CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

MEMORANDUM

Date: April 28th, 2009

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application TR1-01267

Enclosed is a letter from Dr. Evan Snyder of the Burnham Institute for Medical Research, an applicant for funding under RFA 08-05, CIRM Early Translational Research Awards. Although this letter was not received at CIRM at least five working days prior to the April ICOC meeting, we were able to review the extraordinary petition. We are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with Dr. Csete and the scientific staff, and concluded that the petition does not present compelling evidence that should alter the recommendation or score of the Grants Working Group (GWG).

Reviewers were "enthusiastic about the potential impact of this proposal", which "focuses on the development of a stem cell-derived therapy for Parkinson's disease (PD)". CIRM believes that the competitive score and rank order of this proposal appropriately reflect the enthusiasm of the GWG while accounting for some of the scientific concerns of the GWG members. We agree with reviewers' concerns that the proposal may be somewhat premature and overly ambitious given the 3-year time frame. Further, reviewers had justifiable concerns over some aspects of the research plan, and noted that some of the collaborators did not have essential roles or were unclear in their commitment to the project. These concerns collectively impacted the score and rank of this application.

With regard to the applicant's concern that his proposal was not evaluated as a developmental candidate proposal, we do not agree with the conclusion that the "GWG felt enjoined from making that choice [to review the application as a developmental candidate proposal] without knowledge of the applicant's actual intent". Based on the GWG discussion, reconsideration of the 'category' of the application (bottleneck vs. development candidate) would have had absolutely no impact on the review process. The GWG provided the recommendation and score of this application based on the overall scientific merit of the application, and gave careful consideration of the strengths and weaknesses of the proposal. We support the GWG recommendation of this Tier I proposal.

CIRM staff will be prepared to provide further analysis should that be requested by any member of the committee.

Redactions, if any, have been made pursuant to the policy in consultation with the author(s) of the letter. An unredacted version will be available for review in closed session.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

EXTRAORDINARY PETITION **FOR CIRM GRANT APPLICATION TR1-01267**
FOR RFA 08-05 (CIRM EARLY TRANSLATIONAL AWARDS)
"Developmental Candidates" for Cell-Based Therapies for Parkinson's Disease (PD)

Distinguished Members of the ICOC:

Our proposal, entitled **" 'Developmental Candidates' for Cell-Based Therapies for Parkinson's Disease (PD) "** was *recommended for funding* & ranked 12th of the top 15 proposals. In the summary we received 6 days ago of the Grant Working Group's deliberations, it was indicated that this proposal would have been ranked even higher had certain points been clarified. Since some of these points were either errors-of-fact, the result of a clerical error/computer glitch, or simply the lack of space in the original application form to provide a detail that proved pivotal to one reviewer or another, we thought it prudent to bring these simple facts to the ICOC's attention, "filling in the gaps", as it were. The Summary State suggests that the inclinations of the GWG were to upgrade this grant (which was termed a "tour-de-force"), placing it more securely in the "funding zone". We now briefly provide the clarifications that validate those inclinations, hoping that this information may guide the ICOC in making their funding decisions. Thank you for this opportunity to provide additional information.

1. As indicated by its title, this proposal *is* in the prioritized "Developmental Candidates" Category.

As indicated in the summary of the GWG's deliberations, one of the reviewers recognized – based on the actual title of our application where this designation was specified as well as in the substance of the research plan -- that this proposal fit under the prioritised "Developmental Candidates" designation, despite the fact that a clerical error or computer glitch in the PUFF had mistakenly left the "Bottleneck" box errantly checked on the face page prepared by secretarial staff. The GWG felt enjoined from making that choice without knowledge of the applicant's actual intent. To be clear, it always was our intent to be in the Development Candidates category; we specified this intent in the title to safeguard against any electronic or secretarial errors. In fact, another computer/clerical error also existed in the face page – the Victorian Collaborative Partner box either failed to be check or did not retain its keystroke check; nevertheless, GWG understood the error & reviewed their science & budget. We hope the ICOC will similarly make this same common-sense adjustment & prioritize this proposal as the GWG had hoped.

