TOTAL BUDGET RFA 1305	
TIER 1	$25,611,294
TIER 2	$9,109,832

						Range				Number of Scores in Tier		
	APP #	PROJECT TITLE	SCORE	Median	SD	Low	High	BUDGET	TIER	TIER 1	TIER 2	TIER 3
	RT3-07692	Small molecule tools and scale-up technologies to expand human umbilical cord blood stem and progenitor cells for clinical and research use	86	85	5	80	95	$1,416,600	1	15	0	0
	RT3-07949	Embryonic stem cell-based generation of small animal models for assessing human cellular therapies	82	85	10	50	92	$1,499,400	1	14	0	1
	RT3-07804	Injectable Macroporous Matrices to Enhance Stem Cell Engraftment and Survival	82	82	4	74	86	$1,452,708	1	13	1	0
	RT3-07914	Skin-derived precursor cells for the treatment of enteric neuromuscular dysfunction	82	80	7	65	90	$1,818,751	1	14	1	0
	RT3-07848	Site-specific gene editing in hematopoietic stem cells as an anti-HIV therapy	81	83	5	64	85	$1,500,624	1	14	1	0
	RT3-07893	Optimizing the differentiation and expansion of microglial progenitors from human pluripotent stem cells for the study and treatment of neurological disease.	81	81	1	80	84	$1,147,596	1	15	0	0
	RT3-07800	Engineered Biomaterials for Scalable Manufacturing and High Viability Implantation of hPSC-Derived Cells to Treat Neurodegenerative Disease	77	80	7	55	82	$1,380,557	1	12	1	1
	RT3-07683	Identification and isolation of transplantable human hematopoietic stem cells from pluripotent cell lines; two steps from primitive hematopoiesis to transplantable definitive cells, and non-toxic conditioning of hosts for hematopoeitic stem cell transplants	77	80	10	45	90	$1,452,708	1	11	2	1
	RT3-07798	Advanced animal model for predictive preclinical testing of engineered cardiac autografts and allografts	77	80	22	10	98	$1,936,944	1	11	1	1
	RT3-07948	Injectable Hydrogels for the Delivery, Maturation, and Engraftment of Clinically Relevant Numbers of Human Induced Pluripotent Stem Cell-Derived Neural Progenitors to the Central Nervous System	77	76	2	75	80	$1,452,708	1	15	0	0
	RT3-07763	A suite of engineered human pluripotent stem cell lines to facilitate the generation of hematopoietic stem cells	76	80	10	45	83	$1,382,400	1	13	0	2
	RT3-07655	User-friendly predictive molecular diagnostic assays for quality control of stem cell derivatives for transplantation and drug discovery	76	79	8	60	90	$1,784,052	1	10	2	2
	RT3-07879	Multimodal platform combining optical and ultrasonic technologies for in vivo nondestructive evaluation of engineered vascular tissue constructs	76	75	10	50	90	$1,838,337	1	11	3	1

						Range				Number of Scores in Tier		
	APP #	PROJECT TITLE	SCORE	Median	SD	Low	High	BUDGET	TIER	TIER 1	TIER 2	TIER 3
	RT3-07907	Technologies to improve in vivo function of transplanted stem cells	75	75	10	50	86	$1,393,200	1	12	1	2
	RT3-07796	A Chromatin Context Tool for Predicting iPS Lineage Predisposition and Tissue Graftability	75	75	4	64	82	$1,452,708	1	11	3	1
	RT3-07616	Development of Relevant Pre-clinical Animal Model as a Tool to Evaluate Human Stem Cell-Derived Replacement Therapies for Motor Neuron Injuries and Degenerative Diseases	75	75	8	60	85	$1,308,711	1	10	3	2
	RT3-07670	Development of a clinical-grade extracorporeal liver support system using human induced pluripotent stem cell-derived hepatic cells	75	75	9	50	95	$1,393,290	1	10	4	1
	RT3-07678	A small molecule tool for reducing the malignant potential in reprogramming human iPSCs and ESCs	74	75	9	62	85	$1,341,161	2	9	1	5
	RT3-07899	Development of 3D Bioprinting Techniques using Human Embryonic Stem Cells Derived Cardiomyocytes for Cardiac Tissue Engineering	73	75	8	50	85	$1,368,517	2	9	4	1
	RT3-07838	Development of a scalable, practical, and transferable GMP-compliant suspension culture-based differentiation process for cardiomyocyte production from human embryonic stem cells.	72	75	9	50	87	$899,728	2	9	3	2
	RT3-07981	Multi-modal technology for non-destructive characterization of bioengineered tissues	72	75	7	60	80	$1,846,529	2	9	2	3
	RT3-07887	New materials and methods to instruct hematopoietic stem cell fate from human pluripotent precursors.	69	70	6	60	78	$1,373,966	2	4	6	5
	RT3-07808	A novel experimental procedure to generate large-scale cultures of human multipotent progenitors	66	65	6	55	75	$1,161,000	2	3	6	6
	RT3-07832	Survival and Function of Individual Stem Cells Measured Longitudinally in Small Animal Model In Vivo	66	64	5	60	75	$1,118,931	2	2	5	8
	RT3-07836	Multivalent growth factor conjugates for improved efficiency of stem cell expansion and differentiation	64	63	5	60	75	$1,313,489	3	1	2	11
	RT3-07974	MRI reporters to noninvasively image long-term stem cell engraftment in large animal spinal cord injury model	64	61	7	50	78	$1,857,600	3	2	5	8
	RT3-07632	Tools to harness in vivo signals for the generation of definitive hematopoiesis from human pluripotent stem cells	63	64	5	50	75	$1,373,180	3	1	1	13

						Range				Number of Scores in Tier		
	APP #	PROJECT TITLE	SCORE	Median	SD	Low	High	BUDGET	TIER	TIER 1	TIER 2	TIER 3
	RT3-07870	Recapitulating the 3D Microenvironment for Directing Vascular Fate	62	63	5	50	74	$1,064,703	3	0	1	13
	RT3-07633	Bioengineering personalized functional lungs using induced pluripotent stem cell technology	61	64	10	45	80	$1,363,544	3	1	1	12
	RT3-07880	Mitochondrial genome editing tools for the generation of novel animal and stem cell models	61	60	8	50	77	$1,743,120	3	1	2	12
	RT3-07859	3D Bioprinting for Stem Cell Delivery	61	60	4	50	64	$2,182,176	3	0	0	14
	RT3-07662	Engineering instantly integrated vascularized tissues for enhanced engraftment and tissue regeneration	<60					$1,440,426	3	1	0	12
	RT3-07756	Delivery of stem cells for muscular dystrophy	<60					$1,735,180	3	0	0	15
	RT3-07864	The generation and expansion of fully functional human hematopoietic stem cells by cellular delivery of RUNX1a transcription factor	<60					$1,857,599	3	0	0	15
	RT3-07805	A scaffolding system to enhance lineage-specific differentiation of pluripotent stem cells by on-demand mechanomodulation of the cell niche	<60					$1,060,025	3	0	0	14
	RT3-07738	High-fidelity genome engineering to treat genetic disease	<60					$1,708,560	3	2	1	12
	RT3-07898	Skeletal Muscle Regeneration by Direct Cellular Reprogramming of Human Fibroblasts to Satellite Cells with Myogenic and Cell-penetrating Peptides	<60					$1,570,500	3	0	0	14
	RT3-07813	Magneto-endosymbionts; in vivo translational tools for stem cell imaging and ablation.	<60					$900,000	3	0	0	15
	RT3-07883	Developing novel genetic neurological disease monkey models with and without stem cell transplantation	<60					$1,152,000	3	0	0	15
	RT3-07851	Development of an optimized stem-cell-seeded xenogeneic extracellular matrix construct and delivery system for cardiac repair following myocardial infarction	<60					$1,809,305	3	0	0	15
	RT3-07901	Label-free analysis and purification of cell-based therapies for cost-effective regenerative medicine	<60					$1,237,352	3	0	0	15

						Range				Number of Scores in Tier		
	APP #	PROJECT TITLE	SCORE	Median	SD	Low	High	BUDGET	TIER	TIER 1	TIER 2	TIER 3
	RT3-07975	A comprehensive microfluidic platform for the production of stem cell micro-beads for therapeutic transplantation	<60					$900,000	3	0	0	15
	RT3-07841	Advancing Stem Cell Replacement Therapies through Precision Single-Cell Profiling	<60					$1,327,648	3	3	0	11
	RT3-07962	A large animal model of mucopolysaccharidosis I for stem cell therapy development.	<60					$1,669,272	3	0	0	15
	RT3-07855	Neuronal Precursor Cell Therapy in Large Animals: Delivery, Dosing, Safety, and Efficacy	<60					$1,651,680	3	0	0	15
	RT3-07891	Enhancing thymic epithelium differentiation with three dimensional matrices and small molecule libraries	<60					$1,429,200	3	0	0	15
	RT3-07750	Development of Clinical Tools for Predicting and Evaluating Immune Responses to Regenerative Cellular Therapies	<60					$1,374,020	3	0	0	15
	RT3-07881	iPSC-based Bioartificial Liver device	<60					$1,934,352	3	0	0	13
	RT3-07965	Optimizing safety and efficacy of transgenic human induced pluripotent stem cell-based personalized cellular therapeutics	<60					$1,345,730	3	0	0	15
	RT3-07990	Joint surface regeneration: Deliver stem cells and control their fate with novel intelligent clinical grade biomimetic materials	<60					$1,025,839	3	0	0	15
	RT3-07900	Bioactive thermo-reversible polymers as adjunctive therapy to stem cell treatment of heart failure	<60					$1,904,077	3	0	0	15

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

M E M O R A N D U M

January 16, 2015

From: Patricia Olson, Ph.D., Executive Director, Discovery
To: Application Review Subcommittee, Independent Citizens Oversight Committee (ICOC)
Subject: CIRM Team Recommendations re applications submitted under RFA 13-05, Tools and Technologies Awards III

In accordance with Section 7, Article V of the Bylaws of the Scientific and Medical Research Working Group and Section 6, Article VI of the Board's bylaws, both as amended on 3/19/13; the President and scientific team members, following internal review and consideration would like the Application Review Subcommittee to consider the following in making it's funding decisions.

Authorized by the ICOC:	$35.0 MM	~ 20 awards
Tier 1 applications (GWG)	$25.6 MM	17 awards
Tier 2 applications recommended by CIRM	$ 3.6 MM	3 awards
Proposed for funding	$29.2 MM	20 awards

CIRM recommendations and rationale

All Applications in Tier 1: The CIRM team supports the Grants Working Group's recommendations to fund the 17 Tier 1 applications

Tier 2 Applications: CIRM is recommending that three applications, which received average scores placing them in Tier 2, be funded. The overriding rationale for CIRM's recommendation is that each of the three applications had a median score of 75 (Tier 1) and in each case a strong majority (9 of 15) of the voting members of the GWG scored the applications in Tier 1. A fourth application also met these criterion but is not being recommended for funding due to overlap in technology and leadership with another application that is being recommended for funding in Tier 1.

Application #: RT3-07678
Tier, Average Score: Tier 2, 74
Title: A small molecule tool for reducing the malignant potential in reprogramming human iPSCs and ESCs.
Requested Funding: $ 1,341,161

Points for Consideration:
- The proposal addresses the safety of human pluripotent stem cell (PSC) derived cells for transplantation – a bottleneck to clinical application of stem PSC-derived cell therapies. The applicant has identified a small molecule tool that selectively kills pluripotent cells but not differentiated derivatives. The application proposes studies to further characterize the specificity and selectivity of the cell killing and to define the mechanism of action. If successful, such a molecule could be used *ex vivo* to remove cells that could form teratomas or potentially tumors. This could accelerate the development of stem cell therapies for patients by reducing the teratoma and tumorigenicity risk.

- There are no active grants in the CIRM portfolio that address this translational bottleneck.
- The small molecule approach is a potentially cost effective way to address this safety bottleneck.

CIRM Recommendation: Fund.

Application #: RT3-07899
Tier, Average Score: Tier 2, 73
Title: Development of 3D Bioprinting Techniques using Human Embryonic Stem Cells Derived Cardiomyocytes for Cardiac Tissue Engineering
Requested Funding: $ 1,368,517

Points for Consideration:
- Three-dimensional (3D) printing is a disruptive new technology that has been a driver of innovation in many disciplines, including engineering and manufacturing. This technology also has the ability to transform regenerative medicine.

- Although CIRM has funded projects that involve 3D microenvironments and/or 3D hydrogels, none of these projects involve 3D printing technology.

- This application intends to establish design criteria for printing vascularized cardiac tissues from pluripotent stem cells, which may have implications beyond this project. For example, the emphasis on creating vascularized cardiac tissue with the appropriate cell types may have implications for developing vascularized tissues in general.

- This is an opportunity to fund a leader in the field to further develop and apply a cutting edge technology to address the bottleneck of cell survival and engraftment. If successfully developed, such a technology would accelerate the development of stem cell therapies and increase their likelihood of success.

CIRM Recommendation: Fund.

Application #: RT3-07838
Tier, Average Score: Tier 2, 72

Title: Development of a scalable, practical, and transferable GMP-compliant suspension culture-based differentiation process for cardiomyocyte production from human embryonic stem cells.
Requested Funding: $899,728

Points for Consideration:
- This proposal represents a unique opportunity to investigate the bottleneck of scalable production systems for cell therapies. The ability to make cell therapies at scale is critical to accelerating the development of cell therapies that could benefit patients. There are no projects within CIRM's grant portfolio exploring this production system.

- The proposal builds on and leverages previous CIRM funded investments by this PI in GMP manufacture at a smaller scale of pluripotent stem cell derived cell therapies

CIRM Recommendation: Fund.

Application #: RT3-07981
Tier, Average Score: Tier 2, 72
Title: Multimodal technology for non-destructive characterization of bioengineered tissues
Requested Funding: $1,846,529

Points for Consideration:
- There is another application recommended for funding in Tier 1 that proposes to optimize and apply the same imaging technology platform to a different test system. In addition, the scientific leadership of the two applications is the same.

CIRM Recommendation: Do Not Fund

All Remaining Applications in Tier 2: The CIRM team recommends, "do not fund".

All Tier 3 Applications: The CIRM team supports the Grants Working Group's recommendations to **not** fund.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07616: Development of Relevant Pre-clinical Animal Model as a Tool to Evaluate Human Stem Cell-Derived Replacement Therapies for Motor Neuron Injuries and Degenerative Diseases

GWG Recommendation: Recommended for Funding
Final Score: 75

Public Abstract (provided by applicant)

Motor neurons degenerate and die as a consequence of many conditions, including trauma to the spinal cord and its nerve roots and degenerative diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. Paralysis and in many cases death may result from a loss of motor neurons. No effective treatments are available for these patients. Most cellular therapy studies for motor neuron disorders are done in rodents. However, because of the dramatic differences between the rodent and human spinal cord, translation of these studies to humans is difficult. In particular, the development of new stem cell based treatments is limited by the lack of large animal models to test promising candidate therapies.

This bottleneck will be addressed by developing a new research tool in which human embryonic stem cell-derived motor neurons are transplanted into the spinal cord of large animal model after injury and surgical repair of motor nerve roots. This injury and repair model mimic many features of motor neuron degeneration in humans. Microscopic studies will determine survival and tissue integration of transplanted human cells in the large animal model spinal cord tissues. Evaluations of walking, muscle and bladder function, sensation and magnetic resonance imaging (MRI) will test for possible benefits and potential adverse effects. This new research tool will be available for future pre-clinical testing of additional stem cell-based therapies that target motor neuron loss.

Statement of Benefit to California (provided by applicant)

Paralysis resulting from motor neuron loss after cauda equina and conus medullaris forms of spinal cord injury and from neurodegenerative conditions, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), are devastating and affects thousands of patients and their families in California (CA). These conditions also create a significant financial burden on the state of CA. No effective treatments are available for these underserved patients. Development of a clinically relevant research tool is proposed to evaluate emerging stem cell-based motor neuron replacement therapies in translational studies. No such models are presently available to the global research community. As a result, the proposed research tool, which will remain based in CA, may

attract interest across the United States and abroad, potentially being able to tap into a global translational research market of stem cell-based therapies and contribute to a positive revenue flow to CA.

Future benefits to people in CA include: 1) Development and translation of a new CA-based research tool to facilitate and expedite clinical realization of emerging stem cell-based therapies for devastating neurological conditions affecting motor neurons; 2) Reduction of health care costs and care giver costs for chronic motor neuron conditions with paralysis; 3) Potential for revenue from intellectual properties related to new cellular treatments entering clinical trials and human use.

Review Summary

Proposal Synopsis
This proposal addresses a major bottleneck in regenerative medicine by developing a preclinical large animal model for cellular therapy strategies to combat motor neuron injury, degeneration, and loss. Such therapies could be useful for the treatment of motor neuron conditions, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), or for trauma and associated cell death due to spinal cord injury (SCI). The investigators will use a reproducible model to mimic SCI that will involve removal of lumbosacral ventral roots followed by their reimplantation. Then human embryonic stem cells (hESCs) that have been differentiated to motor neurons will be transplanted into the spinal cord. A regimen of immunosuppressive therapy will be optimized to allow for engraftment of the human cells into the animal host and both short-term and long-term measures of successful engraftment will be measured. Potential favorable or adverse outcomes assessed in the short-term include transplanted cell survival and integration into spinal cord tissues, whereas long-term outcomes will involve analysis of functional survival of transplanted neurons as assessed by muscle and bladder control, walking, and detection of sensation. Although small animals, such as rodents, have been used previously, they are limited in their utility as a preclinical model because of the extremely short distance axons would have to travel. In addition, such animals cannot be evaluated well for the same functional measures of recovery. The successful completion of the study could provide a means to evaluate promising new cellular therapies for a wide variety of motor neuron conditions that currently cannot be treated.

Significance and Rationale
- The rationale for use of the large animal model is sound as it should increase translation potential due to the longer distances involved as compared to rodents and the possibility of more sophisticated analysis of functional recovery.

- This approach seeks systematically to learn how transplanted stem cells function in an established reproducible model of injury.

- The injury and repair methodology while feasible and fairly straight-forward does not represent an actual injury that occurs in humans and the investigator did not sufficiently describe the applicability of the model to other types of injury or disease. Both of these concerns impacted the perceived significance of the proposed animal model.

- A great portion of the proposed work has already been done and the primary novel component is the addition of the motor neuron cells.

Feasibility and Experimental Design

- The work was considered feasible based on prior publications and the strength of the provided preliminary data. The principal investigator (PI) has recently published on this injury model, mimicking many features of AML and SMA.

- The sophisticated measures of functional recovery following transplantation with the proposed animal model were considered a great strength because current animal models cannot provide such data.

- Insufficient data were provided to support dosing of immunosuppressive (IS) regimen, the IS regimen is not adequately described in the proposal, and concerns regarding experimental design of this component of the application were raised. Failure to dose appropriately could lead to death due to endogenous viral infections and the total number of animals to assess the regimen likely needs to be increased to properly identify doses.

- There is insufficient data in the application as to whether cell transplantation into the proposed animal model will yield functional recovery and with respect to other behavioral functions in which motor neurons are of clear importance. The absence of certain controls in the study was deemed of some concern, though baseline information from similar animals outside the study would be obtained.

- Little attention was paid to the problem of movement of the spinal cord (in association with breathing) during and shortly after the time of cell delivery. This has been a subject of great concern in the development of transplantation approaches suitable for use in ALS patients. There was also some discussion that other large animal models may be more appropriate for dosing studies due to the larger diameter and length of the spinal cord, though it was acknowledged that the proposed model has greater value in terms of outcome measures.

Qualifications of PI and Team

- The teams involved all have appropriate expertise and the respective environments are excellent for carrying out the proposed research.

Responsiveness

- The application is responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07632: Tools to harness in vivo signals for the generation of definitive hematopoiesis from human pluripotent stem cells

GWG Recommendation: Not Recommended for Funding
Final Score: 63

Public Abstract (provided by applicant)

Hematopoietic (blood forming) stem cells (HSC) produce all the cells of the blood including those of the immune system. These versatile stem cells are typically obtained from the blood or bone marrow of healthy donors, and transplanted into patient recipients to treat many acquired and inherited diseases such as leukemia and sickle cell disease. In recent years, laboratory research has opened up exciting new opportunities to use HSC to treat an even broader range of diseases such as organ rejection and autoimmune diseases such as multiple sclerosis, diabetes and lupus. However, mismatches between donor-recipient tissue types can cause severe illnesses that require hospitalization and can be fatal. To provide an unlimited source of perfectly matched HSC, scientists have tried to create HSC from pluripotent stem cells (PSC). However no current culture methods can produce the potent HSC that can be used for clinical transplantation. We propose a completely novel approach to overcome this bottleneck. We will create a system to mimic the process of blood formation that occurs in normal human fetal development, in which HSC are created in specialized tissue over many weeks. We will develop bioengineering technology that will allow us to transplant cells derived from PSC so that they create the same tissues and complex signals inside the body that produce HSC during normal development. This technology could also be adapted for differentiation of PSC into other tissues.

Statement of Benefit to California (provided by applicant)

This proposal has direct benefits for Californians who suffer from diseases of the blood and immune system, and will also have broad impact on the safe and effective delivery of stem cells to Californians in general. In 2009, the California Department of Public Health listed lymphoma and leukemia among the 7 most common cancers in California, and together they are the fourth leading cause of death from cancer. Our State's wonderful ethnic diversity also complicates the ability to match donors to recipients. Those from ethnic minorities are severely under-represented in the National Marrow Donor Program registry. For the Latino population, the largest ethnic group in our state, matching is often difficult because their genetics reflect very diverse contributions of European, African and Native American ancestry. Our goal is to create new technology to safely produce perfectly matched, blood-forming stem cells from pluripotent stem cells. If successful, this tool would provide new opportunities for

patients of diverse genetic background who currently lack suitable transplant donors. In addition, the tools and concepts developed from these studies will have great relevance for the production of other tissues from pluripotent stem cells to address other major health problems that afflict Californians. All scientific findings and tools developed in this proposal will be available to researchers throughout California, under CIRM guidelines.

Review Summary

Proposal Synopsis
This proposal aims to address technical obstacles that currently prevent the efficient differentiation of pluripotent stem cells (PSCs) into definitive hematopoietic stem cells (HSCs) with long-term engraftment potential. The investigators propose to combine PSC-derived embryonic mesoderm progenitors (EMPs) and bioengineered scaffolds to create an artificial microenvironment that mimics the process of blood development that occurs during early human development, thereby providing an experimental model for investigating human hematopoiesis and potentially informing the development of clinical approaches for the generation and engraftment of functional HSCs.

Significance and Rationale
- This application addresses a significant translational bottleneck. A successful outcome could have great impact and might eventually lead to more efficient and possibly safer HSC transplantation methods.

- While reviewers believed that the proposed system might offer experimental advantages over existing in vitro systems as a research tool for investigating human hematopoiesis, they were uncertain of its clinical translatability.

- Reviewers considered the technology to be novel.

Feasibility and Experimental Design
- While interesting, the preliminary data were not sufficient to convince reviewers that a highly artificial system with PSC-derived EMPs, engineered microenvironments, and expansion in an immune-deficient mouse would be adequate to support the generation of definitive HSCs. This is a significant weakness of the proposal.

- The research plan lacks details on how experimental outcomes would be quantified, for example, to determine the number of definitive HSCs from a single construct that would constitute success, or to determine how much better, if any, the proposed approach is over standard in vitro methods.

- Reviewers appreciated the modular design of the proposed experiments for enabling diverse combinations of factors to be tested for their contribution to hematopoiesis.

Qualifications of PI and Team

- The PI is a well-established scientist with a wealth of relevant experience in HSC biology, and the Co-PI brings complementary expertise in biomimetic scaffolds.

- The research team is qualified to undertake the proposed studies.

Responsiveness

- Reviewers agreed that the proposal is responsive and addresses a key objective of the RFA: identifying methods to expand isolated human HSC and/or generate HSC from human pluripotent stem cells resulting in long term, multilineage engraftment.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07633: Bioengineering personalized functional lungs using induced pluripotent stem cell technology

GWG Recommendation: Not Recommended for Funding
Final Score: 61

Public Abstract (provided by applicant)

For patients with end stage lung disease currently the only treatment option is lung transplantation but there are not enough lungs available for everyone who needs them. In addition, rejection after lung transplantation is very common and the medicines taken to suppress the immune system often result in serious infections. We have developed a bioengineering strategy that allows us to pattern airway cells derived from patients specific induced pluripotent stem cells into lung sacs with a blood vessel supply to allow for the exchange of oxygen and carbon dioxide. This technology also has the potential to be used for other organs where the cells are patterned around the blood supply, such as the liver. We are proposing to use patient specific induced pluripotent stem cells and this novel bioengineering approach to make functional patient specific lung tissues for patients with poor lung function and perform preclinical proof of concept to show that these bioengineered lungs function in a small animal lung transplant model.

Statement of Benefit to California (provided by applicant)

There are millions of people in the USA who suffer from lung diseases. The lungs have tremendous reserve, so that it is not until many years after lung injury that respiratory symptoms become manifest. California, the most populous state, is also the state with the largest number of people 65 years of age and over (3.6 million people in the year 2000) and therefore the prevalence of severe lung diseases in the USA is highest in California with a conservative estimate of over 100,000 cases. Of these, the worst cases develop end stage lung disease and either succumb to their disease or undergo a lung transplant. There were 190 lung transplants in California in 2012 with many more patients on the waiting list. Yet, most of the patients who were fortunate enough to receive a lung transplant will not be disease free, as 50% of them will develop rejection of their lung and they are all at risk for severe infections from the medications that reduce the immune system to try to prevent rejection. The development of a patient specific lung that could help patients breathe without any risk of rejection represents a ground breaking new treatment for patients with severe lung diseases. This proposal outlines the progress we have made in developing functional lung organoids from induced pluripotent stem cells and details the work and milestones required in order to move this cutting edge technology closer to the clinic.

Review Summary

Proposal Synopsis
The objective of this proposal is to use tissue engineering to generate autologous, functional lungs from induced pluripotent stem cells. This technology would address an unmet medical need for the treatment of end-stage lung disease for which the only current treatment is lung transplantation. Lung transplantation is severely limited by the shortage of available donor organs, and by complications of immune rejection and of long-term immunosuppressive medications. This proposal attempts to address these critical roadblocks by: 1) differentiating patient-derived iPSC to mature cell types of the airway that would not require immunosuppression if transplanted, and 2) developing the appropriate construction of functional iPSC derived tissue and vascular architectures that can be integrated into the host lung. The engineered lung tissue will be tested for oxygen exchange in vitro using biosensors and in vivo in a small animal model. If successful, this technology platform could be used to generate other types of vascularized organs for transplantation.

Significance and Rationale
-The proposal addresses significant bottlenecks to translation of human stem cell therapies as it aims to develop a functional organ-like structure, in this case a lung organoid. If successful, the project would have a major impact upon the field and for patients.

-The proposed technique will use synthetic matrices, which might offer advantages over the competing decellularized matrix technologies which are, however, further along in development.

-The rationale for the approach is sound, but feasibility issues may be limiting.

Feasibility and Experimental Design
-There were mixed reviews regarding the strength of their preliminary data to support the feasibility of this project. Reviewers were concerned about the ability to sufficiently scale-up the organoids to functional tissue.

-There were concerns regarding the feasibility of producing functional lung tissue with gas exchange capacity. There was insufficient preliminary data to support that the cells could withstand cyclic mechanical stretch and that they would orient and integrate correctly into the host vascular and respiratory circuitry.

-A considered strength is that the investigators have access to the necessary tools and reagents to complete most of the proposed work.

Qualifications of PI and Team

-The PI of this proposal is an accomplished investigator, well-recognized within the lung regenerative medicine community and well-positioned to lead the proposed studies.

-The collaborators of the PI are internationally recognized experts in their respective fields and bring outstanding relevant collaborative strengths.

-The expertise of the team ranges from stem cell biology to synthetic chemistry and biomaterials and also includes pulmonary and critical care medicine.

Responsiveness

-The proposal is responsive to the objective of RFA in that it provides an approach for engineering synthetic lung tissue that will be reconstituted using iPSCs.

-The applicants provide a plan describing how the technology will be made accessible to the stem cell community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07655: User-friendly predictive molecular diagnostic assays for quality control of stem cell derivatives for transplantation and drug discovery

GWG Recommendation: Recommended for Funding
Final Score: 76

Public Abstract (provided by applicant)

Three years ago, with help from CIRM funding, we developed an assay. This is a genomics-base diagnostic assay, similar to those now used for diagnosing cancers; but in our case, it is designed to analyze human ES and iPS cells. The assay is very simple to use; researchers use microarrays to profile the genes that are active in their cells. They upload the microarray data to the website, and in a few minutes they find out whether or not their cells are pluripotent. Our assay is replacing the old method for proving pluripotency, which involves producing tumors in animals. Our assay has been extremely popular, with 9,386 samples analyzed by 581 research groups in 29 countries so far. In this proposal, we plan to take the same concept and apply it to translational stem cell applications. Our new assay will allow researchers to easily detect DNA damage in their stem cells, and will enable the detection of undifferentiated or other abnormal cells (which potentially could form a tumor) in populations used for cell replacement therapy. We are also designing specific assays for quality control of neuronal cells to be transplanted to Parkinson's disease patients and for other neurological therapies. Finally, with our European partners, we will develop an assay for ensuring reliability of drug screening assays using stem cells. Our tools will greatly simplify translation of hESCs and iPSCs to the clinic.

Statement of Benefit to California (provided by applicant)

California is at the leading edge of development of stem cell therapies to treat previously untreatable diseases. It is critical at this important stage, when treatments are being transferred from the lab to the clinic, that the cells used for therapy are carefully produced and qualified. Our project combines two of California's best scientific assets: genomics and stem cells. Our quality control assays for stem cell production are based on our long experience in genomic analysis of stem cells and development of genomics-based diagnostic tests. The assays will ensure that stem cells used for therapy are consistently of high quality. This will speed the development of stem cell therapies for Californians.

Review Summary

Proposal Synopsis
The applicant's team has developed a web-accessible database that stores information from hundreds of human pluripotent stem cells and their differentiated/derivative cells analyzed by a suite of gene expression tools. This database currently generates predictions of pluripotency for stem cells, based on uploaded gene expression data. The applicant proposes to extend the current capabilities of the database to include additional functionalities requested by its users, including next-generation RNA sequencing data; quality control assays for cell preparations differentiated from stem cells for transplantation, especially in neurological diseases; assessment of the genomic stability of stem cell lines; and guidance in selection of the most appropriate lines for high-throughput drug screening. The bottlenecks addressed by this application are preclinical evaluation and cost efficient production of stem cell therapies.

