CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

MEMORANDUM

Date: June 21, 2010

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RM1-01733 (**LATE**)

Enclosed is a petition letter from Dr. Shizuru of Stanford University, an applicant for funding under RFA 09-03, CIRM Stem Cell Transplantation Immunology Awards. This letter was received at CIRM on June 21, 2010 after the requested deadline of 5 business days prior to the ICOC meeting, but we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

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June 18, 2010

Alan Trounson, Ph.D., President of CIRM
Robert Klein, J.D., Chairman of the ICOC
210 King Street
San Francisco, CA 94107

Dear Distinguished Officers of CIRM:

Please find attached an extraordinary petition for the application entitled "Purified allogeneic hematopoietic stem cells as a platform for tolerance induction" sent in response to RFA 09.03 CIRM Stem Cell Transplantation Immunology Awards. On behalf of my team we wish to thank you for the opportunity to submit this petition, and for the extensive efforts that you, members of the Grant Working Group, ICOC and CIRM have devoted to the review of these applications.

Please do not hesitate to contact me with any questions.

Sincerely,

Judith A. Shizuru, M.D., Ph.D.
Associate Professor of Medicine
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Extraordinary Petition For Grant RM1-01733

This document is an extraordinary petition regarding the application entitled "Purified allogeneic hematopoietic stem cells as a platform for tolerance induction". Our reasons for writing this petition are that we believe the primary purpose of the study and the novelty of our approach was not understood by the reviewers. The major concerns summarized in the Review were: the application lacked novelty; the experimental reductionist approach did not have relevance to human transplants; and rigorous purification of blood stem cells is not necessary to prevent a major complication of transplant as current methods exist which can achieve these goals. Thus, it was judged that the relevance and impact that this work would have on human transplantation is unclear. In this petition we outline why these concerns demonstrated a failure to understand the significance of our proposal for tolerance induction and stem cell replacement therapy.

Our application is directed at overcoming the fundamental obstacle to the use of blood stem cell transplants as a method to induce immune tolerance to donor tissues and for the treatment of autoimmune diseases [multiple sclerosis, childhood diabetes, systemic lupus erythematosus and many others]. This obstacle is simply the robust and long-lived engraftment of purified blood stem cells [called hematopoietic stem cells or HSC] from a genetically different [allogeneic] donor transplanted into patients that have been prepared to accept the HSC using safe and non-toxic treatments. Success in this endeavor would be ground-breaking and open the field of transplantation biology and regenerative medicine. Transplants of pure HSC from a donor has never been attempted because of concerns that, without 'helper' lymphocyte populations, HSC will not engraft. However, these 'helper' lymphocytes can cause the sometimes deadly complication called graft-versus-host disease (GVHD). HSC do not cause GVHD. GVHD is the major reason why blood/marrow transplants are high risk procedures and such transplants are not usually performed for tolerance induction. Even low numbers of lymphocytes can be deleterious to recipients causing a syndrome called subclinical GVHD, a syndrome that, while not obvious to clinicians, can seriously impair the recipient's ability to fight infections. Thus, establishment of safer ways to permit HSC engraftment would benefit the many patients who currently must undergo allogeneic transplants, and in particular, those patients who have only half-HLA matched (haplo-identical) donors available. Only ~25% of people that need a transplant have an HLA-matched sibling donor, thus world-wide searches are performed to identify unrelated HLA-matched donors. This process takes time – time that some patients cannot afford. The ability to safely use haplo-identical donors would expand the related donor pool to parents, children and imperfectly matched siblings, thus opening the door for many more transplants that could be expediently performed.

The review states that we "fail to acknowledge that purified $CD34^+$ cells have been used in the allogeneic setting with no GVHD development". The importance of contaminating lymphocytes must not be underestimated. This statement that grafts processed by CD34 selection do not result in GVHD is not supported by the existing clinical literature. At least two experienced teams that are skilled in CD34 cell selection for haplo-identical transplants reported an incidence of clinically measurable GVHD of 10-20% (Handgretinger et al, *Pediatr Transpl* 2003; 7 (Suppl 3), 51; and Aversa et al. *J Clin Oncol* 2005; 23, 3447). These numbers are concerning since, as noted above and as we have shown (Tsao et al. *PNAS* 2009; 106, 3288), even in the absence of clinically obvious GVHD, subclinical GVHD can be highly damaging to immune function. Thus, if 10-20% of patients have clinical manifestations of GVHD, then we predict that many more have compromised immunity on the basis of subclinical GVHD.

In addition to the points raised above, the primary purpose of our proposal is to develop ways to permit engraftment of purified adult HSC as an essential step towards the transplantation of HSC derived from human embryonic stem cells (hESC) which will not contain lymphocytes. This goal is directly aligned with the mission of the CIRM, and it is clear that this intent was not considered by the reviewers. Indeed, the CIRM RFA which solicited studies for this grant

explicitly stated "the derivation of HSC from hESC opens the possibility of donor specific tolerance induction via hematopoietic chimerism. The recipient immune system is reconstituted with ES-derived HSC followed by transplantation of therapeutic tissue derived from the same hESC line. However, while research to differentiate hESC into HSC is making considerable progress, the efficiency of engraftment of HSC in the recipient remains a challenge." Our application responds directly to this need, a point which was unrecognized by the reviewers.

Below, is a point-by-point discussion of the specific concerns raised in the review. The underlined sections were extracted directly from the CIRM Report and are followed by our response.

Although the rationale underlying the project was judged to be sound, the reviewers did not find sufficient merit to support this proposal. The overall experimental approach was found not to be particularly novel, which left them unconvinced that project will have a strong impact on the field. They felt that some of the strategies described in the proposal have been previously investigated in separate studies with minimum success and therefore combining these elements would only have an incremental impact on human transplantation.

