TRAN 1-11628 "Human neural stem cells (hNSCs) for neuroprotection in perinatal hypoxic-ischemic brain injury (HII) – Pre-IND-enabling studies" – PI: Evan Y. Snyder, MD, PhD, FAAP

Dear Members of the Application Review Subcommittee of the ICOC:

It has taken me 25 years to get here, to the threshold of possibly helping a subpopulation of kids who suffer from HII, a disabling but, based on our data, potentially modifiable cause of cerebral palsy (CP).

As a stem cell & developmental biologist, pediatrician, neonatologist, & child neurologist, I am *passionate* about this project, having used this condition since the early 90's as a guide both **(a)** to unveil what proved to be fundamental concepts of stem cell biology (migration/homing/pathotropism [even before I discovered it to brain tumors], injury niche-directed differentiation, neuroprotection, periventricular germinal zones, "windows-of-opportunity" for plasticity, recapitulation of developmental cues by acute/subacute injury, molecular differences between acute & chronic injury), & **(b)** to exploit these properties for the benefit of this & other conditions. Our goal is to obtain data critical for a *successful pre-IND application which, in turn, is essential for advancing approval of a Phase 1b/2a clinical trial within 2-3 yrs. using hNSCs for neuroprotection against acute/subacute perinatal HII***.** I am familiar with every aspect known-to-date about the science & clinical realities of this condition (having cared for scores of asphyxiated babies) -- giving me confidence that a clinical trial can be seamlessly piggybacked upon the routine care & assessment of these neonates. Beyond the pre-IND-enabling work (hopefully to be funded by this TRANS1 mechanism), I intend to shepherd this approach, at every step, to & through each clinical trial phase to insure the highest degree of rigor. We have compelling data supporting **(a)** the efficacy & safety of an hNSC-based intervention that ***salvages the penumbra*** (& the neural networks therein) via multiple mechanisms, leading to histological & behavioral "rescue" & **(b)** the feasibility of using *MRI* & *spectroscopy* for *selecting* patients whose molecular profile indicates they should respond to hNSC's *neuroprotective* mechanism-of-action (MOA), as well as *tracking both hNSC fate & therapeutic impact*.

I know you must make the difficult choice of funding 4 of 7 meritorious proposals. I will make a case for why this project should be among the 4. At the end of this letter, I will briefly point out some *factual* errors made by the GWG that hopefully will enhance your enthusiasm for this approach, making your passion as great as mine.

CIRM, to my knowledge, has not funded many projects addressing pediatric neurologic diseases & few, if any, in babies. *Yet the neonatal brain is where NSCs can have their greatest impact*. If you accept that NSCs are components of developmental programs – the brain's building blocks & its natural chaperones -- & that the newborn brain is still a developmentally plastic organ, then NSCs placed (in a minimally-invasive manner) into a developmental region (e.g., the periventricular germinal zone, where they intermix seamlessly & migrate with – indeed, *augment* – endogenous NSCs – unlike in the adult -- performing their normal homeostasis-restoring function), then there may be no more ideal situation for a stem cell/developmental approach. In this patient population, we have an otherwise normal immature brain subjected to an acute insult -- the perfect niche. It is here, I submit, that stem cells can yield their greatest impact.

HII represents the most prevalent neonatal neurological problem &, in some ways, the most challenging. Unlike a rapidly progressive genetic neurodegenerative disease – which, while tragic, is time-limited – CP, as a non-progressive, static condition, can place a decades-long burden of terrible impairment, instability, & dependence upon the child (as well as upon families/caretakers & society). *Perinatal HII* occurs in 2-4/1000 births. Despite *therapeutic hyperthermia* (which is only marginally effective in a subset of babies), 80% of HII infants develop neurologic signs with 10-20% remaining significantly impaired (e.g., CP; cognitive, sensory, emotional, motor impairment; learning disability; autistic behavior; epilepsy). The cost to the economy is $1MM-per-child in terms of life-long medical & rehabilitative care; the indirect costs based on impact on family dynamics is 2-5-fold greater. *Conversely*, *if HII's severity were lessened early*, in the neonatal period, as we believe we can do in properly-selected responsive patients, the *positive* impact can also be *decades* long. Any improvement that will enable a kid to engage with family, friend, & the environment, self-care, ambulate, work a job, live independently, be creative & inquisitive, learn can potentially improve the quality-of-life not just of the patient, but also of all who share his/her life for years going forward. Such is the joy of being a pediatrician.