Significance and Rationale
- Given the broad user base, improvements to the existing database functionality could have widespread impact in the stem cell field, though some reviewers questions the degree of added value to the existing database.

- Reviewers considered community access critical to the potential impact of the proposed work but were not clear from the proposal that the databases will be freely accessible.

- Reviewers agreed that the current suite of bioinformatics tools will eventually have to be updated, but had differing opinions as to whether the proposed list is most appropriate.

-Most reviewers deemed the list of expanded features logical for advancement of the stem cell field. In theory, the new features could greatly increase accuracy of stem cell assessment. However, because stem cell phenotype changes constantly, some reviewers questioned how effective the proposed tools could be in translational activities and drug screening.

- The contribution in fund matching by a European granting agency offers added confidence to the potential impact of the project for development.

Feasibility and Experimental Design
- The project builds on a successful platform and the aims are clearly and logically laid out. Success criteria, some potential pitfalls, and timelines are well described. Reviewers were confident that the project is realistic but ambitious.

- Reviewers were concerned that the proposed RNA sequencing development is not sufficiently supported by preliminary data, rendering it difficult to determine the likelihood that the tool will work as planned to improve the database.

Qualifications of PI and Team

- The applicant team has worked successfully together in the past on similar projects. They have appropriate experience and resources to perform the proposed work.

- The scientific and funding collaboration is very complementary.

- The extent of effort for the collaborator addressing the analysis, presentation, and sharing of RNA-seq data is unclear.

- Some reviewers expressed concern that the applicant may be overextended/over-committed.

Responsiveness

- The proposal is highly responsive to RFA and addresses a specific need by proposing to develop clinically relevant tests for human stem cells

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07662: Engineering instantly integrated vascularized tissues for enhanced engraftment and tissue regeneration

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

This project aims to engineer a clinically-applicable pre-vascularized bone graft to accelerate repair and regeneration of large bone defects. Lack of vascularization and engraftment continues to be a major challenge and the bottleneck in translation of bone tissue engineered constructs to clinical practice. Pre-vascularized large bone grafts capable of rapidly integrating with native bone tissue would significantly advance currently available treatments. To this end, we have developed a number of novel synthetic scaffolds, vessel grafts, and cell-laden hydrogels that are promising for tissue regeneration. Thus, in this project, we will combine these three components in one single device, which will then be used to repair both vascular and bone critical defects. Our integrated, streamlined approach centers on enabling rapid microcirculation across the large synthetic graft and the graft-host tissue interface. This will significantly improve on stem cell survival and tissue engraftment, accelerating the bone healing process. Completion of our work is expected to provide a more effective platform for accelerated bone repair by achieving functional vascularization rapidly.

Statement of Benefit to California (provided by applicant)

In California, musculoskeletal disease cases affect more than 50,000 people in the private and public sectors, causing extensive losses in lost days and decreased productivity. Workers compensation losses in the state amounted to more than $15 billion last year, in a trend increasing at an alarming rate. From these, approximately 20% corresponded to musculoskeletal injury claims. Large bone defects remain a significant clinical problem among all musculoskeletal disorders. Our fully vascularized graft is expected to promote repair and regeneration of large bone defects by improving instant and effective microcirculation. A platform that can accelerate bone repair can decrease patient and government burden by limiting the rate of failure of current treatment options, minimizing the time for healing, and reducing the impact of lost productivity. Success of this design would make the technology available to clinical environments in California and beyond, potentially revolutionizing the field of tissue engineering and regenerative medicine.

Review Summary

Proposal Synopsis
The applicant proposes to engineer a pre-vascularized bone graft that can accelerate repair and regeneration of large bone defects. This proposal would address a bottleneck faced by current tissue-engineered bone constructs that do not engraft and vascularize well. The applicant will engineer a graft by combining novel scaffolds, hollow fibers and stem cell-laden gels and test the ability of such a device to repair vascular and bone critical defects both in vitro and in preclinical models.

Significance and Rationale
- The proposal uses novel technology for the scaffold which is exciting and interesting, but also overly complex without preliminary data to support the complexity.

- There are no preliminary data to suggest that the scaffold can stand up to the pressure of real vasculature without leaking. Thus, the scaffold may not be able to support tissue growth. It is unclear how the proposed technology is superior to others that have been attempted recently and in the historical literature.

- This proposal is unlikely to make an impact on stem cell-based therapies and the studies to examine the effect of vascularization on stem cell behavior are underdeveloped in the proposal.

Feasibility and Experimental Design
- The proposed scaffold is overly complex and it seems unlikely that it will be able to be scaled up to a size suitable for human use. Thus, the clinical impact is limited.

- The components proposed to make the scaffold are novel, but there is no justification as to why these components are used instead of known, previously tested components.

- Reviewers questioned whether it was really feasible to use the vascular implants for certain bone breaks given the possibility for thrombosis.

- The second aim proposes using mesenchymal stem cells, but the experiments are limited with insufficient characterization of the stem cells.

- The animal model proposed shows good results with currently available scaffolds, and thus is unlikely to show additional benefits using the novel technology proposed.

- The in vitro and in vivo optimization experiments are complementary toward achieving project goals.

Qualifications of PI and Team

- The team is qualified with appropriate expertise in scaffold engineering.

- The team lacks stem cell expertise.

Responsiveness

- Although the proposal suggests using mesenchymal stem cells for some experiments, experiments with stem cells are limited and will yield little information to be gained for the field of stem cell therapy.

- No plan to disseminate the findings is proposed, and it is likely that it will be difficult for others outside this laboratory to benefit from the work.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07670: Development of a clinical-grade extracorporeal liver support system using human induced pluripotent stem cell-derived hepatic cells

GWG Recommendation: Recommended for Funding
Final Score: 75

Public Abstract (provided by applicant)

Liver failure is the fourth leading cause of adult death in California. Because liver cells can regenerate, some patients with liver failure could be saved without having to undergo organ transplantation if their liver function could be supported temporarily. Here, we propose to develop a device to support these patients called the extracorporeal liver support system (ELS).☐

Numerous pre-clinical studies and clinical trials have demonstrated the therapeutic effectiveness of ELS using human or animal liver cells housed in a device outside of the patient's body but connected to the patient's circulation. The device removes toxins and prevents irreversible brain damage while the patient regenerates his or her own liver cells. However, the limited availability of human cells and insufficient functionality of animal cells prohibits this therapy from being widely adopted.

For this project, we will develop ELS using human stem cell-derived liver cells (hPSC-Hep) that will overcome two major bottlenecks in the translation of human stem cell therapies: scalability and safety. The unlimited supply and consistent quality of hPSC-Hep will allow us to make ELS scalable. By keeping the hPSC-Hep in a device separate from the patient's body, we will also be able to allay any safety concerns about these cells forming tumors.

The result will be a widely available, safe and effective treatment that will alleviate the need for liver transplants for certain patients.

Statement of Benefit to California (provided by applicant)

Liver disease is a leading cause of death in California. California's rate of 10.6 deaths per 100,000 people exceeds the national average of 8.8. To mitigate this problem, we propose developing a clinical device that can temporarily perform liver functions until a patient's own liver cells recover. The device will use stem cells as a source of unlimited and quality controlled liver cells. Because the device is outside of the patient's body, these stem cell-derived liver cells will remain separate from the patient's blood stream, overcoming any risk of tumor formation. If successful, the device will be the leading choice for treatment, and will allow patients to recuperate without undergoing costly

liver transplantation, which places an economic burden on patients' families as well as society.

Furthermore, the production of this device could constitute a novel industry that would provide job opportunities to the citizens of California. If successful, our industrial partner plans to launch a new California-based company in the near future.

The benefits of this new regenerative therapy will have a tremendous impact on the state of California and the thousands of patients suffering from liver diseases.

Review Summary

Proposal Synopsis
This proposal is to develop an improved extracorporeal liver assist device using human pluripotent stem cell-derived hepatocytes (hPSC-Hep) which can be obtained in unlimited quantities, to treat patients with acute liver failure. The proposal will address two bottlenecks preventing the large-scale clinical implementation of hPSC-Hep in liver assist devices: 1) the poor functionality of hPSC-Hep derived in conventional 2D culture and (2) the limited scalability of bioreactor devices. The first bottleneck will be addressed by co-culturing the hPSC-Hep cells with non-hepatocyte liver cells in a 3D culture system, an approach that is expected to improve the functionality of the hPSC-Hep. To address the second bottleneck, the applicant has teamed up with an industrial partner who has produced a scalable bioreactor.

Significance and Rationale
- Reviewers concurred that this is a strong proposal that addresses an unmet medical need and if successful, it would have impact on the field of stem cell-derived hepatic cells and a potential for clear clinical impact in liver disease.

- Reviewers agreed that the scientific rationale for co-culturing stem cell-derived hepatocytes with other cell types to achieve improved function is sound and innovative.

- Some reviewers noted that a significant strength of the proposal is the fact that a previous iteration of the proposed bioreactor has already undergone testing in a Phase I trial, demonstrating feasibility and the capacity of the team to move product development towards the goal of clinical application.

Feasibility and Experimental Design
- All reviewers found the experimental design to be feasible and supported by preliminary data.

- Though reviewers noted that the project is highly translatable for clinical application, some reviewers expressed concern that the use of non-hepatocyte cell types has the

potential to compromise the uniformity and reliability of the process thus making the device more complex and expensive.

- Reviewers highlighted innovative aspects of the approach, the thoughtful consideration of potential pitfalls and the excellent endpoints that will be used to evaluate liver function, which they viewed as a significant, clinically relevant strength of the proposal.

- Some reviewers would have appreciated the inclusion of additional data around the co-culture system and the functionality of the hepatocytes.

- Reviewers concurred that the facilities and scientific environment are adequate for the project.

- A common note of concern was that this is a highly competitive commercial area, leading to some questions about the tool's commercial viability.

Qualifications of PI and Team

- Reviewers concurred that the PI is well qualified and has committed the appropriate effort for completing the proposed work.

- Reviewers concurred that the collaborative team is excellent.

- Reviewers recognized the very good external partners.

Responsiveness

- Some reviewers questioned whether the proposal was developing, testing, or improving a broadly applicable tool or technology as called for in the RFA. Some reviewers thought the proposal responsive to the RFA as the development of a tool to improve expansion and maturation of hepatocyptes is broadly applicable to that community. Others thought the proposal was focused on development of a therapeutic device.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07678: A small molecule tool for reducing the malignant potential in reprogramming human iPSCs and ESCs

GWG Recommendation: Tier 2
CIRM Recommendation: Recommended for Funding
Final Score: 74

Public Abstract (provided by applicant)

This research project aims to solve a key bottleneck in the use of differentiated human embryonic stem cells and induced pluripotent stem cells for the regeneration and replacement of diseased or damaged tissues. This bottleneck is the potential of unintended transplants containing failed-to-differentiate stem cells developing into benign growths called teratomas, or worse, malignant teratocarcinomas. It is essential to overcome this safety concern before stem cell-derived therapies can become acceptable for human use. Stem cells and cancer cells have many common properties. Both can replenish themselves indefinitely, and can potentially grow in different parts of the body. Before they are administered to patients, stem cells must be forced in the laboratory to turn into more mature cells that are programmed to become neurons, heart cells, beta cells of the pancreas, and other differentiated cell types. The mature cells, unlike the stem cells, do not grow indefinitely, but rather can replace a specific function that is defective in disease. We have identified a specific small molecule tool that selectively kills pluripotent stem cells but does not damage differentiated lineage cells. We will investigate the mechanism of action of the tool and test the tool for specificity in a variety of pluripotent stem cells and their differentiated lineages. The end goal is to develop a technology that will minimize the potential of developing unexpected tumors from stem cell therapies.

Statement of Benefit to California (provided by applicant)

Our proposal benefits California by adding new essential knowledge on mitochondrial mechanisms that control human pluripotent stem cell (hPSC) function to support the taxpayers' commitment to personalized cell therapies. This work builds on highly successful CIRM Seed & Basic Biology I awards. CIRM funds to date resulted in 20+ publications and training of 14 individuals including post-docs, graduate students, undergraduates, and CIRM Bridges to Stem Cell Biology program trainees, some of whom have entered the California workforce. Here we have identified a small molecule modulator of a mitochondrial redox protein that selectively kills pluripotent stem cells but not their differentiated lineages. Because contamination by hPSCs in transplanted donor cell pools is a key concern for regenerative cell therapies, there is a critical need to develop methods for reproducibly eliminating potentially cancerous cells. Our small

molecule is an exciting candidate tool and will be characterized extensively. Our ongoing work underpins therapy development in California's major academic centers and will provide data for many of California's biotechnology companies in the growing stem cell industry, whose success will propel hiring and increased economic prosperity for the state. With success, tangible health and economic impact on California, its academic institutions and companies, and the rest of the nation will be achieved as California leads the way forward with personalized medicine.

Review Summary

Proposal Synopsis
The applicant proposes to develop a method to selectively eliminate undifferentiated human pluripotent stem cells (hPSCs) in cell therapy transplants. To accomplish this goal the applicant has identified a small molecular tool that recognizes and removes hPSCs, but does not affect differentiated cells. The proposal aims to investigate the mechanism of action of this molecule and to test it for specificity on varied populations of human embryonic stem cells, induced pluripotent stem cells, and their differentiated progeny. Their ultimate goal is to develop a technology that will minimize the potential of tumor formation with pluripotent stem cell-derived therapies.

Significance and Rationale
- The applicant proposes to eliminate the residual undifferentiated pluripotent cells from the population of differentiated cells by using a small molecular probe. If successful, and able to demonstrate the removal of any residual probe in the screened transplants, this would be a significant advancement in human stem cell therapy.

- Reviewers acknowledged that the rationale was supported by convincing preliminary data provided by the applicant. A concern was expressed that the applicant did not demonstrate the advantages of this approach over other methodologies.

Feasibility and Experimental Design
- The applicant will perform experiments on various undifferentiated and differentiated stem cell lines to determine the effective doses of the compound, as well as a potential mechanism of action. The experiments are logical and well thought out. The preliminary data that is provided supports the experimental design and approach.

- Reviewers would have preferred that the investigators propose testing the compound on additional differentiated cell types beyond the neuronal lineage to assess the breadth of its applicability.

- A concern was raised that potential downstream effects of the small molecule on the function of differentiated cells need to be assessed to know if this could be used clinically.

Qualifications of PI and Team

- The principal investigator and collaborators are well qualified to conduct the research with appropriate institutional commitment and excellent resources.

- The collaborators are well established investigators in stem cell biology and small molecule chemistry, which improves the outcome for success on the project.

Responsiveness

- The proposal is responsive to the RFA in that it addresses a significant bottleneck in enabling the safe use of pluripotent stem cells in regenerative medicine applications.

- Human ESCs and iPSCs will be used in this proposal. If successful, the results and techniques can be used throughout the stem cell community.

- The reviewers had some reservations as to the direct translation of the tool /technique for clinical applications.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07683: Identification and isolation of transplantable human hematopoietic stem cells from pluripotent cell lines; two steps from primitive hematopoiesis to transplantable definitive cells, and non-toxic conditioning of hosts for hematopoeitic stem cell transplants

GWG Recommendation: Recommended for Funding
Final Score: 77

Public Abstract (provided by applicant)

A goal of stem-cell therapy is to transplant into a patient “tissue-specific” stem cells, which can regenerate a particular type of healthy tissue (e.g., heart or blood cells). A major obstacle to this goal is obtaining tissue-specific stem cells that (1) are available in sufficient numbers; and (2) will not be rejected by the recipient. One approach to these challenges is to generate tissue-specific stem cells in the lab from “pluripotent” stem cells, which can produce all types of tissue-specific stem cells. The rationale is that pluripotent stem cells that will be tolerated are easier to directly obtain than tissue-specific stem cells that will be tolerated. Furthermore, descendants of a tolerated pluripotent stem cell will also be tolerated and can be produced abundantly.

The goal of the proposed project is to develop techniques for generating transplantable blood-forming stem cells from pluripotent stem cells. In pursuit of this goal, we will study how blood-forming stem cells arise during development. We will also test new methods--less toxic than current chemotherapy and radiation--for preparing recipients for transplantation of blood-forming stem cells.

Additional benefit: Successful transplantation of blood-forming stem cells allows the recipient to tolerate other tissue or organ transplants from the same donor. Thus, transplanted blood-forming stem cells could allow people to receive organs that they may otherwise reject, without taking immune-suppressing drugs.

Statement of Benefit to California (provided by applicant)

We aim to generate from stem cells that can produce all tissues of the body those stem cells that specifically form blood. We will also test new methods--less toxic than current chemotherapy and radiation--for pretreatment before transplantation of blood-forming stem cells. A large number of patients in California could benefit from advances in this field, primarily those with diseases affecting the production of blood and immune cells: leukemia, lymphoma, thalassemia, certain types of anemia, immune deficiency diseases, autoimmune diseases (e.g., lupus), etc. For leukemia and lymphoma alone, in 2014 in California, there will be an estimated 12,060 newly diagnosed cases, 103,400

existing cases, and 4,620 deaths (per the California Cancer Registry). The cost of these blood cancers are difficult to estimate but they account for 6% of cancers in women and 9% in men in California, where the estimated cost of cancer per year is $28.3 billion.

The reagents generated in these studies can be patented, forming an intellectual property portfolio shared by the state. The funds generated from the licensing of these technologies will provide revenue for the state, help increase hiring of faculty and staff (many of whom will bring in other, out-of-state funds to support their research) and could reduce the costs of related clinical trials. Only California businesses are likely to be able to license these reagents and to develop them into diagnostic and therapeutic entities.

Review Summary

Proposal Synopsis
A major bottleneck addressed by this proposal is the production of hematopoietic stem cells (HSCs) that can be engrafted safely into hosts without rejection. Currently, HSCs derived from embryonic stem cells are more like primitive HSCs which do not produce all of the necessary blood-forming cells and which do not durably engraft in a host. One of the goals of this proposal is to determine how during embryogenesis, definitive, multi-potent, and engraftable HSCs that can give rise to all of the necessary blood cell lineages arise. The investigators will dissect the factors and processes involved in the development of definitive blood cells first using HSCs from a murine model and then attempt to differentiate hESCs to transplantable HSCs using analogous factors or processes. A second goal of the proposal is to develop a safer means for conditioning the host to accept the cells without immune rejection. The current method of choice is to massively irradiate the host, which has toxic consequences. After the team assesses their differentiation protocol in vitro, resulting multi-potent cells will be labeled and transplanted into relevant model animals for optimization of the low-toxicity conditioning protocol.

Significance and Rationale
- The proposed research addresses a significant bottleneck to stem cell therapy and has the potential to significantly contribute toward the development of cost-efficient techniques to produce large quantities of engraftable stem cells. The proposal would also has promise to identify new markers for HSC precursors, thus contributing to the development of cell tracking techniques.

- This proposal addresses an important unmet need and strategies capable of providing a ready supply of suitable cells in addition to well-tolerated conditioning regimens are warranted. This proposal addresses both of these challenges.

-In theory, the proposed approach may offer an advantage over currently used conditioning regimens. The hESC-derived HSCs could become a reliable source of engraftable cells for recipients with common tissue types.

- Reviewers thought the significance of this proposal to be somewhat diminished by an incomplete understanding of the systemic effects that may be caused by the proposed treatments to be used in the low-toxicity conditioning protocol described.

Feasibility and Experimental Design

- Strong preliminary data were provided that positively reflect on the high level of expertise of the principal investigator and team and suggest that much of the ambitious work proposed will yield desirable results. However, critical preliminary data to support much of the proposed work is lacking, and reviewers were forced to rely on the strength of the investigator rather than the strength of the data in evaluating feasibility.

- Though the experiments proposed for generating HSCs from hESCs were considered high risk, they were also associated with possible high reward.

- The low-toxicity conditioning regimen was based on strong preliminary data and was considered feasible and could result in collection of efficacy data to be used for clinical translation. This helps to mitigate the high risk associated with the other goal and represents a viable alternative to success.

- The data obtained by analysis of animal HSC development may not be completely translatable to the generation of human HSCs. There is a lack of sufficient direct evidence that evaluating murine development will generate insights into identifying human precursors. Some reviewers noted that this risk is somewhat mitigated by the excellence of the investigator and the appropriateness of the experimental design.

Qualifications of PI and Team

- The principal investigator (PI) is a pioneer in HSC developmental biology with an exceptional record of success and his/her involvement in the project is pivotal to and necessary for success.

- The co-PI and other personnel are well-qualified.

-Despite some of the high risks associated with the proposed research, the reputation and prior success of the PI suggests the potential for valuable information for the stem cell community to be generated by the proposed work.

Responsiveness

- The proposal addresses several priorities of the RFA, including the development of better tools for the generation of engraftable HSCs and for improving the conditioning of the host for better success and safety for transplantation.

- Some resources used to differentiate stem cells into HSC were not described in terms of availability to the scientific community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07692: Small molecule tools and scale-up technologies to expand human umbilical cord blood stem and progenitor cells for clinical and research use

GWG Recommendation: Recommended for Funding
Final Score: 86

Public Abstract (provided by applicant)

Tens of thousands of patients need bone marrow transplants (BMT) every year, some for bone marrow (BM) cancers and some for inherited diseases such as sickle cell anemia and thalassemia, but many lack a BM donor. African Americans, Asian Americans, and people of Hispanic descent are more likely than others to lack a stem cell donor.

BMTs provide hematopoietic (blood) stem and progenitor cells (HS/PCs) that replace the patient's diseased BM with healthy BM. The new BM provides all the circulating blood cells throughout life.

Many BMTs use HS/PCs that do not come from the BM. One such 'other' source is umbilical cord blood (UCB). UCB HS/PCs have many advantages over other HS/PC sources (i.e., BM or peripheral blood). For example, we can easily obtain UCB HS/PCs without any risk to the donor, and we can keep the cells stored in freezers to be available when a patient needs them. However, most UCB samples contain too few HS/PCs to be used to treat people.

Expanding the number of HS/PCs in UCB samples will increase the number of clinically usable UCB samples, offering new hope for thousands of patients who currently lack a donor. We previously screened >120,000 compounds for their ability to expand UCB HS/PCS, and identified a short list of lead candidates. This grant will fund the next step in our effort to develop a novel, clinically-useful UCB HS/PC expansion protocol. Successful completion of this proposal will result in life-saving treatment for thousands of patients.

Statement of Benefit to California (provided by applicant)

Our proposal seeks to establish a novel method to expand umbilical cord blood hematopoietic stem/progenitor cells (HS/PCs) to make bone marrow transplants (BMTs) available to thousands of patients who currently lack a stem cell donor. The benefits to California are wide-ranging:

• Grow California's skilled workforce and create jobs: This project will train scientists in stem cell research and technology, and our success will attract more talent from outside California.
• Increase innovation: This proposal is highly translational, with a goal to move rapidly from bench to bedside. However, our research will also provide basic insights into stem cell biology that can be applied by other scientists to help patients more broadly.
• Enhancing the medical treatment of California residents: Compounds that expand UBC HS/PCs have the potential to improve clinical benefit and reduce health care costs by increasing the success rate of stem-cell transplants. Given California's diverse ethnic population, we have many patients who need a BMT yet lack a donor, so our residents will directly benefit from our success.
• Attracting venture capital and commercialization: We aim to develop technology that will be highly attractive to the biotechnology industry. We have identified GE as a partner to commercialize our reagents and processes. Furthermore, commercially viable compounds will attract venture capital to fund cell therapies and create new biotech jobs for the California economy.

Review Summary

Proposal Synopsis
Many patients need bone marrow transplants which provide a source of life-saving hematopoietic stem and progenitor cells (HS/PCs), but there continues to be a shortage of available donors. Umbilical cord blood (UCB) is an alternative source of HS/PCs but many UCB samples contain too few HS/PCs to be clinically useful. The major objective of this proposal is to develop a method for expanding UCB HS/PCs for clinical applications. The technology being proposed is the utilization of small molecules (a short list has been identified) that can significantly expand UCB HS/PCs to clinically relevant levels. The applicant proposes to characterize the compound-expanded cells and develop standard operating procedures to scale-up production for future clinical use. The significant bottleneck addressed by this proposal is the current lack of patient-relevant human UCB for therapeutic usage, especially for people with ethnic minority backgrounds. The clinical product will contain therapeutically sufficient levels of HC/PC to replace a patient's diseased bone marrow (BM) with healthy BM. Successful completion of this proposal could result in the potential for life saving treatments for many patients who otherwise wouldn't receive a transplant.

Significance and Rationale
- Reviewers thought that UCB is an ideal source of stem cells for transplantation and the need for new UCB HS/PC expansion molecules is high as a clinically-relevant approach to safely and efficiently expand UCB HC/PCs would address a major bottleneck, with outcomes that would be particularly relevant to ethnic minority patients.

- Given that only one expansion molecule is currently in use for this type of expansion, reviewers felt that the discovery of new chemical cell-expanders with higher potency and efficacy would be a major advance. The rationale for this approach is well supported.

- The development of standard operating procedures to scale-up production of cells from UCB could be beneficial for many patients and is highly significant.

- The applicants have established partnerships that will provide additional expertise, funding and support. This is arrangement that improves the likelihood for a successful outcome.

Feasibility and Experimental Design

- Reviewers noted that the experimental design and aims are logical and accomplishing the project goals appears feasible within the time frame given.

- Given that the research team already has a short list of candidate compounds, the probability is high that a successful optimized lead compound will be produced out of this research.

- It is likely that the partnerships developed will provide strong support for the scale-up and marketing of the UCB HS/PC expansion technology.

Qualification of PI and Team

- The reviewers indicated that the principal investigator (PI) has the requisite expertise and experience to successfully conduct this project.

- The co-principal investigator has experience appropriate and beneficial expertise in drug discovery and development.

-Favorable comments were made by some reviewers about the particular expertise of the collaborators assembled for this project, which they thought significantly strengthened the probability for a successful outcome.

Responsiveness

- Reviewers indicated that the proposal was responsive to the RFA, and some indicated that it addresses “one of regenerative medicine’s unique and significant translational challenges”, that is to safely and efficiently expand UCB HS/PC for clinical use. The proposal will use human stem and progenitor cells.

- Some reviewers commented that the partnerships established for commercializing the new technology developed from this project will provide the potential to develop other technologies for advancing clinical applications.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07738: High-fidelity genome engineering to treat genetic disease

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

We are producing better tools to correct genetic liver diseases that would otherwise require patients to get a liver transplant. Liver transplants are difficult and they force patients to take medication for life that suppresses the immune system. Recent advances in stem cells (iPSCs or "stem cells from skin cells") can be combined with molecular scissors to preform "genome editing" to cure human genetic disease. We are particularly focused on genome-editing tools to repair genetic mutations that cause liver failure, because the liver has regenerative properties that may allow the repaired liver cells to cure the disease. By correcting a patient's own cells, liver transplants and lifelong immune suppression would be obsolete. We will focus on the three most common mutations that cause liver failure. Two of the mutations are in the ATPB7 gene, which cause Wilson disease, and one is in the A1AT gene, causing alpha-1 antitrypsin deficiency in a combined total of >80,000 people world-wide. We will determine which gene-editing method is best at repairing each mutation, while making sure that it does not damage the rest of the genome. We will also try different methods to speed up the process, since prolonged cell culture can cause DNA damage. All of our aims will help us evaluate gene-editing tools in stem cells, which will be essential for future human clinical trials.

Statement of Benefit to California (provided by applicant)

We are building tools to cure specific genetic diseases that cause liver failure, normally requiring liver transplantation. We are focused on two severe genetic diseases (Wilson disease and alpha-1 antitrypsin deficiency (AATD)) that affect 1:30,000 people—roughly 1,000 Californians. Curing these diseases would avoid costly transplantations and eliminate the need for lifelong immune suppression. Also, treating AATD with protein injections costs >$100,000 per year. Each year in California, >700 liver transplants are preformed, while >3000 people remain on the waiting list (http://www.unos.org/). Our goal is to improve quality of life and increase productivity for the thousands of Californians who suffer from genetic diseases.

We are also hopeful that this project will also help build upon California's leadership in biotechnology. Therapeutic genome engineering will likely become a global enterprise that could be dominated by leading medical centers in California. Although we are focused on genetic liver diseases, many other diseases could be targeted by therapeutic

genome editing. As this type of therapy comes to fruition, we anticipate that California medical centers will lead the way.

Review Summary

Proposal Synopsis
This proposal addresses a current lack of rapid, efficient, and accurate methods for directly introducing specific single-base substitutions ("genome editing") into human induced pluripotent stem cells (iPSCs). By overcoming this roadblock, it may eventually be possible to correct certain disease-related genetic mutations in a patient's own cells for developing autologous cellular therapies as an alternative to organ transplantation. This procedure that can be significantly hampered by limited availability of donor organs and the side effects associated with long-term immune suppression. The applicant predicts that using the most efficient gene correction technique can reduce culture time and the incidence of spontaneous mutations that occur during the gene correction process and during prolonged time in culture. Studies are proposed to test this hypothesis by comparing the accuracy and efficacy of 4 commonly used gene-editing techniques to correct various point mutations associated with two different liver diseases in iPSCs, and exploring methods to reduce the overall time needed for gene correction.

Significance and Rationale
- If successful, the development of advanced genome editing techniques could provide an important and significant step towards the use of autologous cell therapy for treating certain genetic disorders, in the liver area and more broadly.

- While they do not necessarily provide a new type of technology, the proposed studies offer a new approach for comparing existing technologies.

- Optimization methods may be context or cell-type dependent, and thus the extent to which the results would be generalizable is unclear.

Feasibility and Experimental Design
- The major weakness of the proposed studies is the lack of attention afforded to statistics, which is critical for interpreting the large amount of data that would emerge from these studies. In the opinion of the reviewers, without proper statistical analysis, efforts will be wasted.

- Reviewers were concerned, especially for aims 1 and 2, wether the experiments were properly powered to detect expected differences and answer questions adequately. There is no clear description of the power of the proposed experiments to identify significant or meaningful differences between the four gene editing techniques. Similarly, reviewers were uncertain whether the parameters chosen for assessing

genome-wide nuclease fidelity were optimal, as there were few specifics provided on the experimental design.

