth pointing out that many countries introduced either primary law or ethical guidelines complemented by relevant law. This indicates that guiding document with or supported by legal force is important to ensure compliance (Goold, 2004). Nevertheless, legislated law may fail to ensure compliance due to inadequate and ineffective mechanisms of enforcement. The revision of the South Korean law due to the nation's controversial scandal of embryonic stem cell research (Mason, 2006) underscores the increasing importance of adequate safeguards and effective compliance mechanisms (Jhalani, 2008). Since Malaysia aspires to advance this area ethically, another significant question is how should the regulatory framework be changed to ensure ethical compliance?

**Hypothesis**

Research question: How should the current regulatory framework be changed to facilitate stem cell research and to ensure ethical compliance in Malaysia?

## **Research**

Qualitative interviewing was the most suitable and flexible approach to data collection for this study since it requires in-depth information and the subject matter is potentially sensitive. Respondents could not be as open in other methods (Bryman, 2004). This method was chosen because it would enable the interviewer to get in-depth insight into the meaning and significance of what is happening (Wilkinson et al., 2003). The respondents for this project chosen were among the stakeholders: stem cell scientists; religious scholars; members of ethics committees; government officials; policymakers; and NGOs. The interviews started off with established contacts followed by more respondents traced using a 'snowball' sampling technique (Bryman, 2004). They were 23 respondents, an appropriate sample size since this area is not vastly developed in Malaysia. Semi-structured and open-ended questions were chosen for this study to allow sufficient flexibility than that of structured model (Wilkinson et al., 2003). The interview questions were designed with an aim to explore with the interviewees the aspects of the current regulatory system and research development, and the changes needed, which could facilitate stem cell research and ensure ethical compliance. A thematic analysis based on the modified grounded theory was used to analyse the data obtained (Bryman, 2004).

## **Observations**

The analysis of the findings from the interviews is still at a preliminary stage. For now it is suffice to say that it is obvious from the data obtained that there are changes needed as expressed by the respondents, which could be learned from other experienced countries. However, this summit would be a good platform to get some insights and comments from relevant parties on the following questions arose out of the interviews : (i) Should stem cells used for clinical trials or therapies be regarded as drugs and should they be regulated as such?; (ii) Is it necessary to enact primary law to govern basic stem cell research or guidelines are sufficient? ; (iii) Should Malaysian researchers focus on embryonic stem cells or adult stem cells or both? ; and (iv) Is it effective to regulate basic stem cell research through research funding only?

**Submission ID: 34233**

**Submission Title: Ethnically diverse pluripotent stem cells for drug development**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Drug development is a lengthy and expensive process that can last more than 10 years and can cost in excess of \$500 million. One reason for these exorbitant costs is “post-marketing drug failure” in which drugs are recalled after hundreds of millions of dollars have been spent getting the drugs to market.

**Background**

The primary reason for post-marketing drug failure is idiosyncratic drug induced liver injury (DILI). DILI may frequently be caused by genomic variations in genes encoding drug metabolizing enzymes as there is extensive evidence showing that these variations affect drug toxicity and efficacy. However, there is currently no practical means to screen candidate drugs *in vitro* for genome variation-associated toxicity early in the drug development pipeline. Current methods utilize *in vitro* testing, animal studies and human clinical trials, all of which are suboptimal for capturing population-based genetic variation.

## **Hypothesis**

We propose that the development of a human induced pluripotent stem cell (iPSC) biobank that captures the most common genetic variations in drug metabolizing enzymes can provide the pharmaceutical industry with a renewable source of cells for early stage toxicology screens.

## **Research**

We are building an ethnically diverse panel of iPSCs that can fulfill this need. We derived iPSCs from fibroblasts obtained from skin biopsies and from a multiethnic cohort of healthy individuals, including several Caucasians, African Americans, Asians and Middle Easterners.

## **Observations**

Using immunocytochemistry, embryoid body-based differentiation techniques and teratoma assays, we have demonstrated that the ethnically diverse iPSC lines analyzed thus far are truly pluripotent. In addition, extensive characterization by whole genome single nucleotide polymorphism (SNP) analysis indicates that the reported ethnicity of the lines matches that determined by SNP analysis. This project is ongoing, and we hope that it will lead to more efficient toxicity screening for drug development.

**Submission ID: 34262**

**Submission Title: A Regenerative Approach to the Treatment of Multiple Sclerosis**

## **Author(s)**

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The Scripps Research Institute

## **Co-Author(s)**

## **Topic**

Basic Research, Medicine, and Health

## **Problem**

Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination of axons and subsequent neuronal dysfunction. Current treatments for MS focus on modulating the pathological immune response, but are only moderately effective. Herein, we describe the identification of drug-like small molecules by a high throughput screen, that help regenerate the myelin sheath by inducing the differentiation of oligodendrocyte precursor cells into mature oligodendrocytes.

## Background

MS is primarily mediated by T-lymphocytes, which trigger inflammatory processes targeting myelin-producing oligodendrocytes (OLs), causing lesions in the myelin sheath, eventually leading to impaired neuronal conduction. Modulating the pathological immune response is the primary focus of current therapeutic approaches for the treatment of MS. While these treatments are moderately effective in the early stages of MS, none have proven to modify the course of primary progressive or relapsing-remitting MS.

In the central nervous system, a widespread proliferating population of nerve/glia antigen-2 (NG2), platelet-derived growth factor receptor (PDGFR $\alpha$ ) positive adult stem cells, termed NG2-glia or oligodendrocyte precursor cells (OPCs), are the major source of newly formed mature OLs required for remyelination. Remission in MS is largely dependent upon OPCs migrating to sites of injury, and subsequently differentiating to a mature cell fate capable of repair. However, even with immune suppression, progressive phases of MS are associated with inhibited differentiation of OPCs, wherein OPCs migrate to lesions in the spinal cord, but either fail to differentiate or form immature OLs that do not produce myelin.

## Hypothesis

As such, the identification of drug-like small molecules that selectively induce differentiation of OPCs into mature myelin-producing OLs at sites of demyelinated lesions may provide an effective alternative strategy of enhancing remyelination as a treatment for MS. These molecules either alone or in combination with immune suppression could improve the prognosis for MS patients.

## Research

We developed a High Throughput-High Content Imaging based screening platform with the expression of myelin basic protein (MBP) as a readout, to rapidly identify molecules that induce the differentiation of rat primary OPCs into functional myelinating OLs. This platform was used to screen large libraries of drug-like small molecules, natural products and other pharmacologically active agents, and led to the identification of several classes of novel compounds that induce robust differentiation of OPCs *in vitro*. The best compounds were then tested in rodent models for MS- the experimental autoimmune encephalomyelitis model and the Cuprizone model. Further, rigorous *in vitro* and *in vivo* analysis of the immune system as well as compound myelination was conducted.

