MEMORANDUM

Date: January 21, 2009

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RT1-01073-1

Enclosed is a letter from Dr. Susan Fisher, of the University of California San Francisco, an applicant for funding under RFA 08-02, CIRM Tools and Technologies Awards. This letter was received at CIRM at least five working days prior to the January ICOC meeting, and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

As required by that policy, I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with Dr. Csete and the scientific staff, and concluded that the petition does not present compelling evidence that should alter the recommendation or score of the Grants Working Group (GWG).

We agree that reviewers considered this a meritorious application. The proposal to create an "antibody tool kit for human embryonic stem cells and their progeny" was highly regarded but not unique. To clarify, there is at least one other application in Tier 2 that proposes the development of an antibody tool kit.

In regard to the reviewer criticisms, we believe that reviewers were justified in raising concern about the PI's overall commitments as the application lists 15 currently active projects that consume 87% of the PI's effort. In addition, the PI lists 3 pending projects that would consume 40% effort. Regardless of additional personnel that can help lead the project, the PI is ultimately responsible for managing and ensuring that the project is carried out as proposed. We disagree that the only way to increase the percent effort of a given individual is to remove another individual from the project. The CIRM Grants Administration Policy limits the annual salary requests for each key person to $207,000. It does not, however, limit the percent effort that an investigator can commit to a project. In fact, a key person may contribute any effort between 1 and 100 percent without requesting any salary support from the CIRM grant. Therefore, the budget cap alone should not prevent an investigator from contributing greater effort when appropriate. We recognize that effort contributed to a project should be appropriately compensated, but the CIRM grant should not necessarily be viewed as the sole source of support.

CIRM staff will be prepared to provide further analysis, should that be requested by any member of the committee.

Redactions, if any, have been made pursuant to the policy, in consultation with the author(s) of the letter. An unredacted version will be available for review in closed session.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

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January 22, 2009

Robert Klein, Chairman, Independent Citizen's Oversight Committee
Alan Trounson, President
Marie Csete, Chief Scientific Officer
California Institute for Regenerative Medicine
210 King Street,
San Francisco, CA 94107

Re: Extraordinary Petition for ICOC Consideration of Funding Application RT1

Dear Sirs and Madam,

We are writing to seek your support for funding of our CIRM Tools and Technologies Award Application, RT1-01073-1: "Production of an Antibody Toolkit for Characterizing Human Embryonic Stem Cells and Their Differentiated Progeny."

Prior to making a funding decision, we respectfully ask that you consider several unique elements/strengths of our proposal as well as our response to administrative issues that were raised in the critique:

*Antibodies are essential tools for defining the differentiation stages of cells in distinct lineages. Although this approach has been beautifully used to define hematopoietic stem cells and their progeny, the precise phenotype of "stemness" and differentiation stages in other lineages remains rudimentary. Our application is the only grant that was not triaged that proposes to provide an antibody tool kit to define human embryonic stem cells (hESCs) and their differentiated progeny. In fact, the reviewers unanimously agreed that we had achieved "a near optimal congregation of expertise."

*Our grant proposes a dual approach. Using a comprehensive screening strategy, we will identify commercial antibodies already available to investigators that define hESCs and their progeny. In addition, using conventional and phage display monoclonal antibody techniques we will generate new antibodies that well be screened on very low passage nonfederal hESCs and, in a handful of cases, on human embryos.

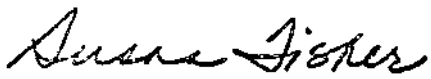
*In partnership with a major company with the largest collection of monoclonal antibodies in the world, we will identify currently available antibodies that can be used NOW to identify hESCs and their differentiated progeny. Specifically, we will combine several antibodies using distinct fluorochromes. This approach will enable the identification of unappreciated subsets of cells, a proven approach for hematopoietic stem cells that we will apply to hESCs and their progeny. We note

that the latter cells include pancreatic beta cells, cardiomyocytes, and neurons that can be used in regenerative therapies for diseases that are a high priority for CIRM. Finally, we are leveraging the resources of a California company, which anticipates maximizing its return on its investment.