- Reviewers strongly supported the rationale for reducing culture time by simultaneously combining gene correction techniques with improved iPSC production methods (Aim 3), although they were not convinced from the preliminary data that a goal of 50% would be achievable.

- The application provides a clear timeline with specific milestones that are feasible based on the proposed level of support. Another strength is the availability of mutant iPSCs and fibroblasts for study.

Qualification of PI and Team

- Reviewers agreed that the applicant team is superbly qualified in the proposed methodologies, but weaker in the area of statistics. Availability of the bioinformatics core is essential but not sufficient to perform the necessary statistical analysis.

- Inclusion of a project manager adds strength to the team.

Responsiveness

- The reviewers indicated that the proposal was responsive to the RFA by meeting the objective of improving existing technologies to address translational bottlenecks to stem cell therapies.

- Human iPSCs will be used in the proposed studies.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07750: Development of Clinical Tools for Predicting and Evaluating Immune Responses to Regenerative Cellular Therapies

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Regenerative cellular therapy, the restoration of human organ function by the transplantation of progenitor cells capable of re-building the damaged organ, has significant potential to improve medical treatment of disease. However, transplantation of progenitor cells requires overcoming the body's immune defenses which may recognize these tissues as foreign.

Statement of Benefit to California (provided by applicant)

The research proposed in this application is designed to define the role of the immune system as a barrier to clinical regenerative cellular therapies and develop diagnostic tools to assess immune responses in cellular therapy patients. The successful completion of this project will provide significant insight into the immunology of regenerative cellular therapies and generate diagnostic tools to support clinical applications. These outcomes will directly benefit the State of California by advancing the mission of CIRM, promoting the intellectual leadership of CA in the fields of regenerative medicine and stem cell biology, and attracting additional governmental and philanthropic funding for continuing cutting-edge biomedical research.

Review Summary

Proposal Synopsis

Restoring organ functions in the clinic by the transplantation of progenitor cells that could repair and re-build the organ is an attractive use of stem cell therapy. In some cases, the transplantation of progenitor cells may trigger the host immune response and recognize the transplanted cells as foreign. The application proposes to develop novel diagnostic tools to monitor potential immune responses to human pluripotent stem cell (hPSC)-derived cells and tissues. A series of experiments are proposed to define the antigenic molecules on the transplants that are seen by the recipient's immune system. To accomplish this objective the investigator will attempt to catalog all of the different molecules seen by the immune system on the iPSC cells and tissues, and then develop markers for such reactions to allow for compatibility testing and post-transplant monitoring. Successful completion of the project could provide clinically useful tools to

increase our understanding of multipotent cell immune reactivity during the differentiation into replacement tissues.

Significance and Rationale

- Immune system recognition and rejection of stem cells and stem cell-derived tissues is a significant bottleneck in regenerative cellular therapies. However, in order to address this bottleneck, the immunogenicity of the actual cells and functional tissues that will be transplanted into patients must be characterized - not the intermediate stage products currently proposed by the applicants.

- Though it was acknowledged that iPSC could be used in allogeneic transplant, when using autologous iPSC or histocompatibility-matched iPSC, it is not apparent that these immune responses are really a hindrance (i.e., bottleneck) to clinical translation of such therapies, and the applicant did not sufficiently describe this aspect of the proposed bottleneck.

- In general, the reviewers were of the opinion that the final cell product should be utilized in the proposed studies for the research to have the potential for significance. This lack of significance is a primary weakness of the proposal.

Feasibility and Experimental Design

- A concern of the proposed research is the lack of sufficient data presented to verify that the tissues are clinically-relevant transplants. If these tissues are not the intended clinical material, then the data generated could be misleading and/or require repeating of the studies proposed with the appropriate transplant material.

- The proposal would be strengthened by defining which antigenic molecules are to be evaluated. For given cell product, the specificity of recipient immune complexes could be variable and therefore hinder the utility of this tool in the clinic.

Qualifications of PI and Team

- The investigators are well qualified and productive with the appropriate experience and training needed to accomplish the research described in this application.

- The research team is well qualified to carry out the studies in the proposal.

Responsiveness

- While reviewers agreed that the descried bottleneck is important for clinical translation of stem cells and addressing the bottleneck would be of value to the field, the reviewers thought that the application was not really responsive to the RFA in that the experimental design did not address the described bottleneck of the translation of stem cells into the clinic.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07756: Delivery of stem cells for muscular dystrophy

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Over the past several years, there has been great progress in our knowledge about how to create stem cells from adult cells, how to correct mutations in stem cells, and how to differentiate the stem cells into the types of cells needed for various therapies. In the case of muscle diseases, we now know how to create corrected muscle stem cells from the skin cells of muscular dystrophy patients. The major roadblock in translating these advances to the clinic is the development of safe and effective methods to get the stem cells into the muscles of patients, so that the cells can do their job of repairing the diseased muscle. The delivery issue is truly a bottleneck to moving stem cell therapies to the clinic. This project attacks that bottleneck by developing methods to use the existing blood system of the body to carry the stem cells to their intended body tissues. Starting with experiments in a relevant small animal model, we will inject patient-derived stem cells into key arteries that will distribute the cells to limb muscles. We will use various drugs and treatments to stimulate the cells to leave the blood vessels more effectively and engraft into the muscles. The work will be extended to a larger animal model to scale up in size by ten-fold. We will then carry out experiments to scale the methods up to the size of patients. Solving the stem cell delivery problem in muscle is likely also to accelerate progress to the clinic of stem cell therapies in many other tissues and organs.

Statement of Benefit to California (provided by applicant)

The proposed research on delivery of muscle stem cells could lead to a safe and effective stem cell therapy for limb girdle muscular dystrophy type 2D, as well as other forms of muscular dystrophy. This outcome would deliver a variety of benefits to the state of California.

There would be a profound personal benefit to the Californians affected directly or indirectly by muscular dystrophy. In addition, development of effective methods to deliver stem cells in muscle is also likely to accelerate the development of delivery methods for stem cell treatments involving degenerative disorders affecting many other tissues and organs.

Safe and effective stem cell therapies for muscular dystrophy and other disorders would also bring economic benefits to the state by reducing the huge burden of costs associated with the care of patients with long-term degenerative disorders. Many of these patients would be more able to contribute to the workforce and pay taxes.

Another benefit is the effect on the business economy of the state of novel, cutting-edge technologies developed in California. Such technologies can have significant effects on the competitiveness of California through the formation of new manufacturing and health care delivery facilities that would employ California citizens and bring new sources of revenue to the state.

Therefore, this project has the potential to bring health and economic benefits to California that are highly desirable for the state.

Review Summary

Proposal Synopsis
Cellular therapies offer a promising approach to treating muscle diseases such as muscular dystrophy. However, a major bottleneck in translating these advances to the clinic is the development of safe and effective methods to deliver sufficient numbers of cells into multiple affected muscles. The applicant proposes to advance this bottleneck by developing methods to use the circulatory system to more efficiently deliver genetically corrected cells to limb muscles. Intravascular delivery of muscle precursor cells differentiated from genetically corrected induced pluripotent stem cell (iPSCs) derived from muscular dystrophy patients will be used in animal models of the disease. Engraftment of the corrected muscle precursor cells will be assessed, as will changes in muscle function after administration. Various approaches are also proposed that may enhance vascular delivery of cells and engraftment into the target muscles. Plans also include using a large animal model for scale-up studies to support future clinical use. Information gained from these studies could be beneficial for the delivery of stem cell therapies in other large tissues and organs.

Significance and Rationale
- The application proposes to develop safe and efficient methods for vascular delivery of cell therapy products, which, if successful, could translate to a broad clinical benefit for many diseases of solid tissues.

- Reviewers were concerned that the team had not yet identified a cell population for administration with sufficient potential for engraftment; if intramuscular injection of cells has not resulted in sufficient cell engraftment, it is difficult to predict that superior engraftment of the cells would occur following vascular delivery.

Feasibility and Experimental Design

- Preliminary data from small animal models were presented to support the utility of using genetically corrected cells injected into skeletal muscle to address muscle disease, and to support the feasibility of using a vascular delivery approach to deliver cells to skeletal muscle. However, reviewers felt that more robust quantitative engraftment data will be required to support moving to larger animal models.

- Reviewers commented that the proposal presented an overly ambitious number of variables to test in multiple experimental models, and it was unlikely that the studies could be accomplished in the proposed time frame.

- Reviewers questioned whether the most appropriate animal models of disease had been chosen for the proposed studies.

- Reviewers described the proposed studies in a large animal model as premature. While scale up studies will be required to support clinical translation, a number of potential technical issues were not adequately addressed, such as ways to minimize the potential for host immune responses and demonstration of measurable endpoints.

Qualifications of PI and Team

- The principal investigator was recognized as an expert in developing gene editing tools and has the necessary experience to oversee the project.

- The composition of the research team was described as a major strength of the application and reviewers felt that members have appropriate expertise and access to resources to conduct the proposed studies.

Responsiveness

- Successful delivery of a cellular therapeutic to large areas of skeletal muscle is a significant technical challenge and the proposal aims to address this by developing a more effective method for vascular delivery of cellular therapeutics; the application was viewed as highly responsive to the RFA.

- Human iPSCs will be used in the proposal.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07763: A suite of engineered human pluripotent stem cell lines to facilitate the generation of hematopoietic stem cells

GWG Recommendation: Recommended for Funding
Final Score: 76

Public Abstract (provided by applicant)

Our goal is to develop tools that address major bottlenecks that have prevented the generation of blood forming stem cells in culture for therapeutic use. To help overcome these bottlenecks, we will generate a suite of human embryonic stem cell reporter lines that can be used to monitor key milestones in blood stem cell development. These lines will serve as tools to identify factor combinations to improve the in vitro differentiation of hESCs to functional blood stem cells. Once individual lines have been validated, lines that contain multiple fluorescent reporters will be generated, and a multi factor screen will be performed to optimize conditions that induce these blood stem cell regulators. To track the location and quantity of transplanted cells in recipient small animal model, we will generate hESC lines with in vivo reporter system that combines bioluminescent or PET imaging, and serum-based assay. Our in vivo tracking tools will be broadly relevant and not restricted to studying the in vivo biology of blood forming cells. These tools will help translate the promise of stem cells to cell based therapies to treat human disease.

Statement of Benefit to California (provided by applicant)

This project will help improve California economy as many of the vendors used for reagents and supplies are located in California. This project will also help create and maintain jobs for skilled personnel and helps train post-doctoral fellows who will become the next generation of stem cell scientists. The long-term goal of this project is to improve in vitro differentiation protocols to create transplantable blood forming stem cells for therapeutic use. If we, or others who will use our reporter lines generated in this study, achieve this goal, there will be new, theoretically unlimited sources of HLA-matched or patient specific blood stem cells that can be used for treating many serious blood diseases, including leukemias and inherited immunodeficiencies or anemias. Availability of patient specific blood stem cells for transplantation would be a major benefit in California, as there is currently limited availability of suitable bone marrow donors for individuals from mixed ethnic backgrounds.

Review Summary

Proposal Synopsis
This proposal will use gene targeting methods to generate a suite of reporter human embryonic stem cell (hESC) lines to mark the expression of genes that indicate hematopoietic stem cell (HSC) specification. Current differentiation systems are unable to efficiently produce from pluripotent stem cells (PSCs) definitive and engraftable HSCs with long-term multi-lineage reconstituting capacity. The project may also provide for monitoring of engraftment in animal models by reporting of the location and/or quantity of engrafted cells. The bottleneck addressed by this application is cost efficient production of stem therapies and improving cell tracking of engrafted cells.

Significance and Rationale
- Reviewers concurred that this project was highly significant and addressed an important problem in the field. The approach was considered high risk but impressive with potential to address the described bottleneck.

- Some reviewers noted that the reporters developed for in vivo tracking could have uses in monitoring location and survival of other stem cell-derived cell types.

Feasibility and Experimental Design
-Reviewers found that the proposed experiments were supported by sound preliminary data.

- The application did not sufficiently describe the experimental plan for screening and validation of screen hits.

- Reviewer considered the project to have straightforward achievable aims.

-Reviewers noted that the use of multiple reporter lines to identify lineage progression during PSC differentiation to HSCs was a strength.

- Reviewers did not think the proposal sufficiently addressed the prospect of off target effects.

-Reviewers noted concerns that there may be limited resolution of in vivo imaging of fluorescently marked cells.

Qualifications of PI and Team
-The PI has a strong track record and experience in HSC and PSC biology.

-Reviewers concurred that this was a strong multidisciplinary team and that the facilities and environment were outstanding in supporting this project.

-Reviewers concurred on the importance of the international collaborators, though some reviewers noted that the same external team was involved in similar projects with others.

- Some reviewers noted that the PI has several pending grants, with some potential for overlap.

Responsiveness

-With its focus on cost efficient production of stem therapies and improving cell tracking of engrafted cells this project was found to be highly responsive by reviewers.

-The reviewers concluded that reporter lines proposed could be very helpful to the HSC derivation field.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07796: A Chromatin Context Tool for Predicting iPS Lineage Predisposition and Tissue Graftability

GWG Recommendation: Recommended for Funding
Final Score: 75

Public Abstract (provided by applicant)

Induced pluripotent stem (iPS) cells are cells derived from skin that closely resemble embryonic stem (ES) cells and can be coaxed into many different types of cells such as nerve cells, heart cells, liver cells, and also back to skin cells. One major bottleneck in the field is our ability to coax the cells into sufficiently pure and mature cell populations. One recognized reason for this difficulty is that every individual iPS cell line behaves slightly differently and the protocols optimized and refined to work well in one particular line do not work as well for another iPS cell line. Since robust differentiation protocols are needed for generating transplantation tissues, this line-to-line variability represents a major stumbling block for the realization of such a therapeutic approach. We here propose to develop a tool, to prospectively identify lines that will work well in a given differentiation protocol. Specifically we will develop a prediction tool whether a given iPS cell line will be able to efficiently give rise to skin cells. The tool is based on the observation that critical lineage-determination factor that regulate genes need to access the proper target sequences and that these gene sites require a permissive epigenetic configuration to be properly accessed. Validation of the approach for skin will make the tool usable in principle for predicting the differentiation of iPS cells into other medically important tissues.

Statement of Benefit to California (provided by applicant)

Cell transplantation-based therapies are heralded as new treatment options with often curative aspirations for many different kind of diseases. In particular, induced pluripotent stem (iPS) cells are intriguing potential donor cells because they can be derived from individual patients circumventing transplant rejection, and they can theoretically be differentiated into an unlimited numbers of specialized donor cells. A major hurdle limiting their usefulness is the observation that iPS cell lines are heterogeneous and that iPS cell lines from the same patient behave differently when scientists attempt to differentiate them into one particular tissue. Our chromatin context tool will provide a solution to this problem as it seeks to predict which iPS cell line will be useable for efficient differentiation. The tool is based on the observation that critical lineage-determination factor that regulate genes need to access the proper target sequences and that these gene sites require a permissive epigenetic configuration to be properly accessed. Successful development of the tool will eliminate

many weeks of costly trial and error and help prospectively identify those lines that can be used for therapy. Therefore, our tool will close a critical gap that hinders clinical application of these exciting new cell-based therapies and therefore will benefit all Californians that suffer from a disease that could be treated with iPS cell-based transplantation.

Review Summary

Proposal Synopsis
Induced pluripotent stem cells (iPSCs) are cells derived from tissues that can be guided into many different types of cells. One major bottleneck in the field is our ability to guide these cells into sufficiently pure and mature cell populations. Protocols optimized and refined to work well in one particular iPSC line generally do not work as well for other iPSCs. The major objective of this proposal is to devise an efficient method of genetically altering iPSCs to produce cell sheets for use in treating skin diseases. The proposal hypothesizes that the variability between different cell lines is the result of chromatin modifications occurring during differentiation of the iPSCs. Gain and loss function of a transcription factor will be evaluated to determine what influence this may have on differentiation variables. A series of iPSC lines with different differentiation efficiencies will be examined to determine if the transcription factor binding site can influence skin cell differentiation. If successful, this series of studies could generate mechanistic insight into iPSC line-to-line variability in skin cell differentiation and offers the potential to efficiently screen iPSC lines for use in skin cell tissue engineering applications. Validation of this approach in skin cells may offer the ability to predict the differentiation of iPSCs into other medically important tissues.

Significance and Rationale
- The applicant has proposed a project that will address the current roadblock for screening iPSC lines to identify those with a higher efficiency for differentiation into skin cells.

- The ability to screen iPSCs as a quality control measure is very attractive. Some reviewers indicated that the specific tool developed would initially be limited to skin cell differentiation but could have the potential to be further modified for use in predicting iPSC differentiation capacity in other cell lineages.

- The working hypothesis is very focused on a specific transcription factor pathway rather than a more global approach. Reviewers expressed concern that results could be limited to skin cell pathways.

Feasibility and Experimental Design
- There was some degree of discussion regarding the preliminary data that linked the transcription factor to variations in iPSC lines. Experimental design was strong and proposed techniques were considered valid.

- In general, the reviewers commented that the research plan was clear and logical. A concern was noted that the final objective was entirely dependent upon the success of the previous research aims.

- A concern was raised that the proposal would have benefited from the addition of more details regarding pitfalls and alternative approaches.

Qualification of PI and Team

- The research team has the requisite expertise and experience to successfully conduct this project.

- Favorable comments were made by some of the reviewers about the facilities and environment to conduct the research.

- A reservation was expressed that one of the key personnel may be overextended or overcommitted.

Responsiveness
- In general, the reviewers indicated that the proposal was responsive to the RFA. Some reservations were noted regarding the idea that the proposal seemed to be primarily addressing a biological question and that the tool being developed would be dependent upon verifying the biological hypothesis of the proposal.

- The tool being developed from the proposal could generate mechanistic insight into iPSC line-to-line variability in skin cell differentiation and offers the potential to efficiently screen iPSC lines for use in skin cell tissue engineering applications, which is responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07798: Advanced animal model for predictive preclinical testing of engineered cardiac autografts and allografts

GWG Recommendation: Recommended for Funding
Final Score: 77

Public Abstract (provided by applicant)

Heart disease is the number one cause of death in the US. Heart muscle injured during a heart attack does not regenerate, and the resulting damage leads to heart failure, which inflicts almost 6 million people in the US alone. Recently, several studies have shown that direct injection of stem cell-derived heart cells may offer regenerative potential in the damaged heart. However, injected heart cells often lack the spatial and temporal organization required to create uniform tissue with synchronized beating, while rapid donor cell death poses another key limitation. For these reasons, we propose to transplant engineered heart muscle (EHM) that is spatially and temporally organized into a relevant large animal model. Our proposal addresses unique translational challenges pertaining to tissue engineered heart repair by scaling our established human induced pluripotent stem cell (iPSC) differentiation protocol to create one billion human and large animal model cardiomyocytes for each EHM, in order to meet clinical demands by: (1) adopting our established human EHM tissue engineering process to the large animal model; (2) defining conditions for EHM implantation; and (3) performing a pivotal feasibility, safety, and efficacy study in the large animal model with chronic heart failure. Our studies will establish long-term safety and efficacy of iPSC-EHM therapies in a clinically relevant large animal model, which will overcome a major unresolved bottleneck to the translation of stem cell therapies to humans.

Statement of Benefit to California (provided by applicant)

Cardiovascular disease (CVD) affects more than 1.7 million Californians. The societal and financial costs are tremendous, with CVD accounting annually for an estimated $8 billion in California health care costs alone. Following a heart attack, the endogenous regenerative process is not sufficient to compensate for heart tissue death. Thus, using regenerative therapies with human stem cells to form engineered heart tissue is emerging as a promising therapeutic avenue. Engineered tissues are already being used in patients needing artificial blood vessels, bladders, and tracheas. Our multidisciplinary team proposes to create human engineered heart tissue (EHT) for treatment of post-attack heart failure in a clinically-enabling large animal model, and we are confident we will be able to move our potential therapy into preclinical human trials. Development of therapies for diseases such as CVD could potentially improve the

California health care system by reducing the long-term health care cost burden on California. In addition, our research may provide an opportunity for California to benefit from royalties, patents, and licensing fees, which will create cutting-edge projects, attractive jobs, and innovative therapies that will generate millions of dollars in new tax revenues and opportunities in our state. Finally, our research could further advance the flourishing biotech industry in California, serving as a crucial engine to power California's economic future.

Review Summary

Proposal Synopsis
In this application, this international collaborative team proposes to develop a large animal model to enable the study of engraftment and efficacy of pluripotent stem cell (PSC) derived therapies in the absence of xenograft rejection. The team will generate engineered heart tissues (EHT) from both human induced pluripotent stem cells (hiPSC) and from large animal model iPSC. They will then test comparability of these heart tissues to determine relevance of the model-derived tissue to the human counterpart. In parallel, the team will develop large animal model of heart failure. The team will then use the model to establish immune monitoring and immune suppression protocols for; to assess immune responses to; and to assess the efficacy of autologous, allogeneic, and xenogeneic iPSC-EHTs.

Significance and Rationale
- The proposal addresses the engraftment bottleneck and generated reviewer enthusiasm. If successful, this will allow for a comparison of differences in transplantation, survival and integration of human xenografts versus model derived allografts and autografts. This has not been previously tested and is important work that will have significant translational implications.

- The proposed work, if successful, could enable the study of immune response to autologous large animal model PSC-EHT in immune competent hosts. Such study has not previously been possible.

- The development of immune suppression regimens for xenografts is important for the field, so if the proposed research is conduct, there is potential for impact. However, due to concerns regarding the experimental design, some reviewers were unsure that the proposed work would advance development of such regimens.

Feasibility and Experimental Design
- The team can perform the techniques required to execute the proposed studies, including use of large animal models.

- The collaborator has generated iPSC-derived cardiomyocytes from the model cells.

- Reviewers found the description of the experimental plan to lack the necessary details to evaluate the likelihood that useful, interpretable data will be generated, particularly related to the large animal immunosuppression studies. The application did not clearly describe how many animals would be included per experimental condition nor how data across groups would be compared and analyzed to inform developing protocols and models.

- The application presents little data supportive of efficacy or safety of the proposed EHT, and surgical delivery of EHT bears increased risk over injection techniques used to deliver cell suspensions.

- It is not clear from the application where large animal surgery will take place. It appears that most of the animals will be in Europe. The number of large animals per group to be tested in California appears small, and reviewers were not certain that adequate data for analysis will be generated.

- Reviewers found the timeline to be confusing as some dependent activities are proposed prior to completion of necessary foundational activities.

Qualifications of the PI and Team

- The PI and collaborators are superb, are renowned world experts in all of the required areas, and are capable of conducting all aspects of the studies.

- The proposed collaborations are already in place and are a strength of the application.

Responsiveness

- The proposal is highly responsive to the large animal section of the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07800: Engineered Biomaterials for Scalable Manufacturing and High Viability Implantation of hPSC-Derived Cells to Treat Neurodegenerative Disease

GWG Recommendation: Recommended for Funding
Final Score: 77

Public Abstract (provided by applicant)

Cell replacement therapies (CRTs) have considerable promise for addressing unmet medical needs, including incurable neurodegerative diseases. However, several bottlenecks hinder CRTs, especially the needs for improved cell manufacturing processes and enhanced cell survival and integration after implantation. Engineering synthetic biomaterials that present biological signals to support cell expansion, differentiation, survival, and/or integration may help overcome these bottlenecks. Our prior work has successfully generated synthetic biomaterial platforms for the long-term expansion of human pluripotent stem cells (hPSCs) at large scale, efficient differentiation of hPSCs into dopaminergic progenitors and neurons for treating Parkinson's Disease, and modulation of stem cell function to promote neuronal differentiation within the brain. We now propose to advance this work and engineer two synthetic biomaterial platforms to treat neurodegenerative disease, in particular Parkinson's Disease and Retinitis Pigmentosa. Specifically, our central goals are to further engineer biomaterial systems for scalable hPSC differentiation into dopaminergic and photoreceptor neurons, and to engineer a second biomaterial system as a biocompatible delivery vehicle to enhance the survival and engraftment of dopaminergic and photoreceptor neurons in disease models. The resulting modular, tunable platforms will have broad implications for other cell replacement therapies to treat human disease.

Statement of Benefit to California (provided by applicant)

This proposal addresses critical translational bottlenecks to stem cell therapies that are identified in the RFA, including the development of fully defined, xenobiotic free cell manufacturing systems and the development of clinically relevant technologies to enhance the survival and integration of human stem cell therapies. The proposed platform technologies for expanding and differentiating pluripotent stem cells in a scaleable, reproducible, safe, and economical manner will initially be developed for treating two major neurodegenerative disorders - Parkinson's Disease and Retinitis Pigmentosa - that affect the well-being of hundreds of thousands of Californians and Americans. In addition, the biomaterial platforms are designed to be modular, such that they can be re-tuned towards other target cells to even more broadly enable cell

replacement therapies and enhance our healthcare. This work will thus strongly enhance the scientific, technological, and economic development of stem cell therapeutics in California.

Furthermore, the principal investigator has a strong record of translating basic science and engineering towards clinical development within industry, particularly within California. Finally, this collaborative project will focus research groups with many students on an important interdisciplinary project at the interface of science and engineering, thereby training future employees and contributing to the technological and economic development of California

Review Summary

Proposal Synopsis
The investigators propose to develop biomaterial scaffolds engineered to present micro environmental cues ("tunable" scaffolds) to improve the efficiency and scalability of human pluripotent stem cell expansion and differentiation and to develop bio-degradable, tunable biomaterials for cell delivery to improve the efficiency of cell survival and engraftment. This application thus addresses the bottlenecks of cell survival/engraftment and scalable production of stem cell therapies. The focus of this application is on scaffolds and biocompatible delivery vehicles for cell therapies for Parkinson's Disease and retinitis pigmentosa, but the resulting modular, tunable technologies will enable adaptation to cell therapies for other diseases.

Significance and Rationale
- The application addresses substantial technical obstacles that impede progress in cell replacement therapy, which, if successful, could be a significant advance in enabling cell therapy development and commercialization.

- Some reviewers considered the proposed studies to be a significant and innovative expansion of previous work; others thought it to be derivative of previous work and represented "fine-tuning" of previously developed technology.

- Current technologies for efficient scalable expansion and differentiation and efficient, effective cell engraftment are not optimal; the rationale is strong for use of 3D biomaterials functionalized with cues that mimic the microenvironment.

Feasibility and Experimental Design
- Abundant preliminary data clearly demonstrate the expertise of the investigators/team and support the feasibility of the proposed work.

- The underlying biomaterials technology is strong; applicants are proposing a systematic approach for assessing biomechanical and biochemical parameters, including use of in vivo disease models.

- Concerns were raised about the ambitious nature of the proposed work. These were partially mitigated by the quality of the investigators.

- Concerns were noted regarding the long-term biocompatibility of the delivery biomaterial, lack of assessment on cell migration and integration (in addition to engraftment and survival) and the related diffusion and spatial distribution of biochemical cues.

Qualifications of PI and Team

- Reviewers acknowledged that these are highly competent investigators with relevant expertise, strong track records of achievement and existing fruitful collaborations.

Responsiveness

- This application was perceived by all reviewers as highly responsive to the need for improved technologies for production and delivery of stem cell based therapies.

- No dissemination plan was included in the application.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07804: Injectable Macroporous Matrices to Enhance Stem Cell Engraftment and Survival

GWG Recommendation: Recommended for Funding
Final Score: 82

Public Abstract (provided by applicant)

Despite the great promise stem cells hold for regenerative medicine, the efficacy of stem cell-based therapies is greatly limited by poor cell engraftment and survival. To overcome this major bottleneck, the goal of this proposal is to validate the efficacy of novel microribbon (μRB)-based scaffolds for cell delivery. These scaffolds combine the injectability and cell encapsulation of conventional hydrogels with macroporosity, which facilitates nutrient transfer, cell survival, proliferation, and tissue formation. In preliminary studies, our μRB-based scaffolds markedly enhanced the survival of human stem cells and accelerated bone repair in vivo. Thus, here we propose to validate the efficacy of μRB-like hydrogels with tunable stiffness and macroporosity as cell-delivery matrices that enhance the engraftment and survival of stem cells for both soft and hard tissue reconstruction using relevant animal models in vivo. Our results will significantly accelerate clinical translation of stem cell-based therapy by enhancing cell delivery, survival, and integration, thus improving therapeutic outcomes, reducing the number of cells needed for transplantation, and reducing the associated time and cost to produce these cells. Our validated platform will be broadly applicable to diverse cell types, and its wide dissemination will crucially advance the translation of stem cell-based therapies to combat both acute and degenerative human conditions

Statement of Benefit to California (provided by applicant)

Tissue loss and organ failure represents a substantial socioeconomic burden to the State of California, with increasing medical costs for treating patients suffering from various degenerative disease, trauma and congenital defects. Furthermore, the average life-span and percentage of aging population in California is expected to grow, with increasing needs for better therapeutic strategies for caring these patients. Stem cell-based therapies hold great promise for treating tissue loss and enhancing tissue regeneration, often via direct injection of cells at the target site. However, the majority of transplanted cells die shortly after transplantation, which greatly diminishes the efficacy of stem cell-based therapies. Poor cell engraftment and survival remain a major bottleneck to fully exploiting the power of stem cells for regenerative medicine. Here we propose to validate the efficacy of novel μRB-like hydrogels as cell-delivery matrices that enhance the engraftment and survival of stem cells for both soft and hard tissue reconstruction. Our results will significantly accelerate clinical translation of stem cell-

based therapy for residents in California by enhancing cell delivery, survival, and integration, thus improving therapeutic outcomes. Our validated platform will be broadly applicable to diverse cell types, and its wide dissemination will crucially advance the translation of stem cell-based therapies to combat both acute and degenerative human conditions.

Review Summary

Proposal Synopsis
The efficacy of stem cell-based therapies depends on efficient cell engraftment and survival following transplantation. The applicants plan to address this bottleneck by further improving and testing an injectable scaffold system they have developed to enhance cell survival and engraftment in vivo. They will optimize the matrix stiffness of the scaffold, and test the effects of this matrix on the survival and engraftment of adipose-derived stromal/stem cells (ASC) and their efficacy in both soft and hard tissue reconstruction using relevant animal models.

Significance and Rationale
- The reviewers strongly supported the notion that this proposal targets a critical bottleneck for development of stem cell therapies.

- Reviewers were very enthusiastic about the proposed scaffold design. They deemed it to be very interesting and novel, and some characterized it as a paradigm shift.

