MEMORANDUM

Date: August 29, 2012

From: Ellen Feigal, MD
CIRM Senior Vice President, Research and Development

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RB4-06256 (**LATE**)

Enclosed is a petition letter from Dr. Hanna Mikkola of University of California Los Angeles, an applicant for funding under RFA 11-03, CIRM Basic Biology IV Research Awards. This letter was received at CIRM on August 29, 2012 (less than 5 business days prior to the ICOC meeting) and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

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August 29th, 2012
Extraordinary Petition for RB4-06256: Mechanisms protecting self-renewal of human hematopoietic stem cells
Principal Investigator: Hanna K.A. Mikkola, MD PhD. University of California Los Angeles

To the Chairman of the ICOC and the President and Chief Scientific Officer of CIRM,

CLINICAL TRANSLATION BENEFITS: The potential clinical benefits of this work include expansion of autologous and allogeneic HSC grafts and developing strategies for programming functional HSCs from pluripotent cells or other sources, thereby overcoming a major clinical roadblock of insufficient HSC supply and broadening the therapeutic use of successful stem cell transplants.

BUILDING ON PREVIOUS CIRM FUNDED WORK: Despite the growing literature on the regulation of mouse HSCs, mechanistic understanding of human HSCs is minimal, largely due to lack of model systems in which human HSCs and/or their niche can be manipulated, and the need for specific markers to purify HSCs for molecular studies. We have overcome these bottlenecks in human HSC research through work funded by my CIRM New Faculty Award (RN1-00557) (Figs. 1-3), and are already using these tools and knowledge in CIRM disease team award (DR1-01430) to screen for potential toxic effects of leukemia stem cell drugs on HSC self-renewal. We hope that CIRM recognizes the importance of building on this progress to not only uncover fundamental mechanisms of human HSC regulation, but also to use these discoveries to develop novel translational approaches to utilize the full potential of HSCs in treating disease.

RESPONSE TO REVIEW: We thank the reviewers for acknowledging the compelling preliminary data, the highly feasible experimental plan and the expertise of the PI in HSC biology that all speak for high probability of success. However, we strongly believe that the reviewers judged incorrectly 1) the significance of understanding human HSC self-renewal mechanisms and the clinical importance of these findings and 2) the validity of using fetal liver HSCs as an outstanding model for understanding mechanisms of human HSC self-renewal, especially as it relates to expanding cord blood HSCs or generating HSCs from pluripotent cells, as well as 3) missed to give appropriate credit to key elements that had been clearly outlined in the proposal.

Summary: *The goal of the proposed research is to identify factors and understand mechanisms involved in the expansion of HSC in culture. In preliminary research, the applicant identified a surface protein on human HSCs, GPI80, that serves as a marker for transplantable HSCs and is required for HSC maintenance. Proposed studies will focus on the role of this protein. The first specific aim will be to further examine the effects of altered expression of GPI80 on HSC function in vivo and identify cellular pathways affected by changes in the protein's level. The second aim is to identify and validate components functioning downstream of GPI80 to facilitate HSC maintenance. The third aim is to identify stromal factors that support HSC expansion.*

1) -Significance and Innovation - The proposed study could provide new insights into HSC-niche

interactions and lead to the identification of novel factors that facilitate HSC expansion. ***One reviewer questioned the project's rationale and suggested that the need for expansion of autologous HSCs for therapeutic purposes is fairly limited, although another reviewer considered HSC expansion to be a key clinical problem.***

Response: ***We concur with the second reviewer who considered HSC expansion a key clinical problem.*** Although HSCs have been used for decades for treating hematological malignancies, inherited immune and red cell disorders, bone marrow failure syndromes and other life threatening blood diseases, ***because of lack of HLA-matched bone marrow donors and low number of HSCs in cord blood, only up to 35-50% of patients can benefit from this curative treatment*** (p.1). *Moreover,* ***in many cases, transplantation is carried out using mismatched donors*** (leading to graft-versus-host-disease and need for immunosuppression) ***or too low number of HSCs*** (leading to slow recovery with high risk for fatal infections and bleeding). Therefore, there is a major effort to develop methods for HSC expansion and generation from pluripotent cells. However, these efforts have not been successful due to poor understanding of the mechanisms that establish and maintain human HSC self-renewal - the focus of this proposal. ***This work will be important for achieving expansion of both autologous and allogeneic HSC grafts and for developing strategies to differentiate HSCs from pluripotent cells or reprogram from other sources.*** Thus, although this is a basic biology grant and not specifically focused on clinical applications, this work may lead to the development of broadly applicable tools for generating HSCs to fulfill the unmet clinical need.