## Observations

Amongst these classes of effective compounds was Benztropine (EC<sub>50</sub> ~500 nM), which showed excellent brain exposure properties and *in vivo* safety profile. Benztropine showed excellent *in vivo* efficacy in pre-clinical animal models for MS both alone and in combination with immune suppressants FTY720 and Interferon-beta. Evidence derived from *in vitro* and *in vivo* T-lymphocyte assays, adoptive transfer models and the Cuprizone model with Benztropine indicates that the observed efficacy of this compound results from an enhancement of remyelination rather than immune suppression.

Identified molecules from other classes, possessing suitable drug-like properties are also being evaluated using *in vitro* and *in vivo* models for MS and could serve as potential candidates for a regenerative therapy for MS. In addition, pharmacological and mechanistic studies using these novel regulators of OPC differentiation could serve to elucidate new pathways and targets regulating the remyelination process. These molecules that harness endogenous adult stem cells (OPCs) to effectively

regenerate the myelin sheath *in vivo*, could have a significant impact on the development of clinical combination regimens with existing immuno-modulatory drugs for the treatment of MS and other demyelination related diseases.

Results published in Nature (October 2013) and previously presented at the ISSCR conference, 2012.

**Submission ID: 34268**

**Submission Title: The Public-Private Collaboration Models for Successful Development of Next Generation Stem Cell Therapy Product for Cardiovascular Disease**

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**Topic**

Industry Infrastructure

**Problem**

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**Background**

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**Hypothesis**

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**Research**

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**Observations**

According to the World Health Organization (WHO), approximately 50% of the global death toll of 35 million per year is attributed to cardiovascular diseases. Currently, there are few treatment options available for cardiovascular diseases including drug therapies and surgical procedures, but these can only treat the symptoms without actually curing the diseases. Especially for damaged myocardium, cell regeneration is impossible thus making heart transplantation the only treatment method. In order to satisfy such unmet medical needs, there have been active clinical applications of stem cells as an alternative treatment method to cure cardiovascular diseases. In 2011, the Ministry of Food and Drug Safety Korea (formerly known as Korea Food & Drug Administration) has approved the launch of acute myocardial infarction (AMI) stem cell therapy product, Hearticellgram-AMI, as the world's first stem cell therapy product. In addition, 76 relative clinical trials, of which 26 cases are US trials, are currently under progress by 27 stem cell companies worldwide. Among these trials, 59.2% (45 cases) are phase II clinical trials, implying that the trials for cardiovascular diseases are active, and one corporation has already published a result of a clinical trial, which proves the efficacy of stem cells. Despite such active R&D, there still exist numbers of objection in terms of effective therapeutic approach other than the mechanism of stem cell's pharmacological action. Stem

cell and regenerative medicine, as a key alternative medicine, need much more effective and scientific clinical approaches in order to succeed in commercialization, and this seems to be able to be solved through clinical research activation.

Hence, this research aims to analyze the current private investment and development trends, focusing on cardiovascular disease, in order to find the methods to overcome unmet needs that are difficult for corporations to solve alone through collaboration between the government and private sectors.

**Submission ID: 34269**

**Submission Title: Introducing induced pluripotent stem cells to African medical trainees**

**Author(s)**

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**Topic**

Patient Advocacy and Communications

**Problem**

In March of 2012 we invited a researcher from The Scripps Research Institute Center for Regenerative Medicine (TSRICRM) to The University of Stellenbosch, Department of Hematology, Tygerberg Hospital, Cape Town, South Africa to give a ten day lecture series about human induced pluripotent stem cells (hiPSCs) to medical residents and hematology fellows

**Background**

hiPSCs are of interest to medical professionals because they have the ability to self renew and generate all types of tissues in the body. As such, they may provide a novel platform to study disease, develop drugs and generate cells for regenerative medicine.

**Hypothesis**

The goal of the lecture series was to familiarize participants with hiPSC literature, technology and laboratory techniques.

**Research**

In the lecture series, a unique set of slides was presented which are used for training at TSRICRM and were developed by leaders in the field. The invited researcher also presented additional content on the applications of hiPSCs for infectious diseases. In addition, she presented on the need for development of biorepositories on the African continent as well as room temperature stabilization of extracted nucleic acids as an energy efficient approach to biobanking. In order to familiarize the trainees with hiPSC culture techniques she supplemented the lectures with video demonstrations using content from *The Journal of Visualized Experiments*.

### **Observations**

To our knowledge, this lecture series was the first in all of Africa designed to introduce medical trainees to hiPSCs and we are pursuing further training opportunities in collaboration with TSRICRM. Our center is one of two H3A (Human Heredity and Health for Africa) biorepositories in Africa, which may be developed into a full scale biobank. Along with the biobank, this training will help to create the required infrastructure to carry out iPSC development and research on the African continent.

**Submission ID: 34271**

**Submission Title: Maintenance of Undifferentiated Human Embryonic Stem Cells on Amniotic Fluid Cells in Humanized Culture Conditions**

**Author(s)**

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human embryonic stem cells

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**Topic**

Basic Research, Medicine, and Health

**Problem**

For therapeutic purpose, hESCs must be almost excluded the use of animal-derived materials from the feeder cells, substances and medium containing animal serum.

**Background**

Human embryonic stem cells (hESCs) have been cultured on mouse embryonic fibroblast (MEF or STO) feeder cells with a medium containing animal products.

**Hypothesis**

We propagated hESC lines cultured on human feeder layers with humanized culture condition

**Research**

In this study, we propagated hESC lines, SNUhES32 and H1 were cultured on feeder layers derived from human amniotic fluid cells (hAFCs) with humanized culture condition as a Knockout SR XenoFree and CELLstart

**Observations**

All hESCs were maintained up to 10 passages at this culture system and then were analyzed characteristics of their pluripotency. HESCs kept positive alkaline phosphatase enzyme activity and expressed pluripotent stem cell markers, SSEA-4,

TRA-1-60, TRA-1-81, Oct-4, and Nanog like hESCs cultured on conventional conditions. Also, we investigated contamination of xenoantigenic components such as nonhuman N-glycolylneuraminic acid (Neu5GC) in cultured hESCs by flow cytometry, and the results was not observed on hESCs under humanized conditions. In conclusion, we performed that hESCs (SNUhES32 and H1) could be successfully maintained on hAFCs using of humanized materials instead of animal components. Therefore, this study suggested that the humanized culture conditions for hESCs and it could be more clinical use in therapeutic applications and regenerative medicine.