Currently, patients that must undergo blood/marrow transplants receive radiation or chemotherapy as the basic component of treatment to permit engraftment of donor cells. These therapies are unspecific and are delivered at the cost of damaging normal cells and tissues. Thus, realization of a strategy using antibodies only that specifically target recipient cells to permit engraftment of purified HSC is highly novel. The review states that some of the strategies described in our proposal have been previously investigated and thus our approach is not novel. In fact, we authored some of those studies. Specifically, the central component of our proposal is the use of an anti-cKit reagent to eliminate endogenous HSC and thus clear niche space for donor HSC. This finding was reported by our team in *Science* (Czechowicz et al, 2007; 318, 1296), and was the first demonstration that antibody treatment could achieve HSC clearance.

Our studies over the last 10 years have revealed that the cellular barrier to HSC engraftment is a combination of lymphoid cells and endogenous HSC. These cells express unique surface molecules that can be targeted by antibody treatment. Combining antibodies to target multiple host barrier cells has never been tested. Thus, the argument that "combining these elements would only have incremental impact" is speculative. The field of antibody therapy to effectively target cells in humans is undergoing rapid expansion as the understanding of how best to generate these therapeutic reagents grows. Exchanging toxic radiochemotherapy for antibody therapy would have more than incremental impact on human transplantation, it would radically change and invigorate the field.

The reviewers identified several problems with the research plan, which diminished their enthusiasm for the proposal. For example, reviewers were unconvinced by the reductionist approach in Aim 1 because it fails to account for the complex interactions that multiple cell populations have in an intact immune system. The clinical correlate would need to block or ablate simultaneously multiple effector cell populations, which would be to some extent equivalent to a myeloablative-immunosuppressive regimen.

The science of how best to achieve engraftment of HSC without causing harm to the recipient is complex. Because of these complexities we have taken a reductionist approach, using as recipients, mice that genetically lack specific immune populations in order to reveal which cells are responsible for preventing HSC engraftment. This approach has taught us that elimination of recipient T, B and NK cells eliminates the immune barrier to engraftment, and the major remaining barrier is the resident HSC population. Antibodies that specifically recognize these four cell subsets exist, and hence we proposed to systematically test and optimize this approach in mice with an intact immune system. Antibody therapy is much more specific than radiation and/or chemotherapy. Thus, we strongly believe that there is no basis for statements to be

made in such a dismissive and categoric fashion that our approach would "to some extent be equivalent to a myeloablative-immunesuppressive regimen".

There is a population of children who could immediately and directly benefit from this reductionist approach. Children with primarily immune deficiencies disorders, a chief example of which is the syndrome of severe combined immune deficiency (SCID), lack lymphocyte subsets, and thus have deficiencies analogous to the mice that we study. Transplantation of blood/marrow cells is the only proven cure for these diseases. However, these children either engraft poorly because their own HSC are not eliminated, or they receive toxic conditioning that can be deleterious to development, including neurocognitive function. Thus, once an appropriate anti-human HSC reagent is identified and vetted by our studies, we could move directly to a clinical trial of transplants of pure HSC and antibody conditioning for children with SCID. This strategy has been enthusiastically received by the pediatric transplant community.

Reviewers commented that complex immunodeficient humanized model experiments described in the 2nd aim may not accurately model human mixed chimeras or resistance to engraftment. They felt that before embarking on studies to enhance engraftment proposed in the project, establishment and better characterization of the immunodeficient mouse model is needed.

Members of our team have recently developed a robust mouse model in which human blood forming cells engraft at high levels. Details of this model were published in *Nature Protocols* (Park et al, 2008; 3, 1932). "Humanized" engrafted mice have all of the mature components of the human blood system and are thus a unique and valuable preclinical tool for testing reagents aimed at depleting human cells. We described in our application our proven use of these animals to screen for candidate anti-cKit antibodies that will eliminate human HSC. Since details of the model have already been published and we have demonstrated the value of this model in generating the information needed to move to a clinical trial, this criticism is unjustified.

Furthermore, the proposal fails to acknowledge that purified $CD34^+$ cells have been used in the allogeneic setting with no GvHD development. Instead, the problems have primarily been poor reconstitution after transplant and the proposal is not clear how its approach would circumvent this problem. Reviewers felt that the proposal would generate basic biology results in the preclinical model, but its relevance to human applications is unclear.

The inaccuracy of the statement that grafts composed of $CD34^+$ selected cells do not result in GVHD was discussed in the third paragraph of this petition. Since such CD34-selected grafts contain low numbers of T lymphocytes, the poor immune reconstitution observed following transplants of $CD34^+$ cells may be a consequence of subclinical GVHD rather than due to a lack of mature lymphocytes. Regardless, the point about poor reconstitution is irrelevant to our studies since we propose to generate mixed hematopoietic chimeras, not to replace the recipient immune system. When pure HSC are transplanted into animals conditioned for transplant with non-myeloablative treatments, the recipient blood system does not convert to donor type and the immune system of the recipient continues to function along side the donor HSC derived immune cells. Along these same lines, comparison with $CD34^+$-selected grafts is irrelevant as we aim to establish the path of non-morbid conditioning to permit stable engraftment of pure HSC from adult sources, thereby providing a critical link to the future when pure HSC derived from hESC sources, which will not be mixed in with mature cells, will be transplanted as a strategy to induce tolerance to tissues from the same hESC donor.

In closing, we believe that the novelty of our research has the potential to have a dramatic impact on not only the science of stem cell engraftment but can lead directly to clinical trials for patients in need of safer therapies. We thank the members of the CIRM Grant Working Group and the CIRM staff for their efforts and respectfully submit this petition for consideration.