2. A concern was raised that our Victorian Collaborative Partner does not have the expertise to perform homologous recombination (necessary for the Lmx1a knock-in cells that will allow simple FACS isolation of committed human dopaminergic progenitors, "cell type #6" in the proposal). Similarly there was a query about whether such cells should included among the comparisons, & that insufficient detail was provided about the planned mouse studies.

In 2008, our Australian collaborators (Pouton & Haynes) established 2 genetic reporters of dopaminergic (DA) neuron development in mouse ESCs using homologous recombination (targeted to *Lmx1a* & *Msx1*), using standard techniques & are in the process of establishing a bank of other related reporter lines (particularly designed for FACS isolation). The methods used for producing the human ESC *Lmx1a* knock-in line will be identical. Although they recognize that working with human ESCs is more labor-intensive than with mouse ESCs, they, indeed, have experience with human ESC culture & envisage no major problems. . In addition, their Monash University collaborators (the Elefanty/Stanley group) have successfully produced 3-4 knock-in reporter lines in human ESCs.

Although 1 referee suggested removing this cell type from the relevant animal model studies, we thought it critical to emphasize that this portion of the plan would *provide the first study of its kind using a population of committed DA precursors*. This approach may well be the best opportunity to successfully replace DA neurons.

The major activity of the Victorian-funded node will be to carry out implantation studies using a variety of ESC-derived neural progenitors/precursors in the MPTP *mouse* model of PD (addressing another concern raised by a reviewer that preliminary rodent work should also be included). The aim of these studies will be: **(i)** to establish which is the most appropriate genetic marker to select for DA precursors, & **(ii)** to investigate the extent to which implants of DA precursors become established as functional DA neurons *in vivo*, making such work ideally-suited for CIRM's "*early*" translational funding mechanism. The mouse studies & their analysis (with which the Australian group has extensive familiarity) will be analogous to the procedures detailed in the relevant animal models, but with the advantage that the reduced cost of the mouse model will allow screening of a variety of different genetic markers prior to advancing to the true "proving ground" the animal model. Our current understanding is that expression *Lmx1a* is the most specific marker of mitotic DA precursors. However there are other such markers (e.g., *Msx1*) that warrant scrutiny as well as ruling out more mature post-mitotic DA precursors, or even mature neurons, as effective implants.

3. Subcontractee Dr. Eugene Redmond is willing & able to devote 30% to this project.

The GWG praised the fact the PI (Snyder) would devote 30% effort to this project but indicated that they were uncertain about the subcontractor's commitment. We clarify that he, too, will devote at least 30% effort to this important work. The reviewers noted that the subcontractor "contributes a unique resource to the project". We agree. We in California have fiduciary responsibility to use our citizen's money most effectively & parsimoniously to bring them therapies. The subcontractor's involvement helps meet that goal. The Michael J. Fox Foundation has made a similar assessment in designating Dr. Redmond's resource as its animal model facility & investing ~$1MM in expanding its capacity for Parkinson's Disease research.

4. A query was raised about the proper time to enlist the powerful relevant animal models for PD, its validity, & how one would look for tumors in such an animal.

We wish to endorse the view of those GWG reviewers who felt this model needs to be pursued now. The use of systemic MPTP in this model, the most authentic extant model of actual human Parkinson's Disease, is extremely critical for any informative study exploring therapeutics for this condition. Multiple independent published papers have supported the predictive power of this model. Particularly with regard to the action of human stem cells, the similarities between humans & this model are of particular relevance with regard to tropic & trophic factors & gene regulatory mechanisms which are simply different from rodents. In all of our published & preliminary studies with human stem cells in the model – and we have recently published an exhaustive study of one such stem cell type -- cell survival has not been an obstacle; and proper circuit restoration/preservation & function on human-like tasks has always been our measure success.