- Some reviewers felt the proposed technology would have broad applicability for use with various cell types, while others cautioned that different cell types would necessitate adjustments to the scaffold.

Feasibility and Experimental Design
- The experiments are well designed and well described.

- The proposed studies are supported by extensive preliminary work and build on broad expertise developed by the group.

- Some reviewers felt that the application did not appropriately address the complexities inherent in manipulating material stiffness.

- Some reviewers noted that preliminary experiments lacked an ASC alone control, while others felt this was mitigated by the use of ASC with materials other than those under investigation.

- Some reviewers would have liked to see more analysis of the biodegradability of the scaffold and how that relates to long-term cell retention.

Qualifications of PI and Team

- The principal investigator was recognized as a well-accomplished expert in the field of biomaterials.

- An excellent team is assembled for this project; very accomplished team members cover all necessary aspects of the proposed research.

Responsiveness

- This proposal is quite responsive to the RFA. There was some debate as to whether this represents a stem cell project, but this was considered to be of minor concern.

- Dissemination of the technology among researchers in the stem cell field may be challenging as it involves specialized biomaterial expertise.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07805: A scaffolding system to enhance lineage-specific differentiation of pluripotent stem cells by on-demand mechanomodulation of the cell niche

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

An individual patient can donate cells to be induced to form stem cells that are "pluripotent," that is, they can divide and differentiate into any type of tissue, a promising option for wound repair and regeneration. However, stem cells also can divide into unwanted types of tissue such as tumors, a risk that currently limits their use. Controlled transformation, called "differentiation", before therapeutic stem cells are transferred to the patient could minimize these adverse effects. This project uses electro-active nanofiber scaffolds, essentially webs that hold colonies of stem cells while they divide and mature. Having discovered that the stiffness of the web can control cell fate and that the stiffness can be electrically controlled, this project seeks to mass-produce desired types of cells for clinical application. Each nanofiber has a core whose stiffness can be "tuned" using an electric field, and an outer shell that is biochemically treated to stimulate stem cells to differentiate to a target cell type. The goal is a high-volume cell factory using programmable nanofiber webs to instruct stem cells to differentiate into many target cell types in numbers useful for regenerative medicine.

Statement of Benefit to California (provided by applicant)

This project seeks to advance the safety and effectiveness of the use of induced pluripotent stem cells (iPSCs) for regeneration of damaged tissues in the patient's body. California's previous and ongoing investment in stem cell research will be leveraged by a novel technology to control how iPSCs differentiate – maximizing the formation of desired cells and reducing the growth of undesirable cells and their attendant risks. The developed high-throughput device offers a cost-effective, efficient approach to determine optimal protocols for differentiating iPSCs to many different cell types applicable to various diseases. The project speaks directly to the mission of California Institute for Regenerative Medicine, particularly in the use of stem cells for new therapies to improve human health and well-being for California's rapidly growing population. The project will provide the technology base for a scalable stem cell culture system, in the process advancing stem cell science, including cell differentiation responses to controlled mechanical and biochemical environmental stimuli. The commercialization and marketing of the full-scale system would benefit the people in California with the financial impact of increased employment and tax revenues. This

proposal will develop and test a novel technology platform and a scaled-up prototype to mass produce stem cells for clinical application 慳潰口濼獩桔

Review Summary

Proposal Synopsis
This proposal addresses a key bottleneck in generating stem cell-based therapies-namely, controlling the cell lineage during the differentiation process. Failure to accurately control this process could lead to the generation of cells which would not be functionally useful or which could proliferate uncontrollably into tumors. One key factor that affects stem cell differentiation is the stiffness of the surrounding environment. The premise of this proposal is that the degree of stiffness may direct the differentiation of inducible pluripotent stem cells (iPSCs) into target cell types. The application proposes the development of a substrate comprised of nanofiber mats of which the stiffness can be changed in response to electrical currents. The substrate will be optimized for biocompatibility and integrated into a high-throughput system for thorough evaluation. This will be followed by scale-up in a modular set-up, resulting in a novel tool for the screening of mechanical conditions capable of directing the production of target cell types in large numbers. The effects of mechano-modulation on stem cell differentiation will be evaluated in a cell culture system within an electrically tunable environment. The long-term goal of the proposal is to develop a novel scalable technology that will augment current techniques to differentiate iPCSs into chondrocytes and, ultimately, other cell types.

Significance and Rationale
-The differentiation of iPCSs may be influenced by many possible types of variations in substrate, which could influence differentiation. These additional parameters are not adequately considered in the proposal, and reviewers were not fully convinced that variation in stiffness would be sufficient to significantly influence differentiation of a broad number of cell types.

-The ability to dynamically modulate the mechanical properties of a substrate would be very useful in studies optimizing differentiation of a stem cell to a desired lineage. However, it is not clear how universal such a technology would be, so there is a doubt that the proposed research would benefit the stem cell biology field in a broad sense.

-The proposed tool for stem cell differentiation is interesting and novel and reviewers were generally enthusiastic regarding the proposed the materials systems.

-The competitiveness and advantages of this approach over others available or in development was not adequately described.

Feasibility and Experimental Design

-The fabrication of nanofibrous substrate with tunable stiffness is supported with preliminary data and this approach is a strength of the proposal.

-The mechanical properties of the substrate may be dynamically tuned by a number of techniques. It is not clear whether the range of variation in the stiffness of the proposed substrate would be sufficient to induce cell differentiation into the desired lineage. Further, the rapidity of the substrate response claimed but may not be relevant to stem cell applications.

-The integration of the screening platform into a high-throughput system is an important addition. However, the system and its operation were not considered to be well described in the proposal.

-The work considers a combination of soluble and matrix-related cues in a dynamic fashion. However, the proposal does not properly account for the possibility that cells will deposit their own substrates over time, thus, changing key properties of the system.

-The experimental design does not adequately allow dissection of the effects of the system mechanics from the effects of the electrical currents.

-Scaling up of the chemically-mediated cell differentiation processes is challenging. Adding electrically tunable substrates to the systems is likely to be even more challenging and may result in lack of success in the scale-up.

-The design of the optimization experiments is sound.

Qualifications of PI and Team

- The PI of this proposal has a strong background in material science. The PI has a good publication record in the areas of electrospun polymers and cell responsiveness to mechanical stimuli as a trainee.

- The project will be performed by a large research team that includes experts in material science, electronics, cell biology and to some degree, in pluripotent stem cells.

- There was some concern about the lack of expertise on chondrocyte differentiation and characterization.

Responsiveness

- The proposal is responsive to the objective of the RFA. If successful, it may lead to the development of a new tool to direct the differentiation of iPSCs using an electrically tunable nanofiber substrate. This tool could become a useful addition to existing stem cell differentiation systems.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07808: A novel experimental procedure to generate large-scale cultures of human multipotent progenitors

GWG Recommendation: Tier 2
Final Score: 66

Public Abstract (provided by applicant)

The long-term goal of the studies proposed here is to generate large numbers of cells that can regenerate deficiencies in the hematopoietic cell compartments of patients that are immune-deficient. For example, CD4 helper T cells are critical in modulating the immune response against viral and bacterial pathogens. During HIV infection, the CD4 compartment is selectively reduced, suppressing the activity and response of cytolytic CD8 T cells, needed to abolish cells infected with the virus. Pharmaceutical therapies have been developed but they are not consistently effective and multidrug resistant viral strains are increasingly prevalent. Consequently, different approaches are needed to enhance T cell reconstitution. Aging individuals contain large deficiencies in the TCR repertoire caused in part by homeostatic proliferation and consequently do not respond well to vaccination. Again, new approaches are needed to increase the size of the TCR repertoire in patients. In vitro manipulated human dendritic cells are now being explored to tolerize against autoimmune disease or to stimulate anti-tumor responses. However, the number of dendritic cells that can be isolated from patients using current technologies is small. Hence, here we would generate novel experimental tools and strategies to provide new avenues for the treatment of human hematopoietic disease.

Statement of Benefit to California (provided by applicant)

Our studies would provide new tools that can be utilized to generate large numbers of hematopoietic progenitors. This research would directly impact novel approaches that are being developed to apply basic research findings to the area of regenerative medicine. It would permit new avenues for reatment of human disease and help to maintain the position of California as a leader in basic and applied biomedical research.

Review Summary

Proposal Synopsis

The goal of this proposal is to develop methods to generate large numbers of multipotent hematopoietic stem or progenitor cells from human embryonic stem cells (hESC) or cord blood (CB), for use in the treatment of cancer patients and those with compromised immune systems. The applicant will attempt to achieve this goal by

suppressing the expression or inhibiting the activity of a transcription factor that induces differentiation of hematopoietic progenitor cells. This proposal addresses the bottleneck caused by inability to expand human hematopoietic stem cells (HSC) in culture for subsequent multi-lineage engraftment in patients.

Significance and Rationale

- Some reviewers felt that the preliminary data, obtained in a murine system, supported the idea that inactivation of a specific transcription factor would allow unlimited generation of multi-potent hematopoietic progenitors, while others felt that many important details, such as quantitation of the progenitor cells, were missing from the preliminary data.

- Reviewers cautioned that preliminary data obtained using the murine system may not predict results in human cells. Providing some preliminary data on human cells would have strengthened the application.

- There are many others attempting to achieve this goal, with some other methods already being tested clinically. The applicant did not acknowledge the highly competitive state of the field in the proposal.

Feasibility and Experimental Design

- Promoting cell proliferation brings with it the risk of malignant transformation. This possibility is not addressed in the proposal, and is a serious concern.

- Some reviewers expressed concerns that the differences between hematopoietic stem and progenitor cells were not clearly delineated in the application, making it difficult to determine whether stem or progenitor cells would be transplanted.

Qualifications of PI and Team

- The PI has considerable expertise in transcriptional regulation of lymphocyte development, however the team would benefit from greater expertise in stem cell biology.

Responsiveness

- The proposal is responsive in trying to develop methods of expanding human hematopoietic stem cells capable of multi-lineage engraftment.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07813: Magneto-endosymbionts; in vivo translational tools for stem cell imaging and ablation.

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Many types of diseases are currently treated with the transplantation of cells. Soon, diseases such as Parkinson's, diabetes, and heart failure will also be treated by the transplantation of stem cells into the body, also known as "cell therapy." These cells may repair tissues, replace the function of diseased cells, or help attack disfunctional tissue or tumors. Tracking and controlling these transplanted cells is crucial in the overall outcome for a patient. However, it is not always possible to know where the transplanted cells migrate in the body, how long they persist or to easily remove them if something goes wrong. We propose development of a living magnetic particle as a tool that allows tracking of newly transplanted cells and selective destruction if needed. Importantly, our particle undergoes cell division within the transplanted cell, ensuring that the label stays inside the cells of interest. With traditional passive MRI contrast agents, the signal is reduced as cells divide. In this project, these magnetic particles will be examined in multiple types of stem cells, validated in an animal model, and assessed relative to the currently used imaging particles. These magnetic particles are non-invasive, are translatable to the clinic for treatment of stem cell-based diseases and will help guide the development of new cell-based therapy. This technology could have a significant impact on the treatment of many diseases.

Statement of Benefit to California (provided by applicant)

The work described in this proposal has many benefits to the State,, including new technology to track transplanted cells. Transplanted cells that have been labeled with novel particles have a wide variety of therapeutic applications, such as aiding in the treatment of cancer, organ failure, diabetes, neurodegenerative, and orphan diseases, all of which affect Californians in large numbers. We are based in California, thus this work will support current jobs and provide new ones directly and indirectly through the California-based collaborations we have at public and private research institutes and with industry. California businesses could use this technique in basic science laboratories, translational studies, and, ultimately, routine cell-based therapies in hospitals all over the state. Our technology can accelerate progress in many California-based biotechnology companies. With the California Institute for Regenerative Medicine funding, California is already leading the country in stem cell research, and this technology would connect basic stem cell research and the the fields of chemistry,

molecular imaging, hematology, oncology, and immunology. Cell-based therapies are a major player in nearly 2,000 ongoing clinical trials and approximately 60,000 stem cell transplants are performed each year in the field of oncology, indicating a large clinical market for sensitive, specific cell-based labeling tools.

Review Summary

Proposal Synopsis
The ability to track and monitor cells after administration to patients remains a challenge for the development of stem cell-based treatments and the field of regenerative medicine. The goal of this proposal is to develop a method of transferring a magnetic label to cells that would allow visualization over time by MRI of cellular therapeutics after delivery and which also could be used to later destroy the cells using heat, if required. The specific objectives are to further develop the labeling system in multiple cell types, perform toxicology testing with labeled cells, and validate the methods to track and destroy labeled cells in vivo.

Significance and Rationale
- The development of a non-invasive, clinically-relevant tool that allows the tracking and long-term monitoring of cells after administration would be of broad value to the field of regenerative medicine and the development of cellular therapies.

- Current methods of labeling cells for imaging are limited due to a common requirement for genetic modification of the cells, progressive signal dilution with cell division, and the potential that the label itself could alter the biology of the manipulated cell. The proposed labeling method, if successful, would minimize the dilution of signal over time and would not require genetic modification of the cells. However, doubt that a self-replicating label can be achieved, that the iron load may prevent high quality MRI images, and that iron accumulation might be toxic to cells limit potential significance of the proposed work.

- If the technology were able to provide a mechanism to selectively eliminate transplanted cells, this would be significant. However, reviewers were concerned that the proposed methods for ablation were not sufficiently specific and could damage nearby cells and host tissues.

Feasibility and Experimental Design
- Reviewers had multiple concerns with respect to the cellular ablation component of the technology. Insufficient data were presented to support the ability of the label to selectively damage target cells when stimulated or to describe the effects of the ablation technology on a migratory/widely-distributed cell population. In addition, it was unclear whether the ablation methods proposed would damage surrounding tissue via a significant bystander effect.

- Reviewers felt that the application did not sufficiently address the immunogenic potential of labeled cells, or the possibility that the high levels of label that would need to accumulate in a cell to facilitate the proposed ablation method could be toxic to the cell.

- Reviewers considered the preliminary data that demonstrated the ability to deliver and monitor the label in adherent human and murine cell types a strength of the proposal and appreciated that in the proposed studies, the new technology will be compared to results obtained using existing technologies.

- Reviewers were concerned that the clinical feasibility of adopting this method for labeling cells would be low, as patient MRI scans were likely to provide inadequate resolution of the intracellular label, allowing only large accumulations of cells with poorly defined borders to be detected on imaging.

Qualifications of PI and Team

- Reviewers assessed the team as having appropriate capabilities to conduct the proposed research but suggested that adding expertise in magnetic stimulation of cells could strengthen this component of the proposal.

- The team is spread among a number of locations, which could complicate sharing tools and materials. It is unclear how the multiple sites will be managed to allow the studies to progress as projected.

Responsiveness

- The proposal is responsive to the RFA because it addresses an imaging hurdle in stem cell research and the proposed approach could be broadly applicable to many therapeutic areas and cell types.

- The applicant provided a clear plan for how the technology would be made accessible to the stem cell research community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07832: Survival and Function of Individual Stem Cells Measured Longitudinally in Small Animal Model In Vivo

GWG Recommendation: Tier 2
Final Score: 66

Public Abstract (provided by applicant)

Lack of information about the survival and function of individual transplanted stem cells constitutes a major bottleneck to the application of stem cell therapies to neurodegenerative diseases. We have created a state-of the art laboratory for non-invasive imaging of the structure and function of individual cells in the eyes of live small animal model. This imaging technology, paired with the rich toolbox of "optogenetic" probes (genetically programmable fluorescent proteins) available for characterizing the morphology and function of different cell types, makes the eye an ideal organ for tracking the fate of neurological therapeutics. This research will overcome the bottleneck, tracking the survival of individual transplanted stem cells in a live small animal model over time, determining how the stem cells integrate into the tissue, and how they function in relation to the nearby neurons and vasculature. The eye, as a transparent window into the central nervous system with its own imaging optics, provides a unique opportunity to study the fate, form and function of individual transplanted stem cells.

Statement of Benefit to California (provided by applicant)

California has become a national leader in the development and application of stem cell therapeutics. This research will for the first time in living mammals non-invasively track the survival, morphology and function of individual transplanted human-derived stem cells in a portion of the central nervous system. Using state-of-the art imaging technology that enables visualizing single cells in a live small animal model, the research will provide a means of efficiently testing many aspects of stem-cell based neuronal therapeutics, and build a platform for visualizing stem-cell therapeutics applied to many ocular and systemic conditions with ocular manifestations. The research will provide quantitative, visually accessible information about stem cell therapeutics to the California stakeholders of stem cell research.

Review Summary

Proposal Synopsis
This application addresses a major bottleneck in the clinical translation of stem cell therapies: a lack of adequate tools for tracking the survival and function of transplanted stem cells over time. Towards this end, the applicant proposes to improve the resolution of an existing imaging platform, thereby enabling individual stem cells to be noninvasively monitored after transplantation into the living eye. The goals of the project are to quantify morphology, survival, and tissue integration of transplanted stem cells at single cell resolution, to establish an animal model system for testing the technology, and to evaluate the ability of stem cells to repair tissue damage in this model.

Significance and Rationale
- If successfully executed, the ability to noninvasively image individual transplanted cells over time would be highly significant.

- Similar instruments are in use clinically, but they do not allow resolution at the cellular level.

- Reviewers thought that some aspects of Aim 1 lack novelty, as the applicant had previously conducted similar experiments with another cell type that showed long-term viability without detrimental effects. Additional studies with stem cell types that are not expected to incorporate into the ocular surface (due to lack of injury) were considered to be of limited value.

Feasibility and Experimental Design
- In the preliminary data, administered cells appeared clumped, which limits the ability to track single cells longitudinally, the major goal of this proposal. Moreover, there is little clear evidence provided that the imaging technique can distinguish dead cells from viable ones.

- The proposal does not adequately address several factors that might confound ability to quantify cell survival, such as the possibility of surrounding cells taking up reporter tags leaked from dead cells or released from exosomes, or how cells migrating from peripheral parts of the field to the central part would affect counts.

- Reviewers thought that the cell image provided in the preliminary data depicted a generally quite large cell type, and they questioned the current capabilities of the technology for visualizing other cell types of interest that are typically much smaller.

- Reviewers did not believe the proposed techniques would be effective in deeper parts of any tissue and would therefore limit the use of the technology to specific regions.

- The use of an immune deficient animal model may limit the ability to assess inflammatory responses to stem cell transplantation and its affects on the imaging technology.

Qualifications of PI and Team

- The principal investigator, who was recognized as having the necessary expertise and experience to oversee the proposed studies, has assembled an excellent team of collaborators.

- A concern was raised that the surgical experiments appear to rely on a single member of the team, which could be a limitation.

Responsiveness

- The proposal addresses a key bottleneck in stem cell technology in that better tools are needed for tracking the survival and function of transplanted stem cells, which is highly responsive to the RFA.

- The proposal does not include a concrete plan to disseminate the findings or share the technique with the research community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07836: Multivalent growth factor conjugates for improved efficiency of stem cell expansion and differentiation

GWG Recommendation: Not Recommended for Funding
Final Score: 64

Public Abstract (provided by applicant)

Biomanufacturing of stem cell products is fundamentally different from the manufacturing of vaccines and biologics, since in stem cell biomanufacturing the cells are the product. Most of the CIRM-funded research to date has focused on the upstream steps of the biomanufacturing processes such as stem cell isolation, culture conditions for cell maintenance and expansion, and this productive research has yielded viable solutions, but at significant per-patient costs. For example, we are part of a CIRM-sponsored collaboration to develop a stem cell-based engineered heart tissue, which will be used to for treatment after an infarct. For this device to be effective, approximately one billion stem cells are required to achieve sufficient cellularity in the graft. A substantial component of the overall cost for this type of stem cell treatment is due to the chemical reagents that are used to direct the stem cell to replicate and to differentiate into the specialized cell types that can provide effective treatment. My laboratory has developed methods to engineering these reagents to increase their potency. As a result, we can reduce the amount of these reagents that are required for stem-cell bio manufacturing to as little as 10% compared to the amounts that are currently required. Therefore, we anticipate the findings of this project should dramatically reduce the costs associated with stem cell therapy and to improve the access to these therapies for patients who need them.

Statement of Benefit to California (provided by applicant)

Tens of thousands of Californians suffer from diseases that CIRM researchers are currently developing stem-cell based therapies as effective treatment options. Despite the clinical successes achieved using stem cells, access to these new therapies are currently limited by the high cost associated with stem cell biomanufacturing. In this project, we will specifically address the costs associated with the growth factors used to promote stem cell expansion, differentiation and functional recovery after cryopreservation. By engineering growth factor conjugates that are approximately an order of magnitude more potent that unconjugated growth factors, we will dramatically reduce the amount of these reagents that are required, as well as their associated costs. Therefore, we will lower a critical price-barrier that currently exists and currently limits the wide-spread clinical use of emerging stem cell therapy breakthroughs.

Review Summary

Proposal Synopsis
This proposal aims to address two bottlenecks in the development of stem cell-based therapeutics: the large expense of manufacturing stem cell products at a clinically relevant scale; and the decreases in functional capacity that can occur in these products after cryopreservation. To meet these objectives, the applicant proposes to investigate the impact of growth factor (GF) conjugates on key steps in the growth and differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs). He/she argues that such conjugates can be engineered to exhibit equivalent bioactivities as their typical, unconjugated counterparts at much lower concentrations, thereby reducing the amount of GF needed for the various stages of cell processing and dramatically lowering the costs associated with manufacturing hESCs and iPSCs for cell-based therapies. Research objectives are to 1) develop and characterize a specific GF conjugate system for expanding hESCs and iPSCs in suspension culture; and 2) evaluate the cost-effectiveness of using GF conjugates to differentiate hESCs and iPSCs to cardiomyocytes, a critical cell type that is lost after heart attack.

Significance and Rationale
- Developing more efficient and cost effective methods for generation and expansion of hESCs and iPSCs is highly significant. If successful as envisioned, the project could have major impact.

- The rationale for improving cardiomyocyte survival after cryopreservation with the proposed GF conjugate is not well supported by the preliminary data. Moreover, reviewers were skeptical that varying GF concentrations would lead to mature cardiomyocytes.

- Application of the proposed technology to a suspension cell culture system is novel.

- Concerns were raised that extending the conjugate technology to a cardiac differentiation procedure that requires multiple media components or exchange steps could render the overall process quite expensive for further clinical applications.

Feasibility and Experimental Design
- While there were strong preliminary data supporting the utility of GF conjugates in other contexts, reviewers were doubtful that this information could be directly extrapolated to the proposed system for cardiac differentiation and thus questioned the overall feasibility of Aim 2.

- The proposed time frame is reasonable, and the investigators have ready access to needed reagents, protocols, and an established large scale manufacturing technology.

- Reviewers suggested that if the proposed technology is to be widely useful, it should be tested with additional systems/protocols for producing cardiomyocytes.

Qualifications of PI and Team

- The PI is a renowned, established investigator with a successful track record in the areas of biomaterials, stem cell differentiation and the cardiac lineage.

- The assembled team of collaborators provides outstanding, complementary expertise.

Responsiveness

- The proposal aligns with an RFA objective aimed towards reducing the costs of human stem cell manufacturing processes.

- Reviewers perceived the overall proposal as a series of small projects that were pieced together to create a responsive proposal.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07838: Development of a scalable, practical, and transferable GMP-compliant suspension culture-based differentiation process for cardiomyocyte production from human embryonic stem cells.

GWG Recommendation: Tier 2
CIRM Recommendation: Recommended for Funding
Final Score: 72

Public Abstract (provided by applicant)

As ongoing CIRM-funded development of regenerative medicine (RM) progresses, the demand for increasing numbers of pluripotent stem cells and their differentiated derivatives has also increased. We have established a scalable suspension culture system for the production of large quantities of hESC for banking and to seed production of a number of regenerative medicine cell types, notably retinal pigmented epithelia, neural stem cells, dopaminergic neurons and cardiomyocytes, that support a number of CIRM and NIH-funded groups. In addition, we have adapted this system for the suspension production of several hESC derivative cell types, notably cardiomyocytes. While our system has provided unprecedented production capability for a number of cell products in pre-clinical and imminent clinical studies, it has proven impractical to scale up to the level that will be required for clinical trials for some hESC cell products, notably cardiomyocytes, due to high expected human doses. This project will resolve this scale-up challenge by adapting our suspension cell culture system, that is limited to 1-3L spinner culture flasks, to a more readily scalable and controllable suspension bioreactor system that utilizes “bags” capable of volumes up to 500L. Achieving this objective will remove a key barrier to progressing RM for cardiac applications as well as open the door to large clinical trials and commercialization of other regenerative medicine cell products in the years to come.

Statement of Benefit to California (provided by applicant)

We have developed GMP-compliant suspension cell culture processes for scalable production of hPSC and derivatives. These processes have been invaluable in our support of CIRM- and NIH-funded regenerative medicine projects, including those with RPE, NSC, DA neurons and cardiomyocytes (CM), as well as for production of GMP banks of hPSC for various projects. Our GMP-compliant suspension culture CM production process has made pre-clinical animal studies and small early clinical trials practical. However, while our current CM system is readily transferred to other groups and is meeting current production requirements, the scale requirements for anticipated high dose clinical trials is beyond the practical limitation of our spinner flask-based system.

hPSC and CM are sensitive to changes in shear encountered at every scale-up step and re-optimizing conditions at each step is prohibitively expensive. Our experience using bag-based bioreactors for non-hESC products suggests that scale-up in bags will be more controllable and predictable than spinners or stir-tanks reactors. It is also a readily transferred technology. We propose to adapt our suspension hPSC and CM processes to a bag system, optimize conditions at a small scale, then demonstrate scalability at a moderate scale. Success in this project will remove a key barrier to developing many regenerative medicine products, and in particular those where high human doses are anticipated, such as CM.

Review Summary

Proposal Synopsis
An existing cell production method is able to produce large quantities of human pluripotent stem cells (hESC) and their differentiated derivatives, but is unsuitable and impractical for scale up to the level that will be required for clinical trials for some hESC-derived cell products such as cardiomyocytes, due to high expected human doses. The proposed project will address this scale-up bottleneck by adapting the existing suspension cell culture system to a more readily scalable and controllable bag bioreactor system. The applicant will optimize a number of critical parameters in order to provide a Good Manufacturing Practice (GMP)-compliant platform for the large-scale propagation of hESC-derived cardiomyocytes for clinical applications.

Significance and Rationale
- Reviewers agreed that this proposal directly addresses a critical bottleneck in the field. However, there was disagreement regarding the degree of impact the proposed technology would have if successful.

- Reviewers thought that the rationale for the approach is logical and scientifically sound.

- Some concerns were raised over the lack of discussion of the perceived advantages of the proposed method compared to other GMP-compatible methods for manufacturing hPSC-derived cardiomyocytes currently in use.

Feasibility and Experimental Design
- The aims of the research are logical and the experimental plan is carefully designed and should yield meaningful data. All of the necessary resources appear to be in place.

- Reviewers generally thought the proposed research to be supported by the strong preliminary data and the prior successes of the PI in achieving scale up of pluripotent stem cells and differentiation of hESC to cardiomyocytes.

-Some reviewers were unsure if the proposed bag bioreactor would indeed address several specific limitations of the existing method and thought additional preliminary data in this area would have strengthened the proposal.

- Some concerns were raised over a lack of discussion regarding potential pitfalls and alternative strategies.

Qualifications of PI and Team

- The PI and team are well-qualified and have prior success in developing suspension culture systems

- The PI has extensive expertise in GMP-compliant cell manufacturing processes.

-The proposed collaborations are supportive of the project.

Responsiveness

- The proposal, which utilizes human pluripotent stem cells, was viewed as relevant and responsive to the RFA.

- A plan is in place to make both the methodology and the cells available to the stem cell community.

-Some reviewers expressed some reservation that the proposed scale up is very product specific.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07841: Advancing Stem Cell Replacement Therapies through Precision Single-Cell Profiling

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

To overcome well-founded safety concerns regarding pluripotent stem cell replacement therapies (CRTs), this CIRM Tools & Technologies project provides never-before-realized precision in quality control of any CRT product. While a population of replacement cells may appear to be uniform, issues with variation among the cells still plague our development efforts. Cell-to-cell variation leads to contaminating cells that can result in clinically devastating effects, such as tumor formation. Our present approach to flagging contaminating cells early in protocol development relies on limited information, primarily on lineage markers. But much information regarding cellular decision making is not in these markers, but locked inside the cell in intracellular protein signaling - the decision logic of a cell. To add intracellular "decision state" to our understanding, we propose to develop a new single-cell protein measurement tool for rigorous intracellular characterization during CRT process development. The deep cell profiling will guide protocol optimization, to maximize production of desired cells. Rational, informed optimization of the protocols to create cell replacement therapies will reduce the chances of later adverse side effects in patients, thus surmounting an epic bottleneck to clinical advancement of cell replacement therapies.

Statement of Benefit to California (provided by applicant)

The proposed CIRM Tools & Technologies project will benefit California from accelerating clinical stem cell therapies, to bolstering industry, to ensuring our research universities are on the cutting edge of research & training for the next-generation of stem cell researchers & patients. The studies will contribute novel & needed analysis tool for ultimately controlling the generation of safe & suitable stem cell replacement products. Success in the research will advance development of safe & effective cell replacement therapies, reduce long-term neurodegenerative tolls by moving towards new cell replacement options, catalyze commercialization of intellectual property, and bolster the nascent biological manufacturing sector and job creation by considering GMP compliance at an early research stage. The research will contribute to California's pioneering role in stem cell research for clinical impact by establishing world-class fundamental biological and advanced engineering expertise in California and by training of the next cadre of California's innovation leaders, thought leaders, and business leaders. Outcomes will directly support a mandate levied by the voters of California to

support stem cell research towards the development of cures to benefit patients. While developed here for neurodegenerative conditions, the tool will be designed for wide applicability and dissemination to research & manufacturing centers.

Review Summary

Proposal Synopsis
This proposal is focused on a single cell protein profiling (proteomics) technology developed by the applicant, and its use for improving cell maturation (differentiation) protocols to maximize production of desired cell types from stem cells. It is suggested that this type of approach will enhance stem cell replacement therapies by allowing the creation of cell populations free of undesired cell types. The investigators propose to profile markers of differentiation and signaling pathways and other factors as cells progress from stem cell to dopaminergic neuron fate, and to use the information gained to optimize differentiation protocols.

Significance and Rationale
- The development of a tool to more efficiently optimize protocols for the generation of specific cell types could be of significant value. However, the single cell proteomics tool already exists, and only minor tool improvements are proposed.

