RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 3-5, 2007

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

Contents

[Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at www4.od.nih.gov/oba/rac/protocol.pdf.]

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

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The Recombinant DNA Advisory Committee (RAC) was convened for its 110th meeting at 8:00 a.m. on December 3, 2007, at the National Institutes of Health (NIH), Building 31-C, Conference Room 10, Bethesda, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 1:40 p.m. on December 3, from 8:00 a.m. until 5:15 p.m. on December 4, and from 8:30 a.m. until 10:45 a.m. on December 5. The following individuals were present for all or part of the December 2007 RAC meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania
Jeffrey S. Bartlett, Columbus Children's Hospital (present on Days 1 and 2; *via teleconference* on Day 3)
Stephen Dewhurst, University of Rochester Medical Center
Hildegund C.J. Ertl, The Wistar Institute (present on Days 1 and 2; *via teleconference* on Day 3)
Hung Y. Fan, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center (present on Days 1 and 2)
Jane Flint, Princeton University (*via teleconference*)
Ellen E. Grant, HealthNow New York Inc. (present on Days 1 and 2; *via teleconference* on Day 3)
Jeffrey P. Kahn, University of Minnesota
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation (present on Days 1 and 2; *via teleconference* on Day 3)
Prediman K. Shah, Cedars-Sinai Medical Center
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities (present on Days 2 and 3)
Scott E. Strome, University of Maryland (present on Day 1)
Richard G. Vile, Mayo Clinic (present on Day 2; *via teleconference* on Day 3)
David J. Weber, The University of North Carolina at Chapel Hill (present on Days 1 and 2; *via teleconference* on Day 3)
Lee-Jen Wei, Harvard University
David A. Williams, Children's Hospital Boston/Harvard Medical School
John A. Zaia, City of Hope National Medical Center (present on Days 1 and 2; *via teleconference* on Day 3)

Office of Biotechnology Activities (OBA)

Jacqueline Corrigan-Curay, Office of the Director (OD), NIH
Amy P. Patterson, OD, NIH

***Ad Hoc* Reviewers and Speakers**

Abdu Azad, University of Maryland
Barry J. Byrne, University of Florida
Odile Cohen-Haguenauer, Ecole Normale Superieure de Cachan
J. Stephen Dumler, The Johns Hopkins Medical Institutions *(via teleconference)*
Steven A. Goldman, University of Rochester Medical Center *(via teleconference)*
David W. Hackstadt, National Institute of Allergy and Infectious Diseases (NIAID), NIH *(via teleconference)*
Katherine A. High, Howard Hughes Medical Institute/The Children's Hospital of Philadelphia

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Joseph Kanabrocki, University of Chicago
Nancy M P King, Wake Forest University
Jay Lozier, Warren Grant Magnuson Clinical Center, NIH
Claudia A. Mickelson, Massachusetts Institute of Technology
Michael J. Miller, Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services (DHHS) *(via teleconference)*
Nicholas Muzyczka, University of Florida *(via teleconference)*
Didier Raoult, World Health Organization *(via teleconference)*
Naomi Rosenberg, Tufts University *(via teleconference)*
Leonard B. Seeff, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH
Daniel J. Sexton, Duke University *(via teleconference)*
Sander van Deventer, University of Amsterdam
David H. Walker, The University of Texas Medical Branch at Galveston *(via teleconference)*

Nonvoting Agency Representatives

Daniel M. Takefman, Food and Drug Administration (FDA), DHHS

NIH Staff Members

Dennis M. Dixon, NIAID, NIH
Linda Gargiulo, OD
Mary Groesch, OD
Bob Jambou, OD
Mary Joyce, National Heart, Lung, and Blood Institute (NHLBI), NIH
Elizabeth Kang, NIAID, NIH
Robert Kotin, NHLBI, NIH
Laurie Lewallen, OD
Harry Malech, NIAID, NIH
Maureen Montgomery, OD
Stuart Nightingale, OD
Marina O'Reilly, OD
Roland Owens, NIDDK
Paul Plotz, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIH
Louise Rosenbaum, NIAMS, NIH
Gene Rosenthal, OD
Tom Shih, OD
Bruce Whitney, OD

Others

There were 136 attendees at this 3-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, *ad hoc* reviewers and speakers, and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III is a list of abbreviations and acronyms used in this document.

I. Day 1 Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff, RAC Chair, called the meeting to order at 8:00 a.m. on December 3, 2007. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was initially published in the *Federal Register* on November 13, 2007 (72 FR 218) and subsequently in the *Federal Register* on November 26, 2007 (72 FR 226) (showing the corrected December 4 meeting

time). Issues discussed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB) (a subcommittee of the RAC), followup on a serious adverse event (SAE) in a human gene transfer trial using an adeno-associated viral (AAV) vector, public review and discussion of five protocols, update on synthetic biology by the RAC's Biosafety Working Group, presentation on and discussion of the use of immunosuppression with AAV vectors, and followup on the discussion of proposed experiments involving deliberate transfer of chloramphenicol resistance to *Rickettsia conorii* and *Rickettsia typhi* that would require a Major Action under *Section III-A-1* of the *NIH Guidelines*.

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as special Federal Government employees, read into the record the conflict of interest statement, and suggested that questions be addressed to the OBA committee management officer.

II. Minutes of the September 17-18, 2007, RAC Meeting/Drs. Flint *(via teleconference)* and Kirchhoff

Dr. Kirchhoff noted that the minutes of the September RAC meeting were carefully reviewed.

A. Committee Motion 1

Dr. Kirchhoff moved and Dr. Flint seconded that the RAC approve the September 17-18, 2007, RAC meeting minutes. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

III. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Albelda, Federoff, and Strome

Dr. Strome reported that of the 12 protocol submissions received by the OBA in the past 3 months, 8 were not selected for public review at this RAC meeting; one of the protocols reviewed at this RAC meeting had been selected for review in September 2007, but its review had been deferred to this meeting. Of the 8 protocols not selected, 6 are for cancer, and 2 are for infectious diseases (human immunodeficiency virus [HIV] type 1 infection). The 8 protocols employ a variety of viruses, including retrovirus, ribonucleic acid (RNA) transfer, canarypox, *Saccharomyces cerevisiae*, and a plasmid vector.

Dr. Strome discussed the adverse events (AEs) that were reported to the OBA during this 3-month reporting period. A total of 124 AEs were reported from 30 clinical trials, of which the majority were unrelated to the gene transfer products; there were 23 new reports in which the AE was possibly related to the gene transfer products. The GTSAB reviewed 20 initial and 20 followup AEs from 12 trials.

During the reporting period, the OBA received 130 amendments. Three M1CI responses were discussed briefly. In Protocol #0410-677, the investigators increased the time interval between dose cohorts from 3 weeks to 6 weeks, and in agreement with the RAC's recommendation, the investigators will produce an audiocassette version of the informed consent document for this visually impaired population. In Protocol #0510-731, the investigators will follow the RAC's recommendation that a stent be placed if the parotid duct is obstructed, and in the informed consent document, the investigators will address the RAC's concern about gender differences. In Protocol #0704-853, the trial has not begun, but the responses to the RAC's recommendations were provided: The investigators plan to administer all four doses to at least two participants prior to proceeding to the next cohort. In addition, the third participant in a cohort will receive at least three doses prior to escalation to the next cohort. The investigators also agreed to exclude potential participants who are anticipating surgery or other procedures requiring general anesthesia (local anesthesia will be allowed), and magnetic resonance imaging (MRI) or computerized tomography (CT) scans of the central nervous system (CNS) will be performed to look for brain metastases in patients with primary pulmonary adenocarcinoma.