While one would could plausibly spend a few years vetting in rodents the proposed human stem cell types described in the proposal, one would still need to repeat most of these studies with the same array of cells in this animal model because human biology more closely approximates that of a the relevant animal model than a rodent. A rodent cannot reliably predict a human cell's behavior, whether positive or negative. Similarly, rodents do not develop appropriate side-effects – e.g., dyskinesias, one of the most concerning potential side-effects in humans following cellular therapies in Parkinsonism. In other words, one would still need to pre-screen cells in relevant animal models before rationally advancing to an IND. The unique

features of this animal facility (detailed in the proposal) & of this team allows for parsimony in time, resources, & expense in being able to do critical preclinical research in a model that is also so relevant to the human condition. As noted above, we have already published the extensive screening of one human neural stem cell type &, hence, have established baseline parameters allowing the proposed work to proceed quite expeditiously. The cells to be used have been extensively characterized & we know precisely the outcome measures to be assessed making this in no way a "fishing expedition"; indeed the reviewers praised us for "justifying almost all of the proposed cell comparisons".

We and our colleagues have published recent reviews on the importance of relevant animal models for human disease [e.g., Wakeman DR, Crain AM, Snyder EY, *Regenerative Med* 1(4): 405, 2006; Capitanio JP, Emborg ME, *Lancet* 371: 1126, 2008].

Regarding searching for tumors, we will perform full necropsies on all animals. The team has extensive experience in pre-clinical safety studies for drug trials &, therefore, routinely employs GLP for FDA-submitted materials. Reliable safety data will be acquired by our routine full histology of the brain & of all standard peripheral organs & tissues, evaluating for tumor formation, for inappropriate differentiated cell types, & for distribution of labeled stem cells & their progeny (including assessment for markers of incompletely differentiated pluripotent cells).

5. A request for more detail about the human ventral mesencephalic (hVM) precursor cell line (cell population #4 in the proposal) & a statement about its tumorigenicity.

A paper confirming the described origin & properties of the new hVM neural stem cell line has just been published (*Exp Cell Res, 2009; doi:10.1016/j.yexcr.2009.03.011*) Space precludes repeating those characterizations here. Briefly, the DA neurons generated by these cells mature to the point of acquiring the electrophysiological properties of true substantia nigra pars compacta neurons (including firing trains of action potential). The cells not only respond to depolarization, but also to a battery of agonists of GABA, glutamatergic & DA receptors (appropriately blocked by corresponding antagonists). Therefore, one can state that the DA neurons mature functionally & are well-equipped with the correct set of neurotransmitter receptors & ion channels. Tumors have never been observed. This human stem cell-derived cell type warrants careful testing as have proposed.

5. Proposal includes an investigator who fulfils CIRM's partnership with Spanish institutions.

It is also worth noting that, although this application was submitted before CIRM announced its plans to partner with Spanish institutions, our original research plan includes contributions from long-term collaborator, **Dr. Alberto Martinez-Serrano**, Assoc. Prof. in the Center of Molecular Biology "Severo Ochoa" at the Autonomous University of Madrid. As noted above, Dr. Martinez-Serrano will provide important test human stem cell populations & perform important characterizations – specifically, the above-mentioned *"human stem cell population #4: the hVM precursor cell line" [Milestone 4]*). If desired & appropriate, we can now formulate (& formalize) this involvement to conform to the recent "Collaborative Funding Partner" plan that CIRM has now forged with Spain.

Thank you for your kind consideration of this essential project which employs an authentic animal model of Parkinsonism that will be *required* for advancing stem cell-based therapeutics to clinical trial – *but at a fraction of the cos*t that would be incurred under any other arrangement.

Sincerely,

Evan Y. Snyder, M.D., Ph.D., F.A.A.P

TR1 - 1267: Recommended for funding (79)

EXECUTIVE SUMMARY

This proposal focuses on the development of a stem cell-derived therapy for Parkinson's disease (PD). For this purpose, the applicant proposes a comprehensive characterization and comparison of six human cell populations. The human cell populations to be compared head-to-head are: a) hNSCs derived from fetal central nervous system (CNS), b) from human embryonic stem cells (hESCs), c) from reprogrammed human fibroblasts, d) human ventral mesencephalic (hVM) cells isolated from fetal CNS, e) hVM precursors generated from hNSCs and f) hVM precursors generated from hESCs based on gene expression. In Aim 1 the applicant plans to characterize these six cell preparations by transcriptosomal and phosphoproteomic profiling and assess their capacities for expansion, differentiation and secretion of neurotransmitters and trophic factors. In Aim 2 the applicant proposes to transplant these six cell preparations into a predictive preclinical animal model of PD and assess relative safety, fate and therapeutic efficacy. In Aim 3 the applicant will attempt further optimization of the safest and most efficacious cell preparation in combination with a strategy for optimizing the milieu for DA neuron survival and process outgrowth. The best hNSC derivation strategy (with or without adjunctive strategies) will then be advanced for final safety & toxicity studies, GMP scale up, and other preclinical development activities necessary to move a product candidate forward.