- The development and use of single cell proteomics technologies is a very important goal for the stem cell field.

Feasibility and Experimental Design
- The reviewers felt that some demonstration should have been provided as to how competitive the proposed technology is in terms of throughput and cost in comparison to other single cell proteomics tools that are currently available.

- It remains unclear as to whether this tool is sensitive enough to achieve the goals outlined by the applicant.

- The reviewers were unconvinced that the proposed analyses will yield relevant information useful for protocol optimization, especially since analysis of authentic primary dopaminergic neurons is not included.

- There is no mention of how many cells will need to be analyzed to achieve the goals of the study, and there is no indication that large numbers of cells can be analyzed by the methods proposed.

- A reviewer acknowledged that this proteomics technology is unique in its ability to provide some, albeit limited, subcellular information.

Qualifications of the PI and Team

- The PI and the Co-PI have the expertise to complete the studies.

Responsiveness

- The responsiveness of this application to the RFA is unclear. The technology is so general that it is not specific to stem cells. However, its application to stem cell research is very desirable.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07848: Site-specific gene editing in hematopoietic stem cells as an anti-HIV therapy

GWG Recommendation: Recommended for Funding
Final Score: 81

Public Abstract (provided by applicant)

The overall goal of this proposal is to develop new methods and technologies to improve our ability to engineer hematopoietic stem cells. These are the adult stem cells found in the bone marrow that give rise to all of the components of the blood and immune systems. Being able to engineer these cells provides potential treatments for diseases of the blood including genetic diseases, such as sickle cell disease or severe immune deficiencies, as well as serious infections such as HIV/AIDS. We work with a new class of genetic engineering tools called targeted nucleases that have the potential to make genetic engineering of stem cells much more precise and therefore safer. In addition, we are exploring methods to deliver these reagents directly to the stem cells in the body, without the currently necessary steps of first removing the cells and performing the genetic engineering in a lab. Such capabilities would greatly improve the safety of human gene therapy, as well as facilitate its practical implementation.
HIV/AIDS is our disease of focus, and we will use these techniques to develop new treatments that go beyond the current use of targeted nucleases in patients, where HIV's co-receptor gene, called CCR5, is being disrupted. Our goal is to develop a next-generation of anti-HIV therapies and we expect that the techniques we develop will be broadly applicable to other disease of the blood and immune systems where stem cell therapies could be of benefit.

Statement of Benefit to California (provided by applicant)

HIV/AIDS is a major social, economic and health burden to California and its citizens. The numbers are sobering: California has 14% of all US cases of HIV, second only to New York, with 220,543 cases reported through June 2014, including 98,161 deaths. With the advent of improved antiretroviral drugs, mortality has significantly decreased, but so has the length of time people need to take the drugs, and the economic burden to the state is revealed by the cost of drugs representing 85% of all AIDS-related costs. Both federal and state laws require that the AIDS Drug Assistance Program be the payer of last resort for these medications, and its budget is underwritten by the General Fund. Beyond the fiscal concerns, patients live with the potential for developing side effects to the drugs or drug-resistant virus, and accessing these life-long drug regimens is a daily struggle for many. Consequently, the development of stem cell based therapies for HIV brings the potential of one-shot and long-lasting treatments that could arm a patient's

own immune system with the capability to suppress HIV in the absence of drugs. Such an outcome would provide economic returns over the long-run by reducing spending on drugs, as well as improving the quality of life for individuals with HIV/AIDS. Beyond HIV, the development of technologies to improve the efficiency, safety and implementation of hematopoietic stem cell therapies will benefit other diseases where such cells could be curative.

Review Summary

Proposal Synopsis
This proposal addresses a major bottleneck in the use of stem cell-based therapies—that is, in performing in vivo site-specific gene editing. The investigators propose to utilize a technology for gene editing with which they have had success in vitro. The work proposed is centered predominantly on optimizing both the genes and the gene delivery vehicle for efficiency and specificity of gene modification of hematopoietic stem cells (HSCs). The principal investigator (PI) proposes to achieve site-specific editing using a specialized class of targeted gene editing reagents that have the potential to make genetic engineering of stem cells much more precise and therefore safer. Once the PI and team have optimized the design and delivery of targeted genes to achieve increased efficiency of gene-editing of HSCs in vitro, they will then test their optimum reagents in vivo using a humanized small animal model. The focus of the proposed work is to develop technology to address challenges in gene editing in HSCs. In addition, the proposed research will advance development of human immunodeficiency virus (HIV) therapies based on altering the HSCs in such a way as to make them resistant to the virus. If successfully developed, the proposed technology poses a potential functional cure to HIV, and the approach could potentially be applicable to the treatment of a variety of blood diseases.

Significance and Rationale
- The plan of work addresses some significant challenges that exist for clinical translation of gene editing in HSC. If successful, the work would be highly significant for the field.

- The proposal addresses the current inefficiencies of site-specific gene editing methods and aims to use precision gene editing in HSCs to develop new anti-HIV therapies. However, if successful, the technology has the potential to be broadly applicable to other diseases of HSCs that could be cured by successful gene-editing.

- The choice of vector technology is well justified and appropriate.

Feasibility and Experimental Design
- All the proposed aims were clearly delineated, well- reasoned, and supported by extensive preliminary data.

- The team has already developed highly efficient methods to introduce targeted genes into HSCs and has experience in the use of the target DNA editing reagents for site-specific editing.

- Little attention was paid to off-target gene-editing with the nuclease technology proposed or to off-target uptake by vectors in vivo, particularly since higher efficiencies of uptake might actually increase off-target effects.

- The PI assumes that viral tropism will predict in vivo targeting, which is a minor weakness of the proposal.

- The PI has already established collaboration with a biotech firm that has agreed to provide them targeting reagents with tested specificity for human HSC in vitro.

Qualifications of PI and Team
- The PI is well-known for HIV studies and work with HSCs and has assembled important collaborations with a company that will provide vectors.

-The team overall is small with only the PI and some postdoctoral fellows to perform the work.

Responsiveness
- The application was deemed responsive to the RFA.

- Although the company provided a letter stating it will provide vectors, it was not clear whether or how such vectors might be distributed to the scientific community if the work proved successful.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07851: Development of an optimized stem-cell-seeded xenogeneic extracellular matrix construct and delivery system for cardiac repair following myocardial infarction

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Heart attack results in 1 in 6 deaths in the US. Although patients benefit from initial treatment, they commonly develop chronic heart disease, with scarring and degeneration of heart muscle cells, and ultimately death. Advancement of promising regenerative medicine therapies into clinical practice is currently hindered by lack of appropriate animal models in which to test these potential cures. This proposal aims to develop a novel large animal model of heart attack which will for the first time allow for accurate assessment of novel treatments for heart attack related chronic heart disease. Previous heart attack models result in high death rates and extremely variable heart lesions, which prevent accurate assessment of potential treatments. Our team has developed a consistent reproducible large animal heart attack model, which overcomes all of the limitations of previous models. We aim to utilize this model to firstly identify novel blood borne markers of heart muscle cell degeneration. Such a marker will allow for more accurate assessment of patients following heart attack, and also provide a sensitive method for assessing novel treatments in the large animal model. Finally, our group has developed novel animal derived biomaterials which have the potential to dramatically increase healing following heart attack. We aim to test the ability of these stem cell seeded animal derived biomaterials to improve heart healing and function following heart attack.

Statement of Benefit to California (provided by applicant)

Center for Disease Control statistics indicate that myocardial infarction (MI) is the leading cause of morbidity and mortality in the US, accounting for ~100,000 deaths per year in California alone. Lack of an appropriate large animal MI model has hindered clinical translation of novel regenerative medicine therapies. This proposal aims to develop a large animal model of MI, overcoming limitations of current models and providing California with an important recourse for future testing of regenerative medicine MI cures. Furthermore, we aim to utilize this model to identify novel microRNA biomarkers which correlate with post-MI cardiomyocyte pathologic gene reprograming and progression to heart failure. Such a biomarker would provide California with an important tool for monitoring of post-MI disease progression and therapeutic interventions in both animal models and clinical patients. Finally, our team

has developed novel patent-pending technology for antigen removal (AR) from animal-derived tissues. Our team's preliminary data indicate that mesenchymal stem cell (MSC) seeded extracellular matrix (ECM) scaffolds produced using AR have significant advantaged over traditionally decellularized scaffolds. Such MSC/ECM constructs are expected to exhibit significant regenerative potential in the porcine MI model. We anticipate technology transfer to an existing California-based start-up company, providing further benefit to the state through commercialization of our AR method.

Review Summary

Proposal Synopsis
A preclinical large-animal model of myocardial infarction (MI) is proposed. The goal of the model is to produce low mortality, reproducible, and clinically-relevant MIs. The applicant also proposes to apply this model to detect novel biomarkers for MI. Finally, the team propose to develop antigen depleted xenogeneic scaffolds for cell delivery in order to both reduce antigenicity improve regeneration in their MI model.

Significance and Rationale
- Reviewers concurred that development of a large animal model for MI is not a critical bottleneck; alternative large models in the selected species exist and a direct comparison to these models was not provided.

- Some reviewers agreed that generating antigen free xenogeneic novel biomaterials addresses an important bottleneck.

- Identification of novel MI biomarkers might be an important tool, and could potentially serve as a potency assay for cell therapy, though this was not perceived to be a major emphasis of the proposal.

Feasibility and Experimental Design
- The aims are logical and achievable. Much of the preliminary data support the feasibility of the study.

- It was noted that though the proposed model is reported to deliver a more consistent injury, no data are provided showing its improved reproducibility over standard models.

- Ischemia reperfusion injury is more clinically relevant than the proposed MI induction method.

- The planned method for tracking specific cells in vivo may be complicated by background signal (noise) in host tissue, particularly in the heart.

Qualifications of PI and Team

- The PI is productive and has appropriate surgical and other expertise to conduct the project.

- The team is well qualified and has committed the appropriate effort to complete the proposed work.

- The environment and collaborations were found to be good.

Responsiveness

- Reviewers did not agree that the novel large animal model addresses a significant bottleneck in the field.

- Development of antigen-depleted scaffolds is responsive.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07855: Neuronal Precursor Cell Therapy in Large Animals: Delivery, Dosing, Safety, and Efficacy

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

To realize the full potential of neural stem cell therapies, bottlenecks must be overcome, including delivery of cells into the nervous system at an optimal dose that is both safe and effective. We will evaluate novel cell delivery devices, establish a tolerable dose, and assess safety and efficacy of transplanted stem cell-derived human nerve cells. Large animal models will be studied because transplanting cells in a large and more complex brain will allow us to investigate bottlenecks to stem cell therapy in humans that cannot be addressed in smaller animal models. We will test two clinically compatible delivery devices and will compare cell survival, distribution, and needle tract size among other measures. We will also determine the optimal dosing range for cell delivery in adult large-brain mammals, a critical step toward a clinical trial in humans, and will assess long-term safety including absence of tumor formation and immune tolerance among other outcome measures. In collaboration with an Australian team, we will test inhibitory nerve stem cell therapy for efficacy in suppressing seizures in a primate epilepsy model. The specific aims of our proposal are essential steps in preparation for an eventual human trial.

Statement of Benefit to California (provided by applicant)

Our proposal will determine optimal parameters for neural stem cell-based therapy (intra-cranial delivery, dosing, and safety measures) that may translate to more effective treatments for patients with a broad range of disorders, such as epilepsy, Parkinson's disease, Alzheimer's disease, stroke, and multiple sclerosis, among others. In addition to developing parameters to aid cell therapy in the human brain in general, we propose enabling studies leading toward the use of cell grafts of inhibitory interneurons for epilepsy therapy. There are an estimated 1,000,000 individuals in the United States who currently live with disability associated with medically refractory focal seizures. As the country's most populous state, California has the largest number of patients with intractable epilepsy (approximately 120,000). The potential savings in medical care costs, and improvement in quality of life will therefore have a disproportional benefit to the state of California. The estimated economic cost to California in lost productivity and medical expenses from patients with medically refractory epilepsy amounts to $1.2 billion annually. Moreover our proposal will provide key data on cell delivery, dosing, and safety measures that will facilitate cell therapy for a wide range of brain disorders

for which there is currently no cure and which take an enormous economic toll to California.

Review Summary

Proposal Synopsis
This application is focused on two issues that relate to moving stem cell therapies from the laboratory to the clinic for treating neurological diseases - intracranial cell delivery and predictive animal models. First, the applicants propose to assess two recently designed clinical-grade intracranial delivery systems. The investigators will use the new delivery systems to transplant human neuronal precursor cells into two large animal models and will assess optimal dose, safety, and efficacy of the cells. Second, the California team will collaborate with an international team to test inhibitory neuronal precursor cell therapy for efficacy in suppressing seizures in an animal model of epilepsy. The investigators will optimize inhibitory cell delivery, define tolerable dosing, perform long-term safety studies, and evaluate efficacy in an animal model of stroke-induced focal epilepsy.

Significance and Rationale
- This application proposes to assess two intracranial delivery systems that have already been developed for delivery of cells into animals with larger brains. It was unclear to reviewers that whether the application was focused on device development or if the application was focused on the next stage of research for the epilepsy cell therapy project.

- Reviewers noted that the delivery device criticized by the applicant is widely used and works well, which reduces the overall significance.

-There is a strong rationale for pursuing this inhibitory neuronal precursor cell therapy.

- Reviewers noted that one of the species proposed is not that much larger than a rodent and does not provide value in terms of scale-up.

Feasibility and Experimental Design
- Reviewers questioned the value of the large animal studies proposed in Aim 1 and whether they will sufficiently inform those proposed in Aim 2, with respect to immunosuppressive regimen and/or dose extrapolation.

- Reviewers noted a deficiency of details on seizure phenotypes (on which the therapeutic experiments rest) presented, and that there are no publications on seizures in this animal model.

- There was concern regarding the lack of discussion of logistics to deliver the cells from California to the location of the international team for the proposed collaborative studies.

- All methods to identify and differentiate the cells have been developed.

- The delivery devices are developed.

Qualifications of PI and Team
- The team is excellent and well-qualified to perform the studies proposed.

Responsiveness
- Reviewers questioned this application's responsiveness to the RFA. They noted there are no tools or technologies being developed and it is not clear that testing the existing devices addresses a translational bottleneck.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07859: 3D Bioprinting for Stem Cell Delivery

GWG Recommendation: Not Recommended for Funding
Final Score: 61

Public Abstract (provided by applicant)

3D bioprinting is an emerging technology that can print cells with the potential to generate live functioning bioartificial tissue. As a demonstration of the wide range of applications for bioprinting in regenerative medicine, we aim to explore the utility of a platform to print stem cells in an organized manner to overcome existing deficiencies in cell based therapies for joint injury and arthritis, and retinal diseases.

In Aim 1 we will develop a platform for 3D biofabrication. We will deliver several types of cells including human embryonic and pluripotent stem cells in printable bio-inks made from naturally occurring components of tissue.

In Aim 2 we will develop a technology for printing layers of retinal tissue. We will engineer the spatial pattern of retinal ganglion cells which are cells that conduct the signal from the light receptors in the retina to the optic nerve and the brain. We will assess the ability of printed cells to integrate with retinal tissue that has lost these ganglion cells.

In Aim 3 we will develop a technology for directly printing cells in live knees

We will directly print cells in a cartilage defect created in the femoral condyle of a large animal model. We will assess cell viability; mechanical properties; microscopic structure and integration of regenerated tissue into host tissue.

Successful achievement of our aims will lead to novel stem cell therapy for arthritis and retinal diseases such as glaucoma.

Statement of Benefit to California (provided by applicant)

The proposal will likely result in the clinical application of tissue engineered products from stem cells.

Over 10% of Californian residents suffer from some form of arthritis, the most common being osteoarthritis. This number will increase dramatically as the entire population in general and the “baby boomer” segment in particular, ages. The socioeconomic burden of medical treatment combined with lost productivity is close to $20 billion a year in

California alone. Specifically, a biological replacement for arthritic joints will address the existing weaknesses and complications of traditional joint replacement with artificial biomaterials. A biologic replacement would also allow younger patients to benefit from this surgery and dramatically reduce the number of complications from artificial replacement.

Retinal diseases affect all ages of Californian residents. Retinitis pigmentosa affects children and young adults, diabetic retinopathy strikes in middle-age, while older individuals suffer from retinal vessel occlusions, glaucoma, and age-related macular degeneration. The latter two affect millions of people worldwide and ultimately lead to retinal cell death and blindness. Transplantation of the ganglion cell layer is complex requiring guidance of axons into the optic nerve head. Printing stem cell-derived retinal sensorineural cells to enhance visual function will be present a major step advance towards functional stem cell replacement therapy.

Review Summary

Proposal Synopsis
This proposal describes a study that will utilize 3D bioprinting of i) stem cells derived from human embryonic stem cells as a therapy for joint injury and ii) retinal ganglion cells in an ex vivo small animal model of degenerated retinal tissue. The research team will optimize stem cell bioprinting conditions with multiple biomaterials (bioink) laid down in an organized pattern. They will use this information to integrate retinal ganglion cells that have been bioprinted using a scaffold. They will then assess the appropriate spatial implantation of the cells into ex vivo cultures of retinal tissue from small animals. Finally, they will perform in vivo bioprinting of derived stem cells into defective knee joints in a large animal model.

Significance and Rationale
- Reviewers thought the aims were disconnected and not well focused because of the proposed use of two different cell types.

- It was unclear what advantage bioprinting would have over conventional methods for cell implantation into cartilage tissue. A convincing rationale for utilizing this approach in the two proposed tissues was not presented.

- Retinal and cartilage diseases affect large populations and there is a major unmet clinical need for stem cell based therapies for functional regeneration of these tissues.

Feasibility and Experimental Design
- Reviewers raised concerns over the lack of experimental detail presented throughout the application. The reviewers felt the application was unclear or lacked sufficient details such as the plans to optimize the concentrations of components, the resolution

of the 3D bioprinting, the time required to print, density and architecture of bioprinted retinal cells, and details for maintenance of ex vivo cultures.

- Reviewers were unclear how the methodologies used from cartilage bioprinting would be relevant for the retinal project.

- The in vivo studies were deemed premature. Some concerns were also raised over the lack of justification for using human embryonic stem cells vs. stem cells derived from adult tissue and whether bioprinted cartilage cells would actually implant better in vivo than using current methods. Reviewers also questioned the widespread applicability of in vivo bioprinting, given the advanced set-up required.

- There was a general absence of alternative approaches and statistical evaluation. Experiments will largely be trial and error.

- The proposal was supported by strong preliminary data, in particular the established technical success of bioprinting procedures that are to be utilized throughout the proposed studies.

Qualifications of PI and Team

- Reviewers expressed enthusiasm over the strong collective expertise of the team. There is published expertise covering a broad range of disciplines including stem cells, 3D printing, bioengineering, tissue regeneration, retinal and cartilage biology, and translational applications.

- The feasibility of team collaboration was viewed as a key asset to the success of the proposal.

- Some minor concerns were raised over the perceived lack of expertise with large animal models and the high costs associated with the study. However, investigators have secured additional funds from non-CIRM sources for some of the proposed studies.

Responsiveness

- The proposal was viewed as responsive to the RFA as it addresses methods to improve cell delivery, implantation and long-term survival/function. The proposal also utilizes a preclinical large animal model. However, there was some doubt that new technology would be developed as a result of the studies.

- The proposal utilizes human stem cells, though it is not clear that the investigators require human cells for the studies.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07864: The generation and expansion of fully functional human hematopoietic stem cells by cellular delivery of RUNX1a transcription factor

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Blood stem cell transplantation is a lifesaving cell therapy for many blood disorders, such as aplastic anemia, sickle cell anemia, thalassemia, AIDS, and cancer. One major unsolved bottleneck of human stem cell therapies is lack of sufficient numbers of functional blood stem cells from matched umbilical cord blood cells or from conversion of patients' own induced pluripotent stem cells (iPSCs) to blood stem cells. Many current transplant recipients are suffering from immunosuppressive treatment. Thus, the ultimate goal of the proposed research is the development of reliable methods to get sufficient numbers of therapeutic blood stem cells from donor cells or from patients' own iPSCs. Importantly, iPSCs are much easier than blood stem cells to grow and to maintain stem cell features in cell culture. Therefore, genetic defects of patients can be first corrected by gene therapy in iPSCs without changing their potential to become different types of somatic cells; then, efficient methods need to be established to convert iPSCs to blood cells to treat patients' blood disorders. Here we propose to develop the recombinant cell-penetrating transducible RUNX1a protein and the polycistronic RUNX1a co-expressing srRNA to promote expansion and production of blood stem cells from cord blood and patients' own cells safely and reliably for stem cell therapy. These methods will not create unfavorable mutations since there is no disruption of genomic DNA using these methods.

Statement of Benefit to California (provided by applicant)

Thousands of Californians are suffering from blood-related diseases that may be cured with blood stem cell transplantation and/or blood transfusion. However, these life-saving measures are limited by a lack of eligible donors, the necessity of finding correctly matched blood products, and insufficient numbers of blood stem cells from donors. Current treatments for some of these conditions can cost patients tens of thousands of dollars per year. Despite current treatments, many patients die from their disease waiting for a lifesaving transplant. Furthermore, many transplant recipients suffer from immunosuppressive regimen related complications. We propose to develop novel approaches of using recombinant cell-penetrating RUNX1a protein and the polycistronic RUNX1a co-expressing srRNA to enhance the specificity and efficiency of making therapeutically useful blood cells from umbilical cord blood and from human iPSCs. Therefore, the long-term benefit of the proposed work is to improve the

treatment of thousands of Californian patients who need to receive healthy and functioning blood cells to alleviate their disease conditions, to reduce complication related to transplant rejection and graft-versus-host disease, and to expand utility of cord blood. In turn, this will benefit California's financial status in reducing the cost of treating related patients with expensive yet ineffective methods.

Review Summary

Proposal Synopsis
Stem cell transplantation is a lifesaving cell therapy for many blood disorders. The inability to produce functional hematopoietic stem cells (HSC) from induced pluripotent stem cells (iPSCs) in sufficient quantity is a bottleneck that, if solved, could enable the treatment of many patients with HSC transplantation therapy (HSCT). The efficiency of such conversion and expansion of HSCs has been neither sufficient nor reliable for actual clinical applications. The goal of this proposal is to establish methods for controlling the expression of a transcription factor to enhance the generation and expansion of functional HSCs from human iPSCs without disruption of genomic structure. In order to accomplish this goal, the applicant plans to first develop methods to deliver the recombinant transcription factor, and then to apply those methods to iPSCs and characterize the differentiating iPSCs. Secondly, methods will be developed to co-express the transcription factor with other positive regulators to promote HSC production from the iPSCs. Results from these proposed studies could provide new tools for generating HSCs in sufficient numbers to treat patients that would benefit from HSCT.

Significance and Rationale
- The applicant proposes to utilize novel combination approach for generation and expansion of HSC. If successful, the reviewers expressed support that the strategy could significantly impact therapies requiring HSCs for transplantation.

- The applicants propose to use a nucleic acid based technology to deliver the transcription factor into cells without genomic integration. This was considered as an efficient, versatile and potentially significant method of transducing stem cells although the implications for clinical risk are unknown.

- In order to try to advance clinical translation, the project includes a collaboration to evaluate iPSC-derived HSC in a preclinical animal model. However, this part of the project was considered a weakness in the proposal because of limited data and an inadequate justification/rationale for the inclusion of the proposed preclinical model.

- A concern was raised that the proposal did not provide an adequate rationale regarding how the proposed technique would be superior to current methods for HSC expansion.

Feasibility and Experimental Design

- The reviewers were concerned that even though some technology design work is essential, the testing on HSCs is not planned until year 2. Thus, significant effort could be spent before the investigators know if the transcription factor will work as hypothesized.

- Although the preliminary data was considered to be supportive of the experimental approach proposed, reviewers considered the experimental outcomes to be insufficient and the justification for the effect size weak. Overall, it was agreed that improvements in the statistical basis for sample size determination and statistical analyses to be performed would strengthen the application.

- Quantitative evaluation of testing will be required and there was not enough detail provided on how this will be achieved.

- The feasibility of accomplishing the tasks proposed for the preclinical model was questioned as limited data was presented regarding the engraftment potential of modified HSCs and whether the vector delivery system will apply in this model.

Qualifications of PI and Team

- The proposal includes a strong team of investigators, each contributing relevant scientific expertise.

Responsiveness

- The proposal is targeted to address a significant barrier in stem cell transplantation, which is the availability of sufficient HSCs for transplant. This is highly responsive to the RFA.

- Human iPSCs will be used in this proposal.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07870: Recapitulating the 3D Microenvironment for Directing Vascular Fate

GWG Recommendation: Not Recommended for Funding
Final Score: 62

Public Abstract (provided by applicant)

Vascular endothelial cell (EC) or endothelial progenitor cells (EPC) derived from stem cells are a promising cell source in a number of clinically relevant therapeutic applications. Specifically, these applications include building new blood vessels in dead heart tissue caused by a heart attack, and for revascularization of peripheral limbs aged or diabetic patients. However, specialized arterial-specific EC are perhaps most desirable for lining the lumens of small diameter vascular grafts, known to reduce thrombosis/arteriosclerosis normally associated with replacement grafts. Unfortunately, the arterial specification of EC during vascular development is not well understood and current ESC-derived EC exhibit properties and markers consistent with venous-specific endothelium. The proposed project will address this chasm by exploring the combination of parameters, including mechanical signals like shear stress and material stiffness, in order to direct human stem cells into arterial-specific endothelial cells.

Statement of Benefit to California (provided by applicant)

This project will have many benefits to the state of California. The most important benefit is the contribution to fundamental understanding and development of stem cell-derived products for advancing healthcare in cardiovascular disease (the #1 killer in the state and country). In addition, these studies will train new graduate students and postdoctoral researchers in the stem cell field, contributing to our state-wide expertise in the area. These studies will also spend supply dollars in the state of California, supporting our state's economy. The proposed studies are expected to lead to new products that can be launched into a start-up company. This will bring income into the state of California, as well as, secure more biotechnology jobs in the state.

Review Summary

Proposal Synopsis

This proposal is focused on the development of a technology to differentiate between arterial and venous endothelium. The underlying hypothesis is that arterial-specific endothelial cells will be needed to successfully create tissue engineered vascular grafts

for clinical use. To accomplish this goal, the biological and mechanical signals required to specify arterial endothelium need to be elucidated. The proposed studies will explore the signals that are involved in directing the fate of arterial and venous endothelium. Aim 1 will focus on biochemical and mechanical signaling. Aim 2 will incorporate surface-related mechanical signals that mimic native arteries. Aim 3 will translate the information gained in the first two aims to in vivo studies using silk-based tissue engineered vascular grafts. The applicants suggest that the findings from these studies will generate new tools/technology that will advance health care for those suffering from cardiovascular-related diseases.

Significance and Rationale

- The ability to generate arterial endothelial cells from embryonic stem cells and the characteristics of those cells was not viewed as a major bottleneck to the translation of engineered vascular grafts. The bottleneck to translation of this kind of approach may be more related to getting the cells to adhere to scaffolds.

- The reviewers appreciated the importance of defining the inducing signals that would generate embryonic stem cell-derived endothelial cells to their ultimate fate (i.e., arterial or venous).

- The proposal considers many different signals by which vascular cells respond to their environment. Although these features are important, it was felt that the major advance of the study is lost due to the large number of conditions tested.

- There are too many parameters to be examined without any new technology being introduced. Reviewers did not feel that the scaffold to be used would replicate the properties of native blood vessels, as claimed.

Feasibility and Experimental Design

- A concern was that none of the studies introduce new technology. The technology to be used in this application was developed by collaborating laboratories.

-Some of the experiments proposed, such as the modeling studies, were well designed and appreciated by reviewers. However, some of the methods proposed, such as the method for seeding cells on the scaffold, were not viewed as current with the state of the art in the field.

- The PI proposes to examine biochemical, environmental and mechanical signals that may propagate the formation of arterial-specific endothelial cells but there was not a logical flow of the knowledge gained between the proposed studies.

- The PI did not explain why only changing the topography will change the endothelial cell characteristics towards arterial versus venous.

Qualifications of the PI and Team

- The PI and the team have the necessary expertise in vasculogenesis, stem cells, modeling and biomaterial fabrication to conduct the studies.

Responsiveness

- Reviewers did not feel that the proposal was focused on developing new technology to address a translational bottleneck, and so was minimally responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07879: Multimodal platform combining optical and ultrasonic technologies for in vivo nondestructive evaluation of engineered vascular tissue constructs

GWG Recommendation: Recommended for Funding
Final Score: 76

Public Abstract (provided by applicant)

Current vascular replacement materials are far from ideal, with all available biomaterials exhibiting significant clinical complications. The development of novel biocompatible decellularized vascular grafts holds great promise for functional restoration of vascular tissues suffering from trauma or disease. However, the need for destructive analysis at multiple in-vitro and in-vivo time points creates a costly critical bottleneck in development of such vascular biomaterials and regenerative medicine approaches. We propose to research, test and validate a tissue diagnostic technology combining optical and ultrasound imaging techniques. This platform will enable label-free, real-time, non-destructive analysis of composition, structure, function and site specific cellular repopulation of extracellular matrix of engineered vascular tissue constructs. This technology is expected to alleviate the need for destructive assays across multiple time points, which are costly and frequently impractical. The technology will facilitate (a) in-vitro rapid screening of vascular scaffold production methods; and (b) in-vivo assessment across multiple time points of vascular constructs. This technology can improve our ability to produce functional engineered vascular tissues in the laboratory for in-vivo implantation which can accelerate the integration time of the vascular implant with the surrounding host tissue, thus to contribute to restoring the desired quality of life to the patient.

Statement of Benefit to California (provided by applicant)

Cardiovascular disease is the leading cause of death in western societies (about 1 in 5 deaths); which in combination with the prevalence of peripheral artery disease in aging population (12-20% in individuals >60 years of age) and ischemic stroke due to atherosclerosis of carotid artery make this disease the most prominent health problem in California and in the United States. New therapeutic and diagnostic technologies including advancements in vascular tissue engineering and materials for blood vessel replacement are needed. The proposed multimodal technology has the potential to improve our ability to produce functional engineered vascular tissues in the laboratory and thus to significantly impact treatment for coronary and peripheral artery disease, and to provide solutions for California's citizens greatest health problem. In addition, the global market for vascular grafts and patches is expected to significantly increase

over the next 5 years in both United States and Europe due to the prevalence of cardiovascular disease and increased number of interventional vascular procedures. Since both the imaging technology and vascular materials proposed to be evaluated in this CIRM application have potential for commercialization, advancement of this technology has the potential to contribute to California's economic growth. Moreover, a tool for non-destructive label-free engineered tissue analysis as proposed here can accelerate research in all areas of interest to CIRM.