A. Presentation by Dr. Kang Regarding Amendment to Protocol #9802-231

One amendment from Protocol #9802-231 was discussed in more depth. Dr. Elizabeth Kang, NIAID, NIH, discussed the two research participants dosed to date in this gene transfer trial for X-linked chronic granulomatous disease and the proposal to administer the immunosuppressive agent rapamycin.prior to gene transfer and for up a month after the gene transfer.

Participant #1 was a 28-year-old male with chronic granulomatous disease who had developed liver abscesses refractory to antimicrobial treatment. He was enrolled in the trial and received the gene transfer with busulfan preconditioning. He made a gradual recovery from his prolonged thrombocytopenia, and his neutrophils dropped for a maximum of 11 days but never to zero. Participant #1's cells began expressing $gp91^{phox}$ at 24 percent but slowly declined. At his 1-year followup, gene correction remains steady at about 1 percent. Although this level of correction is not curative, he did have good response in that each cell expresses an almost normal amount of $gp91^{phox}$. Participant #1 was enrolled in this trial due to the liver abscesses; at 4 months posttransplant, the abscesses were resolving, by 6 months they were almost gone, and by 10 months they were completely gone. At the 1-percent expression level, participant #1 continues to have some infection, but his infection frequency rate has decreased.

Participant #2 was a 31-year-old Caucasian male who had phoma and paecilomyces infections of the lung and chest wall eroding into a rib, which had been ongoing for about 2½ years at the time of his enrollment in this trial. This was despite treatment with multiple antifungal agents.

Following gene transfer, his clinical course was quite similar to the first subject however his initial marking levels were slightly lower; his initial increase in $gp91^{phox}$ expression was 5 percent. However, the decline in expression was much more rapid and the investigator proposed that this was due to an immune response against the transduced cells, Data using dihydrorhodamine (DHR) analysis of oxidation in peripheral blood neutrophils was presented. The results suggested that there was something in the patient's serum that was causing stimulation in this subject's cells. Dr. Kang acknowledged that they are unable to categorize this immune response as T-cell mediated versus antibody mediated. Moreover, because this subject ultimately succumbed to *Cytomegalovirus pneumonitis* and died further studies could not be done.

Dr. Kang noted that a third potential participant is awaiting transplant. He has an ongoing pelvic abscess, and he expresses some $gp91^{phox}$ protein.

In summary, for the two participants treated to date, little toxicity has been associated with the conditioning regimen, and mild thrombocytopenia has occurred. Measurable levels of marking and protein production occurred in participant #1 with clinical improvement, and participant #2 exhibited possible immunologically mediated rejection. Accrual to this clinical trial is ongoing with the amended protocol, which adds rapamycin to prevent further immune mediated destruction of the transduced cells.

B. RAC Discussion

Dr. Strome expressed the concern of the GTSAB—adding a drug in the absence of data, with only a suggestive effect in one participant and no hard evidence of an immunologic response. This agent has the potential to be toxic and in the absence of those data, the GTSAB hoped to wait for information that an immunologic response was occurring.

Dr. Kang explained that the investigators are planning to dose with rapamycin for a maximum of about 1 month; rapamycin has been shown to be well tolerated. Although it is an immunosuppressant, rapamycin does not have the same complications as prednisone or cyclosporin. Rapamycin is currently being used in another transplant protocol and has not produced any additional unusual infections.

Drs. Albelda and Ertl were concerned that beginning to use rapamycin at this point, without enough data, would compromise the investigators' ability to draw conclusions from their trial.

C. Committee Motion 2

Dr. Federoff summarized the RAC general consensus that, in the absence of data to select the most appropriate agent, such data should be collected. Once those data are analyzed, they will provide evidence as to whether rapamycin or some other approach might be recommended.

It was moved and seconded that this consensus of the RAC be approved. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Followup on a Serious Adverse Event in a Human Gene Transfer Trial (OBA Protocol #0504-705) Using an Adeno-Associated Viral Vector: Analysis of Its Scientific and Safety Implications

RAC Reviewers: Drs. Albelda, Bartlett, Dewhurst, Ertl, Federoff, and Strome

Dr. Federoff explained the purpose of this discussion, which was to bring to closure the analysis of the scientific and safety issues surrounding the death of the research participant known as "Subject 1209." The September 2007 RAC meeting featured an in-depth discussion of these issues but without benefit of all of the supporting data, so the RAC was unable to come to definitive conclusions at that earlier meeting. At this time, Dr. Federoff stated that the RAC's opinion is that the primary cause of death of Subject 1209 was an opportunistic infection—disseminated histoplasmosis with subsequent bleeding complications and multiorgan failure. Her apparent risk factor for such an infection was her systemic rheumatoid arthritis (RA) therapy, chiefly the tumor necrosis factor (TNF) antagonist drug adalimumab. In addition, the RAC is unable to rule out definitively, due to lack of data, the possible role of the gene transfer in her clinical course. Dr. Federoff expressed the hope that generalizable lessons could be learned from this SAE to enhance the safety of other gene transfer trials and provide potential strategies to quickly gather the data needed for safety evaluations in case of an SAE.

Dr. Corrigan-Curay summarized the portion of the September RAC meeting devoted to reviewing this protocol and the associated death of Subject 1209. She also reviewed the results of the product retesting performed by Targeted Genetics Corporation, the results of coagulation studies performed by the University of Chicago, and other data received since the September 2007 RAC meeting. Product retesting by Targeted Genetics Corporation showed no evidence of contamination (no adenovirus [Ad] was detected), a culture for herpes simplex virus (HSV) was negative as of day 14, and a histoplasmosis culture was negative. PCR data on the levels of vector in multiple tissues at autopsy was reviewed. Positive but non-quantifiable levels were found in the spleen, liver and brain with quantifiable amounts found in the tonsils and right knee. The remaining autopsy samples were negative. PCR for the wild-type AAVrep gene was also done on these tissues. The right knee had quantifiable levels and the heart and trachea were positive but not quantifiable. Of note, the heart and trachea were not positive for the vector used in this protocol, so the finding of wild-type AAV may indicate natural infection. Per the protocol sponsor, Targeted Genetics Corporation, the presence of wild-type AAVrep gene in the right knee where the vector was injected was expected from preclinical data.

Regarding the possible role of the transgene product, data from eight individuals who received the same dose as Subject 1209, but who were not also taking systemic TNF-antagonist drugs, indicated that the transgene product was not detectable in their sera at 4 and 12 weeks after vector administration. Although an assay was not available to distinguish the transgene product from the systemic TNF-antagonist the subject was taking, levels of TNF-antagonist in this subject's serum never exceeded the expected steady state concentration for an individual on the systemic TNF-antagonist adalimumab. In addition, the levels rapidly declined after July 2nd the day the subject received the gene transfer and after which subject did not receive any additional systemic TNF-antagonist.

