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January 17, 2014

Dear Members of the CIRM ICOC:

We are writing this letter to express our concern regarding a significant gap in the portfolio of grants awarded by CIRM. This gap is a lack of attention to important problems relating to Child Health. Notably, a total of only 7 grants with a Pediatric Disease Focus have been funded by CIRM. In Child Health (and adult health), one of the most important areas in stem cells that have been neglected is congenital sensorineural hearing loss. Only 3 grants relating to hearing loss have ever been awarded by CIRM. Yet the incidence of just congenital hearing loss (not including hearing loss from other causes or associated with aging) is 400 in 100,000 newborns. Moreover, the disability will last for life, causing a significant burden to the patient and family and a high economic cost to society. By comparison, there have been 19 grants on ALS, where the incidence is 2 in 100,000 people.

The single most important etiology of congenital sensorineural hearing loss is prenatal cytomegalovirus (CMV) infection, responsible for an estimated 30-40% of congenital hearing loss. This may be an underestimate, as most newborns are not routinely screened for CMV infection. CMV is a herpesvirus that targets the central nervous system of infants prenatally and is the most common cause of birth defects and childhood disabilities in the U.S. Like other herpesviruses, it remains within the individual for life with periodic reactivation. The infection of the fetus can occur not only from primary infection of the mother during pregnancy, but also from reactivated virus. Approximately 1 in every 150 children is born with congenital CMV. Of those children, 1 in 5 will be born with or will develop permanent neural disabilities, the most common of which is hearing loss, which is observed in 10-15% of all infected children. Each year in the U.S., approximately 35,000 children are born with congenital CMV infection.

Most members of the public know that Rubella (German Measles) infection of women during pregnancy causes deafness in the newborn, but few, including pregnant women, have heard about CMV. Yet, every year CMV causes more deafness than Rubella ever did in its worst epidemic year prior to introduction of the vaccine. More children are permanently disabled each year by Congenital CMV than by Down Syndrome, Fetal Alcohol Syndrome or Spina Bifida. What is particularly disturbing is that many babies are born with no apparent symptoms of congenital CMV. The symptoms can appear at birth or later on as the baby develops. The hearing loss often associated with congenital CMV may occur months, if not years, after the baby's birth!

There is no drug or vaccine to prevent congenital hearing loss due to CMV, and thus it is essential develop new therapeutic strategies, including stem cells, gene targeting, and drug discovery. Unfortunately, there is no accepted mechanism by which CMV infection leads to hearing loss, and a lack of public awareness regarding the devastating effects of congenital CMV Infection has made it difficult to garner support for studies to identify an etiology.

The mammalian cochlea, which contains the sensory hair cells and spiral ganglion neurons responsible for hearing, lacks the ability for endogenous regeneration and self-repair. Thus,

implantation of exogenous stem cells that can proliferate and differentiate holds great therapeutic potential. Although there have been studies in small animals with rodent ES cells, until recently there has been no successful model to study hearing restoration using human ES cells. **What has changed the landscape is a transformative and ground-breaking paper from the laboratory of Dr. Marcelo N. Rivolta in the UK that was published in Nature at the end of 2012.** They describe a unique differentiation protocol whereby human ES cells were differentiated to auditory progenitors that could be further differentiated to auditory neurons and hair-cell like cells. What was most significant, however, is that they showed that when these human otic progenitors were transplanted into the cochlea in a gerbil model of auditory neuropathy, the cells engrafted, differentiated, and restored auditory function. This had never been achieved before with cells derived from human stem cells.

We are two senior well-established professors in related fields at the University of California San Diego, who each have over 35 years of experience as respected independent scientists. Dr. Deborah Spector is a Distinguished Professor of Cellular and Molecular Medicine, who has extensive knowledge of the molecular/cell biology and pathogenesis of human cytomegalovirus infection and experience in human neural stem cell differentiation. Dr. Allen Ryan is a Professor of Otolaryngology and Neurosciences, who has expertise in the molecular, cellular, and physiological basis of inner ear function and dysfunction and has years of experience studying CMV-induced cochlear damage in animal models.

When the landmark paper from Dr. Rivolta's lab was published a year ago, we appreciated the tremendous importance of this work, and knew that we now had the tools to determine how CMV compromises the differentiation and function of human ES-derived inner ear cells and to begin to investigate therapeutic strategies involving implantation of exogenous stem cells. In addition, the lab of Dr. Spector had recently found by RNA deep sequencing analysis of the transcriptome of the CMV infected neuroprogenitor cells that there was strongly enhanced expression of three microRNAs (miR-96, miR-182, and miR-183). These microRNAs specifically play a critical role in auditory development by altering the expression of more than 100 genes critical to inner ear function. We also discovered that other genes involved in cochlear sensory and neural development were dysregulated by CMV infection. These findings implicate a novel molecular mechanism of damage to the developing inner ear of congenitally infected children.

The results of our studies and the exciting paper from Dr. Rivolta's lab provided the impetus for us to submit a grant proposal in response to the RFA 13-02: CIRM Basic Biology Awards V. In the period between submission of the pre-application and full application, we were able to successfully replicate the differentiation protocol, and showed in the application the pictures of the cells that had the properties of the otic progenitors, hair cell-like cells and auditory neurons. In addition, we discussed our proposed studies with Dr. Rivolta, and he provided us with a strong letter of support, indicating his enthusiasm for our studies and willingness to provide advice and assist us in developing his successful in vivo model of cochlear implantation of human ES cells for our work.

The CIRM ICOC will be making funding decisions at its meeting on January 29th regarding proposals submitted for CIRM Basic Biology Awards V. Our application "Viral damage to otic progenitor cells - a model of congenital cytomegalovirus hearing loss", which received a score of 60, is one of these proposals. We hope that we have provided you with sufficient information to recognize that the science has dramatically progressed with respect to human stem cell transplantation to the inner ear. The technology is now available to do basic research using human stem cells to understand the mechanistic basis of congenital sensorineural hearing loss due to CMV, and the level of gene regulation. Moreover the foundation has been laid to

investigate novel stem cell therapeutic strategies. We encourage you to now include these studies as part of CIRM's portfolio.

Thank you for your consideration of this letter.

Sincerely,

Deborah Spector, Ph.D.
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