**Application #: DISC2-10473**
**Project Title: Homing/Efficacy of a Novel Pluripotent Non-Tumorigenic Human Adult Stem Cells Isolated from Adipose Tissue in Acute Myocardial Infarction Mice Models**
**PI Name: Gregorio Chazenbalk, Ph.D**

ICOC Governing Board:

I am sending this letter regarding the rejection on my grant application recently submitted (DISC2-10473). I am respectfully disagree with most of the reviewers' concerns. I strongly believe that CIRM should support this innovative research that could have a tremendous impact in regenerative medicine and cell therapy. I would like to briefly explain orally my point of view on this topic during the next CIRM public hearing on Dec 14 2017.
Ischemic heart disease, including acute myocardial infarction (AMI), is a common cause of morbidity and mortality worldwide, and its prognosis is poor. More than 1.5 million Americans suffer from heart attacks in the U.S. every year with an annual cost of 300 billion dollars (1). Extensive tissue damage with functional cardiomyocyte loss leads to heart failure, the primary cause for hospitalization in patients 65 year old and older and uses more Medicare dollars than any other diagnosis. Many different types of stem cells with some potential for cardiomyocyte regeneration have been investigated in clinical trials. While the use of these stem cells has been shown to be feasible and safe, the major drawback is modest efficacy and inconsistency.
Recently, a new population of pluripotent stem cells has been isolated from bone marrow and skin cells. These cells, named Multilineage Differentiating Stress-Enduring (Muse) cells are intrinsically capable of lineage plasticity, generating cells representative all three germ layers from a single cell, as well as self-renewal. Tumorgenicity is commonly understood to accompany pluripotency. The fact that Muse-AT cells do not undergo teratoma formation when transplanted *in vivo*, makes them inherently unique amongst their ESC and iPSC counterparts. This lack of tumorgenicity can be explained in part by their intrinsically low telomerase activity, low level of expresion of Lin28 and high levels of expression of Let 7 (critical factors for tumorigenesis), eradicating tumorigenic risk through unbridled cell proliferation. Muse cells can efficiently home into damaged tissues and differentiate into specific cells leading to tissue regeneration and functional recovery as described in different animal disease models (i.e. fulminant hepatitis, muscle degeneration, skin ulcers, liver cirrhosis, cerebral stroke, vitiligo, focal segmental glomerulosclerosis). Recent studies by Dr Dezawa's/Dr Minatoguchi's labs have demonstrated that Muse cells repair heart damage (50 % reduction) and function recovery (36 % increased in ejection fraction) in acute myocardial infarction (AMI) mice and rabbit models. These results have been reproduced in other AMI animal models. Based on these results, the companies Mitsubishi/Life Science Corporation haves announced that they will start in 2017 the first clinical trial to treat AMI patients with Muse cells, with the aim of approval in 2021.
Our laboratory has developed a novel, fast and very efficient methodology for the isolation of human Muse cells form adipose tissue (Muse-AT cells). Approximately 25 million highly purified Muse-AT cells can be obtained from 100 grams of lipoaspirate material in 12 hours in a reproducible manner without the need of cell expansion, or special devices and are ready for infusion. Based in all of this, we strongly believe that the technology for Muse-AT isolation and their use to treat AMI patients is feasible with a high potential rate of success.

The recognition by the scientific community of the high potential of Muse-AT cells for tissue regeneration is absolutely critical so that they may be further studied and put into clinical trial as soon as possible. Success of Muse-AT cell therapy will not only significantly reduce the number of AMI patients with disability or death, improving their quality of life and reduce healthcare cost but will also open new avenues for Muse-AT cell treatment in many other disorders, significantly improving the quality of life of countless patients.

Looking forward to meet you this Thursday.

Many thanks for your attention

Sincerely,

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I am sending this letter regarding the rejection on my grant application recently submitted (see above).

**Concerns**

We respectfully disagree with most of the reviewers' concerns and here are my responses to them.

1- It is not clear whether the proposed cells will integrate - major concern given all the other studies using stem cells

It is already well known that Muse cells efficiently home into damaged tissues and differentiate into specific cells leading to tissue regeneration and functional recovery as described in different animal disease models [i.e. fulminant hepatitis, muscle degeneration, skin ulcers, liver cirrhosis, cerebral stroke, vitiligo, focal segmental glomerulosclerosis and acute myocardial infarction (AMI)]. Based on Dr Dezawa's/Dr Minatoguchi's labs studies, Muse cells repair heart damage (50 % reduction of heart infarct size) and function recovery in mice and rabbit AMI models. These results have been reproduced in other AMI animal models. Based on these results, the companies Mitsubishi/Life Science Corporation haves announced that they will start in 2017 the first clinical trial to treat AMI patients with Muse cells, with the aim of approval in 2021.

2- Injecting cells has a low likelihood it will work based on historical type

The great challenge in the cell based regenerative medicine is the efficacy of tissue regeneration. For example, after intravenous infusion of stem cells, only 0.04% of the cells reach the infarct region, whereas most of injected cells was found in other organs, especially in the lungs. Furthermore, within the infarct area, integrated stem cells die within days or weeks after. In contrast, more than 20% of infused Muse cells are capable to integrated and survived, and then differentiated into functional cardiomyocytes in the infarct area, leading to tissue regeneration and functional recovery. This is, in part, due to Muse cells has a unique characteristic of high resistance to severe cellular stress.

3- Insufficient preliminary mechanistic data showing that the cells are better than all the other cells which have been used before for treating ischemic heart disease.