Regarding a possible immune reaction, no data were available in this case to comment on whether there was a CD8+ mediated T-cell response against the vector capsid. There was some agreement that if an

immune reaction had occurred, it was unlikely to have played a significant role in the clinical course. Moreover there was consensus that an immune reaction was not the primary cause of the death of Subject 1209 because the primary cause of her death was histoplasmosis and retroperitoneal bleed.

Additional findings from the various studies undertaken since September 2007 included the following: Factor II, X, and XI levels were approximately 20 percent to 25 percent of normal; Factor IX (FIX) was about 30 percent; Factors V and VII were depressed at 50 percent; and fibrinogen was low. Factor VIII and von Willebrand factor were the only elevated coagulation factors. The D dimer level was elevated, and a weakly positive lupus anticoagulant was present. The resulting global decrease in clotting factors partly explains the type and degree of hemorrhage seen in Subject 1209; it does not rule out the possibility of another anatomic factor such as a mycotic aneurysm that was never identified.

A. RAC Conclusions

Dr. Federoff read the RAC conclusions regarding the assessment of the role of gene transfer, an update on the status of this protocol including revisions incorporated, and RAC recommendations on trial design and lessons learned:

- After review of all of the information available, we conclude that there was no evidence of contamination of the gene transfer product. As expected, the majority of vector remained in the knee at the injection site with extremely low levels of vector present elsewhere outside the injection site. The AAV serotype 2 (AAV-2) rep gene, which may indicate replication-competent adeno-associated virus, was found only in very low levels in the heart and the trachea but the vector itself, tgAAC94, was not detected in these sites. The evidence does not support the theory that a helper virus led to widespread dissemination of replication-competent AAV.

- The declining levels of TNF antagonist in the serum do not support the theory that transgene production led to excessive systemic levels of TNF antagonist. They were consistent with the decline in the systemically administered Humira. The fact that this does not appear to have been a factor in this case does not diminish the importance of having an assay to specifically detect levels of the transgene product. The degree of functional TNF inhibition cannot be determined.

- The absence of significant vector in the liver and spleen at autopsy and the high anti-AAV titers do not exclude an immune response. Whole-blood samples from before and after administration of the product were not available for CD8+ capsid-specific T-cell assays. In the absence of these data, an immune response cannot be definitively ruled out. If such a response had occurred, it may or may not have been a contributory factor, but it was not a primary factor in this patient's death. The inability to exclude an immune reaction to the vector underscores the importance of obtaining samples for T-cell assays.

- A possible role of the gene transfer in this clinical course cannot definitively be excluded due to the lack of data. If the interarticular injection of the gene transfer vector did play a role, it was very unlikely to have been a significant contributor, if at all, to Subject 1209's clinical course and was not the cause of her death.

- It is the RAC's opinion that the unfortunate death of Subject 1209 was primarily a result of an opportunistic infection, disseminated histoplasmosis with subsequent bleeding complications, and multiorgan failure. Her apparent risk factor for such an infection was her systemic RA therapy, chiefly the TNF-antagonist adalimumab.

- Update on the status of this protocol: The FDA has removed the trial from clinical hold; enrollment is complete, but not all research participants have received their second dose. The protocol was revised as follows:

    - The study product will not be administered to participants with temperatures greater than 98.6 Fahrenheit, localizing signs and symptoms, or unexplained fatigue or malaise on the

day of administration—which are considered sensitive indicators of possible infection and, therefore, consistent with excluding these individuals from an injection. Participants who have a history of opportunistic infection are also excluded, and participants are required to have failed at least one disease-modifying anti-RA drug. Participants who will receive a second dose will first sign a revised informed consent document.

- o Additional monitoring: Blood will be drawn at additional timepoints after administration of the study agent for complete blood count, serum chemistry, vector deoxyribonucleic acid (DNA), and TNF receptor:Fc protein. Blood will be drawn at multiple timepoints to obtain data on potential T-cell responses to the AAV-2 capsid.

- Regarding trial design and lessons learned, this case underscores the importance of developing assays to distinguish the gene transfer product from treatments the participant is receiving. The RAC recommends that AAV trials monitor for anticapsid T cells since these may help in the interpretation of AEs and may shed light on the safety of AAV vectors generally. The FDA currently recommends such monitoring for AAV trials. The potential role for immunosuppression in altering the risks to participants enrolled in gene transfer trials needs to be carefully considered.

- Clinical criteria for the timing of the second dosing, especially in safety trials, should be thought out in advance and articulated in the protocol. To enhance the safety of participants in gene transfer trials, investigators should consider developing a medical card that would include a brief description of the vector and a Web-based link to find out additional information, a 24-hour series of contact numbers for study investigators, and a list of samples that should be collected upon admission to a hospital. Protocols should plan for additional blood and other samples that may need to be collected in the event of an SAE, and investigators should think through the logistics of such collections should the subject be under the care of physicians who are not involved in the trial. The logistics of an autopsy should also be developed in advance, including a detailed protocol that could be shared with an outside institution as well as considering mechanisms for transfer from an outside institution to the institution conducting the trial.

- With respect to informed consent, in early-phase trials it is critical to take steps to actively prevent therapeutic misconception in the informed consent process, especially when the investigator is also the participant's physician. Investigators need to recognize that their belief in their study may also lead to therapeutic misconception. A discussion of the importance of an autopsy is critical in clinical trials, and it may be prudent to involve the family, if possible, in that discussion. The *NIH Guidance on Informed Consent for Gene Transfer Research* provides a potential resource for information on the issues related to consent; it can be accessed online at http://www4.od.nih.gov/oba/rac/ic/.

B. RAC Discussion

Dr. Kahn asked how the informed consent document was revised. Dr. Corrigan-Curay explained that the OBA would receive the revised informed consent document along with the amended protocol after it has been reviewed by the GTSAB. She offered to circulate the informed consent document to everyone involved.

Dr. Zaia asked whether there was a conflict of commitment regarding Subject 1209's rheumatologist (who was the study investigator), stating his belief that if her physicians had diagnosed histoplasmosis earlier, Subject 1209 might still be alive. Dr. Corrigan-Curay explained that the physician who consented Subject 1209 was also her rheumatologist. From the time she got sick, the investigator was not involved in the hospitalization or the diagnosing at the local and university hospitals.

C. Committee Consensus

Noting the importance of the RAC's consensus on this issue, Dr. Federoff stated that, with the input of Dr. Ertl, Ms. Shapiro, and Dr. Strome, the RAC recommendations would be revised for complete concordance of language and would be distributed to all RAC members. A full RAC vote will be held at either the March 2008 RAC meeting or a special RAC meeting scheduled to be held on January 14, 2008.