This research was supported by grants (2012M3A9C6049722) from National Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning.

**Submission ID: 34279**

**Submission Title: Application of Droplet Digital PCR Technology to Detection of Residual Undifferentiated Cells in Cardiomyocytes Derived from Human iPS Cells.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human pluripotent stem cells (hPSCs), i.e. human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are able to self-renew and differentiate into multiple cell types. Thus, numerous attempts have been made to utilize hPSCs in regenerative medicine/cell therapy. However hPSCs have tumorigenic potential. Therefore, residual undifferentiated hPSCs in products that would eventually proliferate and form a teratoma is one of the most obvious safety issues to develop cell therapy hPSC-derived products.

**Background**

In our previous studies, we have demonstrated that the qRT-PCR assay can successfully detect as low as 0.002% residual undifferentiated hiPSCs in hiPSC-induced retinal pigment epithelial (RPE) cells using LIN28 as a target gene (the LIN28/qRT-PCR method). This is equivalent to that present in a mixture of a single hiPSC and 50,000 RPE cells.

**Hypothesis**

In this study, we used the LIN28/qRT-PCR method to test the hypothesis that the LIN28/qRT-PCR method could be applied to other hiPSC-derived product, e.g. cardiomyocyte (CM) cells. Furthermore, to establish more sensitive assay than the LIN28/qRT-PCR methods, we examined droplet digital PCR system instead of qRT-PCR (LIN28/ddPCR method).

**Research**

To establish a high sensitivity assay for detection of residual undifferentiated hiPSCs in the final product, we compared two in vitro assays: the LIN28/qRT-PCR method and the LIN28/ddPCR. To achieve this goal, these assays were used on cell mixtures that contained defined numbers of undifferentiated hiPSCs in CM cells, and we also tried to determine the lower limit of detection (LLOD) of each assay by using multiple lots of primary cells as backgrounds.

**Observations**

The LIN28/qRT-PCR method detected 0.01% undifferentiated hiPSCs spiked in CM cells. Moreover, The LIN28/ddPCR methods were able to detect as low as 0.001% undifferentiated hiPSCs in CM cells, which is equivalent to a single hiPSC contained in 100,000 cells.

The LIN28/ddPCR method detecting residual undifferentiated hiPSCs is the most sensitive method that previously reported. This highly sensitive in vitro method with ddPCR is expected to contribute to process validation and quality control of cell therapy products derived from hPSC.

**Submission ID: 34299**

**Submission Title: A DNA methylation biomarker panel for assessment of the neural differentiation status in human neural stem cells (hNSCs) derived from embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) lines.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

The use of iPSC - derived hNSCs to replace ESC - derived hNSCs for regenerative medicine and neurological diseases could greatly reduce the risk of immune rejection in patients receiving these treatments. iPSC - derived hNSCs have been shown to follow the same neural differentiation program as ESC - derived hNSCs, but show more variability in differentiation efficiencies.

**Background**

DNA methylation biomarkers can be used to generate an epigenetic neural lineage signature of hNSC derived from iPSC or ESCs. Epigenetic characterization of these derived hNSCs will become a necessary prerequisite for determining whether derived hNSC will be able to be used for future cell-based transplantation therapy.

**Hypothesis**

A panel of DNA methylation biomarkers can be used to characterize the variability and differentiation efficiencies of hNSCs derived from embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) lines.

**Research**

Human multipotent stem cells from ESC or iPSC were generated using the EZ spheres protocol and then cultured as an adherent monolayer for 3 passages before undergoing differentiation. Differentiation was performed for 14 days with normal differentiation media, or differentiation media that contained inflammatory factors from either polymorphonuclear neutrophils (PMNs), or macrophages, to mimic the effect of inflammation on neural differentiation. Undifferentiated cells and cells differentiated for 14 days were harvested and the genomic DNA was extracted from each cell line. The DNA methylation profiles of the three hNSC lines derived from ESCs and two hNSC lines derived from iPSCs were established using two ectodermal markers (SOX2, and PAX6), one neuronal marker, (Beta tubulin III - BTIII), one astrocytic marker (GFAP), and two oligodendrocytic markers (NG2 and GALC) using the One Step qMethyl kit (Zymo Research). The DNA methylation

profiles of genomic DNA from one embryonic stem cell line (BioTime) and a neural progenitor cell line derived from embryonic stem cells (Millipore) were included as controls for the undifferentiated comparison only.

## **Observations**

In the undifferentiated state, all hNSC derived lines from either ESC or iPSC showed levels of DNA methylation for SOX2, PAX6, and Beta tubulin III below 20%, and levels of DNA methylation for GFAP, NG2, and GALC at or above 20%. The undifferentiated ESC line (BioTime) had a higher percentage of methylation for PAX6 and NG2, and a lower methylation percentage for GFAP as compared to the hNSC lines. The NPC line also had higher methylation levels of PAX6 than the hNSC lines and the highest level of GALC methylation compared to all lines. A comparison of the differentiated hNSC lines with no conditioning, PMN conditioning, or macrophage conditioning showed that the XFS6 derived hNSC line had an increase in DNA methylation markers Beta tubulin III and GFAP on the PMN conditioned media, suggesting that gene expression regulation may be altered by the "inflammatory condition". Different responses in the epigenetic status of DNA methylation affecting the gene expression of these cell lines will help to select lines with the most promise for cell-based transplantation therapy.

**Submission ID: 34300**

**Submission Title: Assessment of Genomic Stability in NINDS Repository Patient-derived Fibroblasts: Implications for Biobanking of Neurodegenerative Disease Fibroblasts and iPSCs.**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Skin fibroblasts are an important source of somatic cells for the reprogramming and subsequent differentiation of induced pluripotent stem cells (iPSCs) into human neural progenitor cells and other cell types. Access to a well-characterized set of patient-derived fibroblasts is therefore required for successful iPSC reprogramming, implementation of disease-in-a-dish models, and other stem cell-related studies.

**Background**

The NINDS Repository at the Coriell Institute (<http://ccr.coriell.org/NINDS>) has established a large collection of fibroblasts derived from donors diagnosed with amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, frontotemporal dementia, or Huntington's disease, as well as neurologically normal population controls. In an effort to ensure the quality of these valuable resources, the NINDS Repository has established a vigorous quality assessment program including G-band karyotyping on primary fibroblasts.

