MEMORANDUM

Date: March 14, 2013

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application IT1-06584

Enclosed is a petition letter from Dr. Andrea Ghetti of AnaBios Corporation, an applicant for funding under RFA 12-02, CIRM hiPSC Tissue Collection Awards. This letter was received at CIRM on March 14, 2013 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

San Diego, March 14, 2013

Dear ICOC members,

We are appreciate the opportunity of presenting this "extraordinary petition" to the ICOC for three principal reasons: 1) to ensure that the committee fully understands the scope, depth, potential and novelty of the donor heart project for the collection and validation of the IPSC stem cell work for LVH; 2) to clarify operational questions related to organ procurement, and the quality and use of donor tissues; 3) to bring to light critical advancements and methods development since the time of submission of our proposal that could significantly improve capabilities and the probability of project success.

1) **Scope, depth and novelty of the project**

The goal of our project is to provide a truly validated reference data for the stem cell research on human hearts with left ventricular hypertrophy (LVH). The proposal goes far beyond the collection and banking of fibroblasts from skin samples of normal and LVH organ donors; the proposal offers the potential of also banking the adult progenitor cells derived directly from the normal and diseased hearts of the organ donors, which may prove to be a more mature and relevant starting point for differentiation of the myocytes into an adult like phenotype. Even more importantly, the project proposes to fully investigate the physiological profile of each of the normal and diseased hearts. Such an offering represents a completely novel and needed approach to discovery in this field. While the cost of such an undertaking seems to be great for the apparent "quantity" of hearts being studied, the potential "quality" of data generated from such an in-depth undertaking is immense. Such data will allow researchers to create relevant models with the IPSCs or the adult cardiac progenitor cells that reflect the true state of differentiation of the normal and diseased phenotypes because the exact phenotypic endpoints of each donor will be understood and quantitatively defined. The model will further enable the scientific community to generate in vitro models of the disease, therefore facilitating the elucidation of the pathophysiology of the disease, the triggering events that express or influence manifestation of the disease and most importantly, create the platforms for the focused search for therapies and disease management strategies. The proposal is especially innovative and relevant because in the absence of the functional profiling reference data from the original diseased hearts, the scientists differentiating iPSC will be left guessing at which point they will have produced a cellular model faithfully replicating the original diseased tissue.

2) **Clarification of operational questions related to organ procurement and use of donor tissues**;

a) **Donor Criteria and medical assessment of LVH:** It is standard procedure that all brain dead organ donors undergo echocardiography to determine the transplantability of the heart, unless there are unusual medical circumstances. By default, all organ donors in the study will have undergone an echocardiography and the results will be used to determine whether there is a certified medical diagnosis of LVH.

b) **Ethics and oversight of donation:** Every donor in the study will meet the standards of ethical consent in the US: 1) specification of the tissues, ie heart and skin, 2) utilization in a research environment, and 3) utilization by a for-profit company. All donors are fully anonymized. There are no restrictions pertaining to use of the tissue for genetic analyses, as long as HIPPAA regulations are observed.

c) **Validation of the cardiac tissue preservation methods and physiological relevance of the ex-vivo functional data:** AnaBios has worked on over 350 human hearts and has perfected "cardioplegic" formulations for recovery and reperfusion of hearts for experimentation and transport times up to 15 hours post cross clamp. AnaBios extensively validates the preservation of the tissue function. As shown in the example in Figure 1, the cardiac action potential measured ex-vivo in the human heart samples tested in our laboratory is indistinguishable from the action potentials measured in vivo in human hearts by Franz and colleagues.

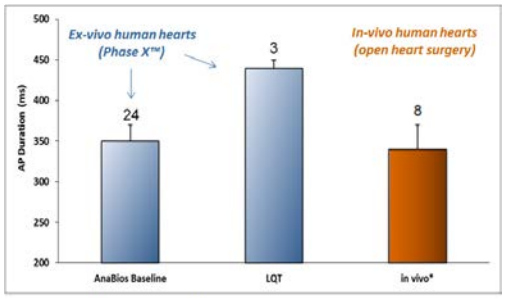

Figure 1: Left, average action potential duration measured ex-vivo in ventricular trabeculae from 24 human donor hearts. Center, average action potential duration measured in ventricular trabeculae from 3 human hearts from donors with long QT syndrome (LQT). Right, action potential duration measured from endocardial tissue during open heart surgery.