V. Discussion of Human Gene Transfer Protocol #0710-877: A Phase II Safety and Efficacy Study Evaluating Glutamic Acid Decarboxylase Gene Transfer to the Subthalamic Nuclei in Subjects with Advanced Parkinson's Disease

Principal Investigator:	Peter LeWitt, M.D., Henry Ford Health System/Neurologix, Inc.
Additional Presenters:	Matthew During, M.D., D.Sc., Neurologix, Inc.; and David Eidelberg, M.D., The Feinstein Institute for Medical Research
Sponsor:	Neurologix, Inc.
RAC Reviewers:	Drs. Federoff and Kahn
Ad hoc Reviewer:	Nicholas Muzyczka, Ph.D., University of Florida *(via teleconference)*

Drs. Bartlett and Williams recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Parkinson's Disease is a neurodegenerative disorder with characteristic impairments of motor function such as slowed movement, impaired dexterity, gait disturbance, tremors, and rigidity of muscles. These clinical features are associated with progressive loss of neurons in the brain stem, particularly the substantia nigra pars compacta (SNpc), where more than 60% of a population of dopamine-secreting nerve cells is lost in the brain of PD patients. In PD, additional groups of neurons are affected with neurodegenerative changes elsewhere in the brainstem and in other central nervous system (CNS) regions. Particularly, the subthalamic nucleus (STN) plays a central role in the brain's circuit of cells responsible for regulating movement. Since γ-aminobutyric acid (GABA) plays an inhibitory role at the STN, pharmacological strategies to enhance its actions have been explored as a treatment for advanced PD. In human subjects with PD, infusing the GABA agonist, muscimol directly into the STN can improve motor impairments independently of replacing nigrostriatal dopamine. Similarly, a gene therapy experimental approach has been to enhance local synthesis of GABA in the STN. This strategy involves introducing a recombinant adeno-associated virus (rAAV) carrying the cDNA for 2 isoforms glutamic acid decarboxylase (GAD 65 and 67) into the STN. Animal experiments using this vector have confirmed that GABA synthesis is increased after infusion of the GAD-containing vector into the STN. This experimental approach was tested in an open label, Phase 1 study with 12 PD subjects. After unilateral STN infusion of rAAV-GAD, marked and sustained improvement in PD symptomatology was achieved.

This proposed Phase II protocol is intended to prove further the safety and efficacy results from the Phase I study. In this Phase II study, rAAV-GAD will be infused into each hemisphere of the brain in the STN using the same neurosurgical techniques as in the Phase I study, which is similar to the technique used in deep brain stimulation (DBS). The main focus of this Phase II study is to learn the effects in participants who receive the study agent compared with those who do not. Forty participants will be randomly divided into two groups—20 participants will receive the infusion of rAAV-GAD, and the remaining 20 will receive a partial-thickness burr hole with saline administered extradurally. Participants, care providers, and physicians will be blinded as to which procedure the participant receives. Participant assessment will use the United Parkinson's Disease Rating Scale (UPDRS), and participants will undergo a preoperative and several postoperative positron emission tomography (PET) scans. Based on meeting the 6-month primary endpoint and evaluation of the 12-month data from the treated group, the investigators will offer the same rAAV-GAD treatment to participants in the nontreated group. All participants will be followed for 12 months after surgery.

B. Written Reviews by RAC Members

Twelve RAC members voted for in depth review and public discussion of the protocol. Key issues included the possibility of induction of AAV-specific cytotoxic T-lymphocyte (CTL) response and questions about the dose-escalation strategy, the decision to infuse two regions rather than one as was done in the Phase I study, and the use of the sham procedure.

Two RAC members and the *ad hoc* reviewer provided written reviews of this proposed Phase II trial.

Dr. Federoff asked for an update on the clinical and neuroimaging data from the Phase I trial and data indicating the superiority of GAD65 and GAD67 coadministration over mono-gene transfer. He asked whether antibody titers to AAV would influence enrollment and asked the investigators to discuss the reasons for and the data supporting several deviations from the protocol employed in the Phase I study, including elimination of mannitol in the infused vector preparation. The selection of 6 months as the time period to reach the primary endpoint and establish the risk of AEs should be discussed. Dr. Federoff requested comment on how the protocol would be monitored and managed if clinically asymptomatic intracranial hemorrhages were detected. Questions regarding maintaining the blind should also be discussed, in particular, how the neurosurgical team and the floor clinical staff will remain blinded. He also suggested that peripheral blood mononuclear cells (PBMCs) should be drawn to provide potential information about T-cell responses.

Dr. Kahn focused his review on the informed consent document and ethical issues related to the sham surgery. The risks section of the informed consent document should include a list of the specific risks to the sham surgery group and make clear that this group will receive no benefit from participation in this study. The section regarding disclosure of payment to investigators is currently too general and should include specific information about those payments and the services for which those payments will be made. Clarification is needed regarding the request for autopsy, since the current wording is unclear about giving such consent. Dr. Kahn noted several other portions of the informed consent document that needed rewording for clarity or correctness. In addition, he suggested that the data safety and monitoring board (DSMB) for this protocol include a member with research ethics expertise.

Dr. Muzyczka asked the investigators to comment on why they are proposing to express both GAD65 and GAD67 genes. Dr. Muzyczka asked whether the investigators are concerned about the possible expression of the hepatitis X protein, implicated in hepatic tumor formation, from the woodchuck hepatitis posttranscriptional regulatory element (WPRE). He requested that the investigators provide more information about the replication competent AAV assay to be used, in particular how they plan to deal with the possibility of a false-positive signal. Dr. Muzyczka also asked the investigators to comment on the necrosis seen in the three monkeys injected with virus expressing green fluorescent protein (GFP), especially whether this response could be due to some other component present in the virus preparation.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Dr. Wei questioned the use of the 6-month endpoint for data from the research participants in this clinical trial.

- Dr. Wei requested a justification of the proposed dose.

- Dr. Kirchhoff asked for information about the procedure for determining which potential participants are cognitively impaired and therefore excluded from participation in this trial.

- Dr. Weber asked about the safety of the proposed Medtronic, Inc., device.

- Acknowledging that the use of blinding in this trial is controversial, Dr. Albelda noted that, in addition to efficacy and placebo issues, blinding is important to ascertain whether any side effects are due to the gene transfer.

- Drs. Albelda and Strome asked that given that the phase II trial will differ in multiple respects from the phase I trial, whether an initial safety study should be performed prior to proceeding with the larger phase II trial.

- Ms. Shapiro requested that the surrogate signature line at the end of the informed consent document be eliminated or that it be redesignated more correctly as the signature line for a witness to the research participant's consent.

- Dr. Strome asked whether bilateral injection had been performed before for this type of approach.

- Dr. Federoff asked the investigators whether they plan to add a bioethics expert to their DSMB.

D. Investigator Response

1. Written Responses to RAC Reviews

Dr. During stated that, at this stage, the investigators have no data to suggest any relationship between pretreatment to AAV titers and clinical response. In the Phase I study, as reported in *The Lancet*, the investigators did not see any relationship between preexisting antibody titer and clinical response, so there are no current data to suggest that even high levels of neutralizing antibodies to AAV will influence gene transfer.

Regarding why they propose to express both GAD65 and GAD67 genes, the investigators explained that almost all inhibitory neurons in the brain express both isoforms, which have high homology in their catalytic regions. GAD65 and GAD67 have different kinetics of binding to cofactors and serve slightly different roles in metabolism and neurotransmission. The more active GAD67 provides a mechanism to reduce glutamate as well as being responsible for the bulk of GABA production in the brain; GAD65, which more effectively traffics to the axon terminals and vesicles, is more involved in the axonal release of GABA.

In response to Dr. Muzyczka's concern about the WPRE, the investigators clarified that the WPRE used routinely in their laboratories is obtained from Thomas J. Hope, Ph.D., Northwestern University, who originally isolated and characterized WPRE. The X protein ATG start codon and upstream regulatory sequences of the WPRE sequence have been mutated, therefore preventing the possibility of expression of the truncated X protein peptide.