**Hypothesis**

Although certain human somatic cell populations, such as neurons, have been recently shown to display some extent of chromosomal abnormalities (mosaicism), there is a lack of studies addressing in detail the extent of chromosomal abnormalities present in primary fibroblasts derived from skin explants from neurodegenerative disease patients.

**Research**

Here, we describe the results of G-banding karyotyping of a subset of 39 skin-derived primary fibroblasts collected at the

NINDS Repository. This cohort includes samples from control subjects or donors harboring mutations in ANG, C9ORF72, FUS, GBA, HTT, LRRK2, MAPT, PARK2, SNCA, SOD1 or TDP43. Although a large fraction (~75%) of the fibroblast lines studied were determined to be karyotypically normal, as indicated by G-banding analysis, the remaining fibroblasts displayed various karyotypic abnormalities. Among those fibroblasts with abnormal karyotypes (n=10), our analysis revealed cases of trisomy 8, 12 and 18, and monosomy 15 (n=1 each), as well as chromosomal derivation, deletion, addition and/or translocation involving various chromosomes. The extent of mosaicism observed in fibroblast populations with abnormal karyotypes ranged from 8-72% with an average of 36% (Median=38% and mode=8%) and the overall ratio of normal vs. abnormal karyotypes appeared to be mutation, age, gender and diagnosis independent. These results suggest that a fraction of primary fibroblasts obtained from skin of either neurodegenerative disease patients or healthy controls has either an intrinsic degree of chromosomal aberration and/or a propensity to drift de novo once placed in culture. Further karyotyping on later passages may be useful to detect the selection of emerging dominance conferred by a particular karyotype and DNA microarrays may provide additional information about specific single nucleotide polymorphisms, copy number variation and chromosomal rearrangement details.

### **Observations**

Importantly, we have found that iPSCs reprogrammed from a mosaic fibroblast population can display normal karyotype and remain pluripotent, likely a reflection of the clonal selection process. This observation confirms the value of primary fibroblast from subjects with a unique clinical manifestation and/or mutations even if they display mosaicism. Finally, our findings suggest the cytogenetic analysis is a required step for quality control on fibroblasts derived from skin explants, and should be a checkpoint for chromosomal stability performed by either biobanks before their distribution or by the end-researcher before iPSC reprogramming.

**Submission ID: 34304**

**Submission Title: THE USE OF STEM CELLS FOR A HEALTHY START TO LIFE FOR THE DISABLED CHILD**

**Author(s)**

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**Co-Author(s)**

**Topic**

Basic Research, Medicine, and Health

**Problem**

This poster addresses the use of stem cells to improve the quality of life of the disabled child. It addresses the knowledge gap of clinicians in the new field of regenerative medicine in this context.

**Background**

Early childhood disability is a major pressing problem for clinicians, parents and care givers. This poster provides information on the use of stem cells to improve the milestones of children with disability. It provides a futuristic perspective in dealing with this problem.

**Hypothesis**

Stem cell therapies can provide a completely new dimension to the lives of children with disability and their care givers.

**Research**

An intensive literature review on stem cells and cerebral palsy was undertaken. The author reviewed the experimental work done in animal studies worldwide on the use of stem cells for hypoxia ischemic injury. The poster reviews the international experience of the use of stem cells for early childhood disability. It reviews the ethical issues in this context.

**Observations**

Animal studies have shown promising results in the use of stem cells for hypoxia ischemic injury of the brain. Few studies on the use of stem cells in children with cerebral palsy have shown positive results in improving the quality of life of children with cerebral palsy. There are scores of ethical issues to be dealt with in the context of the use of stem cells for childhood disability. Research in this area, will pave the way to providing good quality life to children with cerebral palsy.

**Submission ID: 34326**

**Submission Title: Spontaneous rescue of Trisomy 21 fibroblasts during reprogramming to pluripotency**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Trisomy 21, also known as Down Syndrome, is a chromosomal disorder in which patients possess an extra copy of chromosome 21 in most of their cells. Trisomy 21 is a devastating developmental disorder for which there are no treatments at this time.

**Background**

The advent of reprogramming technology has opened up many new avenues for the study of diseases like Trisomy 21. The ability to take fibroblasts from a diseased patient, reprogram them into induced pluripotent stem cells (iPSCs), and then differentiate those iPSCs into cell types affected in the patient's disease is leading to many new insights into the etiology of numerous diseases. This approach is based on the assumption that the genetic aberrations that cause the disease in the patient will be present in the iPSCs and can drive disease-relevant cellular phenotypes in the differentiated derivatives.

## **Hypothesis**

We hypothesize that we can model Trisomy 21 in vitro using iPSCs.

## **Research**

We reprogrammed several fibroblast lines derived from Trisomy 21 patients using different reprogramming methods. Characterization of the lines demonstrated that they were truly pluripotent. SNP genotyping of trisomic fibroblasts and disomic iPSCs showed that they were derived from the same person, eliminating the possibility that the disomic clones were derived from contaminating euploid cells from another individual.

## **Observations**

We have made the surprising observation that the reprogramming of Trisomy 21 fibroblasts sometimes led to spontaneous loss of one copy of chromosome 21 in a subset of the resulting iPSC clones, which become seemingly normal, euploid cells. In addition, the spontaneous loss of chromosome 21 occurs regardless of the method used to reprogram the cells. Current studies are aimed at characterizing the differences between disomic and trisomic clones and investigating the mechanism of the conversion of trisomic fibroblast cells into disomic iPSCs. The generation of both trisomic and disomic iPSC lines from the same individual provides us with an unprecedented opportunity to study disease mechanisms at work in Trisomy 21, with the disomic lines serving as the ideal control for the trisomic lines. In the future, these findings may suggest novel therapeutic strategies for the treatment of Trisomy 21.

**Submission ID: 34328**

## **Submission Title: Unraveling the role of MECP2 and X-chromosome inactivation in Rett Syndrome by using patient-derived induced pluripotent stem cells**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Rett syndrome (RTT) is a progressive neurological disorder caused by mutations in the X-linked gene encoding the methyl-CpG binding protein (MeCP2). By virtue of its position on the X chromosome, random X-chromosome inactivation (XCI) in females leads to mosaicism, in which each individual cell expresses only the mutant or only the wild-type version of MeCP2. This mosaicism, and the resulting heterogeneity in cellular phenotype, makes studies of MECP2 dysfunction in Rett Syndrome patients and animal models quite challenging.