Reviewers were enthusiastic about the potential impact of this proposal. PD is a common neurodegenerative disease. It is a progressive disease, usually of unknown etiology for which there are no disease modifying therapies and for which palliative pharmacological treatments have a number of shortcomings. This makes PD a major and rational target for cell-based therapies. Nevertheless, clinical trials on the transplantation of stem cells (mostly fetally-derived) into PD patients have met with mixed success; with some patients showing improvement, some no changes, and others significant side effects of the therapy. However, these trials have pioneered the cell-based treatment for neurodegenerative disease, showing that transplantation of cells into the brain can be safe and technically straightforward and that transplanted cells can survive up to ten or more years. Reviewers agreed that the types of head-to-head comparisons described in this proposal are necessary and critical to advance stem cell-based therapies to the clinic. One reviewer was concerned that it was premature to pursue studies of this scope in this complex and expensive model. This reviewer argued that the best time for head-to-head comparisons is after the various treatments have been fully optimized (short of the proposed animal models) and raised concern that full optimization with NSCs is far from complete. But other reviewers disagreed and felt that the proposed work is needed now.

Reviewers described this proposal as a tour-de-force with an incredibly detailed and ambitious research plan. One reviewer noted that it has some aspects of a large-scale fishing expedition, but the applicant justifies almost all of the proposed cell comparisons. The research design is complex and appears to cover all of the research required to identify a cell therapy candidate (in anticipation of moving forward to preclinical development and clinical trials). Reviewers appreciated the wealth of preliminary data as well as the discussion of pitfalls and alternative approaches. They had mixed feelings about the animal model, with one reviewer praising its validity and another noting its lack of extra dopaminergic abnormalities. Reviewers questioned the inclusion of cell population #6 (hVM precursors generated from hESCs based on gene expression) in the research plan. They noted that generating hESC reporter lines using homologous recombination will be difficult and the rationale for this approach was not well described. In addition, the mouse studies to test this cell population were not well integrated into the proposal. One reviewer was concerned that the research plan is overly ambitious and unrealistic given the three-year time frame. This reviewer also noted that the hVM cell line was not well described, and also wished for more detail on methods for monitoring tumorigenesis in vivo.

Reviewers praised the applicant's qualifications and experience in the neural stem cell field. They also appreciated the proposed PI commitment of 30% effort to the project. Reviewers were impressed by the assembled research team, which includes a strong list of associates and collaborators with considerable

combined expertise. One reviewer noted that the major collaboration with the investigator in charge of preclinical model testing is essential and that this external investigator contributes a unique resource to the project. However, this investigator's percent effort is not listed, which needs to be addressed. Reviewers were less enthusiastic about the collaboration responsible for producing Cell Population #6, noting that it was not well justified. One reviewer recommended eliminating this portion of the proposal. The panel was reminded that CIRM staff would address the budget and its appropriateness as it does for all budgets during administrative review. Reviewers judged the resources and various research environments to be excellent.

Overall, reviewers were enthusiastic about this proposal. Despite finding it overly ambitious, they were impressed and convinced by its detailed, well-organized research plan, strong preliminary data and experienced research team.

PROGRAMMATIC REVIEW

During programmatic review, the Grants Working Group was instructed to consider the specific rank order of all applications in Tier I as an indicator of priority for funding. A motion was made to move this proposal up within Tier 1. The panelist argued that the proposal fits the development candidate category better than the bottleneck category and CIRM is prioritizing development candidates for this RFA. Reviewers were reminded that each proposal was to be assessed on the basis of the category selected by the applicant. The motion failed. Although the panel was reminded of the option for a minority report, no motion for a minority report was made.