In the three monkeys injected with GFP virus, the pallidal lesions were found at a significant distance from the vector injection site, and there was no evidence of significant necrosis or inflammation at the site of injection in these monkeys. Co-investigators have noted that in sensitive monkeys, lesions beyond the nigra occur with MPTP, particularly with high doses. Because these monkeys were not completely naive, having been used in maternal separation studies as infants many years earlier, it might be possible that their history made them more sensitive to the nondopaminergic toxicity of the neurotoxin.

The investigators agreed to make modifications in the informed consent document as suggested by Dr. Kahn in his review of this protocol. Those changes include modifying and clarifying language, including a statement relating to the risks of the sham procedure, and including information about referral to other medical professionals if there are abnormal research-related findings.

In their Phase I study, the investigators used a hollow fused silica fiber to deliver the vector after it was passed down the lumen of a microelectrode sheath. The device created for this Phase II study is designed to retain the advantages of the Phase I catheter while providing many additional benefits that could not be addressed in Phase I due to lack of a suitable device. Specifically, the investigators worked

with Medtronic, Inc., to develop a flexible catheter system that provides significant advantages compared with the Phase I device: It shortens the time in the operating room, reduces the potential for hemorrhaging or lesioning associated with any patient movement, and provides the ability to infuse the vector in the recovery room outside of the operating room.

Regarding the elimination of mannitol from the infused vector preparation, there were data that mannitol was potentially acting as a weak adjuvant, and that mannitol within brain parenchyma could disrupt the normal interstitial space. The investigators decided against the continued use of mannitol despite its strong effect on enhancing local gene expression. To offset the loss of mannitol enhancement, a modest increase in the dose was made. Also a premixed GAD65/67 vector working stock in the final vial could be more consistently delivered without having to add mannitol. Moreover, avoiding the mixing step reduced risks associated with contamination, spills etc.

Six months was chosen for the primary endpoint because peak gene expression is reached by 1 month, and a plateau of both clinical and biochemical imaging effects occurs at 6 months, with no significant differences observed at 12 months. The investigators plan to monitor the research participants for 12 months, with long-term followup to year 5.

Regarding the optimal time to detect intracranial hemorrhage related to brain instrumentation, the investigators explained that, when following a known intracranial hemorrhage, standard protocol at most major medical centers is to repeat the CT scan at 24 hours. If there is no change, then the hemorrhage is considered stable and patients are usually discharged from care. This protocol proposes to obtain a CT scan within 24 hours following surgery. If research participants develop any unusual symptoms more than 24 hours after surgery despite a negative scan, they might be reimaged if the clinicians believe that is justified.

If a clinically asymptomatic intracranial hemorrhage is detected, the individual will remain in the hospital for an additional 24 hours, followed by an additional head CT scan. If the hemorrhage is stable, the research participant will be discharged and followed clinically; if the hemorrhage expands, these standard protocols will be continued and the specific response will be dictated by the clinical condition of the individual and the routine of the particular hospital. A certain rate of asymptomatic hemorrhages is observed in all stereotactic surgical procedures, which is the nature of penetrating the brain with any device, and therefore all experienced centers have routine protocols that would be directly applicable to this situation.

Regarding the role of neurologists in the medical management of PD patients enrolled in this trial, neurologists will attempt to stably maintain participants on their baseline drugs and dosing. However, a participant's neurologist will have the autonomy to make alterations in medical management as needed. All such changes will be documented fully, even though these changes are not a study endpoint.

The investigational sites were chosen because the neurosurgeons have significant expertise and experience in DBS and use similar surgical approaches. Dr. Michael Kaplitt, who will be present at the first case for each site, will train all neurosurgeons. Preoperative neurosurgical planning will follow the DBS routine of each center.

In response to concerns about how the blinding will be maintained, the investigators explained that if a blinded investigator notes an AE that requires unblinding, the principal investigator (PI) will be notified and that participant will be unblinded. In the event of an emergency when the PI cannot be contacted, the blinded investigator will contact the unblinded neurosurgeon, and they will unblind the participant if necessary, although it may be possible for the neurosurgeon to manage the situation without unblinding the participant. The floor clinical staff will not be able to discern any difference between sham and treated individuals because all participants will undergo an identical perfusion, will have the same surgical wound and appearance from an external view, and will have the release cord pulled and the device removed.

2. Responses to RAC Discussion Questions

Dr. During clarified that the investigators will be following the participants for 12 months; the blind will be broken at 6 months, but the data will be collected at 3, 6, and 12 months.

Dr. LeWitt explained that the placebo effect in PD is an important issue; most placebo-controlled clinical studies have shown a waning of the placebo effect at 6 to 12 weeks. In addition, there is often a decline in function as early as 6 months. Therefore, using the 6-month timeframe is appropriate, and there are enough symptomatic and neuroprotective clinical studies that have argued that 6 months is a reasonable endpoint for using PET.

Regarding discerning which potential participants might be impaired cognitively, Dr. During stated that the investigators will use a standardized and validated test based on cognition. They will use a scale that has been accepted in previous studies that will show when an individual is considered cognitively impaired for the purposes of this trial.

Regarding the safety of the proposed Medtronic, Inc., device, Dr. During explained that the FDA is looking carefully at all aspects of this device before it is used in humans.

In response to several RAC members' concerns about bilateral injection, Dr. During stated that bilateral injection in neurosurgery is common and that unilateral injection is rare. There exists significant precedent, with accompanying safety data, for operating on the nuclei bilaterally. Dr. Eidelberg concurred, stating that there is little reluctance to do bilateral subthalamic surgery on a routine clinical basis for DBS and noting, in addition, that Ceregene, Inc., is doing bilateral surgeries into the putamen for delivery of AAV-neurturin. Dr. LeWitt added that, in PD, a major component of the midline structures needs DBS bilateral improvement; it is handicapping for an individual to be treated only unilaterally.

Dr. LeWitt explained that the PI at each site would decide whether an individual patient would be enrolled; that decision will not be made by the neurosurgeon, so the neurosurgeon's financial interest will have nothing to do with the choice of participants or persuasion as to whether to participate. Potential participants will be given the alternative of having DBS, which would likely be performed by the neurosurgeon.

In response to several RAC members' concerns about safety and trial design issues, Dr. During suggested that the investigators start the trial as planned; then, after the first 10 participants have been enrolled and dosed, the DSMB would review the safety issues. The investigators would remain blinded, but the DSMB would be unblinded to make its assessment. This plan was acceptable to the RAC members.

E. Public Comment

Nancy M P King, J.D., of Wake Forest University (and a former RAC member), questioned whether this was the appropriate time to conduct a full Phase II trial. She suggested that the investigators dose three participants first to obtain valid data and then move to the full trial.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

<u>Clinical/Trial Design Issues</u>

- There are several significant differences between the design of the Phase II study and the Phase I trial (e.g., the composition of the infusions, the dose, bilateral infusions, and the delivery catheter). As such, the safety and efficacy data from the Phase I trial are not completely transferable to the Phase II design. An initial safety study of the new approach with a small cohort of research participants should be considered prior to proceeding with a randomized Phase II study design.

- The current plan is to break the randomization code and begin analyzing data from both arms of the study at the endpoint of 6 months. It would be preferable to lengthen the data-gathering by 6 months and maintain the blind until after the data are analyzed at 12 months.