Review Summary

Proposal Synopsis
The applicant proposes the development and testing of a tissue diagnostic technology combining optical and ultrasound imaging techniques to enable label-free, non-destructive, real time, in vitro and in vivo assessment of the composition, structure, function, and stem cell repopulation and remodeling of engineered vascular tissue constructs. The proposed study allows more efficient screening, development and monitoring of engineered vascular grafts by the eliminating the costly requirement for graft destruction during studies requiring analysis at multiple time points. The outcomes will be clinically relevant for laparoscopic or endoscopic determination of implanted engineered vascular constructs in real time, which may allow clinicians to monitor the early signs of rejection or blockage of the vascular constructs. The research team will develop combined optical ultrasound imaging modalities for analysis of vascular biomaterials and evaluate the ability of their multimodal platform to monitor changes in the properties of the vascular construct over time both in vitro and in vivo.

Significance and Rationale
- The proposed study will accelerate the development and clinical application of engineered vascular grafts by using two non-destructive and non-invasive imaging technologies for real time in vitro and in vivo assessment of properties of the graft.

- Successful completion of the proposed study has clinical relevance as it may allow clinicians to determine early signs of rejection or blockage of vascular grafts.

- Reviewers noted that the limitations in resolution and depth of the imaging technologies could limit it's use to smaller vascular grafts, but none the less, considered it potentially a major step forward.

-According to some reviewers, use of stem cells does not seem to be a major focus of proposed study; rather the focus is on imaging technologies for assessing and monitoring vascular grafts.

- Some reviewers noted that the imaging technologies already exist and questioned what was being developed but others noted that the application and characterization in

the context of monitoring changes in engineered vascular grafts was novel and important.

Feasibility and Experimental Design
- The scientific evidence and preliminary data are supportive of the proposed research.
- The proposed aims are logical and achievable, necessary reagents and resources are available to accomplish the aims of the study.

- The proposed collaborative efforts are appropriate and complementary to enhance likelihood of achieving the aims of the project.

- Some reviewers noted that the proposed imaging tools will be limited by spatial resolution and the depth of the tissues and may not necessarily be suited for analysis of larger vascular grafts in vivo.

Qualifications of PI and Team
- The research team is well qualified and has the necessary expertise to accomplish the proposed goals in this study.

- The proposed collaborators have worked together previously and bring together needed expertise.

- All investigators have proven track records in their area of research.

Responsiveness
- The proposal addresses how the outcomes will alleviate a bottleneck to accelerating development and clinical use of engineered vascular grafts.

- The applicant has clearly stated the patenting and commercialization of technology as means of dissemination.

- The applicant has provided a plan for making the technology accessible to the stem cell community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07880: Mitochondrial genome editing tools for the generation of novel animal and stem cell models

GWG Recommendation: Not Recommended for Funding
Final Score: 61

Public Abstract (provided by applicant)

Genetic information in humans and other mammals is encoded in two different genomes, the nuclear genome and the mitochondrial genome. Mutations in nuclear DNA can be efficiently targeted and corrected using current gene-editing strategies. However, strategies for targeting mitochondrial DNA (mtDNA) are lacking. This is important because mitochondrial mutations have been linked to a wide variety of human conditions, including metabolic disorders, type II diabetes, and even aging. Furthermore, mitochondrial diseases can be inherited and current strategies to prevent transmission involve genetic counseling or pre-implantation genetic diagnosis, which cannot guarantee that the newborn child will be free of disease. New strategies for treating mitochondrial diseases are therefore required. We propose to develop technologies for editing mtDNA, which will potentially allow us to repair mutant mtDNA (i.e., cure these diseases) and to generate specific mutations in mtDNA so that we can study these diseases. Importantly, we propose to mutate each mitochondrial gene in stem cells and to make this important resource available to the scientific community. Tools developed in this project may one day allow us to correct mitochondria within stem cells from a patient to treat or to prevent that individual's mitochondrial disease.

Statement of Benefit to California (provided by applicant)

The California Institute for Regenerative Medicine (CIRM) was established to advance stem cell research and to develop therapies to relieve human suffering.

Developing technologies and rapidly translating these advances to the clinic will contribute to CIRM's success. The present RFA on technology development represents an ideal platform for attaining this goal. The UK Human Fertilization and Embryology Authority and the US Food and Drug Administration are currently considering strategies for treating mitochondrial diseases that involve mitochondrial replacement. We propose to develop novel technologies for correcting (i.e., editing) mitochondrial mutations. This strategy represents a complementary form of treatment, which could have profound clinical implications. Funding this research would position California as a leader in developing treatments for mitochondrial diseases.

We envision three major benefits for Californians: (1) California patients will be privileged once therapies are developed and ready to transition to the bedside; (2) California will witness the growth of its technological/industrial infrastructure to develop new forms of treatment; (3) California will establish itself as a worldwide reference for generating stem cell technologies and novel cellular and animal models of these diseases. The combination of the above-mentioned factors is powerful and dividends will be generated in the form of revenues from health care delivery and intellectual property.

Review Summary

Proposal Synopsis
Mitochondrial DNA (mtDNA) mutations have been linked to a wide variety of human conditions, including metabolic disorders, type II diabetes, and aging. Whereas successful strategies to target and edit nuclear DNA have been developed, strategies for targeting mtDNA are lacking. The proposal attempts to address this problem by developing technologies for editing mtDNA. This technology will be used to repair mutant mitochondrial genomes or change the proportion of mutant compared to wild-type mtDNA genomes. It addition, using the technology developed, the investigator will generate a variety of animal models of mitochondrial diseases by mutating the genes in mtDNA within stem cells. These mutated cells would themselves be tools available for distribution to the stem-cell research community and such tools could make it possible to identify the mutant mtDNA genes responsible for type II diabetes. The potential of this technology in preventing mitochondrial disease transmission from mother to child will be investigated using oocytes from small animals. The applicants hope that technologies developed through this project may potentially provide a tool to repair mutant mtDNA within stem cells from a patient in order to treat or to prevent that individual's mitochondrial disease.

Significance and Rationale
- New methods for editing mitochondrial genomes would enable a wide variety of clinical applications. The targeting and editing systems proposed are a reasonable next step, though the potential of off target effects could lead to catastrophic results.

-If successful, the project could have a major impact in treating several mitochondrial-associated pathways and diseases. However, genome editing in oocytes was viewed as unrealistic.

- The principal investigator (PI) failed to acknowledge and cite previous studies using systems similar to those proposed in this application to manipulate mtDNA.

- The genome editing tools, the cell lines with mutated mtDNA-encoded genes, the mitochondrial disease model cell lines developed, as well as the small animal models developed, could be valuable resources for the stem cell community.

- Proposed studies may lead to the identification of novel linkages between mitochondrial functions and diabetes.

Feasibility and Experimental Design

- It is very unlikely that many of the proposed aims could be reasonably accomplished within the timeline of the grant.

- The preliminary data generated were not convincing. No evidence was provided to support the feasibility of one of the proposed editing systems in mitochondria.

-There was also a serious concern with multiple potential technical problems of the proposed experiments.

- The efficiency of the current mitochondrial genome editing tools appears extremely low based on the preliminary data. If this low efficiency is not improved, the studies in the last 2 aims will be difficult or impossible. Moreover, if Aim 1 fails, the other two aims could not be completed.

- Aim 3 is significantly underdeveloped compared to Aims 1 and 2.

- The investigators provided detailed and systematic methods for designing and constructing the genome editing tools.

- The difficulty of targeting nucleic acids into mitochondria is not adequately addressed.

- The comprehensive mitochondrial mutation strategy is a strength.

- The evaluation of the systems proposed is appropriate.

Qualifications of PI and Team

- The PI is a senior scientist who heads a very successful laboratory with broad experience in stem cell biology, vector design, and genome editing.

- There were no proposed collaborations with scientists knowledgeable in the area of mitochondrial molecular genetics.

- There is essentially no research team except for the PI and the post-docs proposed to be hired. No other CVs were appended.

Responsiveness

- The application is responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07881: iPSC-based Bioartificial Liver device

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Acute liver failure (ALF) caused by viral infection, acetaminophen drug poisoning and increased severity of existing chronic liver disease has a critical death rate of 80% of all cases and typically requires a liver transplant. Unfortunately, the amount of liver donors is limited. Employing stem cell technology, specifically induced pluripotent stem cells (iPSC), together with a fiber-based bioartificial liver (BAL) device can benefit patients with ALF that await a donor organ by extending their life until a liver becomes available. In some cases, the additional time may even permit the liver to regenerate to ultimately avoid the need for a liver transplant. The goal of our study is to develop a functional bioartificial liver device consisting of iPSC-derived liver cells that can be used at the bedside of ALF patients to therapeutically compensate their compromised liver function. We have optimized methods to reliably and efficiently generate functional liver cells from iPSC. The following aims will be addressed in the study for developing and validating the bioartificial liver device. Aim 1: Develop and design the bioartificial liver device and test the functionality of the iPSC-derived liver cells in the BAL. Aim 2: Test for safety and effectiveness of the liver device using small and large animal models of ALF. Development of the proposed iPSC-BAL device technology would enable us to translate the therapeutic benefit of BAL device to ALF patients.

Statement of Benefit to California (provided by applicant)

Liver disease is a major health problem and the number of patients wait-listed for liver transplant across national transplant centers is rising, with 16,387 registered for liver transplant and only 1,154 living donors as of May 2014. In California (CA) alone, per the Organ Procurement and Transplantation Network, 3,093 patients are wait-listed with just 108 donors. Globally, over 500 million people suffer from liver ailments and 1 million die annually. Acute liver failure (ALF) from viral hepatitis or drug/alcohol poisoning has a high mortality rate and requires immediate liver transplantation. However, finding a donor liver in short notice is rarely feasible. Alternative therapeutic approaches to liver transplant are critical. A liver support device to functionally compensate a failed liver can serve as a bridge for patients awaiting a donor and provide time for the injured liver to regenerate. A novel iPSC-derived hepatocyte based bioartificial liver (BAL) device would provide great therapeutic benefit for ALF or decompensated chronic liver diseases. National and local health care strain would be reduced. CA is a forerunner in stem cell-based research and technologies. Development

of an iPSC-derived hepatocyte BAL device would initiate at the academic level and likely expand to industrial participation for commercial production. CA would be recognized as the innovator that successfully merged two powerful resources to create a new therapy for liver disease patients.

Review Summary

Proposal Synopsis
Liver disease and specifically acute liver failure is a severe condition that has a critical death rate above 75%. Liver transplantation has been the treatment of choice; however, the amount of liver donors is limited. In this proposal, the applicant proposes combining existing induced pluripotent stem cell (iPSC) differentiation methods with bioreactor technology to generate a bioartificial liver (BAL). The project goals are to develop a functional BAL with resources available to optimize and efficiently produce functional liver cells from iPSC. Once a functional device is available, the safety and effectiveness of the BAL will be assessed in appropriate animal models. Advancements in the development of the proposed iPSC-BAL device addresses a current bottleneck in technology that could advance the field and translate the therapeutic benefits of the BAL device to acute liver failure patients.

Significance and Rationale
- The development of more efficient BAL devices, if successful, could have a major impact on prolonging life in acute hepatic failure and the rationale for the proposed approach is strong. However, due to concerns regarding the preliminary data and experimental design, reviewers did not find it likely that execution of this proposal will have a meaningful survival benefit for patients with liver failure.

- The reviewers felt that this proposal does not address key bottlenecks for this technology in that the preliminary data does not adequately demonstrate relevant functionality of the proposed iPSC-derived cell source for the BAL device.

- Reviewers did not think that the proposed bioreactor design offers sufficient improvements over other BALs that have previously failed.

- The animal models selected for testing of the device did not seem well justified in the proposal.

Feasibility and Experimental Design
- The preliminary data demonstrating hepatic differentiation was not considered strong. Although some gene expression data was included, it lacked sufficient functional outcomes to demonstrate that the differentiated iPSCs would function in an equivalent manner to mature hepatocytes.

- Reviewers were concerned that the large animal model assays did not seem well thought out and lacked appropriate and relevant endpoints.

- Reviewers thought the small animal model studies were not well considered in terms of sample size and timelines and demonstration of efficacy.

- The data included in the application was not convincing that the differentiated iPSCs would have sufficient maturity to function in an equivalent manner to primary hepatocytes in the BAL device.

Qualifications of PI and Team

- The team is qualified with regards to meeting most of the necessary technical skills, although the team and proposal would be strengthened with additional expertise in preclinical animal models and hepatocyte functionality assessments.

- Some reservations were expressed about human iPSC biology expertise and indicated the proposal would benefit from including someone with more extensive iPSC knowledge.

Responsiveness

- This proposal addresses the critical need for a reproducible source of hepatocytes for a bioartificial liver. However, they do not adequately discuss how their proposal will alleviate the true bottleneck of BAL technology, the bioreactor properties and performance.

- The proposal is responsive in that human iPSCs will be used as the cell source.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07883: Developing novel genetic neurological disease monkey models with and without stem cell transplantation

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Animal models carry great promise for developing human disease paradigms for understanding the disease mechanisms and testing therapeutic agents. Transgenic small animal models have been utilized for modeling diseases with genetic components. However, due to significant differences between the genetic make up of these small animal models and humans, many models do not fully recapitulate human neurological diseases. iPSC technology exponentially increased the rate of human disease modeling in vitro (i.e., “disease-in-a-dish”) in recent years. However, the complexity of human neurological diseases (e.g., brain-region-specific pathology) makes it difficult to capture the relevant pathophysiology in a dish. Human iPSC-derived neural cultures contain different neuronal subtypes, and introduction of additional genetic and/or epigenetic variations during iPSC derivation and in vitro differentiation raise additional concerns when modeling human diseases in a dish, underscoring the importance of developing new tools for disease modeling. In this application, we are proposing to develop large animal models in vivo models of human neurological diseases with or without transplantation of neural epithelial stem cells. Given the higher degree of similarities between large animal models and humans, we believe that this approach will significantly facilitate our understanding of the disease mechanisms and serve as a suitable system for development and testing of novel therapeutic interventions.

Statement of Benefit to California (provided by applicant)

Neurological disorders, such as Alzheimer, Parkinson, ALS, stroke or Autism, are chronic debilitating conditions that have enormous socioeconomic implications for not only the residents of California, but also the general population in the US. The utilization of stem cell technologies made it possible to develop models of human diseases in recent years and opened up the exciting prospect of using stem cells for therapy. Unfortunately, neither small animal models nor cell culture models of human neurological diseases can fully recapitulate the underlying pathology due to differences between the genetic make up of these models and humans, and the inherit problems of the cell culture approaches. In this study, using one of the Autism Spectrum Disorders, Rett Syndrome (RTT), as a proof of principal, we propose to develop large animal models in vivo models of human neurological diseases with or without transplantation of stem cells. Our method will be applicable to generate large animal models of many other

neurological disorders, and we believe that this approach will significantly facilitate our understanding of the disease mechanisms and serve as a suitable system for development and testing of novel therapeutic interventions using stem cells or pharmacological agents. The results of our study will generate novel disease models that will facilitate the development of novel therapies that will greatly benefit the residents of California who are suffering from debilitating neurological disorders.

Review Summary

Proposal Synopsis
The objective of this proposal is to create large preclinical animal models of Rett syndrome, which may allow better understanding of human neurological diseases. Since available rodent models have limitations in manifesting phenotypes of human diseases, the proposed models may serve as new avenues to evaluate novel therapies for neurological diseases in humans. To accomplish the objective, the proposal seeks to use three approaches using Rett syndrome modeling as a proof of principle. These are 1) an improved one-cell embryo gene editing approach; 2) a targeted gene editing/knockout in NHP gonads to obtain desired phenotypes; and 3) a transplantation of genetically modified neural epithelial stem cells (NESCs) into brain-specific regions. If successful, the findings may address the bottleneck of preclinical evaluation of neurological diseases.

Significance and Rationale
- The proposal addresses a significant bottleneck to the translation of human stem cell therapies and the need for such an animal model is high.

- It is unclear that the selected disease model (Rett syndrome) is the most appropriate for stem cell therapies intervention, so impact in this disease is uncertain.

- The technology is elegant but this does not seem to be a high yield project with broadly applicable significance.

Feasibility and Experimental Design
- The lack of description of logistics raised serious feasibility concerns for the review panel. The lack of any supporting documentation from the overseas laboratory or description of how efforts will be coordinated exacerbated this concern.

- Some reviewers thought that the second and third aims were not well supported by the preliminary data and further, raised significant concerns which include: the number of animals that can be developed during the funding period, how the animals will be maintained beyond the funding period, and how materials will be transferred between the overseas animal research site and the California laboratory.

- The experimental plan does not adequately describe the potential issues in the animal model related to immune rejection of xenotransplants.

- Since Rett syndrome may be embryonic lethal for males, the number of animals that can be generated in two years is unclear.

Qualifications of PI and Team

- The principal investigator (PI) and research team are well qualified with demonstrated expertise in several key areas.

- Expertise related to behavioral and cognition studies of animals did not seem adequate.

- Documentation listing the expertise, resources, and commitment of the collaborative personnel or institute were inadequate.

- Transfer of materials and logistics of collaboration are not clearly stated.

Responsiveness

- The proposal is responsive to the RFA**'**s objective. However, the application lacks an explicit discussion of how any resulting technology will be made accessible to the stem-cell community.

- The animal model is developed at collaborative institute located abroad, thus accessibility to the stem cell community may be difficult.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07887: New materials and methods to instruct hematopoietic stem cell fate from human pluripotent precursors.

GWG Recommendation: Tier 2
Final Score: 69

Public Abstract (provided by applicant)

Hematopoietic stem cells (HSCs) are an important population of cells that continuously produce and replace blood and immune cells over the course of our lifetimes. These rare, self-renewing cells are the key element of bone marrow transplants, which are used to treat a variety of conditions including many forms of leukemia and solid tumors. Understanding how HSCs are made during embryonic development is important because it could teach us how to make such cells in the laboratory, and possibly allow circumvention of immune compatibility issues between donor and host. In this research we will utilize our knowledge regarding the normal development of HSCs in the vertebrate embryo to replicate HSC formation in vitro from human pluripotent precursors, something that is not currently possible. Our studies will ultimately enable the production of patient-specific HSCs that can be used for lifelong repopulation of the blood-forming system with normal cells free of disease-associated mutations.

Statement of Benefit to California (provided by applicant)

Understanding how hematopoietic stem cells (HSCs) are made during embryonic development is important because it could teach us how to make and amplify such cells in the laboratory. We will translate our knowledge regarding how HSCs are generated in the vertebrate embryo to human pluripotent stem cells (hiPSCs). The creation of hiPSCs holds great promise for new cell-based therapies, including bone marrow transplantation (BMT). These cells have the potential to generate any tissue type, and can be generated in a patient-specific manner. Thus, hiPSCs hold the promise of cellular replacement therapies without the risk of immune rejection, currently a major bottleneck in the clinic. For use in BMT, however, hiPSCs must be coaxed to differentiate into hematopoietic stem cells (HSCs), the rare cells responsible for the long-term, curative effects of BMT. This is currently not possible, due to a lack of understanding of the cues required to generate HSCs in vivo. Insight into the factors needed to instruct and amplify HSCs will be used to provide similar factors at similar timepoints to differentiate hiPSCs into HSCs. Our research will thus lead to great improvements in stem cell therapies to better meet the needs of patients in California.

Review Summary

Proposal Synopsis
This application aims to address the bottleneck of ex vivo differentiation of pluripotent stem cells (PSCs) to hematopoietic stem cells (HSCs) that can self renew engraft and reconstitute all blood lineages in vivo. Presently the self-renewal, engraftment and repopulation efficiency of these cells is poor when compared to the engraftment of adult HSCs. The investigators propose a rational design approach to define key molecules and pathways followed by a screening approach to identify combinations of molecules for optimizing differentiation into HSCs. Finally, HSCs will be tested in vitro and in vivo for function.

Significance and Rationale
- Adult HSCs are easily obtained and engraft with excellent efficiency. However, for patients requiring bone marrow transplantation to treat cancer or blood system disorders where it is difficult to achieve a donor match, successful development of authentic PSC-derived HSC will be a significant benefit.

- The reviewers felt that the overall impact of the proposal is sharply diminished by the fact that differentiation into hemogenic endothelium has recently been accomplished and reported, and that the investigators do not build substantially off this work. The proposed work was not viewed as a good use of resources.

- Reviewers appreciated that the application clearly addresses a substantial obstacle to applying iPSC technology to hematopoietic replacement. Resolving the issue of differentiation to authentic HSC will accelerate clinical application.

Feasibility and Experimental Design
- The preliminary data is strong, the dual approach of rational design of a differentiation strategy and multi-factor screening are strengths of the experimental design; however, reviewers were concerned that others had recently reported on several aspects of the proposed research.

- Enthusiasm by the panel was dampened by the fact that the screening strategy relies on a small and very likely over-simplified panel of reporters and does not take advantage of many of the established markers of HSC differentiation.

- Reviewers were appreciative of the proposed use long-term engraftment in vivo as the true test for authentic HSC

- Reviewers regarded the proposed array technology as an innovative advance although they had some questions on the selection of molecules that will be evaluated.

Qualifications of PI and Team

- The lead PI was recognized as having an outstanding record of productivity and significant expertise in the development of HSCs in vivo.

- Collaborating investigators have significant and critical expertise necessary for the project.

- There was agreement that the team is uniquely qualified to complete the work.

Responsiveness

- The proposed work addresses a significant bottleneck that is preventing the application of iPSC technology to the generation of HSCs that can be used to treat a range of blood disorders.

- Deliverables are listed but there is no explicit discussion of how these will be communicated /shared.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07891: Enhancing thymic epithelium differentiation with three dimensional matrices and small molecule libraries

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Stem cell therapies hold the potential to treat and cure a wide variety of diseases through tissue regeneration and transplantation. A major barrier in the application of these therapies is the need to prevent rejection of grafted tissues by a recipient's immune system. Although suppression of the immune system can achieve tolerance of a graft, this approach also disables many protective immune functions. The immune system is trained to distinguish "self" tissue from foreign tissue or pathogens in large part through the action of the thymus gland. Within the thymus, developing immune cells encounter a wide array of self-proteins to educate them as to what is considered self. Thus, altering what the thymus displays as "self" allows reeducation of immune cells to promote tolerance to new tissues. We have developed a method to generate human thymus tissue from stem cells, which introduces the potential to engineer thymic function and tolerance. Such stem cell derived thymus tissue could be transplanted in conjunction with stem cell therapies to promote tolerance of grafted tissues. We propose to investigate the impact of various small molecules, tissue scaffolds, and transplantation sites on the development of stem cell derived thymus tissue, paving the way to more direct clinical application of such technology. By harnessing the ability to manipulate immune tolerance with engineered thymus tissue, we hope to resolve a major bottleneck in the application of stem cell therapies.

Statement of Benefit to California (provided by applicant)

Stem cell and regenerative medical therapies are still limited by potential rejection of transplanted cells and tissues by the immune system. Given the toxicities and side effects of suppressing immune function, successful transplantation requires promoting more "tolerance" or acceptance of new tissue by the immune system. Our recent development of a technique to generate human thymus from stem cells provides a new tool to promote tolerance of transplanted tissues. Further development of this technology will allow improved implementation of a wide range of stem cell therapies, which will benefit patients both in California and beyond. As we are based in a state institution and a site of a major stem cell research center, the success of our proposed work will advance the level of science in stem cell biology and provide an avenue to further funding from federal and private sources, placing the state of California at the forefront of stem cell research.

Review Summary

Proposal Synopsis
This proposal addresses the bottleneck of immune rejection of transplanted tissue. Although immunosuppressive therapy can help to avoid rejection, continuous and indefinite immunosuppression can result in significant morbidity including increased risks of cancer and infections. The PI proposes to form transplantable thymic organoids for the induction of transplantation tolerance using stem cells. Such stem cell derived thymus could be transplanted in conjunction with stem cell therapies to promote tolerance of grafted tissue. The PI will first attempt to improve the efficiency of thymic epithelial progenitor (TEP) cell generation from pluripotent stem cells via two approaches - small molecule screening and optimization of cell culture conditions. The PI will then test the use of tissue scaffolds for supporting TEP cell self-renewal, differentiation, and development into a 3D structure (organoid). These organoids will be transplanted into animals at different sites to determine their abilities to function as needed to achieve tolerance. It is proposed that the findings from these studies could have generic application to complex organ and tissue differentiation systems. If the findings from these studies are positive, the results could have impact on the development of stem cell medicine.

Significance and Rationale
- The major weakness of the application is that the technology to complete the proposed studies is not sufficiently developed for translation. The PI has not shown that a functional thymus can be generated from pluripotent stem cells. Thus, the application seems to be premature.

- Due to complex nature of the technology requiring multiple components that are at a very early stage of development, the proposed project was considered to be high risk. Also, implementation of the technology in a clinical setting would be challenging.

- If TEP cells could be created from the same pluripotent stem cell source as the therapeutic cells, then long-term immune tolerance to that stem cell donor could be established and this could play an important role in transplant medicine.

Feasibility and Experimental Design
- While the application contains many interesting and novel ideas, very little preliminary data was shown to support that these ideas will result in improved cell numbers or functionality.

- The proposal expects to accomplish more than can be reasonably done within the award timeline. It appears that the first aim could take the full three years to complete.

Significant preliminary work needs to be completed in this aim before other aims can be completed, as they are all dependent upon its results.

- In the proposed pre-clinical model, the applicant has not adequately addressed the major histocompatibility (MHC) matching of the graft with that of the recipient.

- It is not clear if the 3-D system has been validated for the production of functional thymic epithelial progenitor cells.

- It is not clear how the system, once developed in a small animal model, will be scaled for use in larger targets. Further, the use of aged animals may be appropriate.

Qualifications of the PI and Team

- The principal investigator (PI) and partner PI appear to constitute a strong team.

- It is not clear how the team will coordinate activities between the PI's and partner PI's institutions. Some of the work will be split and it is not clear if/why there may be duplication of studies.

Responsiveness

- The proposal is responsive to the RFA in that it addresses the bottleneck of immune rejection of transplanted tissue and the use of stem cells.

- It is not clear how the technology will be accessible to the stem cell community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07893: Optimizing the differentiation and expansion of microglial progenitors from human pluripotent stem cells for the study and treatment of neurological disease.

GWG Recommendation: Recommended for Funding
Final Score: 81

Public Abstract (provided by applicant)

Microglia are a type of immune cell within the brain that profoundly influence the development and progression of many neurological disorders. Microglia also inherently migrate toward areas of brain injury, making them excellent candidates for use in cell transplantation therapies. Despite the widely accepted importance of microglia in neurological disease, methods to produce microglia from stem cells have yet to be reported. Our team has recently developed one of the first protocols to generate microglia from human pluripotent stem cells. We have used several approaches to confirm that the resulting cells are microglia including examination of gene expression and testing of key microglial functions. However, our current protocol uses cell culture supplements that preclude the use of these cells for any future clinical applications in people. The major goal of this proposal is to resolve this problem. We will generate pluripotent human stem cells that have special "reporter" genes that make the cells glow as they become microglia, allowing us to readily monitor and quantify the generation of these important cells. Using these reporter lines we can then streamline the differentiation process and develop improved protocols that could be translated toward eventual clinical use. As a proof-of-principle experiment we will then use the resulting human microglia to study some important questions about the genetic causes and potential treatment of Alzheimer's disease.

Statement of Benefit to California (provided by applicant)

Recent estimates suggest that nearly 2 million Californian adults are currently living with a neurological disorder. While the causes of neurological disease vary widely from Alzheimer's disease to Stroke to Traumatic Brain Injury, a type of brain cell called microglia has been strongly implicated in all of these disorders. Microglia are often considered the immune cell of the brain, but they play many additional roles in the development and function of the nervous system. In neurological disease, Microglia appear to be involved in a response to injury but they can also secrete factors that exacerbate neurological impairment. Unfortunately, it has been difficult to study human microglia and their role in these diseases because of challenges in producing these cells. Our group recently developed an approach to 'differentiate' microglia from human pluripotent stem cells. This enables researchers to now study the role of different genes

in human microglial function and disease. Yet our current approach dose not allow these cells to be used for potential clinical testing in patients. Our proposal therefore aims to develop new tools and technology that will allow us to produce clinically-relevant human microglia. These cells will then be used to study the role of a specific microglial gene in Alzheimer's disease, and may ultimately be useful for developing treatments for the many Californians suffering from neurological disease.

Review Summary

Proposal Synopsis

Microglia are known to play an important role in a variety of neurological diseases, including Alzheimer's Disease (AD) and stroke, and therefore offer potential for use in cell or gene therapy approaches. However, the ability to generate functional microglia from pluripotent stem cells represents a major unmet need. The research team has developed a novel protocol for the generation of microglia from induced pluripotent stem cells (iPSCs), but efficiency remains limited and utilizes reagents that could preclude the use of the derived microglial cells in patients. This application proposes to optimize and refine these protocols by using clinically appropriate reagents for the differentiation and propagation of functional microglial cells from human iPSCs that may be applicable to treating a variety of neurodegenerative diseases. To accomplish this objective, human pluripotent stem cell reporter lines will be generated as a tool to track and select microglial cells differentiated from pluripotent stem cells. The investigators will also assess the potential therapeutic utility of microglia derived using these methods in a small animal model of AD. If successful, the applicants hope to resolve two bottlenecks to the application of stem cells for the treatment of diseases: 1) by developing a means to track and guide the differentiation of human pluripotent stem cells into microglia; and 2) provide a reproducible means to generate microglia from iPSCs using reagents that could facilitate their future use in patients.

Significance and Rationale

- Microglial cells are likely to play a key role in a wide variety of neurodegenerative disorders, giving this proposal broad potential for research and clinical impact.

- Methods to derive large quantities of functionally differentiated human microglia from iPSC constitute a broad, unmet need for translational research.

- The development of the human pluripotent stem cell reporter lines will provide an important new tool that could be distributed to other investigators, regardless of whether the applicants are successful at efficiently differentiating them into functional microglial cells.