2) -Reviewers ***expressed doubts that the results of the proposed studies, which are focused on fetal liver cells, would be applicable to adult HSCs.***

Response: We ***disagree with the criticism on the relevance of using fetal liver HSCs*** in this study. As indicated in p. 2, we specifically chose fetal liver HSCs because i***) Fetal liver HSCs are highly self-renewing HSCs*** that constitute the first major HSC pool in human fetus, and therefore would be a ***functionally ideal and ontologically closer target cell for differentiation from pluripotent stem cells.*** They also ***efficiently engraft the adult bone marrow***. ii) ***Cord blood HSCs -- the prime target for expansion for clinical purposes - are closely related to fetal liver HSCs in their proliferative properties,*** and very different from the quiescent adult HSCs. Our new data shows that ***cord blood HSCs also require GPI80 for self-renewal***. Moreover, iii) as ***the self-renewal of fetal liver HSCs is protected by contact with OP9-M2 adult bone marrow MSC stroma*** *(Fig. 3)*, it is likely that much of the same molecular machinery is also used during adult hematopoiesis.

3a)- Although the ***focus on GPI80 was recognized as novel, the central importance of this component for HSC maintenance and expansion was unclear.***

Response: As indicated in Fig. 1 and 2, we showed that ***GPI80 is not only a novel marker for self-renewing HSCs, but also functionally required for self-renewal***, documenting its central importance for HSC maintenance/expansion. ***The question of how GPI80 protects HSC-niche interactions and self-renewal is the focus of this proposal.*** Using GPI80 as a marker to distinguish HSCs from their closely related, non-self-renewing progeny, we can define the unique machinery that is active only in self-renewing HSCs. As indicated in the proposal, based on the known role of GPI80 in neutrophil homing (p. 2) and the differentially expressed genes between GPI80+ and – HSPCs (p. 5, 6, Table1), ***we hypothesized that GPI80 facilitates HSC-niche interactions that activate key signaling pathways (e.g. integrin and Rho signaling) and govern a transcriptional network required for HSC self-renewal (p. 6).*** By conducting functional assays and microarray analysis for HSCs after GPI80 knockdown (Aim1) and assessing the impact of GPI80 dependent genes for HSC fate using shRNA knockdown (Aim2),

we will validate the molecules downstream of GPI80 that are critical for HSC self-renewal.

3b) - there was little indication about ***how work on new components identified in the study would be prioritized****, and it may result in an unfocused and open-ended project.*

Response: ***We had clearly outlined strategies to choose candidate intrinsic (Aim2) and extrinsic (Aim3) self-renewal regulators for experimental validation***. In Aim 2 (Fig. 4, p.9), we will create "a GPI80 signature" which represents the overlap between genes differentially expressed between GPI80+ and – HSPCs (p.5, 6, Table 1) and genes that also depend on GPI80 for expression (Aim1), and focus on signaling pathways and transcriptional regulators for functional validation. As the supportive effect of MSC stroma on HSPC expansion depends on contact, in Aim3 we will use computational methods to match surface proteins and ECM molecules expressed on OP9-M2 but not the non-supportive stroma, with candidate interaction partners in GPI80 HSCs, to identify niche factors required for self-renewal (p.11, Figs. 3B, 5). ***We intentionally do not limit the functional validation to few known HSC factors to facilitate the discovery of novel self-renewal regulators that could be later developed to therapeutics.*** Using our HSC-MSC *in vitro* system, several genes can be screened for effect on self-renewal and then chosen for detailed studies. Although it is impossible to name the exact list of candidates that will be validated before completing the data analysis, ***we have a track record in using microarrays to identify novel, highly selective surface markers and critical regulatory molecules that govern the fate of hematopoietic and cardiovascular stem/progenitor cells*** (preliminary data, and Van Handel et al. Cell, 150: 590, 2012).

3c) -The applicant ***did not address possible off-target effects in knock-down or over-expression experiments*** *or* ***provide adequate discussion of alternative plans.***

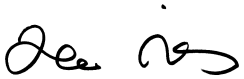
Response: Use of lentiviral shRNA and overexpression is a standard technology in the field and in our lab. ***Possible off-target effects are excluded by verifying that multiple shRNAs show the same effect,*** as indicated in preliminary data (two shRNA vectors for GPI80 demonstrated loss of self-renewal, p.6, 8) and experimental plan (up to five shRNAs will be tested for each gene, p.10). We have used the same lentiviral shRNA methodology in prior publications (e.g. Chhabra et al. Dev Cell 2012). Overexpression is unlikely to cause off-target effects, and if needed, the level of overexpression can be regulated using Tet-On system and varying doxycycline levels (p.10). Notably, ***all experimental methods included a specific section on "feasibility" (p8-11) where we discussed our experience using these techniques, and alternative strategies/pitfalls when less established technology is used.*** Thus, we believe that some of these critical details may have been missed in the review.

-Feasibility/ Experimental Design ***-****The application includes sufficient and compelling preliminary data that support the proposed investigation. The proposed experimental approaches are straightforward, build upon the preliminary data and are designed to yield useful results.*

- Principal Investigator- PI is an excellent, established investigator with much expertise in HSC biology. The team has sufficient experience and expertise to perform the proposed research.

- Responsiveness to the RFA - The proposed research is fully responsive to the RFA.

Sincerely,



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