* We can easily clarify two issues that were the reviewers' biggest concerns, "that the PI is already very highly committed to other programs… and there is low percent effort commitment by many of the key personnel." (1) Although Dr. Fisher is listed as the PI, Dr. Lanier serves as the co-leader of this project. Drs. Fisher and Lanier are very experienced in leading large-scale projects, and will combine their scientific expertise and managerial skills to organize a team of scientists with diverse backgrounds to successfully execute the project goals. (2) The low percentage of effort contributed by key personnel is a direct result of the inherent conflict between the budget cap and the number of key personnel required for this multi-dimensional project. Please note that our group uses the combined expertise of hESC experts as well as leaders in the major branches of regeneration medicine that promise the highest likelihood of success. Given the budget cap, the only way to increase the percent of individual effort is to remove investigators, which would lead to a sub-optimal mix of expertise that will undoubtedly negatively impact the outcome. We think that the strength of our team as a whole is the strongest element of our application.

In summary, we hope that the critical importance of producing antibody kits that will allow investigators to better define hESCs and their differentiated products will outweigh any concerns regarding the ability of this experienced team of investigators to deliver the reagents that the stem cell research community desperately needs. Thank you for considering these mitigating factors.

Sincerely,



Susan Fisher, Ph.D.
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Center for Reproductive Sciences
Department of Obstetrics, Gynecology and Reproductive Sciences
Department of Anatomy
The Eli & Edythe Broad Center for Regeneration Medicine and Stem Cell Research at UCSF
Director, Human Embryonic Stem Cell Program
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RT1-01073-1: Production of an Antibody Toolkit for Characterizing Human Embryonic Stem Cells and Their Differentiated Progeny

Recommendation: Recommended if funds available
Scientific Score: 77
First Year Funds Requested: $444,493 **Total Funds Requested:** $904,172

Public Abstract (provided by applicant)

Monoclonal antibodies are essential tools in many fields of biomedical research. The power of the technology is that each antibody has exquisite specificity able to identify and study single proteins expressed by cells and tissues. This nobel prize-winning technology has revolutionized biologists' ability to analyze and separate individual cell populations. Moreover, monoclonal antibodies have been developed into potent therapeutics that have changed medical care.

Since the early 1980s, when methods for culturing mouse embryonic stem cells (ESCs) were first developed, there has been a need for reagents, including antibodies, that could define different stages of development and differentiation. These studies have met with limited success, in part to do the highly heterogeneous nature of developing tissues. With the discovery of human ESCs in 1998, the challenges have increased. It has become clear that while they share many characteristics of their mouse counterpars, human ESCs also have unique attributes. For example, work from our group shows that master regulators of developmental decisions have different patterns of expression in mouse and human embryos. These data suggest the corollary that the downstream effectors whose actions they control are also expressed in different patterns. In addition to the unique developmental pathways, antibodies made against mouse ESCs rarely cross-react on their human counterparts. These findings led us to the conclusion that th reagent kit that we need for studying human embryos and their derivatives needs to contain species-specific tools.

In this context, our goal is to produce a subset of these tools. Specifically, we propose making antibodies which more faithfully describe hESCs and their differentiated products than the mouse reagents that are currently used for this purpose. How will we accomplish this goal? We will take two approaches. First, a company with offices in California[REDACTED], has made their entire catalog of commercially available antibodies - more than 300 entries - available to us at no cost. These reagents are importanttools in other fields and we suspect that they will have novel applications in hESC research. The advantage of this approach is the speed with which these existing credentialed reagents can be made available to the research community. Second, we propose making brand-new antibodies. These experiments are made possible by the fact that the investigators working on this project have a great deal of experience in hESC research and in the state-of-the-art technologies that are used to produce antibodies. We wil also bank these reagents for distribution to investigators in the state of California, the US, and abroad. We think that the antibody toolkit we propose assembling will have many important applications because human-specific reagents will have higher discriminating power in many different experimental approaches routinely used in hESC research.

Statement of Benefit to California (provided by applicant)

Human embryonic stem cell (hESC) research, only a decade old, is a very young field. In general, it takes many years for a new discipline to mature to the point where scientists have the tools they need to work at full capacity. This is especially true for hESC researchers because the federal government does not fund work that employs human embryos and/or their derivatives. Therefore, prior to enacting proposition 71, scientists in this country lacked the resources required to generate the critical tools that are needed to develop hESC-based regenerative medicine therapies. The proposed project is designed to help fill this gap by rapidly identifying or producing antibodies that investigators can use to characterize hESCs that

are either pluripotent or have differentiated into specific cell types: pancreatic beta cells, cardiomyocytes, or neurons.