### **Background**

Patient-specific induced pluripotent stem cells (iPSCs) are generated by reprogramming patient-derived skin cells into undifferentiated cells. It has been shown that XCI is frequently lost in iPSCs during time in culture. The instability of XCI complicates their use as models for X-linked diseases such as Rett Syndrome. As a solution to this problem, we decided to generate patient derived-isogenic iPSCs cell lines in order to create a model that enables us to study the functional role of MECP2 in Rett Syndrome patients.

### **Hypothesis**

The generation of isogenic homozygous *MECP2* mutant and homozygous *MECP2* wild-type iPSCs derived from a specific Rett Syndrome-derived iPSC line, will provide us with matched lines that can be used to study MECP2 function during neural development.

### **Research**

In order to create pluripotent stem cells from Rett Syndrome patients, fibroblasts from two patients with two different mutations in the methyl-CpG-binding protein 2 (MECP2) were reprogrammed by transient over-expression of reprogramming factors and fully characterized for pluripotency. Several colonies from each patient were selected for confirmation of the *MECP2* genotype and *MECP2* expression. To determine whether the mutant allele, wild-type allele, or both were being expressed in each iPSC clone (which could vary according to the X-inactivation state of the cells), mRNA sequencing was performed on patient-derived fibroblasts and iPSC clones. This characterization let us obtain the proposed paired isogenic iPSC lines to study the MECP2 function in Rett syndrome patients.

MECP2 is involved in mediating DNA methylation, which regulates the activity of many genes. Moreover, glutamatergic and GABA ( $\gamma$ -aminobutyric acid)-ergic neurons derived from Rett Syndrome iPSCs have been shown to have a substantial reduction in their numbers of spines and synapses. We optimized a directed neural differentiation procedure to determine the differentiation capacity of Rett Syndrome-derived iPSCs compared to control cells.

As changes in DNA methylation can be associated with alterations in mRNA expression, we used gene expression microarrays to obtain mRNA expression profiles in the isogenic samples to identify genes that are up-regulated and down-regulated in cells containing the normal MECP2 allele compared to those containing the mutant MECP2 allele. We used pathway analysis to identify biological and functional interactions.

## **Observations**

By using a directed neural differentiation procedure, we have demonstrated that the Rett Syndrome derived iPSCs differentiate into neurons with the same efficiency as control cells. The aim to compare neural progenitors and differentiated neurons from the homozygous wt and mutant isogenic iPSC lines, to determine if the differences are stable when XCI is not present in Rett Syndrome derived iPSCs.

The identification of relevant differentially expressed genes in cells expressing either the wt or the mutant copy of the MECP2 gene as well as the networks in which they are involved helped me predict specific pathways regulated by MECP2 that affect MECP2 mutated cells and their consequences during differentiation. I propose to perform DNA methylation analysis as well and use bioinformatics approaches to correlate the DNA methylation status and gene expression level of each gene and identify groups of genes that have similar correlation patterns between their methylation and transcript levels. This will provide valuable new insights into MECP2 function in healthy as well as affected cells.

**Submission ID: 34329**

**Submission Title: The Human Stem Cell Techniques Course at the Scripps Research Institute Center for Regenerative Medicine**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Advances in biomedical research will require highly trained individuals to fill the demands of the growing field of regenerative medicine.

**Background**

The Scripps Research Institute Center for Regenerative Medicine offers a ten-day intensive techniques course on human pluripotent stem cell culture and experimentation.

**Hypothesis**

Skilled and trained researchers in the stem cell field will be critical for advancing new discoveries in basic research, as well as, in areas of translational medicine.

**Research**

Our courses focus on both understanding pluripotency in a lecture setting including methods, stem cell translation, and ethics, while also actively culturing live hPSCs in our state-of-the-art training lab. Students are taught handling, passaging, cryopreservation techniques, and reprogramming methods, while also learning to properly validate pluripotency through immunocytochemistry, mouse teratoma surgery, and embryoid body formation.

**Observations**

By introducing students and researchers to a variety of stem cell techniques and methodologies, we are further advancing the field by providing participants with the tools they need to make exciting new discoveries in stem cell biology.

**Submission ID: 34331**

**Submission Title: Quantifying the Cellular Response to DNA Damage as a Means of Assessing Human IPS Clone Pluripotency**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Induced human pluripotent stem cells (hiPS) hold much promise as a potential therapeutic agent for an array of maladies, and the production of large repositories of these cells at several institutions are currently underway. However, an efficient means of quality control is needed in order to determine whether a clone is suitable for therapeutic applications.

**Background**

Pluripotency of hiPS cells is routinely determined by a number of techniques, including detection of cell surface markers such as SSEA-3/4 and TRA-1-60/1-80, expression of reprogramming genes, clonal morphology and teratoma formation. These techniques, while useful in determining the pluripotent ground state of a limited number of clones, are cumbersome and expensive when applied to large numbers of samples.

**Hypothesis**

Hypersensitivity to DNA damaging agents is a hallmark of stem cell biology, regardless of tissue source or cellular origin. We have previously demonstrated that mouse IPS pluripotency is directly correlated with sensitivity to the DNA damaging agent etoposide, and we propose here that such sensitivity provides an efficient and superior means of determining pluripotency of hiPS clones.

**Research**

Our laboratory assessed the pluripotent potential of 88 hiPS clones by RNA expression of REXO1, SALL4, TDGF1, SOX2, POU5F1, MYC and ZFP42, FLOW analysis for SSEA-4 and Tra-1-60 and sensitivity to etoposide over a 24 hr. time-course. Differences in overall RNA expression patterns were determined by principal component analysis (PCA), while sensitivity to etoposide was examined as a function of half-maximal concentration (EC<sub>50</sub>).

**Observations**

Clones exhibiting significantly different RNA PCA profiles compared with the collective data set also displayed a significant

decrease in etoposide sensitivity. Interestingly, expression of SSEA-4 and TRA-1-60 were not significantly altered in clones with high etoposide  $EC_{50}$  values or outlier PCA analyses. These results demonstrate the utility in employing etoposide sensitivity as a potential replacement for expensive and/or time consuming assays currently used in the determination of hPS pluripotency.

**Submission ID: 34336**

**Submission Title: The Role of Induced Pluripotent Stem Cells in Conservation of Endangered Species**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Unique plant and animal species are disappearing from the earth at an alarming rate due to a number of factors including habitat loss, climate change and poaching. One approach to stop the loss of these species has been captive breeding programs in zoos and other wild life preserves. While this has been successful for some species, it has not worked for others. A new potential solution to help stave off this loss of species is the use of induced pluripotent stem cells (iPSCs) to generate new individuals of the species.