Ethical/Social/Legal Issues

- Since the protocol involves sham surgery and a progressive neurological disorder that may involve changes in cognitive function, it would be prudent for the DSMB to include a bioethicist, who could help address the complex ethical issues that may arise.

- The consent document currently includes a signature line for a legally authorized representative. However, since the protocol intends to exclude patients who are cognitively impaired, the representative should not be serving as a surrogate decisionmaker. Some research participants may have difficulty signing a consent document due to motor impairments. In such cases, a witness rather than a surrogate decisionmaker would be needed.

- The informed consent document should be revised as follows:

    - Quantitative estimates of the listed risks should be provided (e.g., "the chances of an intracranial bleed is x in 1,000").

    - The discussion of the risks and benefits of alternative treatments should include or be conducted by the patient's physician who does not have a conflict of interest in relation to the study and may be better equipped to provide advice on appropriate alternatives.

    - The request for autopsy section should be revised to be consistent with the language in *Appendix M-III-B-2-c* of the *NIH Guidelines.*

    - The investigators should clarify the statement "If the results are what we had told the FDA we wanted to prove, you will be offered the study agent if you were originally assigned to the sham surgery group." As written, the statement does not make clear that research participants in the control group would need to enroll in a future study to receive the study agent.

    - The investigators should clarify the statement "Any abnormal conditions revealed by physical examination or protocol test results are not the responsibility of the study doctor." It should be made clear that this is a reference to ancillary findings of clinical significance that are not related to protocol procedures. It would make more sense for this issue and the study investigator's lack of responsibility for such findings to be discussed in a separate section rather than with a discussion of the confidentiality of medical records.

G. Committee Motion 3

Dr. Federoff orally summarized the RAC recommendations, which were refined and finalized after this RAC meeting as listed above in section F. These comments and concerns of the RAC were included in a letter to the investigator and the sponsor. Dr. Federoff asked the RAC to approve these summarized recommendations. The vote was 15 in favor, 0 opposed, 0 abstentions, and 2 recusals.

VI. Discussion of Human Gene Transfer Protocol #0710-881: A Phase Ib, Open-Label Trial to Define the Safety, Tolerance, Transgene Function, and Immunological Effects of Intratumoral Injection(s) of Adenoviral-Transduced Autologous Dendritic Cells Engineered to Express hIL-12 Under Control of the RheoSwitch® Therapeutic System in Subjects with Stages III and IV Melanoma

Principal Investigator:	John M. Kirkwood, M.D., University of Pittsburgh
Additional Presenters:	Lisa H. Butterfield, Ph.D., University of Pittsburgh; Costas Loullis, Ph.D., Intrexon Corporation; and Walter J. Storkus, Ph.D., University of Pittsburgh *(via teleconference)*
Sponsor:	Intrexon Corporation
RAC Reviewers:	Drs. Dewhurst, Weber, and Williams

Dr. Kahn recused himself from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

The protocol proposes to assess the safety of injecting dendritic cells transduced *ex vivo* with an adenoviral vector containing the gene for human interleukin-12 (IL-12) into the tumors of patients with stage III and IV melanoma. In the vector, production of IL-12 is under control of a novel gene regulation system called the RheoSwitch® Therapeutic System (RTS). The system was developed to allow for control of time and level of transgene expression. RTS acts as a "gene switch" or conditional promoter of transgene expression. It is activated by binding of an activator drug. The protocol is testing the production of IL-12 under the control of the RTS and activator drug and different doses of the proposed Activator Drug (RG-115932).

Safety and tolerance will be assessed by physical examination, vital signs, serum chemistry, urinalysis, hematology, AEs, and antibodies and cellular immune response to the Ad and the Activator Drug. In addition, this study will analyze hIL-12 levels and cellular immune response (T cells) by using biopsies of the target tumors, draining lymph nodes, and examining peripheral circulation, as well as via a serum cytokine profile. Forty participants will be divided into the two cohorts; all participants will receive a single injection into a melanoma tumor of Ad-transduced autologous DCs. The 12 participants in cohort 1 will be divided into four groups of three participants each, each of whom will receive a single daily oral dose of Activator Drug for 14 consecutive days. Each of the 28 participants in cohort 2 will receive a single oral maximal tolerable dose (MTD) of Activator Drug (as determined from cohort 1) for 14 consecutive days. Additional injection(s) of Ad-transduced autologous DCs in combination with 14 single, once-daily oral doses of Activator Drug may be administered to eligible participants who meet the criteria for retreatment.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of the gene switch method, the need for more information about the feasibility of RTS-mediated regulation of hIL-12 *in vivo* and *in vitro*, the short half-life of the Ad-transduced DCs in relation to the 14-day administration of the Activator Drug, and the need for data to support the presumption that transgene expression would be prolonged.

Three RAC members provided written reviews of this proposed Phase I trial.

Dr. Dewhurst asked eight questions about the preclinical data and the regulatable gene expression system. He requested data from the murine B16 melanoma study and from the experiment in which the Activator Drug was given for 6 days vs. 13 days. Dr. Dewhurst asked the investigators to provide more information about the *in vivo* survival time of the Ad-transduced DCs. He also asked about the availability of information about the human cellular immune response to HSV type 1 (HSV-1), since many participants will be HSV-1 seropositive and may therefore harbor CTLs specific for this protein. Regarding the clinical protocol, Dr. Dewhurst asked for a short list of the most common medications metabolized by the CYP450 3A4 pathway that might be used by participants in this study and suggested providing that list to enrolling clinicians and potential participants. His comments on the informed consent document included whether the preclinical prediction of interference with CYP450 3A4 and platelet activating factor (PAF) receptors might be considered risks that should be disclosed and suggested rewording to avoid the possibility of therapeutic misconception.

Dr. Weber stated eight concerns about this proposed protocol. He requested several different clarifications and additional data for retreatment decisions and the related followup plan. Dr. Weber suggested that pregnancy testing be offered much closer to the point of drug administration and noted that the inclusion criteria should state that males should be encouraged to use contraception. Regarding the Activator Drug, he noted that no information had been provided regarding the safety, efficacy, or pharmacokinetics of this drug and that no safety data for normal healthy volunteers had been provided. Dr. Weber offered several enhancements for clarity and accuracy for the informed consent document and requested that the investigators provide the rationale for choosing 40 research participants.

Dr. Williams posed 13 questions to the investigators. He asked about the evidence that timing of hIL-12 expression would make any difference in efficacy and about the highest dose of DCs injected that express hIL-12. Regarding safety and efficacy of the Activator Drug, Dr. Williams requested data on its effects on tumor cell growth, data on length of treatment, and data suggesting that use of Activator Drug enhances efficacy compared with using no drug. He requested information about the safety record and effects on efficacy of reinjecting research participants already exposed to Ad vectors. Dr. Williams asked the investigators whether transgene hIL-12 expression can be measured in tumor biopsy samples and why only 50 percent of participants would be biopsied and how those individuals would be selected. In addition to several specific wording clarifications, he wondered whether any of the investigators have financial interests in the reagents to be used in this study.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Dr. Weber asked whether the investigators had planned a specific time for reaching the primary endpoints.

- Dr. Williams stated his conclusion that this study is designed to test a proprietary approach that does not have a sufficient amount of data from preclinical studies to proceed to human studies.