How will scientists exploit these valuable tools? We envision a myriad of uses. For example, we now know that we need human-specific reagents for identifying hESCs that are truly pluripotent. Being able to rapidly and confidently phenotype cells that are capable of self renewal would streamline the process of characterizing new hESC lines, which because of the many different types of assays that are required, takes months. In addition, this class of antibodies could be used for the routine assessment of hESC colonies to ensure they retain pluripotency. The methods that are currently used for this purpose are prohibitively expensive to carry out on a regular basis. Consequently, in most laboratories, they are performed at a less than optimal frequency. We think antibodies that react with antigens whose expression is modulated during differentiation will be equally valuable tools. For example, the ability to monitor with great fidelity generation of the three germ layers (ectoderm, mesoderm, and endoderm) would, in turn, enhance the development of methods for triggering these processes. Following the subsequent steps in which the progenitors differentiate into specific cell types is equally important. Numerous other applications exist including examples that have important clinical relevance such as the isolation of pure populations of hESC derivatives for transplantation purposes. Finally, it is likely that a subset of the antibodies that are identified or produced will react with yet-to-be-identified molecules or known antigens not appreciated to be expressed by hESCs that play important functional roles in the maintenance of pluripotency or specific differentiation processes.

How will Californians benefit from research that this toolkit enables? It is difficult to overstate the importance of rapidly generating the tools that are needed for researchers to translate basic discoveries made in hESC systems into regenerative medicine therapies. The proposed project is designed to accomplish this goal as quickly as possible by assembling the antibody portion of the toolkit that every hESC researcher needs.

Review

The goal of this application is to develop a toolkit of monoclonal antibodies (mAbs) that specifically recognize antigens whose expression is restricted to pluripotent human embryonic stem cells (hESCs), or their differentiated derivatives including pancreatic beta cells, cardiomyocytes and neurons. The Principal Investigator (PI) proposes to pursue several approaches to identify such mAbs including systematic screening of a collection of 300+ commercial mAbs (being donated by a company) and generation by two different methods of new mAbs. Different methods of mAb production are expected to increase the likelihood of generating mAbs with differing specificities.

Reviewers noted that the proposed research would increase the available collection of antibody reagents that can be used to label and distinguish primitive stem cell subpopulations, allow monitoring for specific lineage restrictions and differentiation pathways, and potentially identify novel molecular pathway components recognized by these antibodies. They agreed that successful execution of this proposal could greatly enhance experiments addressing basic understanding of hESC biology, and provide tools for both diagnostic usage and therapeutic applications. The reviewers considered the proposed research to be a near optimal congregation of expertise and well thought-out approaches likely to result in an available and useful toolkit of antibodies against hESCs and their derivatives. The reviewers' biggest concern was that the PI is already very highly committed to other research programs and there is low percent effort commitment by many of the key personnel, calling the potential for success of this important but ambitious project into question.

The strengths of the proposal identified by the reviewers include a strong team with significant expertise in all areas covered in the proposal, the proposed three-pronged approach which strongly increases the likelihood of success and preliminary data supporting the ability to execute on the proposal. Most reviewers cited the use of pre-existing reagents through collaboration with a company, documented by a

strong letter of support, to be a strength. This allows immediate screening and potentially makes interesting reagents available sooner to the research community by eliminating the considerable lead time required to generate new mAbs. One reviewer, however, considered this screening of preexisting commercial antibodies a weakness and thought such screening should be undertaken by the company.

The reviewers considered the use of complimentary approaches to generate novel mAbs to be a strength of the proposal. They also considered the screening strategy against multiple hESC lines and/or differentiated progeny to be a strength. They expressed concern that one of the approaches proposed for mAb generation employed few “tricks” and was therefore likely to lead to “common” rather than novel antigen recognition. A reviewer noted that masking with commercial antibodies may be of limited utility and recommended the consideration of other approaches. Reviewers considered the other strategy for mAb generation to have a high probability of success especially since this specific approach has previously been successfully employed by the investigators. However, they expressed concern that this approach could lead to lower affinity antibodies which would be of more limited utility for cell selection/diagnostic applications.

Reviewers uniformly considered this to be a very strong research team. The PI is a very experienced researcher with an excellent track record and the necessary skill set to successfully carry out the proposed experiments. The team consists of many very experienced scientists, albeit several with very low levels of commitment. The power of the proposal also derives from a collaboration between the academic team of researchers and a company. The reviewers uniformly commented that the PI and many of the co-investigators were already generally overcommitted to other research efforts, thus they were concerned about the ability of the research team to manage and be productive in this ambitious project. Reviewers commented that the budget is salary heavy, reflecting salaries being paid to many personnel, most at low percent efforts.

Overall, the reviewers considered this to be a strong proposal but were somewhat concerned about the level of commitment necessary to successfully execute it.

The following Working Group members had a conflict of interest with this application: