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 RB4-05746 (**LATE**)

Enclosed is a petition letter from Dr. Michael Teitell 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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Extraordinary Petition

RB4-05746 Mechanisms in Metabolic Control of Stem Cell Differentiation

Principal Investigator: Michael A. Teitell, M.D., Ph.D.
Institution: University of California, Los Angeles

Dear Chairman Klein, President Trounson, Dr. Sambrano, and members of the ICOC:

Thank you for the opportunity to present this petition requesting that the ICOC support our CIRM Basic Biology IV application. We sincerely thank the reviewers for their consideration and useful feedback. Reviewers commented enthusiastically about our focus on mitochondria as it "***provide[s] critical function[s) in stem cells***" and they "***appreciated the broad significance of the proposed study***". They also commented that "***the project's innovation is its focus on a poorly studied and novel aspect of stem cell biology***" and that the PI and research team has "*expertise*", with a proposal that was considered "*responsive to the RFA*".

Our proposal is clinically significant because mitochondria have been implicated in a broad spectrum of disease, including but not limited to **neurodegeneration** (Parkinson and Alzheimer diseases), **myopathies**, and **immunodeficiencies**. Despite this, reviewers were concerned with the importance of the problem, which lead to comments on feasibility, rationale, and "off target" effects. We believe this concern was because studies of mitochondria and their metabolites in controlling gene expression is new for stem cell biologists, as acknowledged by the reviewers, although it is at the forefront of cancer, metabolism, and epigenetic research (page 2, 3). We also bring to your attention our recent papers on the importance of this question in stem cells (Zhang et al., Nature Protocols, 2012) and its context within developmental and cancer biology (Zhang et al., Cell Stem Cell, invited review scheduled for 11/2012). Below, we respond to the reviewers' comments, which we hope will allow you to fund this project.

Reviewer Comment #1: Reviewers were not convinced that the proposed research would address a major unsolved problem in the field.
Response #1: We respectfully disagree. Many studies focus on epigenetics in controlling pluripotent stem cell (PSC) self-renewal and differentiation at fundamental and pre-clinical levels. Metabolites generated by mitochondria are substrates, cofactors, or inhibitors for the enzymes that regulate chromatin and gene expression (page 2, 8, 10), which is at the heart of PSC self-renewal and differentiation, yet metabolites have been largely ignored- how could this not be important? Metabolite fluxes are controlled by enzymes that are regulated by transcription factors including c-MYC and HIF1α, and by signaling network molecules including PI3K, AKT and mTOR, all shown to be critically important in PSCs (DeBerardinis et al., 2008). Acetyl-CoA generated by catabolism in mitochondria can be transported into the nucleus to increase histone acetylation (Cai et al., 2011; Wellen et al., 2009). Similarly, α-ketoglutarate (αKG) exits mitochondria to function as a cofactor for dioxygenase enzymes including Jumonji-family histone demethylases, TET-family DNA hydroxylases, and possibly prolyl hydroxylases that control HIF1α/2α transcription factor stability (Xu et al., 2011). TCA cycle enzymes, such as

isocitrate dehydrogenase, control DNA and histone modifications, which regulates transcription (Nunnari and Suomalainen, 2012). Deacetylase enzymes, such as Sirtuin family proteins, are sensitive to redox state and can impact histone modifications and post-transcriptional changes through non-histone protein deacetylation (Haigis and Guarente, 2006). S-adenosylmethionine is tightly linked to amino acid metabolism and is a donor for DNA methylation with high levels present in induced PSCs (iPSCs) (Panopoulos et al., 2012). These and additional metabolites exert broad regulatory control over the PSC genome. Because cofactors and modifying enzymes are present, specificity must exist or all genes would be regulated synchronously, which is not observed. Therefore, a hierarchy of target chromatin regions and associated genes, miRNAs, and non-coding elements must exist, which could be regulated by enzyme recruitment with DNA-binding factors, local depletion or excess cofactors, or by modifying enzyme spatial and temporal sublocalization within the nucleus (Katada et al., 2012).

Reviewer Comment #2: Reviewers expressed strong concerns about the experimental approach and project's feasibility.
Response #2: The approaches are common in cancer and chromatin research and are being rapidly adapted by our group and others for human PSCs. We published 1) how to evaluate PSC energy metabolism in Nature Protocols (Zhang, et al., 2012) (page 3, 4); 2) stable gene knockdown and over-expression PSC lines (Zhang, et al., 2011) (page 2-11); 3) ChIP-array and ChIP-Seq methodologies (Chin, et al 2009); 4) GC/MS metabolite tracing (Zhang, et al 2011; Zhang, et al 2012) (page 2-11); 5) and overlapping bioinformatics gene expression profiling (Sherman, et al 2010) (page 10, 11). Given our track record in hPSC metabolism (Zhang, et al 2011; Zhang, et al 2012; Zhang et al, Nov 2012), epigenetics and expression profiling (Wu, et al 2008; Teitell, 2008; Chin, et al 2009; Volinia, et al 2010), and overlapping expression profiling (Sherman, et al 2010), we confess to being confused by these concerns.

