SAMANTHA BUTLER, Ph.D,
DEPARTMENT OF NEUROBIOLOGY
BROAD CENTER FOR REGENERATIVE MEDICINE
AND STEM CELL RESEARCH
DAVID GEFFEN SCHOOL OF MEDICINE AT UCLA
610 CHARLES E YOUNG DRIVE EAST, TLSB
ROOM 3129
LOS ANGELES, CALIFORNIA 90095
PHONE: (310) 206 6816
FAX: (310) 206-0356
EMAIL: BUTLERSJ@UCLA.EDU

17th January 2014

Gilberto Sambrano, PhD
Independent Citizens Oversight Committee
California Institute for Regenerative Medicine
210 King Street
San Francisco, California 94107

RE: Basic Biology (BB) V proposal (RB5-07320): *Assessing the mechanism by which Bone Morphogenetic Proteins (BMPs) direct stem cell fate* (Tier 2, score 73)

Dear Dr. Sambrano and the Independent Citizens Oversight Committee,

Please accept this request to fund my above referenced grant application that was ranked by the Grants Working Group (GWG) at the top of Tier 2 and addresses an important scientific gap in the CIRM portfolio, e.g., **stem cell studies to derive spinal sensory neurons**.

Rationale: People with spinal cord injury commonly seek to regain two critical functions:

1. Motor function
2. **Sensory function**

My project seeks to address the latter, and no less important, function.

Over 10 years ago, while attending a Christopher & Dana Reeve Foundation conference, I was caught off guard by a simple but devastating question from a paralyzed patient. With pain catching in his voice, he asked a distinguished panel of clinicians and scientists "***When will I be able to touch my children? I would like to be able to feel my children when I put them to bed.***" We still have no answer to his question. This project represents the critical first step on the path towards paralyzed patients regaining sensory function.

Unmet Medical Need: **Recovering sensation after spinal cord injury or disease is both an urgent unmet medical need and an objective that would immeasurably improve the quality of patients' lives**. Although important progress has been made towards rewiring the motor circuits that will permit paralyzed patients to walk, very little progress has been made reestablishing the sensory circuits that permit patients to experience their environment, through, for example, the pleasure and pain afforded by the sense of touch. As the patient advocate demonstrated in his question, **many people, faced with the reality of paralysis, place the recovery of sensation - particularly the pleasure of touching another person – above movement**. The ability to detect and respond to the sensation of pain is also critical: it permits us to avoid physical danger. The inability to feel pain can result in terrible consequences for paralyzed patients, such as third degree burns after inadvertently contacting a hot surface.

First Steps: The critical first step towards regenerating sensory spinal circuits in paralyzed patients is described in this proposal. We are seeking to understand, and then apply, the fundamental mechanisms that **direct stem cells to become spinal sensory neurons**. The successful establishment of these cell types in turn makes possible longer-term implantation studies, now in progress with stem cell-derived motor neurons, **seeking to restore sensory function first to injured rodents, and then ultimately to patients.**

Surprisingly, CIRM does not have a strong record of funding basic studies in this area of research: in **four rounds of Basic Biology awards, no grants have been awarded on any aspect of recovery of sensory function after spinal**

cord injury or disease. **The studies in this proposal,** which seek to develop sensory neurons from mouse and human stem cells, **are thus both urgently needed and meet CIRM's programmatic objectives.**

Proposal objective: to determine the mechanism by which different members of the Bone Morphogenetic Protein (BMP) family of growth factors direct stem cells towards the formation of distinct classes of spinal sensory interneurons. Stem cell-derived spinal sensory interneurons are the required first step in the development of human cellular replacement therapies to recover sensory function. This objective is directly in line with the BBV objective to "unravel the key molecular and cellular mechanisms that dictate cell fate".