**Background**

iPSCs can divide indefinitely in culture and can be differentiated into any cell type in the body. Importantly, it was recently shown that iPSCs can be differentiated into functional eggs and sperm.

**Hypothesis**

Using iPSCs, it may be possible to generate new individuals of a particular species.

**Research**

For this project, we made use of the Frozen Zoo at the San Diego Zoo's Institute for Conservation Research, which is an extensive collection of cryopreserved primary fibroblast cultures, collected from over 8600 individual vertebrates from approximately 800 species. We used viral-based vectors to reprogram fibroblasts from a variety of different endangered species including the drill, *Mandrillus leucophaeus*, and the northern white rhinoceros, *Ceratotherium simum cottoni*.

**Observations**

During the course of these experiments we determined that Moloney murine leukemia virus-based retroviral vectors could effectively deliver the reprogramming factors into the northern white rhinoceros fibroblasts when pseudotyped with vesicular stomatitis virus G envelope protein (VSV-G), but not with the amphotrophic envelope protein. The VSV-G

pseudotyped retroviral vectors also efficiently transduced the drill fibroblasts. We were able to reprogram both the rhinoceros and drill fibroblasts using the human OCT4/POU5F1, SOX2, KLF4, and MYC cDNA sequences, suggesting that retroviral vectors carrying human reprogramming factor sequences and pseudotyped with VSV-G may be widely applicable for generating iPSCs from a variety of species.

**Submission ID: 34337**

## **Submission Title: Specific Detection of Stem Cell Biomarkers with RNA probes in Live Cell Populations**

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### **Topic**

Basic Research, Medicine, and Health

### **Problem**

Although cell surface protein markers for embryonic stem cells have been widely employed for detecting and sorting pluripotent and differentiated stem cell populations researchers are currently limited to surface markers for live cell sorting.

### **Background**

Gold nanoparticle RNA probes can be used to detect specific RNA expression within living cells without harming or effecting cell health.

### **Hypothesis**

The use of RNA markers in live cells would allow for greater flexibility in enriching for cells with high and low expression profiles through Fluorescent Activated Cell Sorting (FACS). Live cell detection of RNA markers would leave cells unchanged and unharmed allowing the use of the enriched population for downstream assays.

### **Research**

Here we show the ability to detect specific RNA expression of single cells within live stem cell populations using RNA probes attached to gold nanoparticles. We generated probes to detect mRNA levels of OCT4, UTF1, SOX2, REX1, DPPA2, TERT, KLF4 & TLE1 in human embryonic stem cells (hESC) and hESC-derived neural progenitor cells (NPC).

### **Observations**

We employed this technique to detect changes in Oct4 mRNA in hESC and NPC and thus were able to demonstrate the downregulation of OCT4 mRNA in the differentiated cells by flow cytometry. Fluorescent imaging also revealed the heterogeneity of OCT4 mRNA expression within individual hESC. Finally, we also demonstrated the differential mRNA expression of seven additional stem cell biomarkers in hESC and NPC. This technology enables researchers to analyze and sort live

stem cell populations for additional downstream applications, based on intracellular RNA expression, allowing for new advancements in the fields of regenerative and personal medicine.

**Submission ID: 34339**

**Submission Title: DONOR-DEPENDENT DIFFERENCES IN IMMUNE MODULATORY ACTIVITIES OF HUMAN BONE MARROW DERIVED STROMAL CELLS IN VITRO**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Human bone marrow derived stromal cells (BMSCs), also called mesenchymal stem cells (MSCs) have been in clinical use for the last 7 years to treat graft versus host disease and a variety of other immunological abnormalities.

**Background**

To prepare such cells, bone marrow biopsy samples are placed in tissue culture dishes and the plastic-adherent cell population is cultured. Passage 3 or 4 (P3 or P4) cells are stored in frozen aliquots until used. The heterogeneity of the adherent cell population has been noted and studied by a number of investigators using a variety of methods.

**Hypothesis**

To look for differences among BMSCs derived from several healthy volunteers, we performed in vitro assays of immune modulatory functions of the cells.

**Research**

We used two in vitro assays:

1. Mixed leukocyte reaction (MLR):

Peripheral blood mononuclear cells (PBMC) were plated in 96-well plates (150,000 responders per well). Responders were co-cultured with 2500cGy-irradiated stimulator PBMCs ( $1 \times 10^5$  cells /well). BMSCs were added in the following numbers:  $10^4$ ,  $4 \times 10^4$  and  $10^5$  cells /well. Culture plates were incubated for 6 days and on the day of harvest, 0.5 $\mu$ Ci of 3H-thymidine was added to each well for 4 hours. Counts incorporated into DNA were detected with a liquid scintillation counter and reflected

lymphocyte proliferation. The effect of BMSCs on MLR was calculated as the percentage of the mean suppression compared with the proliferative response of the positive control without BMSCs. The experiments were performed at least three times for each variable described.

2. Monocyte/macrophage derived IL-10 production in co-cultures of BMSCs and two human monocytic cell lines (THP1 and U937) following LPS stimulation: THP-1 or U937 cells (100,000 per well) were plated in 96-well plates. After 3 hours, human 25,000 BMSCs from different donors were added per well and the co-culture was stimulated with phorbol myristate acetate (PMA, 20 ng/ml) to induce the differentiation of monocytic cells into macrophages. After an overnight incubation, the co-culture was stimulated with 1 mg/ml LPS for 6 hours. The supernatants were assayed for IL-10 using the DuoSet IL-10 ELISA kit (R&D). All measurements were performed in quadruplicate.

## **Observations**

Mixed leukocyte reaction (MLR) is an indicator of the BMSCs' ability to suppress T-cell proliferation. We found that all BMSCs affected T cell proliferation even at the lowest cell number used (10,000/well). Using this number of cells, the suppression varied from 52% to 83% among the 6 volunteers studied.

IL-10 induction in monocytes/macrophages reflects the shift that underlies the switch from a pro-inflammatory to an anti-inflammatory state. We found that the BMSC-induced IL-10 concentration varied between 317-1218 pg/ml among the 8 patients studied.

(Part of these findings were presented by Eva Mezey, MD PhD at the ISSCR 2012 meeting)

**Submission ID: 34341**

**Submission Title: Probing the cellular functions of master regulatory microRNAs in stem cell reprogramming by using perturbation-induced cell signature generation**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Stem cell biologists can reprogram human fibroblast cells to pluripotency; however, the current methods work with very low transformation efficiency. The aim of this study is to determine if the existing methodology can be improved by screening the activities of additional key regulatory microRNAs, previously shown to play significant regulatory roles in cellular differentiation.

