MEMORANDUM

Date: October 18, 2010

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application TR2-01763 **(LATE SUBMISSION)**

Enclosed is a petition letter from Dr. Chen of the City of Hope National Medical Center, an applicant for funding under RFA 10-01, CIRM Early Translational II Awards. This letter was received at CIRM on October 18, 2010 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

WenYong Chen, Ph.D.
Assistant Professor
Department of Cancer Biology
Beckman Research Institute

October 15, 2010

Robert Klein, J.D., Chair
Independent Citizens' Oversight Committee
Alan Trounson, Ph.D.
President and Chief Scientific Officer
California Institute for Regenerative Medicine

Re: Extraordinary Petition
TR2-01763: Targeting SIRT1 in leukemia stem cells

Dear Mr. Klein, Dr. Trounson and Distinguished Members of the ICOC,

Thank you for giving us the opportunity to submit a CIRM Early Translational II Award research proposal for funding consideration. We appreciate the peer review of the Grants Working Group, but would like to bring to your attention certain key points of our proposal that may not be fully appreciated by the review panel.

I appreciate the helpful discussions with CIRM scientific staff and your attention in providing me the opportunity to submit an Extraordinary Petition in support of our proposal TR2-01763: Targeting SIRT1 in leukemia stem cells. Attached is a three-page petition highlighting the key points and considerations for your review.

The potential impact of our proposal for developing a novel approach for treatment of leukemia and preventing disease from relapse is substantial. Our pioneering work with epigenetics-mediated chemoresistance has led us to identify that the gene named SIRT1 is uniquely activated in leukemic stem cells, promoting their survival and relapse from chemotherapy. Inhibiting SIRT1 represents a novel approach to eliminate leukemic and potentially other cancer stem cells. Our proposed development of SIRT1 inhibiting drugs has the potential to greatly advance cancer treatment, and leading to improved clinical outcome.

We sincerely appreciate the commitment of CIRM and its governing board in supporting best and novel translational research and finding the effective treatment and cure for human disease, and appreciate your consideration of our petition for funding.

Respectfully,

WenYong Chen, Ph.D.

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Extraordinary Petition TR2-01763: Targeting SIRT1 in leukemia stem cells

Background; Leukemia stem cells (LSC) are responsible for the generation and propagation of several leukemias. LSC resist elimination by current treatments and persist after treatment as a potential source of relapse. There is a pressing need for improved strategies to target and eliminate LSC. We have identified the mammalian SIRT1 gene as a key regulator of LSC survival and drug resistance. We propose to develop potent drug candidates to inhibit SIRT1 to eradicate LSC and enhance cures in leukemia patients. Successful implementation of this approach is expected to lead to vastly improved clinical outcomes for leukemia patients.

Response to critique: I would like to submit the following responses to specific comments made by the reviewers regarding our proposal

1. Rationale for the proposed approach

The reviewers' questions regarding the rationale for the project can be easily clarified based on previous studies and our preliminary data.

The reviewers noted the existence of drugs that already down-regulate activity of the SIRT1 pathway and they questioned whether targeting that pathway further would improve disease outcome. We would like to point out that no SIRT1 inhibitory drugs for clinical use are currently available. Indeed our data indicates that SIRT1 continues to be expressed and activated in CML cells after treatment with Imatinib (data presented in the page 6 of the proposal), SIRT1 activation appears to be resistant to BCR-ABL inhibition after stem cell transformation. Therefore, additional means to inhibit SIRT1 are clearly required to eliminate leukemia cells.

The reviewers also felt that there was over-reliance on mouse CML models for SIRT1 inhibitor efficacy evaluation. They felt that despite serial transplants, the short murine life span cannot accurately model the indolent and unpredictable course of CML in humans and complicates clinical efficacy assessment. It should be noted that evaluation of anti-SIRT1 compounds will be performed using multiple approaches including biochemical tests, thermodynamic assays, crystal structure-based assays, array-based pathway analysis and in vitro culture based assays. However, we feel that testing of the efficacy of these compounds for targeting LSC using mouse models of leukemia is an essential step of the drug development process, since these are currently the best models for studying leukemia cells in vivo. Well characterized large animal models for CML are not available at present. In spite of difference of lifespan between human and mouse, research in the past decades has demonstrated striking similarities between the leukemogenic processes in the two species. Therefore, mouse models are excellent tools for evaluating the drug effects on cancer treatment, in particular, for the early stage of drug development. The CML mouse model we are using was developed by Nobel Laureate Dr. David Baltimore, and is the best characterized CML model today and a state-of-art model system for testing anti-CML drugs. We have used this model to show that SIRT1 gene knockout depletes CML stem cells in this model. We also propose to test compounds in human CML stem cells engrafted in an immunodeficient mice, thus addressing concerns regarding differences in drug effects on human versus mouse LSC (p13-p14 of the proposal). Both model systems are well developed in the PI and co-PI's laboratories.