We believe, based on strong preclinical data [e.g., the **green-circled portion of the histogram** of the penumbra in **Fig. 1**], that salvaging that significant proportion of cerebral tissue – especially the fibers coursing through it & the neural networks within it – *does* improve motor & cognitive behavior. Unlike most stem cell interventions proffered for neurologic conditions – which either make no mechanistic sense or are far from clinical translation -- *this* particular unmet medical need *can* respond to the hNSC's neuroprotective MOA we have characterized molecularly, giving it strong scientific rationale. *Patients with a molecular profile indicating responsiveness to this MOA can be selected objectively via an imaging "biomarker", hence insuring a likelihood of success*. The technology, skilled personnel, equipment, & venues for our prospective clinical trial are in place & engaged. (Indeed, many of the NICUs in our network participated in the initial hypothermia trials, so are experienced in categorizing & following asphyxiated newborns.) Of note, this project may also inform Regenerative Medicine more broadly. The use of a ***penumbra:core ratio*** on routine MRI to select patients responsive to the stem cell's therapeutic MOA for that particular condition (& to exclude patients that will not respond) arguably represents *Regenerative Medicine's 1st biomarker*. Use of such inclusion/exclusion biomarkers will not only help insure greater success in clinical trials, but also help establish standards for safety, efficacy, & ethics.

Returning to the personal nature of this project, because I have been in the stem cell field since its inception, my long-standing commitment to figuring out ways to improve these babies' plight inevitably shaped some of the earliest precepts

& approaches in Regenerative Medicine. With these kids as my motivation, starting in the 80's-90's, I generated the 1st engraftable hNSCs, developed the intraventricular administration technique (now used commonly in the field), offered the 1st insights into the molecular mechanisms mediating stem cell homing, unveiled the existence of NSCs' paracrine actions, & identified the cell-"friendly" post-injury niche. In other words, these kids have helped our field; it would be great for the stem cell field now to help them. I am confident in the safety & efficacy of an ultimate clinical trial.

I greatly fear that, if CIRM does *not* address this condition in a rigorous manner (similar to what we propose), then charlatans will fill the breach. Already they have been making overtures to parents with kids with CP that are groundless & irrational, promote false hope, waste resources, & may pose harm.

Brief correction of a few errors-of-fact in the GWG assessment

• **MRIs are actually routinely performed in neonates.** A referee misunderstood the word *"diffusion"* for *"perfusion".* MRI – T_1, T_2, diffusion weighted imaging, & spectroscopy – require no injection of contrast & are done routinely on all babies with HII or any neurologic symptom. No new technology, additional interventions, or invasive procedures are required. The analysis we will add – *hierarchical region splitting (HRS)* – to distinguish salvageable penumbra from necrotic core **[Fig. 1]** is a *post-hoc* mathematical manipulation on the digital images already obtained via routine MRI (which is performed in all Level 3/4 NICUs).

• **Efficacy in the hands of others?** We were the 1st group to use stem cells against perinatal HII, a "blue print" for cell-based interventions against other CNS conditions. In observing how others subsequently approached HII, we've published 2 observations: **(1)** Only cells of neural origin, or those that have been fully differentiated into rigorously-defined neuroectoderm, belong in the brain. The use of any other cell type, administered by any other route other than via a germinal zone, is ill-advised & will fail. **(2)** There should be no deviation from our well-vetted successful SOPs regarding cell selection, growth, preparation, timing, & administration, as well as the selection of appropriate recipients.

• **We use the FDA-required animal model:** The time-honored *Rice-Vannucci model in rat pups* (not mice, as errantly stated), while not identical to many cases of perinatal HII, *does* encompass both ischemia & hypoxia, and, most importantly, is the one sanctioned by the FDA for approving interventions for HII. The motor, cognitive, sensory, & cognitive tests employed also are those routinely requested by the FDA. For IND-enabling studies, we will, of course, use the *large animal fetal sheep or piglet model*. But their use now is premature. We have successfully transplanted rodents subjected to *global asphyxia (bilateral* carotid occlusion*)*; however, because such models are not yet standardized, the FDA puts little credence in them. Nevertheless, much of our early work entailed showing that NSCs *could* address *global* CNS pathology – e.g., neurodegenerative & demyelinating disorders. We published extensively that integration of NSCs into the ventricular germinal zone enables NSCs to migrate throughout the brain, homing to pathology, achieving safe & effective widely-disseminated functional integration.