Feasibility and Experimental Design

- A major strength of the proposal is the development of pluripotent stem cell reporter lines that the team will use for microglial differentiation. These assets may be broadly applicable tools for the neurodegenerative field. The ability of the investigators to develop these lines appears to be well supported by the preliminary data presented.

- The proposal includes a systematic approach to optimizing the microglial differentiation protocols that is likely to be successful.

- The small animal model of AD proposed could provide a useful platform in which to assess the in vivo functionality of microglia differentiated from iPSC. However, the genetic marker that the applicant proposes to target for confirmatory studies is expressed on a variety of cells, including cell types that may not be involved with AD, which may make interpretation of the data obtained difficult.

- Reviewers commented that it was difficult to fully assess the feasibility of the proposed studies, as experimental outcome measures were not well described and the characterization methods to be used for assessment of the purity of the differentiated microglia cell population may not be sufficiently rigorous.

Qualifications of PI and Team

- Overall, the assembled team is well-qualified to carry out the proposed studies. The PI is well-trained in neurodegenerative disorders and has recruited collaborators with complementary expertise.

- The collective expertise of the research team is validated by strong preliminary data.

- A minor concern was raised over the ability of a relatively junior PI to lead this study and the team of investigators as a clear track record has not yet been established.

Responsiveness

- Overall, the proposal was viewed as highly responsive to the RFA. The human pluripotent stem cell reporter lines will serve as a valuable tool to assess microglial differentiation for the stem cell research community and many groups could benefit from optimized protocols for differentiating microglial cells from iPSC.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07898: Skeletal Muscle Regeneration by Direct Cellular Reprogramming of Human Fibroblasts to Satellite Cells with Myogenic and Cell-penetrating Peptides

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Human skeletal muscles (Skm) are responsible for all kinds of body motions and daily activities. It is difficult to repair or regenerate significant skeletal muscle loss due to severe injury (in nerves, muscles or bones), disuse atrophy, aging and chronic genetic muscular disorders, leading to disability and poor quality of life. The main reason for inability to repair damaged muscles is frequently due to the lack of regenerative ability or number of the so-called skeletal muscle stem cells (SkmSCs). If we can convert patients' skin cells directly to SkmSCs, it would produce host-compatible SkmSCs to repair all muscle disorders mentioned above. We have successfully generated and genetically modified cell-penetrating proteins that could enter the cells spontaneously and transform human adult skin cells to SkmSCs, which have regenerative potentials to repair damaged muscles. Here, we show that these protein-converted SkmSCs can proliferate and differentiate into Skms in muscle-generating conditions. We further propose three Aims to identify the best muscle-generating protein formats and to improve their efficiency of converting human adult skin cells to SkmSCs. We will further explore the potentials of directly applying these muscle-generating, cell-penetrating proteins to the sites of muscle injury/degeneration so as to recover Skm loss in patients' muscles. The overall goal of this proposal is to identify the best cell-permeable proteins for direct Skm regeneration.

Statement of Benefit to California (provided by applicant)

Any sports injury and skeletal muscle disorders can result in muscle loss, leading to poor quality of life and significant disability. Currently, no host-compatible regenerative therapy is available to recover these muscle losses. California is a sunshine state and people are accustomed to various outdoor sports. As a result, sports injuries are frequent events. One of the dismal consequences after these injuries are the loss of skeletal muscle mass due to the degrees of injury severity and prolonged recovery time. One of the potential therapies for these injuries is to booster the endogenous skeletal muscle regenerative potentials. Unfortunately, the number of skeletal muscle stem cells could be limited. If we can convert skin cells to skeletal muscle stem cells, we would be able regenerate skeletal muscle mass and strength to continue meaningful and active life styles. We have developed technology to convert skin cells to skeletal

muscle stem cells. We propose here to improve this technology so that we can have wider applications to repair or regenerate skeletal muscles for all kinds of muscle disorders. If successfully completed, Californians will be benefited most because the active sport events occurring in this sunshine stat and other organ regeneration will soon follow. Successful accomplishment of the proposed research will make California the epicenter of tissue regeneration and will very likely lead to human clinical trials to benefit all Californians.

Review Summary

Proposal Synopsis

Satellite cells are the main skeletal muscle (Skm) stem cells that drive postnatal muscle growth, repair and regeneration. The impaired regenerative capacity of satellite cells has been linked to the onset and progression of neuromuscular degenerative disorders. Current approaches to promote Skm regeneration include myoblast and or satellite cell transplantation, DNA injection, as well as viral transduction. These technologies are limited in their translation into long-term therapies. The authors propose a strategy of in-situ differentiation of local fibroblasts (FB) into functional satellite cells to overcome this translational bottleneck. To achieve this goal, 3 specific aims are proposed: (1) to improve the efficiency of converting FB into satellite cells through the use of novel cell permeable peptides (CPs); (2) to explore an alternative strategy of the CPs in inducing the FB to satellite cell conversion through a novel cleaving technique; and (3) to evaluate the efficiency of FB to satellite cell conversion using (a) an acute muscle injury model and (b) a Duchenne Muscular Dystrophy small animal model.

Significance and Rationale

- If successful, the proposed approach may allow for safer and possibly more efficient satellite cell preparation compared to current approaches.

- Cell-permeable peptides are an established technology, although their applications to direct reprogramming have been few. The applicants did not provide a convincing discussion of the potential advantages of the proposed approach over other current technologies.

Feasibility and Experimental Design

- The aims lack rigorous quantitative analysis of muscle function, and employ analysis of a very limited number of the relevant muscle genes and proteins. Further, critical assumptions of CPs uptake and cell population homogeneity are made, which are not supported by in vivo/preliminary data evidence.

- The preliminary data support the peptide penetration into the host cell, but do not offer any suggestion on the efficiency or efficacy of the process. Some of the

preliminary outcomes were difficult to ascertain due to the small size of the embedded images.

- The bottleneck is identified as the efficiency of cellular reprogramming; however, Aim 1 is focused on the rate of cellular reprogramming. A justification appears to be missing to explain the shift in approach or the direct correlation between efficiency and rate of reprogramming.

- Aim 3 was thought to be underdeveloped in terms of experimental design. It is unclear to the reviewers which disease model the authors propose to use and which cell populations are considered for transplantation.

Qualifications of PI and Team

- The PI and the team have the necessary background to execute the proposed specific aims. A complementarity of expertise ensures that disease models in the field of project focus, protein mediated cell reprogramming, and satellite cell differentiation are appropriately supported.

Responsiveness

- The proposal is responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07899: Development of 3D Bioprinting Techniques using Human Embryonic Stem Cells Derived Cardiomyocytes for Cardiac Tissue Engineering

GWG Recommendation: Tier 2
CIRM Recommendation: Recommended for Funding
Final Score: 73

Public Abstract (provided by applicant)

Heart, stroke and other cardiovascular diseases are responsible for ~17 million deaths per year globally and this number is predicted to reach 23.3 million by 2030. Cardiovascular diseases impose a staggering annual cost of $300 billion on the U.S. health care system. Heart transplantation is the ultimate solution to end-stage heart failure. However, a major limitation in treating cardiac injury is the limited availability of donors; as a result, only a small fraction of patients will benefit from heart transplantation. Tissue engineering holds a great promise to create functional tissue constructs that can reestablish the structure and function of injured tissue with exciting success stories. However, many challenges regarding their development still remain. It is the goal of this project to develop a novel 3D bioprinting technology to fabricate cardiac tissues made from cell-laden hydrogels with engineered microvasculature. By integrating the advanced 3D bioprinting with stem cell technology, functional cardiac tissues will be created with biomimetic 3D microarchitecture and functional vasculature. This novel 3D-printed cardiac tissue will heal the damaged heart and improve its function to pave the way for a superior treatment option for the millions of cardiac patients in the U.S.

Statement of Benefit to California (provided by applicant)

Heart disease and other cardiovascular diseases are the #1 killer in California and remain a leading cause of disability and death. A major limitation in treating cardiac injury is the failure of current therapies to induce myocardium regeneration. Due to the limited availability of donors, only a fraction of individuals who could benefit from heart transplantations actually receive them. One possible avenue for remedying this situation is to artificially engineer cardiac tissues. Tissue engineering techniques have been successfully applied to engineer many types of tissue; however, many challenges regarding their development still remain. This proposal aims to make an advance in tissue engineering by developing a novel 3D bioprinting technology to fabricate tissues made from cell-laden hydrogels with engineered microvasculature. The completion of this work will be a paradigm shift and a landmark achievement in efforts towards clinical

treatments of vascularized cardiac tissue using stem cells. This advanced technology can also have a significant economical impact as heart diseases impose a staggering annual cost of $300 billion on the U.S. health care system. In addition, the development of the 3D bioprinting technology and advanced biomaterials will keep California and the U.S. as a whole in the leading position in this emerging field.

Review Summary

Proposal Synopsis
This proposal addresses two bottlenecks for stem cell-based therapies-1) construction of 3-dimensional (3D) structures with stem cells that more accurately mimic the structure and function of tissues and 2) the retention and survival of these structures after transplantation. The applicant will use a novel 3D bioprinting technique with cardiomyocytes and endothelial cells derived from human embryonic stem cells (hESCs). First, the Principal Investigator (PI) will develop a 3D structure with biomaterials and hESC-derived cardiomyocytes, including incorporation of functional vasculature and control of spatial organization. Then the team will optimize a variety of parameters for tissue survival and function using small animal models.

Significance and Rationale
- 3D bioprinting is a promising new technology that has the potential to provide advancements in the application of stem cell therapies for a number of diseases. The process proposed should offer improvements over current technologies, and the project defines a new technology.

- Reviewers questioned the rationale for selecting the intended scaffolding material and appropriately justified alternatives were not provided.

- Immune responses, also important to tissue survival and retention, will be investigated. However, the investigators did not provide a clear rationale for why their approaches would provide immune protection.

Feasibility and Experimental Design
- Substantial and impressive preliminary data were provided that demonstrate that the investigators have the 3D technology to produce viable cardiomyocytes onto an extracellular scaffold. Their data also convincingly demonstrate their ability to incorporate vascular structures into the tissues.

- The proposal was somewhat lacking in focus and had several aspects that appeared to be tangential to the essential goals of the project. Focusing on the technology development aspects of the proposal would have likely strengthened the project.

- Although some attention was paid to important aspects of the structures such as cell positioning and matrix stiffness, there was a general lack of sufficient tissue characterization proposed.

- The experiments are generally well designed, though the plan is somewhat vague.

- Reviewers cautioned that the work might not be generally applied to other tissues as the design parameters may need to be substantially modified for individual types of tissues.

Qualifications of PI and Team

- The PI is a leader in the field of 3D bioprinting and the expertise required to execute the described project.

- The team has complementary expertise in bioprinting, cardiac tissue engineering, and the use of hiPSCs that are important to the success of this project.

Responsiveness

- The project is highly responsive to the RFA. This is an important technology relevant to the engraftment and integration of human stem cells for therapy.

- Human stem cells are used in an appropriate (though small) animal model.

- A plan is in place to make the technology accessible to the stem cell research community at large.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07900: Bioactive thermo-reversible polymers as adjunctive therapy to stem cell treatment of heart failure

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Heart failure affects nearly 5.7 million Americans of all ages and is responsible for more hospitalizations than all forms of cancer combined. Recent attempts to restore myocardial tissue and improve heart function through the use of cell-based therapies have yielded encouraging clinical results. However, limitations of cell delivery and cell survival of transplanted cells within the heart remain a challenge with less than 10% of the injected cells surviving.

The goal of the proposal will be to develop a bioactive polymer scaffold to provide structural support to the heart and favorably influence the heart's microenvironment to increase cardiac stem cell (CSC) retention and survival, restoration of LV function and ultimately, myocardial regeneration.

The novel approach proposed in this application combines the use of a bioactive polymer and an injection catheter combined to a micro-camera for visualization of the needle entering the myocardium to increase accuracy of delivery, CSC retention and survival as well as provide structural integrity for myocardial regeneration.

At the conclusion of this proposal, the selection of a bioactive polymer with CSCs will have undergone optimization of CSC retention/survival, LV functional restitution and safety/toxicity studies to allow translation to human studies.

Statement of Benefit to California (provided by applicant)

Congestive heart failure (HF) is the fastest-growing clinical cardiac disease entity in the US, giving rise to approximately 600,000 new diagnosed cases each year. The estimated total cost of HF in the United States was $39.2 billion. HF after myocardial infarction has a mortality rate of 50%. Since the only successful treatment for end-stage HF-heart transplantation-is limited by the availability of donor hearts, alternative therapies are needed.

The aims of this proposal is to repair and regenerate the injured heart by injecting stem cells surrounded by a biopolymer scaffold. The biopolymer scaffold will provide mechanical structure to the heart as well as creating a suitable microenvironment that

will enhance cell retention, survival and integration into the heart leading to myocardial regeneration.

The new therapy will lead to better options for residents of California suffering from HF. The therapy can provide an option to end-stage patients with HF that have no options due to the lack of donor hearts. In patients with milder forms of HF, the proposed therapy could provide a means of immediate improvement of HF symptoms and eventual heart regeneration. Improved treatment of HF patients would decrease the burden to California's health system and costs associated with medical treatment as well as lost wages by the patient. Additionally, jobs would be created from the need to produce the stem cells and manufacturing the biopolymer scaffold.

Review Summary

Proposal Synopsis
The applicant proposes to develop a treatment for heart failure consisting of a biopolymer combined with cardiac stem cells (CSC). A percutaneous method to deliver the treatment to the heart muscle would also be developed. After delivery to the heart muscle, the biopolymer is expected to provide structural support and a favorable microenvironment for long-term CSC retention and survival, leading to heart muscle regeneration. The authors propose to optimize the bioactive properties of the polymer in a rodent model of chronic heart disease, and then use a porcine heart failure model to develop the percutaneous delivery method and to test the ability of the biopolymer/CSC therapy to improve left ventricular function. This project addresses the bottleneck of achieving long-term cell engraftment after transplantation.

Significance and Rationale
- The applicant does not offer evidence that the cells to be used in this project are truly CSC that can differentiate into cardiomyocytes, nor does he/she address recent studies that directly contradict this claim. The potential that the cells chosen aren't truly CSC greatly impacts the potential significance of the study; some reviewers considered this a fatal flaw.

- Reviewers weren't convinced that the polymer being used has regenerative properties, as was claimed.

- The proposed technology could positively impact other tissue-engineered therapeutic applications.

Feasibility and Experimental Design
- Reviewers noted that the physical properties of the polymer being used might not be compatible with the percutaneous delivery method that is proposed and felt that there was insufficient evidence that the proposed method of delivery is feasible.

- The applicant failed to address the concern that cells may remain trapped in the polymer. It was unclear what environmental cues would trigger the cells to migrate out of the biopolymer.

Qualifications of PI and Team

- The PIs are established investigators with a track record of excellence and achievement in the field.

Responsiveness

- Reviewers could find no evidence that human cells were to be used in this study. The source of CSC in the animal model is unclear.

- The proposal addresses the call for development of technologies that enhance engraftment of human stem cells. However, reviewers were not confident that results from non-human CSC efficacy studies would be translatable to humans, limiting the potential clinical impact of this project.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07901: Label-free analysis and purification of cell-based therapies for cost-effective regenerative medicine

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Many of the world's major ailments, such as diabetes, cardiovascular disease, and brain injury, can be solved with regenerative medicine, in which cells from a patient or other donor are grown outside the body and exposed to agents to become cell types which can support or replace damaged organs or tissue. However, moving from principle to practice has been hindered by the lack of appropriate methods to purify the therapeutically useful fraction of cells that are grown outside the body. In particular, there are some cells that have the possibility to form tumors when transplanted, and these must be removed in a robust, effective, and cost-effective manner. To overcome this significant hurdle, we propose to develop reliable technologies that will enable (1) the discovery of new, unique physical cell properties for fingerprinting different cell types, and (2) the subsequent use of these cell properties as criteria for quickly separating harmful tumor-forming cells and other cells from the useful cell populations. To achieve these goals, we are in the process of developing two ground-breaking technologies that work in tandem to exploit many of a cell's inherent physical properties: the ability of cells to stick to surfaces and cell stiffness. Importantly, these technologies do not chemically alter the cells, which can lead to unintended side-effects that must be closely studied according to regulations, and can delay and increase costs of bringing treatments to the public.

Statement of Benefit to California (provided by applicant)

Stem cell-based therapies are very attractive options to treat hundreds of thousands of individuals in California suffering from a broad range of ailments such as cardiovascular, neural, and immunological diseases. Besides the therapeutic benefit itself, one major need to realize this goal is new robust and cost-effective approaches to purify differentiated cells in therapeutic cell cultures such that cell behavior post transplantation can be accurately predicted and the risk of potential teratoma formation is reduced. The major benefit that this proposal can bring to the state of California is to hasten research lab-level regenerative medicine applications to clinical trials with less variation in efficacy of treatments and less potential for side effects like tumor-formation. Importantly, our approaches are cognizant of significant financial costs and aim to reduce the economic burden of cell preparation. Because we have partnered with a South San Francisco-based company; we expect that a successful

outcome will position the tool for rapid commercialization, creating jobs within the Bay Area and enabling technology distribution throughout CIRM-funded projects. This presumably will lead to regenerative medicines reaching a broader set of patients in California sooner.

Review Summary

Proposal Synopsis
The primary goal of this proposal is to develop technologies to purify desired cells and remove tumor-forming cells from stem cell derived therapies. The applicant proposes two approaches. The team will develop a cell sorter based on the physical property of cell deformability. To test the applicability of the sorter, they will identify the deformability properties of retinal pigmented epithelial (RPE) cells desired for transplant and use these as gates to sort cells. Function of the sorted cells will be tested in retinal transplantation studies. In the second approach, differences in cellular adhesion properties will be used to separate cell populations.

Significance and Rationale
- The project addresses a significant safety barrier to stem cell-derived therapies.

- Reviewers found the proposed antibody and label free aspect of the proposed cell sorting methods attractive, and appreciated the potential utility of cell deformability profiling as an assay.

- It is unclear how the two sorting methods might synergize.

Feasibility and Experimental Design
- The application lacks convincing data that the deformability sorter could sort cells at clinically relevant scales in a reasonable time frame; thus, this sorter appears more appropriate as a research tool.

- Preliminary data support the applicant's ability to use cell stiffness to characterize and purify cell populations as well as to separate cells based on differential substrate adhesion properties.

- It is unclear that the proposed sorting technologies are superior to existing methods, and preliminary data did not convince some reviewers that all undesirable cells could be removed. Thus, the key goals of the proposal may not be achieved.

- The plans for the differential adhesion studies are underdeveloped.

- The sorting process could impact cell viability and function.

Qualifications of PI and Team

- The PI and team possess an appropriate and relevant track record to perform the proposed studies.

- A productive collaborations is already established between the PI and Co-PI.

Responsiveness

-The proposal aims to achieve a process advance to address a bottleneck in the field.

-The dissemination plan is unclear in its scope and strategy.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07907: Technologies to improve in vivo function of transplanted stem cells

GWG Recommendation: Recommended for Funding
Final Score: 75

Public Abstract (provided by applicant)

Stem cell-based therapy is recognized as a promising therapeutic approach for treating various diseases that are currently intractable. One strategy in regenerative medicine is to transplant stem cells or their differentiated derivatives to regenerate the damaged tissues or halt tissue degeneration. Human embryonic stem cells and human induced pluripotent stem cells having the potential to differentiate into every cell in the human body are highly promising sources of tissue specific cells. Despite the tremendous promise, current stem cell-based therapies suffer from low survival of transplanted cells and limited functional integration of the cells that do survive. In this research program, we address this bottleneck in cell-based therapy by developing tools and technologies to improve the in vivo differentiation, long-term survival, and integration of transplanted cells. Specifically, we will develop clinically translatable biomimicking materials and micro- and nano-technologies to improve the outcome of cell transplantation therapies. A successful cell transplantation therapy that contributes to tissue regeneration, restoring normal tissue function and homeostasis, and halting tissue degeneration will create a new paradigm in cell-based regenerative medicine. For instance, the global stem cell market is forecasted to reach roughly US $63.8 billion by 2015 and technologies such as the one developed here will be a vital component in making this forecast a reality.

Statement of Benefit to California (provided by applicant)

The generous investment of California citizens into stem cell research has made significant advancements in this field. These efforts have substantially improved our understanding of stem cell biology and our ability to differentiate these cells into targeted cell types. However, the technologies to culture and generate large number of stem cells are only one step towards cell-based therapies and the therapeutic outcome of cell-based therapies is dependent upon the ability of the transplanted cells to survive, migrate, and integrate with the host tissue. The proposed research program seeks to develop novel biomimetic materials and micro- and nano-technologies to improve the survival, migration, integration, and the function of transplanted stem cells. The proposed project will benefit the state of California by: (i) improving the efficacy and efficiency of stem cell-based therapies, (ii) maintaining California’s technological leadership in the field of stem-cell technology as well as its applications in healthcare,

(iii) training a highly educated and interdisciplinary work force in biomedical sciences, and (iv) contributing to California's economic leadership by translating technological advancements into commercial applications. The principal investigator and collaborators have a strong record of translating the technologies developed in their laboratory to practice through the formation of startups, and through interactions with industry, particularly within California.

Review Summary

Proposal Synopsis
This proposal aims to develop biomimetic matrix-based tools and nanotechnologies to promote better survival, integration and maturation of transplanted progenitor cells. The investigators will both engineer degradable and growth factor releasing hydrogels and test cell encapsulation approaches towards this goal. To test the applicability of these tools to regenerative medicine, the team will test their impact upon engraftment of human pluripotent stem cell derived (hPSC) muscle precursor cells in mouse models of skeletal muscle injury.

Significance and Rationale
- The proposal addresses a clear and critical bottleneck to regenerative medicine, *i.e.* how to improve stem cell survival and function *in vivo*.

- The panel was universally enthusiastic regarding the promise of the proposed biomaterial approaches for improving post transplantation survival and engraftment.

- These tools are likely to be broadly applicable beyond the specific therapeutic area tested in the application. They will likely benefit the material science field.

- A discussant noted that scalability of this technology for clinical application was not adequately addressed.

Feasibility and Experimental Design
- The bulk of the proposed studies are feasible and well designed.

- There is significant preliminary work to convincingly support most of the proposed studies, including the preclinical proof of concept testing.

- While reviewers were universally enthusiastic about the biomaterial work, there was concern that the application's muscle progenitors possess the repopulating potential of true satellite cells. Reviewer's noted that cell survival is only important if survival of the functional cell type is promoted.

- The experimental design and description of biomaterial chemistry lacks adequate detail in places.

- Studies related to modulating the cell culture environment were less clear than the rest of the proposal.

Qualifications of PI and Team

- The PI is highly qualified to conduct the proposed work; s/he possesses a strong track record in both developing biomaterials and stem cells.

- The team contains numerous collaborators and is strong in micro- and nanotechnologies, but lacks a muscle biologist, which is a clear weakness of the proposal.

- The role of some collaborators is unclear.

Responsiveness

- The application is highly responsive to the RFA.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07914: Skin-derived precursor cells for the treatment of enteric neuromuscular dysfunction

GWG Recommendation: Recommended for Funding
Final Score: 82

Public Abstract (provided by applicant)

The intestine performs the essential function of absorbing food and water into the body. Without a functional intestine, children and adults cannot eat normal meals, and these patients depend on intravenous nutrition to sustain life. Many of these patients do not have a neural system that coordinates the function of the intestine. These patients have a poor quality of life, and the cost of medical care is over $200,000 per year for each patient. Stem cell therapies offer potential cures for these patients while avoiding the risks of invasive procedures and hazardous treatments. A novel approach to treat these patients is to use stem cells derived from the patient's own skin to generate the neural system. This has been shown to be feasible in small animals, and the next step hinges on the demonstration of these results in a large animal model of intestinal dysfunction. We will develop a model in large animals that can be used to test the ability of skin-derived stem cells to form the neural system. Skin-derived stem cells will be isolated from large animal models and human skin to demonstrate their potential to generate a functional neural system. These cells will be transplanted into the animal model to determine the best way for these cells to make the intestine function properly. This research will gather critical information needed to begin a clinical trial using skin-derived cells to treat intestinal dysfunction.

Statement of Benefit to California (provided by applicant)

Gastrointestinal dysfunction destroys the lives of thousands of Californians. These Californians have frequent and prolonged hospitalizations and lost wages due to their chronic illness. It is estimated that the health care cost of Californians with gastrointestinal neuromuscular dysfunction is over 400 million dollars annually. Currently, most of these patients are covered by the state's insurance agency. Stem cell therapies offer potential cures for these patients and reduce this economic burden. The proposed research will obtain critical information needed to begin a clinical trial using skin-derived cells to treat patients with intestinal dysfunction. The California economy will significantly benefit from this research through the reduced costs for health care and increased quality of life of the affected Californians. Additionally, this work will add to the state's growing stem cell industry and will increase employment opportunities and revenue by the state of California. The educational benefit to Californians involved

in this research project will also maintain California's position in leading the stem cell effort in the future.

Review Summary

Proposal Synopsis
The investigator proposes to utilize skin-derived precursor/stem cells for the treatment of neuromuscular dysfunction. The overall objective of the application is to assess the therapeutic potential of the skin-derived stem cells in treating this disease. To accomplish this objective, the proposed studies will develop a large animal model of intestinal neuromuscular dysfunction, as has been done in rodents that will be amenable to stem cell transplantation. The precursor / stem cells will be isolated and characterized from both the animal model and human skin stem cells. The final phase of the proposed studies will optimize cell delivery mechanisms and engraftment in the model system (non-invasive endoscopy). If successful, the proposed research would provide a step toward an improved therapeutic approach for patients with intestinal neuromuscular dysfunction.

Significance and Rationale
- Development of a large animal pre-clinical model in which stem and precursor cells could be delivered therapeutically in a non-invasive fashion targeting intestinal tissue would be a significant achievement.

- The reviewers agreed that the rationale for the development of the technology is logical, adequately justified and scientifically sound. The reviewers indicated that if successfully completed, the project would likely have a major impact upon the field.

- The applicant proposes to use this approach to treat an underserved disease; those patients with neuromuscular dysfunction of the intestine. There is a risk of the proposal being too narrowly focused since the clinical affliction being targeted is somewhat small in numbers of patients.

Feasibility and Experimental Design
- The applicant has presented compelling preliminary data that are supportive of the proposed research.

- The reviewers considered the specific aims of the research project as logical, straight-forward and were confident they could be achieved in the proposed time frame.

- Although the proposal is feasible there is the possibility that the repair strategy is oversimplified as cells other than neurons must be present for functional recovery.

Qualifications of PI and Team

- The principal investigator and the research team appear to be well qualified to complete the proposed work. A concern was indicated that a biosketch provided for all the key personnel would have strengthened the application.

- Some reservations were expressed about the lack of collaboration with other investigators. The proposal would have been strengthened by the addition of someone with expertise in molecular biology.

Responsiveness

- The proposal addresses a current bottleneck in which stem cell therapies for neuromuscular dysfunction have been restricted to small animal models that involve invasive placement of the therapeutic cells. The development of a large animal model of intestinal dysfunction that will allow non-invasive placement of transplants is well within the scope of the RFA.

- Human stem cells derived from skin are used in the proposal.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07948: Injectable Hydrogels for the Delivery, Maturation, and Engraftment of Clinically Relevant Numbers of Human Induced Pluripotent Stem Cell-Derived Neural Progenitors to the Central Nervous System

GWG Recommendation: Recommended for Funding
Final Score: 77

Public Abstract (provided by applicant)

One critical bottleneck in the translation of regenerative medicine into the clinic is the efficient delivery and engraftment of transplanted cells. While direct injection is the least invasive method for cell delivery, it commonly results in the survival of only 5-20% of cells. Studies suggest that delivery within a carrier gel may enhance cell viability, but most of the gels used previously were naturally derived materials that have complex and unknown compositions. In our previous CIRM-funded work, we discovered that pre-encapsulating cells in very weak hydrogels offers the best protection during injection; however, those gels may be too compliant to support long-term cell survival. To address these limitations, we propose the design of a fully defined, customizable, and injectable material that initially forms a weak gel that then stiffens post-injection. We focus our studies on the delivery of human induced pluripotent stem cell-derived neural progenitors for the treatment of spinal cord injury (SCI). There are ~12,000 new SCI patients in the US each year, primarily young adults. SCI commonly results in paralysis, and the estimated lifetime cost for a patient can rise above $4 million dollars. In preclinical models of SCI, stem cell therapies have resulted in partial regeneration; however, reproducible delivery and engraftment of sufficient cells remain difficult and unmet challenges. This award potentially develops transformational regenerative therapies for SCI.

Statement of Benefit to California (provided by applicant)

The annual incidence of spinal cord injuries (SCI) in the United States is estimated at 12,000 new cases per year, with motor vehicle crashes accounting for up to a third of these cases. SCI has devastating impacts not only on the quality of life for the victims and their families, but also on their economic security – the estimated lifetime cost of an SCI patient can rise to over $4 million dollars depending on the severity and age at which the injury was sustained, not including the loss of wages and productivity. Although the most prevalent types of SCIs are those sustained at either the cervical or thoracic vertebrae, there are currently no definitive therapies approved for the chronic management of these SCI. Stem cell-based therapies have recently been shown to be mildly successful in several clinical and pre-clinical trials in various injuries and diseases, and a number of trials are ongoing for applications in SCI. In our proposal, we seek to

advance the stem cell-based approach to the treatments of SCI. The potential benefit of this proposal to the state of California and its citizens include 1) the provision of a better medical prognosis for patients with spinal cord injuries, 2) the improved quality of life for SCI patients and their families, 3) the reduction of the burden of health care costs, 4) the creation and maintenance of jobs in the stem cell technology field, and 5) preserving California's prominence in the field of stem cell research.

Review Summary

Proposal Synopsis
This proposal intends to address the limited survival and function of transplanted cells used in cell therapy applications. Current methods of direct injection of stem cells result in only about 5-20% of the cells surviving. This proposal aims to use bioengineering to develop a novel hydrogel material that will help optimize stem cell survival and function in the mammalian body. The proposed hydrogel will have many unique properties, such as the ability to vary its stiffness, adhesion sites for cell attachments and biodegradability. Stem cells encapsulated in this hydrogel will be used to treat a preclinical model of spinal cord injury. The animals will be monitored and evaluated for cell viability, function and tissue regeneration using standard experimental protocols. This application addresses the CIRM-identified bottlenecks of stem cell engraftment and preclinical evaluation.