The reviewers questioned the inclusion of CML as a disease target, as the LSC population is better characterized in AML than CML. We respectfully submit that CML stem cells are well characterized in a large body of literature, and that the role of SIRT1 in LSC

maintenance and drug resistance is better characterized in CML compared with AML stem cells. In contrast to CML, AML is caused by a variety of different molecular mechanisms, requiring the use of multiple model systems. We therefore plan to initially test compounds on CML LSC and then extend these studies to AML LSC, as discussed in the grant proposal (p13-p14 of the proposal). Importantly, our preliminary studies also support a role for SIRT1 in maintenance of AML LSC (page 7).

The reviewers stated that they were confused as to whether SIRT1 inhibitors would be administered as a first- or a second line therapy after imatinib failure. We would like to clarify that SIRT1 inhibitors are intended for elimination of LSC in CML patients. Therefore following initial safety testing, they would be tested as part of the first line therapy for leukemia with the aim of preventing development of resistance, and eliminating residual disease. At present most patients treated with Imatinib need to take the drug indefinitely in order to prevent disease relapse. The aim of adding a SIRT1 inhibitor to Imatinib or other tyrosine kinase inhibitor treatment would be to eliminate residual leukemia stem cells, allowing discontinuation of treatment without leukemia relapse. It is possible that the SIRT1 inhibitor drugs may also have efficacy as second-line treatment for CML relapsed or refractory to tyrosine kinase inhibitor treatment. Similarly, in AML SIRT1 inhibitor drugs would be used to target AML LSC that resist elimination with conventional therapy, thereby reducing risk of relapse.

The reviewers questioned whether inhibition of SIRT 1 could efficiently ablate LSC, and whether SIRT1 was adequately validated as a target. We believe that our preliminary data showing that SIRT1 is selectively activated by oncogenic transformation in bone marrow progenitor cells, and that SIRT1 inhibition effectively inhibits human CML CD34+ cells in vitro and murine LSC in vivo, provide strong proof that SIRT1 is a valid target for therapy to eliminate LSC.

The above clarifications support a strong rationale for our plans to target SIRT1 as a novel approach to eliminate LSC and our approach for this purpose.

2. Ability to deliver a development candidate product in three years

The reviewers raised several related questions about our approaches and were concerned that the proposed plan would not achieve a development candidate within three years. We would like to bring to your attention that we have already identified a class of water-soluble, potent and novel SIRT1 inhibitors that are active in cell based assays in the 1-5 μM range (preliminary data- p8 of the proposal). The above compounds screened by computational modeling followed by cell-based assay have been validated by ligand docking into SIRT1 structure models and further validated by in vitro deacetylase inhibition assays using SIRT1 recombinant protein and by analysis of change of acetylation of the SIRT1 substrate FOXO1 protein in cells as shown in Figure 6. We have filed a patent application for these compounds (US patent application no. 12/026,554 continuation in parts). Our criteria for the development candidate are to "identify one or more water-soluble SIRT1 inhibitors that work in single digit μM concentration (or lower) to inhibit SIRT1 enzymatic activity biochemically and prevent emergence of BCR-ABL mutations" (p3 of the proposal). The compounds identified are already in the range of our goal, and the main purpose of our proposal is to further modify these compounds to increase their activity and/or reduce toxicity, and identify one lead compound with best potency and specificity and low toxicity for the development candidate.

The reviewers felt the scope of the proposed work was too broad to be accomplished. We agree that the proposal as written may have appeared broad since we have proposed multiple approaches to evaluate SIRT1 inhibiting compounds as mentioned in response 1.

However, we believe these studies are essential to achieve a developmental candidate, and are in line with the requirement by CIRM Early Translational II Research RFA guideline to demonstrate suitability for use in human, compelling disease modifying activity, assessment of safety and stability, research assays developed to characterize the candidate etc. Importantly, the ability to carry out these studies has also been proven by each of our team members. Therefore, we believe we will be able to accomplish these studies and achieve a developmental candidate within 3 years.

3. Team effort and experience

The reviewers agreed that the PI and co-PI have been pioneers in the study of the role of SIRT1 in CML stem cell maintenance and drug resistance, but questioned the level of commitment of the medicinal chemist and whether the team had critical drug discovery and development experience. As indicated in the Part A of the proposal, the medicinal chemist Dr. Horne is a senior professor of synthetic chemistry and will contribute 25% effort to this proposal. We believe that it is adequate for the proposed studies. Dr. Horne is also the director of our Synthetic Chemistry Core facility that will support the proposed studies (p9 and p16 of the proposal). Our multidisciplinary research team will use the translational research infrastructure developed by City of Hope Comprehensive Cancer Center. It is important to note that this translational research setting has already been successful in developing a novel ribonucleotide reductase inhibitor and bringing it to clinical trial. This effort was led by the co-investigator Dr. Yen (p15 of the proposal).

In summary, we have assembled a team with the required expertise and track record, and have the necessary tools to deliver a development candidate within 3 years. We sincerely appreciate the commitment of CIRM and the Members of the ICOC to supporting the best and novel science for translation to clinical applications, and appreciate your consideration of our petition for funding.