Preliminary results indicate that intravenously injected Muse cells selectively homed to the infarct in a rabbit AMI heart through S1P (at damaged heart) and S1P2R (located on cell surface of Muse cells) repairing damage tissue (decreased in infarct size) and restoring heart function (e.g. increased ejection fraction) (paper to be published, data could be provided under request). Actually Aim 3 of our grant proposes to conduct the mechanistic investigation.

**Aim 3: To determine the mechanism underlying homing of Muse-AT cells into a heart damage tissue and the tissue distribution of Muse-AT cells in SCID and CD1 mice AMI models.** Hypothesis: Homing of Muse-AT cells into damaged heart tissue is modulated by the specific interaction of a GPCR ligand (S1P) and its specific receptor (S1P2R). ***Overall experimental design***: 1) For pharmacokinetics of injected Muse-AT cell and distribution studies: inject GFP-labelled Muse-AT cells via tail veins into wild type mice, sacrifice animals and collect the blood samples and different organs (heart, lung, spleen, liver, bone marrows and others) at several time points; characterize the number of GFP positive cells in the blood and in the organs. 2) For the homing mechanism study: 2A) conduct Muse-AT cell migration/ chemotaxis, and attachment to the ischemic damaged heart tissue study in culture dishes combining agonists or antagonists of S1P2R as well as siRNAs to the receptor; 2B) test Muse-AT cells *in vivo* in ischemic-reperfusion animals, combining with agonists or antagonists of S1P receptors.

4- Proposed experiments will not show mechanisms of action

I respectfully disagree with this concern, as stated above (response to concern #3).

5- The idea that pluripotency is identified by marker expression is a flawed concept in the absence of in vivo teratoma formation; this is in fact a hallmark of pluripotency

Muse cell does not form teratoma in an in vivo assay, but do differentiate into three germ layer derived tissues suggesting that Muse Cell is not pluripotent, but rather it is multi-potent. Absence of in vivo teratoma formation could be a great safety indicator for the use of Muse cell in regenerative medicine.

Accepting the definition that teratogenesis is an inequivalent condition for pluripotency, Muse-AT cells should not be considered pluripotent stem cells. However, our major focus in this grant proposal is not to dispute if Muse-AT cells are or not pluripotent of Muse cells based on this definition. The goal of this proposal is to isolate Muse-AT cells by simple, reproducible and efficient technology, to assess the safety and efficacy of intravenously injected Muse-AT cells for heart regeneration and function recovery in AMI ischemia-reperfusion mice models. We also propose to determine tissue-distribution, the pharmacokinetics and the underlying homing mechanism of Muse-AT cells into the damaged heart.

6- Not enough evidence that these are truly pluripotent stem cells

See response to concern #5.

7- It is unclear whether the cells are pluripotent

See response to concern #5.

8- The lack of lineage tracing studies showing trans-differentiation of these cells in vivo into cardiac cell lineage is a weakness

Lineage tracing studies using GFP-Muse cells showing homing, trans-differentiation of these cells in vivo into cardiac cell lineage are demonstrated. These data were not provided in the grant application because of space limitation of the grant proposal.

9- The number of cells administered do not appear to be sufficient to repopulate infarcted region by differentiation

Dr Dezawa's group administered ~ 100,000 Muse cells isolated from bone marrow/mice in the AMI studies showing high efficacy of tissue regeneration and function recovery. Our laboratory has developed a novel, fast and very efficient methodology for the isolation of human Muse cells isolated from lipoaspirate material (Muse-AT cells), under severe cellular stress conditions. Approximately 25 million highly purified Muse-AT cells can be obtained from 100 gr of lipoaspirate material in 12 hours in a reproducible manner without the need of cell expansion, or special devices indicating the feasibility of our proposed studies (dose-response/efficacy/tissue regeneration/function recovery/mechanistics/etc).

10- Sorting on SSEA3 may be a bottleneck in translation to clinic

FACS sorting on SSEA3 is a routine standard procedure for isolating and purifying Muse cells. In addition, 40-70 % Muse-AT cells obtained under our current procedure are SSEA3(+) cells. This will make the use of Muse-AT cells in clinical more practical. Alternatively, we may consider to use Muse-AT cells directly without SSEA3 cell sorting.

11- Scalability seems an issue

Approximately 25 million highly purified Muse-AT cells can be obtained from 100 grams of lipoaspirate material in 12 hours in a reproducible manner without the need of cell expansion, or special devices and are ready for injection. 100 grams lipoaspirate material is easily accessible, abundant, and painlessly, routinely and non-invasively extracted from subcutaneous adipose tissue (30 min procedure) from male/female patients. We believe that the technology for Muse-AT isolation and their use to treat patients is feasible with a high rate of success.

12- Women as donors of adipose cells may be limiting

Muse-AT cells can be isolated from both women and men. Most of our Muse-AT cells studies were performed in women because we obtained lipoaspirate material from cosmetic/plastic surgery clinics in which most of the patients are female. However, we isolated Muse-AT cells from two men that underwent to liposuction and we obtained similar number of highly purified Muse-AT cells/grams adipose tissue. Our intention is to use patient own Muse-AT cells for the treatment in the clinical practice.

13- Four full-time un-named personnel seems high for starting the project off the ground

We agree of this point that three full-time personnel could be sufficient to start the project off the ground. Therefore, budget of the proposal could be adjusted accordingly.

All corresponding references of this document can be send upon request.