Significance and Rationale
- The application addresses an important area in regenerative medicine, i.e. how to improve stem cell survival and function in vivo.

- Design of the novel hydrogel, with the described two-stage gelling process is innovative potentially significant. The approach has the potential to improve the efficiency of cell transplantation by enabling stem cells to survive during injection as well as promote their growth and survival in vivo

- There was some concern that the preliminary data were all with a cell type that is more robust and stress tolerant than the proposed therapeutic cells and may therefore not be a widely applicable approach.

Feasibility and Experimental Design
- The described hydrogel is designed to be tunable to support controlled cell adhesion, growth and degradation.

- The reviewers had split opinions regarding the strength of the provided preliminary data. While some reviewers felt that the proposed research is backed by strong preliminary data and previous publications, others felt that additional preliminary data

were required, particularly noting that some of the preliminary data is not with the cell type proposed.

- Reviewers expressed some concern that the work appears to be in early stages and will require much development before it can be applied clinically.

Qualifications of PI and Team
- The reviewers felt that a strong multidisciplinary team with competent investigators has been assembled.

- The PI and co-PI offer complementary expertise and leveraged funding.

Responsiveness
- The application addresses a bottleneck in the translation of stem cells, specifically the survival, integration, and differentiation of transplanted stem cells.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07949: Embryonic stem cell-based generation of small animal models for assessing human cellular therapies

GWG Recommendation: Recommended for Funding
Final Score: 82

Public Abstract (provided by applicant)

Heart failure, diabetes and neurodegenerative diseases are among the leading causes of death and disability worldwide. These diseases are characterized by the loss of specific cell types and can be treated and potentially cured with stem cell-based therapies. Before human stem cells can be used in clinical trials, however, their safety and efficacy need to be tested in animal models. Currently, immunodeficient small animals are the preferred models. However, it is readily apparent that mouse physiology and behavior is not optimal for studying many human conditions, and this has often led to translation failures. Although larger animal models are useful, they are extraordinarily expensive and, consequently, experimental opportunities and replications are very limited. The rat is widely accepted as more similar to the human in its physiology and therefore superior to the mouse, especially for metabolic, cardiac and neurological studies. We recently developed a technology that allows us to create nearly any type of genetically modified rat. Using this technology, we will develop immunodeficient rats and rat models for heart failure, diabetes and neurodegenerative diseases. We will use these models to assess human cellular therapies. Our project will provide the research community with the tools and technology necessary to overcome the current constraints of mouse models and will serve as a better investigative platform for understanding the progression and treatment of human diseases.

Statement of Benefit to California (provided by applicant)

Heart diseases, diabetes and neurodegenerative diseases affect hundreds of thousands of people in California. These diseases can be potentially improved or even cured by cell replacement-based therapies. Using the small animal model embryonic stem cell-based gene-targeting technology that we developed, we will create small animal models for heart failure, diabetes and neurodegenerative diseases. These small animal models can closely mimic human conditions, and are not expensive to produce. We will also create an immunodeficient rat model to facilitate the assessment of human stem cell therapies in a xenotransplant context. The models developed in this project will be extremely valuable to many investigators in California. We anticipate that, based on results generated using these animal models, new cellular therapies could be developed and many patients in California could benefit from these new therapies. We

also anticipate that the new technologies, cell lines and animal models developed in this project will result in intellectual properties that will bring tax revenue to California.

Review Summary

Proposal Synopsis
The bottleneck addressed by this proposal is the development of a cost-efficient small animal model that can be used to better assess the efficacy and safety of human stem cell products than is currently possible using mouse models. In this proposal, the research team will produce rats that have been genetically engineered to be immunodeficient and/or models for heart failure, diabetes, or a neurological disease. The investigators will then validate these models by testing the safety and efficacy of human pluripotent stem cell derivatives. Lastly, the investigators propose to create a resource by distributing these animals and the genetic engineering technology to the scientific community.

Significance and Rationale
- Reviewers were enthusiastic about the development of a cost-effective small animal model that is better suited for cell dosing studies than widely used mouse models.

- Reviewers were enthusiastic about the plans for dissemination of the rat models and the technology to the stem cell research community.

- Reviewers considered the specific design of the genetically engineered heart failure and diabetes models to be a strong asset of this proposal.

- There was some debate as to whether the proposed immune compromised rats truly provide an advance as compared to the use of currently available rat models. Some reviewers thought the specific approach to establish immunodeficiency represents a major advance and that the proposed rats may provide more complete immunodeficiency. Others thought the current immune compromised rat models sufficient for preclinical testing of cellular therapies.

Feasibility and Experimental Design
- The preliminary data demonstrate success in being able to generate and engineer rat embryonic stem cells and to develop genetically modified rats.

- The experimental plan is detailed and rational.

- The proposal is very ambitious and time lines are aggressive given inherent limitations of animal breeding. It is unlikely that the generation of all the proposed models could be accomplished in the required time frame. However, reviewers felt that the generation and validation of even one model would be a big step forward.

- Some reviewers suggested the applicant employ methods that increase cell retention following injection when validating the rat models.

Qualifications of PI and Team

- The principal investigator (PI) is excellent with a strong track record.

- The PI has secured important collaborators with complementary expertise important for the success of the project.

Responsiveness

- The proposal was seen as highly responsive to the RFA. If successful, it will provide a unique tool that will benefit the stem-cell community.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07962: A large animal model of mucopolysaccharidosis I for stem cell therapy development.

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Mucopolysaccharidosis I (also called MPS I) causes tremendous suffering and early death to affected children. Stem cells hold tremendous promise for treatment of this condition. First, stem cell therapy is permanent, meaning that the child could be essentially “cured” with a single procedure. Second, MPS I is caused by a missing enzyme. The enzyme would be naturally produced by the stem cells, so that we can correct the exact cause of MPS I disease. Third and most importantly, we are already treating children who have MPS I with stem cells, in the form of a bone marrow transplant. Bone marrow contains natural stem cells that provide the missing enzyme when transplanted into children with MPS I. Bone marrow transplant for MPS I is probably more effective than bone marrow transplant for any other condition known, but it is still not a cure. Children who receive a bone marrow transplant for MPS I continue to suffer from loss of intellect (dementia in their teenage or early adult years), because the bone marrow transplant does not get enough enzyme into the brain. In this project, we propose to develop an animal model that will allow testing of brain-directed stem cell therapy for MPS I. The results will allow us to conduct the studies with brain-directed stem cells that will bring this promising new therapy to children with MPS I.

Statement of Benefit to California (provided by applicant)

MPS I is a rare disease, affecting between 1 in 100,000 and 1 in 200,000 children born each year (approximately 2-5 born per year in the state of California). It is not known how many individuals with MPS I currently reside in the state, but the U.S. prevalence is estimated at 1500 people, which would give a rough estimate of 180 people (California accounts for approximately 12% of the U.S. popula-tion). Currently, many (about half) of these children are treated with weekly Aldurazyme enzyme re-placement therapy at a cost of over $200,000 per patient per year, for life (covered by Medi-Cal). In addition, there is an unquantified cost of hospital care and surgical procedures for residual disease that is untreated by current therapies. Stem cell therapy has the potential to greatly alleviate this cost burden, by dramatically improving overall health and removing the need for life-long Aldurazyme treatment. Our program would develop a model to test therapies that would substantially alleviate the burden of this debilitating disease in affected individuals, thus meeting the CIRM mission of alleviating chronic disease and suffering. While specific to MPS I, the stem cells created in this

proposal would aid model development for a vast array of stem cell therapies that would require the use of this species.

Review Summary

Proposal Synopsis
This proposal aims to generate induced pluripotent stem cells (iPSCs) from a large animal model, differentiate them into neural stem cells (nSCs) and test them as a cellular therapy for Mucopolysaccharidosis type I (MPS I), a severe neurological disorder. MPS I is caused by the absence of a single enzyme which is normally secreted and taken up by other cells. The large animal model described for this proposal is one deficient in the same enzyme that causes MPS I in humans and results in a similar disease phenotype. The investigators propose to generate iPSCs from the model animal species, differentiate them into nSCs and characterize them in vitro. The nSCs will be transplanted into the brains of the diseased animals and followed for their distribution, long-term survival, and therapeutic potential.

Significance and Rationale
- Reviewers were not convinced that this proposal would help solve a major bottleneck in this field or necessarily provide information beyond that which is already available from other studies.

- Reviewers were of the opinion that development of a large animal MPS-1 model that supports xenografting of human derived cells would provide more value to the stem cell community. They noted that allogeneic cell transplant is proposed and immunosuppression will still be required, making the value of this approach over human cell transplant unclear.

- The proposal utilizes a potentially advantageous large animal model with relevance to human therapy due its large brain size and longevity. However, reviewers questioned how different the information obtained from large animal models of diseases like MPS I has been from that obtained through studies of mice.

Feasibility and Experimental Design
- Significant concerns were raised that diseased brains may not be conducive for survival or function of implanted cells.

- The preliminary data provides feasibility for i) large animal iPSCs generated from skin fibroblasts and ii) human nSCs derived from iPSCs. However, the ability to generate large animal nSCs, which is a prerequisite for the entire application, remains to be demonstrated.

- It is not until year 2 that phenotypic characterization of nSCs is planned. It is unclear what comparisons will be made with human and murine cells.

- Potential obstacles in generating sufficient animal numbers were viewed as a minor concern.

-The experimental aims are supported by the PI's established experience with the proposed large animal model.

Qualifications of PI and Team

- The PI and team are highly qualified to carry out the proposed studies.

- The proposal is supported by strong individual and departmental commitments.

Responsiveness

- The proposal is more focused on establishing proof-of-concept data for a cell therapy for MPS I than developing tools to address a translational bottleneck. The animal model already exists and generating iPSCs from this species is not novel.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07965: Optimizing safety and efficacy of transgenic human induced pluripotent stem cell-based personalized cellular therapeutics

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Human stem cells have the ability to make almost any cell in our body, thereby providing new and exciting opportunities for research and therapy. The conceptual foundation for addressing this dream of using our own cells to cure diseases, injuries, and disorders stems from delivering custom genetic elements, in a safe and reproducible manner, into human stem cells, unlocking these cells' full potential for personalized regenerative medicine. However, current technology still induces considerable genetic damage in the stem cell product that represents a significant safety concern, and there is a worry that the cells might turn into cancer many years after transplantation.

We propose to integrate state of the art nuclear reprogramming, genetic engineering, in vivo imaging and customized viral technologies to develop, optimize, and standardize a broadly applicable stem cell genomic modification process to address these safety concerns and advance human stem cell-based therapies. The foundation of stem cell genomic modification is the proposed innovative and customizable transgenic vector that can facilitate the establishment of a broadly applicable standardized process for creating transgenic autologous pluripotent stem cells for a range of diseases. Optimized pluripotent stem cell transgenesis involves incorporating novel technologies and stem cell genetic engineering approaches in order to increase the safety and efficacy of future personalized stem cell-based therapies.

Statement of Benefit to California (provided by applicant)

Human induced pluripotent stem cells (hiPSCs) offer great promise to the large number of Californians suffering from injuries, disorders, and diseases, including Alzheimer's disease, cardiovascular disease, diabetes and liver disease. The goal of this project is to develop a targeted transgenesis process to quickly add a customizable triple selection (TS) vector into hiPSCs, without inducing adverse off-target mutagenic events in the hiPSC-derived therapeutic cell product. It is our central hypothesis that hiPSC-derived therapies which incorporate our proposed targeted transgenesis process will demonstrate greater safety and functionality than that observed with equivalent genomically unmodified cells.

The proposed targeted transgenesis process will: 1) benefit California patients suffering from injuries, disorders and diseases, by providing safer and more effective hiPSC-based therapeutics, 2) benefit the California academic community and biotechnology industry, by providing an easily customizable hiPSC targeted transgenesis process for basic research and translational applications, and 3) benefit the California Institute for Regenerative Medicine, by (i) introducing a broadly applicable targeted transgenesis technology to their stem cell translational portfolio, and (ii) providing a significant potential source of future revenue to help support CIRM in its ongoing mission to advance personalized stem cell therapeutics.

Review Summary

Proposal Synopsis
The objective of this proposal is to address a bottleneck in the production of safe genetically engineered human induced pluripotent stem cell (hiPSC) lines to be used for therapeutic transplantation purposes. Current random integration approaches to genetically modify hiPSCs can result in unsafe insertional mutagenesis. The applicant proposes using their newly designed vector system to target safe harbors when introducing transgenes into hiPSCs to prevent the activation of cancer-causing oncogenes. In addition, they plan to optimize culture conditions as a means to reduce DNA damage that occurs during cell division, thereby facilitating genomic stability. Finally, the applicant intends to perform a proof of concept study for the treatment of a genetic disorder in a relevant animal model, using hiPSCs manipulated with the technologies developed in this proposal. The targeted outcome of this project would be to provide a better transgenic vector and improved culture conditions that could increase the safe production of therapeutically relevant hiPSCs to be used in a variety of disorders.

Significance and Rationale
- The idea of targeting safe harbor sites was not considered to be a new approach, and reviewers did not think the applicant sufficiently conveyed how the proposed work surpasses similar studies and approaches.

- Some reviewers commented that it was not clear that clinically relevant mutations arise during culture expansion as asserted by the applicant, so the proposed optimization of culture conditions may be addressing an issue that lacks relevance to stem cell therapies.
- The development of a safe and efficient method for genomic modification could address a major roadblock for producing clinically relevant hiPSCs.

Feasibility and Experimental Design
- Concerns were raised regarding study design for the comparison of hiPSC cultured under conventional conditions versus those developed here, as outcome measures were

not stated and it was unclear whether the studies were sufficiently powered to test differences in tumorigenesis.

- Reviewers thought there was a lack of preliminary data for the proposed proof of concept study.

- Reviewers did not think the potential for off target integration events was sufficiently discussed in the application or addressed in the experimental plan.

- Reviewers considered the vector work feasible based on preliminary data and principal investigator expertise.

Qualification of PI and Team
- The principal investigator has worked in collaborative research projects and seems qualified to oversee the project.

- The reviewers commented favorably about the overall competence of the research team, but some questioned the need for such a large number of collaborators, each with relatively small contributions to the study, which could contribute to difficulties in managing the proposed study.

Responsiveness
- The goal of the proposed studies is to provide a better transgenic vector that could increase the safe production of therapeutically relevant hiPSCs, which is highly responsive to the RFA.

- The proposal will use human-induced pluripotent stem cells (hiPSCs).

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07974: MRI reporters to noninvasively image long-term stem cell engraftment in large animal spinal cord injury model

GWG Recommendation: Not Recommended for Funding
Final Score: 64

Public Abstract (provided by applicant)

Stem cells offer tremendous potential to treat previously intractable diseases. However, the clinical translation of these therapies presents unique challenges. One of which is the absence of robust methods to monitor cell location and fate after delivery to the body. The delivery and biological distribution of stem cells over time can be much less predictable compared to conventional therapeutics, such as small-molecule therapeutic drugs. This basic fact can cause road blocks in the clinical translation, or in the regulatory path, which may cause delays in getting promising treatments into patients. This project aims to meet these challenges by evaluating a new non-invasive cell tracking platform for emerging stem cell therapies. Recent progress in magnetic resonance imaging (MRI) has demonstrated the feasibility of non-invasive monitoring of transplanted cells in patients. We will evaluate new MRI methods that use DNA-based instructions for making cells visible in MRI; when these instructions are programmed into stem cells, one can repeatedly image the location of the cells after they become integrated into tissue. The DNA-based instructions tell the cell to make a specialized protein that accumulates iron in its core and renders the cell magnetic, thereby enabling MRI visualization. We will create specialized neural stem cells that harbor these instructions and evaluate their use in the context of emerging stem cell therapies to treat spinal cord injury

Statement of Benefit to California (provided by applicant)

California leads the nation in supporting stem cell research with the aim of finding cures for major diseases afflicting large segments of the State’s population. Significant resources are invested in the design of novel cellular therapeutic strategies and associated clinical trials. A common need to accelerate the clinical translation of these potentially live saving therapies are methods to non-invasively image the behavior and movement of cells following transplantation. Recent progress in magnetic resonance imaging (MRI) has demonstrated the feasibility of non-invasive monitoring of transplanted cells. These MRI cell tracking methods will be applied to promising new stem cell therapies for spinal cord injury. Overall, MRI cell tracking can accelerate the go/no go discussion making process at the preclinical and clinical trial stages, and can facilitate smaller, less costly trials by enrolling smaller patient numbers. Imaging can potentially yield data about stem cell engraftment success. Moreover, MRI cell tracking

can help improve safety profiling and can potentially lower regulatory barriers by verifying survival and location of transplanted cells. Importantly, MRI cell tracking can help maximize the impact of the State's investments in stem cell therapies by speeding-up clinical translation into patients. This project will lay the foundation for clinical adoption of MRI cell tracking and position California to take a leadership role in driving this technology.

Review Summary

Proposal Synopsis
This application proposes to develop a magnetic resonance imaging (MRI)-based imaging platform to track stem cells in vivo and test this platform in animal models, including a large animal model of spinal cord injury. Neural progenitor cells engineered to express the iron-encapsulating protein will be detected via their paramagnetic signature in MRI. A small and large animal model will be applied to test the technology. Longitudinal studies of cell fate and distribution will be performed using MRI and immunohistochemistry.

Significance and Rationale
- Reviewers felt that the potential for clinical application of the proposed MRI-based imaging technique is limited. The resolution and sensitivity of the proposed tools may not be sufficient to study the survival, distribution, and engraftment of transplanted cells in humans.

- The ability to track the location of injected stem cells is currently limited, and this application addresses an important area in regenerative medicine by proposing to develop a tool to visualize stem cells in vivo. However, alternative reporters and imaging modalities may be more effective than MRI in terms of addressing this bottleneck.

- The reviewers considered the engineering of new protein constructs to be innovative.

-Reviewers acknowledged that although the potential for clinical use might be limited, the potential use of the system as a preclinical tool has significance.

Feasibility and Experimental Design
- Preliminary data in the large animal model showing feasibility in sensitivity and resolution with clinical MRI is lacking.

- High intracellular iron may impair the function of the transplanted stem cells. Reviewers would have appreciated preliminary data addressing the potential for toxicity to cells.

- It is not clear how various potential artifacts will be addressed, for instance how transplanted cells will be distinguished from cells generated endogenously due to injury.

- Development of an inbred large animal model in order to avoid the need to generate autologous induced pluripotent stem cell lines was considered to be a strength.

- The reviewers felt that this application is overly ambitious for a 3-year timeline.

Qualifications of PI and Team
- The PI and the team are well qualified to conduct the proposed work.

- The collaborations, reagents and resources for the proposed studies are in place. Reviewers found the large animal collaboration to be a strength.

Responsiveness
- The proposal directly addresses a bottleneck in stem cell translation.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07975: A comprehensive microfluidic platform for the production of stem cell micro-beads for therapeutic transplantation

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Our project addresses the need for clinically compatible technologies for human stem cell delivery, robust engraftment and maintenance, and in vivo monitoring of cell function and fate at high sensitivity. Transplants of human neural stem and progenitor cells (HuNSPCs) are moving forward clinically for spinal cord injury (SCI) and numerous other central nervous system injuries and diseases. As trials develop, it will be imperative to ensure survival and appropriate differentiation of transplanted cells. Adequate survival and differentiation of cells in an often hostile transplant environment continues to be a significant bottleneck for the field. The current method to check cell viability and differentiation for stem cell therapies is to extract the tissue and stain it with antibodies. Under these conditions, we cannot monitor cell viability and stem cell differentiation in vivo. To address these pitfalls of standard stem cell transplantation we propose to encapsulate HuNSPCs in microbeads to ensure cell viability and use a novel microscopy tool (FLIM) to monitor stem cell health and differentiation without needing to excise or stain the tissue. This project would

Statement of Benefit to California (provided by applicant)

The goal of this project is to to encapsulate HuNPCs into monodisperse beads made of matrix scaffold biomaterials to ensure cell viability and use fluorescence lifetime imaging microscopy (FLIM) to monitor stem cell health and differentiation using autofluorescence without needing to excise or stain the tissue. In the course of these studies, we will address the need for clinically compatible nanotechnologies for human stem cell delivery, robust engraftment and maintenance, and in vivo monitoring of cell function and fate at high sensitivity. We would greatly improve the efficacy and safety of transplants of human neural stem and progenitor cells (HuNSPCs) clinically for spinal cord injury (SCI) and numerous other central nervous system injuries and diseases. The project would also create new technologies for the general stem cell fundamental and clinical research fields. It is likely that companies in California would be interested in commercializing this promising capability that would improve stem cell therapies to benefit patients in California and around the world. As a result, this project will generate new jobs for high-skilled workers and, hopefully, intellectual property that will contribute to the economic growth of California.

Review Summary

Proposal Synopsis
Adequate survival and differentiation of transplanted cells continues to be a significant bottleneck in regenerative medicine. This application proposes to develop a microfluidics system for the encapsulation of cells in droplets and thus provide them with an appropriate microenvironment for increased survival and differentiation. The proposed research aims to encapsulate human neural stem and progenitor cells (HuNSPC) to protect the cells following transplantation. Fluorescence lifetime imaging microscopy (FLIM) will be used to monitor cell health and differentiation using auto-fluorescence without the need to excise or stain the tissue.

Significance and Rationale
-The reviewers felt that the clinical compatibility of the encapsulation process/technology was unclear and not discussed in detail.

-This application addresses a bottleneck prioritized by CIRM i.e., the use of tissue engineering technologies to advance human stem cell delivery.

Feasibility and Experimental Design
-The reagents used for microencapsulation (oil) have not been clearly defined or investigated. There was no data on the effect of the oil on cells or surrounding tissue.

-Reviewers considered the imaging technology weak due to the limitations of resolution, depth and could be an invasive procedure that may involve surgical procedures for access to tissue of interest.

-It was not clear to the reviewers how the encapsulated cells will integrate and function in tissue and contribute to tissue repair in vivo.

-While it was appreciated that the preliminary data demonstrate feasibility of all experimental approaches, no alternative plans were given should the proposed experiments fail to meet their end points.

Qualifications of PI and Team
-The reviewers voiced concern that the collaborators have not worked together in the past.

-It was noted that the PI and individual co-PIs are well qualified to conduct the proposed work.

Responsiveness

-The proposal directly addresses the RFA priorities in cell delivery and maintenance in target tissues and imaging.

- No dissemination plan was provided, although the assumption is that this would be done through publications and out-licensing.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07981: Multi-modal technology for non-destructive characterization of bioengineered tissues

GWG Recommendation: Tier 2
Final Score: 72

Public Abstract (provided by applicant)

Stem cell technologies hold great promise for engineering replacement tissues for repairing functional loss from trauma or disease. Such therapies are particularly important for replacing bone and cartilage in the aging population to maintain an active quality of life. However, the application of stem cells to generate individualized implantable grafts suffers from patient-to-patient variability that is unpredictable and immeasurable without destructive techniques, representing a major bottleneck in translating stem cell technologies to the clinic and delivering a quality product. This process could be markedly improved by the availability of nondestructive, non- or minimally invasive methods to measure dynamic changes in tissue development, thereby reducing the quantity of tissue collection for sufficient cell numbers and cutting costs that do not directly benefit the patient. During tissue formation, cells deposit extracellular matrix molecules that possess a unique fluorescence signature, which can be detected by light, while matrix quantity, detectable by ultrasound, correlates with mechanical strength. We propose the development and application of a multi-modal imaging probe that uses light and sound to detect changes in engineered bone and cartilage, which reflect maturity and mechanical properties. The availability of this tool will advance the personalized medicine aspect of stem cell-based tissue formation while providing new insight into dynamic tissue development.

Statement of Benefit to California (provided by applicant)

The aging population of California, 20% of whom will be over 65 in 2025, will require functional replacement tissues to maintain their quality of life. The promise of using stem cells to generate individualized grafts suffers from donor variability that is unpredictable and immeasurable without destructive techniques. The development of a nondestructive, minimally invasive tool enabling the dynamic assessment of tissue maturation and remodeling would provide users unparalleled insight without destructive biopsies. Herein, we aim to develop a multi-modal imaging probe that uses light and sound to measure the maturity of stem cell-generated bone and cartilage by detecting unique signatures of extracellular matrix components and observing matrix deposition. After optimizing the probe for these tissues, we will characterize maturation of engineered tissues in vitro and after implantation. This tool will reduce the number of cells required to create tissues by eliminating destructive biopsies and provide an

individualized tissue product to maximize clinical outcome, resulting in reduced healthcare costs. The technology will be invaluable to clinicians and biotechnology companies pursuing regenerative medicine in California. Finally, the exposure of trainees to new stem cell-related research may provide the greatest benefit to California by inspiring future scientists to pursue their research efforts within the state or develop therapies at California-based companies.

Review Summary

Proposal Synopsis
The proposal describes the development of a non-invasive optical and ultrasound based technology that can be used to better evaluate engineered tissue. Typically, tissues are analyzed through destructive techniques, which can be an issue due to limited cell numbers and are not useful to analyze what happens after constructs are implanted into the body. The Principal Investigator (PI) proposes two interactive aims. First, the PI will develop and optimize an optical-ultrasound imaging modality and determine its ability to evaluate native and constructed bones and cartilage. Second, the PI will evaluate the ability of this imaging modality to monitor changes to the constructs during cellularization in in vitro and in vivo models.

Significance and Rationale
- It was perceived that the proposal is largely a validation of an established technique and does not introduce a new technology.

- While there may be clinical utility, this proposed work appears to be an incremental step, not the ultimate method.

- The ability to have a noninvasive means to monitor transplants would be incredibly valuable.

-The rationale for the use of the technology is logical and compelling.

Feasibility and Experimental Design
-There was concern with the limited depth of imaging into the constructs using the technologies described. It is conceivable that the engineered tissue may perform differently at the surface than with depth into the tissue.

- The spatial resolution of the technology was not clear.

- There was concern that the PI did not describe how the methodology will circumvent signals from red blood cells, since red blood cells have strong fluorescent signals.

- The preliminary data were compelling and supportive.

- The research plan was carefully designed and logical.

Qualifications of the PI and Team
- The PI and the team are qualified to conduct the proposed studies.

- All of the necessary technologies to conduct the studies are available to the PI and team.

Responsiveness
- The proposal is responsive to the RFA.

- The studies are not particularly focused on stem cells but stem cells are proposed to be analyzed.

REVIEW REPORT FOR CIRM RFA 13-05 TOOLS AND TECHNOLOGIES III AWARDS

RT3-07990: Joint surface regeneration: Deliver stem cells and control their fate with novel intelligent clinical grade biomimetic materials

GWG Recommendation: Not Recommended for Funding
Final Score: --

Public Abstract (provided by applicant)

Our joints degrade with general use throughout our lives. Degraded joints or arthritis not only decreases joint mobility but also causes significant pain that can even wake a person up at night or cause sadness. The gold standard for large joints such as the knee or the hip is an artificial joint replacement made of metal. But such artificial joints do not allow full function of a real joint. Whether old or young, current treatments for arthritis are inadequate. Our goal is to renew the joint surface of damaged joints with real human cells making new cartilage and bone. In this way, people will have normal joints again and enjoy activities of daily living. To achieve a new joint surface, we plan to implant special stem cells called “induced pluripotent stem cells” to the site of damage. The key in making these cells work is to place the cells in a special mesh that will carry and keep the cells in the right place, where the damaged part of the joint needs replacing. These meshes will also instruct the cells what to do (in particular to become a cartilage or bone cell) and also disappear slowly overtime so that meshes are completely replaced only with joint cells. We will focus on making such meshes with desired shapes and to carry the right cells. We combine two major processes to make the meshes 1. 3D printing (where a printer makes a 3D object of our choice) and 2. Electrospinning (a technique to make tiny fibers very similar to fibers found in human organs).

Statement of Benefit to California (provided by applicant)

Bone is the second most transplanted tissue in the body (after blood transfusion). Bone graft for healing and treatment is used in 1 million cases in the U.S. each year. There is an critical need for a biomaterial that can serve as a temporary matrix to support stem cell osteogenesis differentiation (new bone generation) at injury sites. Thus, successful completion of this work will not only provide citizens of California much needed advances in bone healing technology and improvement in health care but it will also provide more high paying jobs and significant tax revenue in California. Our low-cost 3D functionalized scaffolds may also lower the financial burden of the health care system in the state and increase profitability for the California-based companies and health care providers.

Review Summary

Proposal Synopsis
The objective of this proposal is to design a 3D biodegradable scaffold material that facilitates regulated growth and differentiation of induced pluripotent stem cells (iPSCs) or human mesenchymal stem cells (hMSCs). The research team proposes to construct scaffolds for reconstruction of the bone-cartilage interface for transplantation of iPSCs and hMSCs. To accomplish the proposed tasks, they will: 1) design electrospun matrices using biodegradable polymers, 2) test cellular behavior of iPSCs and hMSCs, and 3) validate the translational potential of the proposed technology in a preclinical animal model.

Significance and Rationale
-Although the key goal is to reconstruct bone-cartilage interface, there was no preliminary data and very limited information in the experimental plan addressing cartilage regeneration.

-The proposed studies would not add significantly to similar ongoing work in this area.

-It is not clear that the studies proposed would address a specific bottleneck in the field.

Feasibility and Experimental Design
-The proposal lacks details of the design and rationale of some proposed studies. The experiments are not clearly rationalized, suggesting that approaches are not clearly planned and may not address the proposed goals.

-The lack of preliminary data showing the ability to make matrices with the intended design using the proposed electrospinning technology raised questions about the feasibility that the method could be optimized as proposed.

-It is also unclear why new iPSCs lines need to be generated, and there was not a strong rationale for the outsourcing of iPSCs generation to Australia which may be a limiting factor to accomplishing the proposed goals.

-The indicated preclinical animal model is not considered appropriate for bone/joint diseases.

Qualifications of PI and Team
-There was some concern that the research team does not have sufficient experience and expertise in cartilage cell-matrix biology and iPSCs differentiation.

-The detailed plan for transport of materials and cells between laboratories is not clearly stated.

-The PI and research team have extensive experience in polymeric biomaterials for tissue engineering as well as in osteogenic tissue engineering.

Responsiveness
-The proposal work plans to use pluripotent cells but it is unclear whether these cells are human derived.

-Targeting bone/joint diseases and developing a therapy for ailments like osteoarthritis would address a significant medical need.