- Dr. Ertl requested additional preclinical data to make a convincing case that the proposed approach is as good as or better than using Ad constitutively expressing IL-12.

- Dr. Strome commented that this proposed trial does not seek to address regulatory T cells or any of the regulatory properties that are known to exist as a result of other research.

- Dr. Williams asked about the existence of *in vivo* data suggesting that terminating the Activator Drug results in termination of IL-12 expression.

- Dr. Strome commented that looking for efficacy at day 24 would not be convincing; efficacy analysis at 2 months, 3 months, or a longer timepoint will make the investigators' data more easily interpretable. Data reviewed at day 24 will be difficult to interpret and should not be examined.

- Dr. Zaia queried whether the investigators would be able to answer their research question with the proposed design. If the investigators find that there is no good turnoff, that result may not be due to the ligand and its interaction with the receptor but rather to the Ad or some other cause.

- Dr. Ertl was concerned about the differing temporal lifetimes of DCs after they have been transduced, noting that most of the Ad-transduced DCs would probably be dead within 5 to 8 days. She stated that testing the on-and-off technology would be more convincing if the investigators were to use a stable cell line that does not die rapidly *in vivo*, noting that the investigators are using a turn-on/turnoff system with a cell that is not going to live long enough in humans to produce a clear answer.

- Dr. Williams suggested a straightforward set of experiments that could be done quickly to bolster the investigators' hypothesis that not turning on IL-12 expression for a given period of time will add to the efficacy of constitutively activated hIL-12 in DCs.

- Dr. Albelda suggested that the investigators conduct a preclinical experiment in which they compare turning on the Ad IL-12 vector with the constitutive vector, showing that this approach works better than or at least as well as the other vector. This preclinical experiment should be conducted using an animal that is preimmunized against Ad and should be completed before moving to a clinical trial.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding safety of the Activator Drug, the investigators noted that no cardiovascular, respiratory, or neurobehavioral effects have been observed in good laboratory practices safety pharmacology studies. Toxicology studies in two species for 28 days have been conducted; these studies showed no Activator Drug-related adverse events. However, sporadic test article effects on body weight and food consumption, as well as changes in pathology and organ weight parameters, were observed; these findings were not considered to be adverse due to the small magnitude of the effects.
Possible side effects from the Activator Drug are currently unknown, since no toxicities have been observed in 28-day toxicology studies. No cardiovascular, respiratory, or neurobehavioral effects have been observed in safety pharmacology studies. Complete safety information regarding the Activator Drug will be provided in the Investigator's Brochure, and complete safety and nonclinical pharmacokinetics of the Activator Drug have been provided to the FDA in the investigational new drug (IND) application. The safety, tolerance, and pharmacokinetics of the Activator Drug alone will be tested in healthy male and female volunteers in a Phase Ia trial prior to initiation of the proposed Phase Ib trial; these results will be incorporated into the final protocol and the Investigator's Brochure.

Regarding efficacy of the Activator Drug, the investigators offered data showing that administration of the Activator Drug did cure tumors in mice. Surviving animals in which tumors were eradicated by this therapy continued to survive and remained healthy for at least 50 days following Activator Drug administration.

The safety of reinjecting participants already exposed to Ad vectors includes a clinical study using three repeat administrations of Ad-transduced DCs for constitutive expression of hIL-12; no adverse effects were observed. Data on systematic comparison of single injection vs. repeat injections of hIL-12-transduced DCs are not available. Intrexon Corporation is currently conducting preclinical studies on reinjection experiments in mouse B16 melanoma models.

Approximately 50 percent of participants will be chosen for biopsy at visit 4, and the remaining participants will be biopsied at visit 8 during the inpatient dosing phase. This is being done to ensure that available tissue will remain from most of the participants at the day 14 biopsy and the month 1 biopsy. Participants will be chosen randomly for biopsy on visits 4 and 8.

The appropriate section of the protocol regarding dose-limiting toxicity (DLT) was rewritten for clarity. If DLT is determined at a given dose level, the next group of three participants will be administered the same dose level of Activator Drug. If DLTs are observed in one or more participants in the additional dose group, dose escalation will be discontinued, and the next lower dose will be considered the MTD; otherwise, dose escalation will resume until the MTD is reached or to the maximal dose, whichever occurs first.

The survival of the Ad-transduced DCs in the tumor microenvironment depends on the expression of the IL-12 transgene. The timing of IL-12 expression is also important for DC survival. If hIL-12 is induced in the Ad-transduced DCs at or after 48 hours after DC injection or if the DCs are not provided IL-12 support, they seldom survive in the tumor microenvironment.

Regarding the potential effects of PAF receptor blockade, PAF receptor antagonists are known to have anti-inflammatory and related beneficial effects. In the preclinical studies, the investigators observed only the competitive binding of the Activator Drug on the PAF receptor in a radioligand binding assay at much higher concentrations than those used for induction of IL-12 expression from the Ad-transduced DCs.

Possible side effects from the injection of the DCs are lymphopenia, fever, and malaise. Toxicities associated with intratumoral delivery of hIL-12 and intratumoral delivery of Ad vectors expressing hIL-12 are generally rare; the most common side effects are pain at the injection site, fever, malaise, and chills. Individuals undergoing leukapheresis may experience AEs from this procedure, which occur in 1 percent to 10 percent of people and could cause a decrease in white blood cells, anemia, numbness in fingers and toes, chills, stomach pain, and nausea or vomiting. Rare AEs (less than 1 percent) include seizures and a decrease in blood pressure. The risk of autologous cell therapy is minimal in the absence of genetic modification and when the sterility of the preparation and compatibility of the suspension medium and identity are ensured.

2. Responses to RAC Discussion Questions

Regarding offering retreatment, Dr. Kirkwood clarified that the investigators' intent is to offer a maximum of two retreatments. Day 28 and day 56 would be the timepoints for assessing participants for retreatment—4 weeks and 8 weeks after administration of the Activator Drug. If nonprogression is seen at day 56, then the investigators would consider retreatment. After an additional 56 days, the investigators would reassess response and offer retreatment if needed.

Dr. Kirkwood explained the rationale for including 40 research participants. The primary goal of this trial is safety and tolerability. The choice of 40 participants was made to establish the safety of this approach to a greater confidence before moving to a Phase II trial. The investigators believed they would need 40 participants to produce enough tissue, nodal biopsies, and other samples to establish with reasonable confidence the effect of RTS-regulated IL-12 introduction. The first 12 participants enrolled in the study will determine the safety of the Activator Drug, and the remaining 28 participants will provide additional information on the safety of the MTD of the Activator Drug.

Dr. Kirkwood noted that T regulatory cells will be looked at, and he agreed to add characterization of regulatory T-cell responses.

Although more mouse studies will be conducted and the investigation of murine corollaries will continue, Dr. Kirkwood stated that the investigators will not know the impact on human melanoma until *human* melanoma is investigated. He emphasized that the proposed procedure is likely to be safe, likely to be immunologically and therapeutically interesting, and likely to be of potential benefit to patients.

Dr. Kirkwood reemphasized the investigators' desire to demonstrate this proposed system in which the product can be switched off, a system that has significant future potential. Regarding efficacy of the system, he stated that *in vitro* data suggest that once the Activator Drug has been turned on and the ligand is discontinued through extensive washout, IL-12 transgene expression is eradicated and hIL-12 is secreted. In addition, *in vivo* data in which the agonist is discontinued after 2, 3, or 4 days suggest that slight antitumor effects are belated as a consequence of discontinuation of the ligand.