Reviewer Comment #3: Methods proposed for altering metabolite levels were viewed as indirect, lacking specificity, and likely to have multiple effects, some of which may be off-target.
Response #3: We proposed direct genetic manipulations (gain- and loss-of-function) in the expression of target genes that are published to control metabolite levels and fluxes- this is the most direct method for altering metabolite levels (Refs 8, 11, 12, 23-26). It is correct that altering gene expression to change metabolite levels can have multiple effects, but that is not critical here because our design directly links metabolites to the enzymes they regulate, which is then linked to direct target genes (page 2, 10, 11). We would expect validation from engineering opposing genetic changes or from specific metabolite add-backs and inhibitors, such as oxalomalate to inhibit αKG or addition of cell permeant αKG (Xu, et al 2011) (page 11).

Reviewer Comment #4: Reviewers questioned whether the experimental assumptions based on previous studies of metabolites in cancer cells would translate to studies of stem cells.
Response #4: Our experience and publications by others indicate a very strong connection. Recently, we were invited to pen a review entitled “Metabolism in Pluripotent Stem Cell Self-Renewal, Differentiation, and Reprogramming”, which is tentatively scheduled for publication in Cell Stem Cell in November, 2012. We prepared the manuscript focused on PSCs without mentioning cancer cells or early mammalian development. Both the editor and all three reviewers requested that we revise our paper to directly compare and contrast metabolism in all three settings. The parallels are strong and striking. Without being exhaustive (due to space), common features between PSC and cancer cell metabolism include A) aerobic glycolysis (Warburg, 1956; Ward and Thompson, 2012); B) limited pyruvate entry into the mitochondria, enabling anapleurotic fuels including glutamine and lipids (Varum et al., 2011; Zhang et al., 2011); C) siphoning of TCA metabolites including citrate, αKG, and others to support biosynthesis and rapid cell growth (DeBerardinis et al., 2008; Locasale and Cantley, 2011; Ward and Thompson, 2012); and D) intermediate metabolites as substrates or cofactors for chromatin enzymes that regulate gene expression, which is the focus of our proposal (please see Response #1 above for brief details). It seems logical that strong connections exist between PSC and cancer cell metabolism because if they did not, an entirely different set of

interconnections between metabolites and chromatin regulators would have to exist that are cell state dependent, which would be biochemically infeasible. However, metabolism and gene regulation are not completely conserved between PSCs and cancer cells because, for example, increased reactive oxygen species (ROS) promotes lineage-specific differentiation, whereas in cancer cells ROS promotes proliferation (Crespo et al., 2010; Saretzki et al., 2008; Zhang et al., 2011; Weinberg et al.,2010). The mechanisms for this differential response to ROS in distinct cellular contexts are unknown but raise the central question of this proposal: How does metabolism regulate genes that control PSC self-renewal versus differentiation? In sum, we agree with the published literature and editor and reviewers at Cell Stem Cell that assumptions made for cancer cell metabolism provide a highly valid comparison for studies in PSCs.

Reviewer Comment #5: The rationale for carrying out studies with both embryonic stem cells and induced pluripotent stem cell was unclear.
Response #5: It is known and we co-published that molecular differences exist between individual ESC lines and between ESC and iPSC lines that diminish over culture time (Chin et al., 2009). We also published that similar patterns, within a range, exist for energy metabolism between individual ESC lines and between ESC and iPSC lines (Zhang et al., 2011) (page 2-11). Therefore, it is unknown what the range of metabolic differences are within and between different types of human PSCs, and whether these differences will influence epigenetic patterns and gene expression, which could impact self-renewal and differentiation potential.

Reviewer Comment #6: Some reviewers felt that the preliminary data was supportive of the proposal, while others found that it did not adequately support the proposed study.
Response #6: The field of stem cell metabolism is relatively new with few if any mechanisms establishing connections between metabolites, epigenetic regulators, and gene expression, unlike the situation in cancer biology. It seems, therefore, understandable that some reviewers would be convinced and others not. A main reason for our proposal: The wide gulf in knowledge is a serious liability for understanding and controlling PSCs. With millions of dollars spent on static and dynamic epigenetic and gene expression profiles, it seems odd that the known metabolite regulators of chromatin modifying enzymes that are functioning in PSCs are not studied in this key pre-clinical context. We aim to change this situation through this proposal.

Reviewer Comment #7: It was unclear how alterations in pluripotency or differentiation potential would be quantified or a direct consequence of changes in cellular cofactor levels.
Response #7: We previously generated constitutive UCP2-expressing hPSCs and showed that blocking a metabolic “switch” from aerobic glycolysis to oxidative phosphorylation using differentiation protocols impaired developmental gene expression and reduced the quantity and quality of embryoid body formation, indicating a quantifiable loss of differentiation potential (Zhang, et al 2011) (page 2-11). To be a direct consequence of changes in cellular cofactor levels requires identification of candidate target genes using our study approach, followed by appropriate manipulations of that gene (multi-gene effects would be admittedly difficult in any experimental setting for validating almost any phenotype). We did not expand on these general principles for validated systems because they are standard for many fields and they utilize standard methodologies, which we relied upon referencing to provide (Ref 54, 55, 63).

In summary, it is well established that metabolites and their derivatives are cofactors, inhibitors, and substrates for chromatin modifying enzymes in cancer cells and somatic cells that control gene expression. It is unclear why this fundamental biology would be radically different in PSCs.

Sincerely,

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