Similar methods are commonly used by AnaBios to establish that the contractility of the human donor heart samples is preserved and that tissue excitability, conduction and contractility maintain the same pharmacological properties expected of human cardiac tissue. Furthermore, AnaBios maintains a consistent tissue quality rating showing an RMI rating of 8 or 9+ (evaluated by third parties), which insures that the expression analysis of preserved and banked cardiac samples will be of real value. Thus, the data generated on each heart will provide critical information for the ensuing use of the IPSC and cardiac derived stem cells. If quality data cannot be obtained from a donor heart, then all the tissues from this donor will be excluded from the study and not processed for banking.

3) Critical advancements and methods development since the time of submission of our proposal:

a) **Improved methods for the in vitro derivation of cardiomyocytes with adult-like phenotypes:** Adult ventricular cardiomyocytes are usually quiescent (although excitable) and are characterized by hyperpolarized diastolic potential, relatively long-lasting action potentials and the absence of spontaneous phase 4 depolarization. Compared with adult cardiomyocytes, most hESC- or iPSC-derived cardiomyocytes, exhibit APs similar to fetal or neonatal cardiomyocytes (Sartiani et al. Developmental Changes in Cardiomyocytes Differentiated from Human Embryonic Stem Cells: A Molecular and Electrophysiological Approach Stem Cells 25: 1136–1144 (2007); Chen et al. Electrophysiological Challenges of Cell-Based Myocardial RepairCirculation; 120: 2496-2508 (2009)). An important advancement in this field was recently brought by the discovery made by our collaborator Dr. Chen and his colleagues (Kim et al., Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs Nature 494: 105-110 (2013)). In this work, Chen's team was able to recapitulate the cellular features of an adult onset cardiac disease by inducing metabolic maturation of cardiomyocytes carrying a specific mutation. The extensive biochemical and functional characterization of the cells conducted in their study, clearly showed that the appropriate biochemical and hormonal milieu was able to induce metabolic and electrophysiological maturation of the iPSC-derived myocytes to an extent never achieved

before. AnaBios has licensed this novel in vitro maturation technology and will make it available to all interested researchers. Importantly, this technology will allow the differentiation of adult-like cardiomyocytes from iPSC derived from LVH patients. As described in the original proposal, AnaBios will collect functional data from the donor hearts. By combining the novel Chen's maturation technology and the critical functional reference data that will be provided for each sample, researchers will be in the best position to ensure the relevance of the in vitro model for the study of LVH.

b) **Improved methods for the isolation of cardiac progenitor cells (mesenchymal stem cells) and their differentiation into cardiomyocytes with adult-like phenotype:** The differentiation of adult heart-derived cardiac progenitor cells has been reported to give rise to cells with a more mature and adult-like functional profile (De Boer et al., Human cardiomyocyte progenitor cell-derived cardiomyocytes display a maturated electrical phenotype J Mol Cell Card 48: 254–260 (2010)). In collaboration with Dr. Chen, AnaBios has recently improved the efficiency of isolation and differentiation of adult progenitor cells. This is an important progress in the context of the present proposal as AnaBios has proposed to provide to the derivation facilities and researchers not only fibroblast samples but also cardiac progenitor cells. These samples may in fact prove very valuable in understanding the disease mechanism and may provide a precious material from which the differentiation of disease cardiomyocytes could be started.

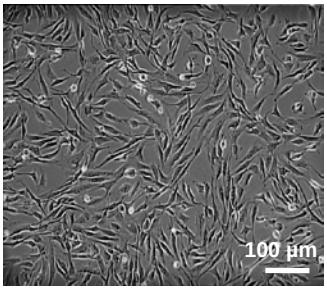

Figure 2: Mesenchymal stem cells from human left ventricle

Conclusion:

LVH is a highly prevalent disorder characterized by increased heart mass which is linked to nearly all forms of heart failure and it is an independent risk factor for myocardial infarction, stroke, arrhythmia and sudden death. The mechanism of the disease and its etiology are poorly understood and a relevant animal model is lacking. Most importantly, no effective therapy is available and the current knowledge gap and lack of models are thwarting the quest for a cure. Genetic factors have emerged as fundamental contributors to the development of the disease.

The proposal by AnaBios is, in many respects, highly innovative and has high potential for generating reagents and knowledge that could significantly advance the quest for an effective treatment for LVH.

We would greatly appreciate your further consideration of the merits, uniqueness and quality of this project.

Sincerely,

Andrea Ghetti, PhD