Transformative potential: The reviews of the proposal were positive, with only subtle concerns that can easily be resolved. Most critically, the **GWG felt that the studies had the potential to be transformational because "sensory neurons are yet to be derived from human embryonic stem cells" and the studies had "broader implications for neuronal stem cell biology" as follows**:

1. Relevance to spinal injury and disease: the need to rebuild the dorsal spinal cord
Damage to the spinal cord can be devastating, with patients losing the ability to move and experience sensory cues in the environment. Thus, an ongoing goal of regenerative medicine is to rebuild injured or diseased spinal cords using stem cell-derived neurons and glial cells. Progress towards this goal has been made with the discovery that the growth factor Sonic Hedgehog (Shh) can direct stem cells to form the spinal motor neurons and other cell types needed to control muscle movements. However, **very limited progress has been made directing stem cells towards the different classes of spinal sensory interneurons that permit us to position our bodies in space, touch our children, and detect and avoid the painful stimuli that can result in serious physical damage**.

To work towards this goal, we propose to test whether the six BMPs present in the developing spinal cord can be used, singly or in combination, to reprogram mouse and human embryonic stem cells into six key classes of sensory interneurons. These stem cell-derived sensory interneurons will be further screened in a novel slice culture assay, to rapidly determine which conditions result in neurons that appropriately integrate into the spinal cord. Together, **these studies**, which have a time-line of only two years, **will facilitate longer-term implantation studies examining first, which classes of stem cell-derived neurons permit the recovery of sensory function in injured mice and then, a human cellular replacement therapy that would allow paralyzed patients to again sense the world**.

2. Rethinking the mechanism through which the BMPs direct cell fate
The BMPs have multiple reiterative roles throughout development; they are thus potent instructive growth factors that can be used to direct stem cells towards numerous cell fates. Classic studies on the activity of other instructive growth factors, such as Shh, have focused on their ability to act in a concentration dependent manner. BMPs have been proposed to act similarly in the spinal cord, largely by analogy with Shh. Moreover, their tendency to be present in overlapping subsets in the embryo has led to the idea that different BMPs may have interchangeable, redundant functions. These models have become textbook dogma; however, there is surprisingly little data to support them. Our preliminary studies have suggested that the BMPs do not act as interchangeable concentration gradients to direct spinal interneuron identity; rather, distinct BMPs have discrete activities specifying the fate of different sensory interneurons. Thus, **the studies in this proposal are likely to overturn the conventional wisdom by which the BMPs are thought to pattern a field of cells in the spinal cord**.

Such a finding has implications for the action of the BMPs throughout development and, as noted by the reviewers, for the utility of BMPs as instructive growth factors in all areas of stem cell biology. If the BMPs are not interchangeable, it will be critical for stem cell researchers to screen through the endogenously relevant BMPs, to find the BMP that is most effective in their differentiation assay. While conceptually simple, this realization has significant transformative implications.

Past allocation of funding: Damage to the spinal cord is surprisingly common; for example, an estimated 1.3 million Americans (~150,000 Californians) are affected by spinal cord injuries. Tens of thousands of Americans, many of which are children, are affected by amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), two degenerative diseases of the spinal cord. Thus, rebuilding damaged or diseased spinal cords is a key goal of regenerative medicine. However, **there have been relatively few projects funded by CIRM in the Basic Biology series that investigate the fundamental principles underpinning the regeneration process in the spinal cord**.

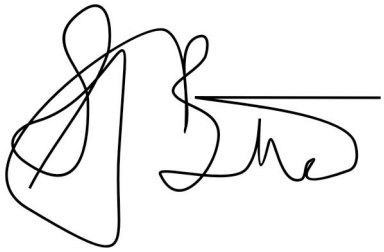
CIRM Basic Biology Program: ~83 grants were approved for funding by the ICOC in four previous rounds of CIRM Basic Biology funding. Of these grants, 17 (~20%) were focused on neural stem cell biology. However, only 4 of these 17 grants (<5% of the total) addressed any issue related to the spinal cord as follows:

BBI: 1 grant: reprogramming stem cells towards the derivation of spinal motor neurons
BBII: 1 grant: spinal cord injury
BBIII: 2 grants: generation of spinal cord disease models (ALS, SMA)
BBIV: 0 grants

There was only one grant (BBII) that examined the derivation of cortical interneurons. **No studies have been approved to derive spinal sensory interneurons**.

In conclusion, the studies in this proposal challenge the conventional view of BMP signaling to address a critical unsolved medical problem, the loss of sensation following damage to the spinal cord. If successful, these studies will significantly advance us along the path to restore sensation after spinal cord injury. I urge you to support them.

Sincerely,



Samantha Butler