**Background**

The perturbation experiments examine the opposing effects of the previously characterized microRNAs: mir-17, mir-302, mir-372, and mir-let7. These microRNAs are proposed to either enhance the stabilization of cells in the pluripotent state, or suppress pluripotency, and so push the cells toward differentiation. Using combinations of factors we have generated 107 microRNA induced cellular perturbation signatures, along with the equivalent gene expression data on illumina HT12v3 microarrays.

**Hypothesis**

Cellular perturbation expression data was generated by the LINCS program using the L1000 technology. After passing quality testing this allowed the imputation of a whole genome signature data set.

**Research**

Preliminary analysis of the data reveals: (1) the individual microRNAs generate distinctive and reproducible cellular signatures; (2) the comparison of the microarray-based expression data and the LINCS data reveals significant similarities and differences .

**Observations**

The investigation of the cellular perturbation signatures using the commercial tools Ingenuity Pathway Analysis, Nextbio and GeneGO Metacore reveals clear similarities to genesets and experiments focused on the control of the cell cycle, oncogenesis and cellular differentiation. We anticipate that the full integration of the stem cell microRNA perturbation signatures with the very large LINCS universe of data types should help guide future improvements to stem cell creation methodology.



**Submission ID: 34344**

**Submission Title: Neurogenic Stem Cell (NSC) and Mesenchymal Stem Cell (MSC)-mediated tumor-targeted gene therapy based on large-scale transfection of plasmid DNA into primary adipocytes**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Cancer continues to be one of the leading causes of mortality and morbidity throughout the world. Current conventional cancer therapies are often symptomatic and passive in nature. It is believed that one of the major obstacles in developing effective cancer therapies is their lack of sufficient specificity for tumors.

**Background**

Human mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells that can be obtained from adipose tissue, expanded and genetically modified *in vitro*, and subsequently used for therapeutic cancer strategies *in vivo*. MSCs are capable of communicating with other cells in the human body and even appear to target areas of injury in response to "homing" signals of cellular damage.

**Hypothesis**

It has been discovered that MSCs possess tumor-oriented homing capacities and are thus a promising option for use as cell therapy carriers in the delivery of therapeutic agents into tumor sites. These MSCs were isolated using human processed lipoaspirate (PLA) cells that are capable of differentiating into multiple mesenchymal lineages and can be induced to form neural stem cells (NSCs).

**Research**

In this study, a nonviral vector, pORF5-codA plasmid containing the *E. coli* cytosine deaminase, was used to introduce the therapeutic gene tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to MSCs derived from adipose tissue. Meanwhile, the characterization, transfection efficiency, cytotoxicity, cellular internalization, and mechanism used by the nonviral vector were evaluated. The *in vitro* expression of this plasmid from the MSCs was demonstrated with luciferase reporter assays and electroporation pulse tests. Additionally, the human NSCs in this study were also an ideal vehicle for cell replacement and gene transfer. The homing ability of the MSC-plasmid was further investigated *in vivo* in the targeting of brain tumors.

**Observations**

The transfected gene targeted strategy is based on the MSCs' capability of tumor-directed migration and incorporation, as well as the *in situ* effectiveness of nonviral plasmids in transferring the therapeutic gene to MSCs. The strategy is also based on the feasibility of using MSCs as targeted gene delivery carriers, indicating that MSCs could be a promising tumor-targeting therapeutic tool in future cancer gene therapies.

**Submission ID: 34348**

**Submission Title: Characterization of dopaminergic neurons differentiated from Parkinson's disease patient-derived induced pluripotent stem cells**

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**Co-Author(s)**

**Topic**

Basic Research, Medicine, and Health

**Problem**

Current treatments for Parkinson's disease temporarily allay the debilitating symptoms but do not reverse the loss of dopaminergic (DA) neurons in the substantia nigra, and eventually become ineffective. Alternative cell therapy-based treatments using patient specific induced pluripotent stem cell (iPSC)-derived DA neurons are a viable option targeting the cause of the problem.

**Background**

Parkinson's disease is a debilitating degenerative disorder of the central nervous system resulting in severe motor deficits caused by the death of dopamine-generating cells in the region of the midbrain called the substantia nigra. Unfortunately, current treatments do not reverse the loss of DA neurons and eventually become ineffective at treating the symptoms. With the development of iPSC technology, it is now possible to generate pluripotent stem cells from the skin of a patient. These patient-derived iPSCs can then be differentiated into the DA neurons that are missing in the patient. Stem cell transplants for Parkinson's disease are currently being investigated and may hold the key to treatment of this intractable disease.

**Hypothesis**

*In vitro* analysis of DA neurons derived from patient iPSCs can be used as a tool to identify potential *in vivo* efficacy for cell therapy-based treatment of Parkinson's disease.

**Research**

iPSCs were generated from the skin of nine Parkinson's disease patients and their differentiation ability was assessed through embryoid body-based differentiation, using immunocytochemistry to verify the presence of the three germ layers. Separately, DA neurons were derived from the iPSCs by a directed differentiation protocol and analyzed for neuronal and non-neuronal gene expression using qPCR.

**Observations**

Embryoid bodies formed from patient specific iPSCs were immunolabeled for mesoderm markers including Brachyury and smooth muscle actin, endoderm markers including alpha fetoprotein and GATA4, and the ectoderm markers Nestin and Tuj1. The embryoid bodies were positive for each lineage, indicating that the cells are pluripotent. In the differentiating neuronal populations, we assessed expression of *PITX3*, *TH*, *DAT*, *SERT*, *GAD1*, *PAX6*, *GIRK2*, *FOXA2*, *LMX1A* and *VMAT* using qPCR. The results so far show that that we have derived DA neurons from patient-specific iPSCs and *in vitro* analysis may indicate which lines will be successful *in vivo* following transplantation.

**Submission ID: 34350**

**Submission Title: Optimizing NIS reporter gene technology for stem cell tracking applications**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

To address the concern that the NIS protein from a given species could be targeted and prematurely destroyed by the immune response of another species, we have developed a non-immunogenic line of lentiviral vectors, which utilize species-specific NIS to eliminate the immune response, and made a panel of lentiviral vectors encoding mouse, rat, dog, pig, rhesus and human NIS proteins.

**Background**

NIS, the thyroidal sodium iodide symporter, is the reporter gene of choice for studies seeking to determine the location, trafficking and long term fate of stem cells at serial time points in living animals or human subjects. In contrast to luciferase, NIS is a nonimmunogenic, nontoxic self-protein that is compatible with tomographic SPECT/CT and PET/CT imaging protocols employing clinically approved and readily available radiotracers.