• **Injection into the ventricles of newborns is actually minimally-invasive.** Because of the open fontanels & skull landmarks, this route-of-administration (ROA) is actually quite easy. In fact, my development of this approach for rodents was based on my prior experience in accessing the ventricles of human babies at the bedside – not the other way around. Indeed, we used to routinely inject antibiotics via this ROA to treat ventriculitis or to insert thin catheters to drain CSF for the relief of post-hemorrhagic hydrocephalus. For the clinical trial, cell injection of the small requisite volume will be performed by neurosurgeons under ultrasonic guidance (which, by experience, *is* precise because of the open fontanels).

• **NSC migration to injury is not limited by brain size.** We long ago established that NSCs migrate long distances to pathology not only in rodents but also in large animals, e.g. Old World monkeys (larger than a human newborn). Our reports that NSCs can transit a whole brain are *not* an artifact of simply observing small rodent brains; the hNSCs migrate to reach whatever terrain needs to be covered.

• **The portion of the infarct that is salvageable is the penumbra, quantifiable on MRI [Fig. 1].** If not rescued, the penumbra progresses to unreclaimable necrotic core. We have shown preclinically that rescue of the penumbra *is* associated with improved motor & cognitive outcome.

• **Dose-response *will* be determined**. Although in our prior publications in rodents we determined an ideal NSC dose, dose-response studies *will* be performed here & in IND-enabling studies. The **statistical tools** used in our papers *will* also be used. Our Institute's Biostatistical Core is involved.

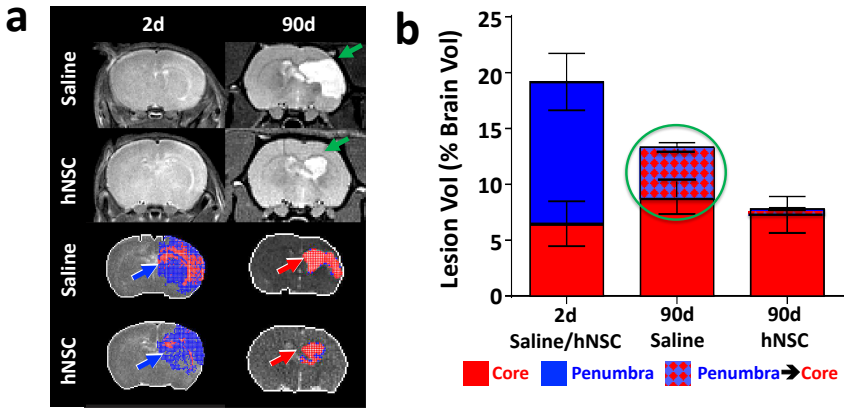

FIG 1.: MRI of "core" & "penumbra" of an HII lesion following hNSC transplantation: Although the core volume does not change in size, the penumbral volume decreases & becomes "normal" tissue by MRI; penumbra that is not rescued progresses to become "core". [A] [Upper Panels] Illustrative T2-weighted images @ 2d post-HII prior to hNSC implantation where the hyperintense (**white**) regions indicate injured brain tissues. At 90 d post-hNSC implantation, the remaining injured tissue has become predominately cystic (**very bright signal**) **(green arrows)**. **Lower Panels** demonstrate relative "core" **(red)** & "penumbra" **(blue)**. Note transition from "penumbra" **(blue) (blue arrows)** @ 2d to primarily "core" **(red) (red arrows)** @ 90d post HII in saline treated brains. **[B]** Quantitative assessment of *total lesion volume (core+penumbra)* demonstrating a significant ($p<0.04$) *decrease in total lesion volume* in *hNSC*-implanted pups due to "rescue" – & hence diminution in size – of the *penumbra* ***(blue)***; the *core* ***(red)***, which contains irreversibly dead cells, is *not* reduced in size. Penumbra that is not "rescued" progresses to become "core" **(red & blue speckling).** The **green circle** suggests the amount of parenchyma that would be salvaged by hNSCs.