**Hypothesis**

To address the concern that the NIS protein from a given species could be targeted and prematurely destroyed by the immune response of another species, we made a panel of lentiviral vectors encoding mouse, rat, dog, pig, rhesus and human NIS proteins.

**Research**

The enormous scientific importance of having a reporter gene that is expressed indefinitely is clear, so it was important to ensure that the non-immunogenic properties of the NIS reporter gene were conserved even when researching a cellular therapy in larger animal species. The work done to create species-specific lentiviral vectors for transducing cells with the NIS gene has proven that NIS retains full benefits in small and large animals, as well as humans.

## **Observations**

Each of these species-specific NIS vectors was shown to transfer a functional NIS protein into cultured cells, permitting them to efficiently concentrate radioactive iodide. To facilitate the customization and optimization of transduction protocols for each stem cell type of interest, from any animal species, we next generated a panel of bicistronic lentiviral vectors encoding both NIS and a selectable marker or fluorescent protein. Each of these vectors was shown to express a functional NIS protein and the expected selectable marker or fluorescent protein. Using some of these tools, we optimized a transduction protocol to transfer the human NIS gene into human fat-derived mesenchymal stem cells (MSCs) and confirmed that MSCs could be accurately and sensitively localized by radioiodine SPECT/CT imaging after they had been inoculated into living rodents. The tools and protocols that have been developed in this project are now being applied to stem cell tracking projects in larger animals, initially pigs.

**Submission ID: 34351**

**Submission Title: Bioculture System: Expanding ISS Space Bioscience Capabilities for Fundamental Stem Cell Research and Commercial Applications**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

With the recent call by the 2011 Decadal Report and the 2010 Space Biosciences Roadmap for the International Space Station (ISS) to be used as a National Laboratory for scientific research, there is now a need for new laboratory instruments on ISS to enable such research to occur.

**Background**

Spaceflight cell culture experiments to date have primarily been conducted in closed system units without the capacity for prolonged culture or experimental manipulation or on orbit.

**Hypothesis**

Since the completion of the ISS and the recent call for it to be used as a National Laboratory for basic scientific research, we now have the opportunity to perform, on orbit, the laboratory experiments we normally conduct on Earth. The Bioculture System supports the extended culturing of multiple cell types and microbiological specimens.

**Research**

The System consists of a docking station that carries ten independent incubation units or 'Cassettes'. Each Cassette contains a cooling chamber (5°C) for temperature sensitive solutions and samples, or long duration fluids and sample storage, as well as an incubation chamber (ambient up to 42°C). Each Cassette houses an independent fluidics system comprised of a

biochamber, medical-grade fluid tubing, medium warming module, oxygenation module, fluid pump, and sixteen solenoid valves for automated biochamber injections of sampling. The Bioculture System provides the user with the ability to select the incubation temperature, fluid flow rate and automated biochamber sampling or injection events for each separate Cassette. Furthermore, the ISS crew can access the biochamber, media bag, and accessory bags on-orbit using the Microgravity Science Glovebox. The Bioculture System also permits initiation of cultures, subculturing, injection of compounds, and removal of samples for on-orbit processing using ISS facilities. The Bioculture System therefore provides a unique opportunity for the study of stem cells and other cell types in space.

## **Observations**

The Space Shuttle predecessor of the Bioculture System, the Cell Culture Module, has previously been used to conduct multiple experiments with stem cells in microgravity conditions, including the differentiation of mouse embryonic stem cells into both embryoid bodies and keratinocytes. These studies have enabled the identification of cellular processes including proliferation and differentiation that are altered in space, and have led to the generation of novel new hypotheses regarding the importance of gravitational stimulation in normal cellular processes. The upcoming validation flight of the Bioculture System on SpaceX5 will enable the study of induced pluripotent stem cells (iPS)-derived cardiomyocytes whilst enabling the key functions of the Bioculture System to be tested and validated. The new functional capabilities of the Bioculture System will enable, for the first time, the study of the response of stem cells and other cell lineages to long-duration spaceflight exposure, whilst enabling normal cell culturing techniques to be automatically conducted within the System on ISS.

**Submission ID: 34600**

**Submission Title: The NINDS Repository Collection of Patient-derived Primary Fibroblasts and Induced Pluripotent Stem Cells for Neurodegenerative Disease Research**

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**Topic**

Basic Research, Medicine, and Health

**Problem**

Induced pluripotent stem cells (iPSCs) reprogrammed from patient-derived primary fibroblasts have become an increasingly utilized and needed resource for the study of human disease and have proven especially valuable in studying neurodegenerative disorders for which disease models are difficult to establish.

**Background**

The National Institute of Neurological Disorders and Stroke (NINDS) Repository is a public resource established in 2002 aiming to provide a centralized and open collection of biological samples (DNA, lymphoblastoid cell lines, fibroblasts, iPSCs, biofluids such as plasma, serum, cerebrospinal fluids, and urine) and associated de-identified clinical data from a diverse population of affected patients and normal controls. Since 2011, the NINDS Repository has added to its web-based catalog (<http://ccr.coriell.org/NINDS>) more than 40 iPSC and 130 fibroblast lines. Most iPSC lines are contributed by investigators from the Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) or Huntington's disease (HD) NINDS-sponsored consortia funded by the American Reinvestment and Recovery Act (ARRA).

**Hypothesis**

By making available to the research community neurodegenerative disease iPSCs and fibroblasts, the NINDS Repository contributes to fulfill the NINDS mission of reducing the burden of neurological disease - a burden borne by every age group, by every segment of society, by people all over the world.

**Research**

To ensure the quality of these valuable resources, all iPSCs and fibroblasts submitted to the NINDS Repository by iPSC Consortia and other investigators undergo rigorous quality assessments (viability, pluripotency, karyotyping, differentiation status, gene expression analysis, sterility) prior to distribution by the NINDS repository. The results are summarized in a Certificate of Analysis displayed on the web-based catalog along with the recommended culturing protocol. The NINDS Repository fibroblast and iPSC collections include mostly cell lines bearing specific genetic mutations associated with PD, ALS, HD, frontotemporal degeneration, or Alzheimer's disease, as well as samples derived from neurologically normal controls. For certain affected individuals, the parental fibroblast, corresponding iPSC line, and whole blood DNA are available.

**Observations**

The NINDS Repository serves as a unique and effective centralized resource where these iPSCs, fibroblasts and their critical phenotypic data, are available to basic and applied research investigators worldwide.