Dr. Storkus explained that the *in situ* analysis of the adoptively transferred DCs indicated that these DCs persist for 15 to 16 days; although the natural lifespan may only be 5 to 8 days, the lifespan is enhanced by the presence of transgenes.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Preclinical

- The hypothesis underlying the trial is that using the RTS system, including the activator drug, to regulate expression of IL-12 will be safer and more efficacious than constitutive expression of IL-12. Currently, there are no *in vivo*-derived preclinical data to support this hypothesis. Without these data and given the potential risks associated with the Activator Drug, whose safety has not been established, it is difficult to justify moving forward to human trials. Animal data demonstrating that IL-12 expression regulated by the RTS is more efficacious than currently used constitutive expression systems should be obtained prior to moving to human trials. Moreover, an assay to distinguish between IL-12 produced by the transgene and endogenous IL-12 needs to be developed. Without it, the study will not be able to achieve one of its primary aims, which is to characterize an optimal dose at which the Activator Drug can induce transgene expression.

The following additional observations and recommendations were also put forth, but they assume that new animal data will be obtained and analyzed and that those data will provide evidence that this approach has the potential to offer a therapeutic advantage compared with constitutive expression. It is also assumed that the investigators will be able to meet their goal of determining the optimal dose at which the Activator Drug can induce transgene expression. Absent such data, it is difficult to justify the potential additional risk of this novel approach.

Clinical/Trial Design Issues

- Biopsy samples are to be tested for the transgene IL-12 expression by RT-PCR. Although the findings from this assay are important, they should be supplemented with direct PCR testing for Ad vector sequences since it is a more sensitive test of the transduced Ad genome.

- In light of the potential for IL-12 to promote a T-regulatory response, a fuller analysis of T-cell responses should be undertaken.

- All research participants enrolled in the trial are to receive a single intratumoral injection, and certain participants are to be eligible for one to two additional injections. One of the criteria for deciding which participants will be eligible for the additional doses is that the participant's disease is stable or showing "clinical or subjective signs of improvement." This criterion is too vague. Specific indicators of disease stability and improvement should be developed and prioritized. In addition, since it may take 2 to 3 months after the first administration to see a response, it is not clear why a determination about additional doses would be made at 1 month or why efficacy in general would be assessed at such an early point.

- Since the study may have three dose cohorts, the protocol should describe the plan of analysis for each cohort.

- The description in the protocol of the plan for following up on research participants who have experienced an AE should be revised. The plan is described more clearly in the Investigator's Brochure and that description should appear in the protocol. In addition, it would be helpful to add information on the expected number and timing of the followup visits and the type of testing that will be conducted during those visits. This information should also be included in the informed consent document.

- The statistical basis for enrolling 40 research participants should be explained. The analysis should take into account the trial design and study endpoints.

Ethical/Social/Legal Issues

The following changes to the informed consent document should be considered:

- Clarify the retreatment criteria.
- Include a discussion of any applicable State HIV reporting requirements.
- Revise or delete the second sentence in this excerpt: "However there is a possibility that the study drug(s) could work for you. This cannot be guaranteed."
- Given that the University of Pittsburgh Medical Center owns stock in Intrexon Corporation, the potential institutional conflict of interest should be discussed in the informed consent document.

G. Committee Motion 4

Dr. Federoff orally summarized the RAC recommendations, which were refined and finalized after this RAC meeting as listed above in section F. These comments and concerns of the RAC were included in a letter to the investigator and the sponsor. It was moved by Dr. Federoff and seconded by Dr. Ertl that the RAC approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VII. Day 1 Adjournment/Dr. Federoff

Dr. Federoff adjourned Day 1 of the December 2007 RAC meeting at 1:40 p.m. on December 3, 2007.

VIII. Day 2 Call to Order and Opening Remarks/Dr. Vile

Dr. Vile, temporary RAC Chair substituting for Dr. Federoff, opened Day 2 of the December 2007 RAC meeting at 8:00 a.m. on December 4, 2007.

IX. Discussion of Human Gene Transfer Protocol #0710-878: A Pilot Feasibility Study of Oral 5-Fluorocytosine and Genetically Modified Neural Stem Cells Expressing *Escherichia coli* Cytosine Deaminase for Treatment of Recurrent High-Grade Gliomas

Principal Investigators:	Karen S. Aboody, M.D., City of Hope National Medical Center, and Jana Portnow, M.D., City of Hope National Medical Center
Additional Presenters:	Behnam Badie, M.D., City of Hope National Medical Center; Michael E. Barish, Ph.D., City of Hope National Medical Center; Carlotta A. Glackin, Ph.D., City of Hope National Medical Center; Mary Danks, Ph.D., City of Hope National Medical Center; and Joseph Najbauer, Ph.D., City of Hope National Medical Center
RAC Reviewers:	Drs. Grant, Somia, and Vile
Ad hoc Reviewer:	Steven A. Goldman, M.D., Ph.D., University of Rochester *(via teleconference)*

Drs. Fan and Zaia recused themselves from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

Brain cancers are difficult to treat because of their location. Surgical removal of the tumor risks permanent damage to nerve tissue and does not eliminate cancer cells that have migrated throughout the brain. In addition, many cancer drugs often cannot travel past the blood-brain barrier, and the drug dose

must be limited because of the risk of toxicity to the entire body. If concentrated cancer therapeutics could be restricted to the tumor sites, damage to healthy tissues would be minimized.

In preclinical models, neural stem cells (NSCs) have demonstrated the ability to seek out and target invasive cancers, even when injected at a distance from the tumor sites. NSCs may be able act as a vehicle to deliver therapeutic agents, potentially targeting malignant cells that have spread beyond the original tumor site. In several neurological tumor models in animals using modified NSCs, a 70-percent to 90-percent increase in survival time or decrease in tumor burden was observed.

The protocol proposes to assess the safety and feasibility of intracerebral administration of genetically-modified neural stem cells (NSC) in combination with oral 5-fluorocytosine (5-FC) in patients with recurrent high-grade gliomas. The neural stem cells have been modified with a *v-myc* oncogene to create an immortal cell line and tranduced with a replication incompetent retroviral vector that contains the *E. coli* gene for cytosine deaminase (CD). Expression of the CD gene in the presence of 5-FC is expected to bring about the production of a chemotherapeutic agent, 5-flurouracil (5-FU), that works against glioma. The hypothesis is that the modified NSCs will selectively attack the tumor while sparing normal tissues.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of the approach, safety concerns about the use of cells containing the v-*myc* oncogene, and the ability of the cytosine deaminase gene to work as a suicide gene in case of proliferation of the modified NSCs.

Three RAC members and the *ad hoc* reviewer provided written reviews of this proposed pilot feasibility study.

Dr. Grant focused her review on the informed consent document. She suggested moving to a more appropriate location the information about the possibility of participants developing depression and the availability of psychological counseling. In addition, Dr. Grant suggested highlighting the possibility of change in liver function and/or kidney function because of the potential permanent nature of those risks. She requested that both male and female research participants be asked to initial the information about the potential risks to a fetus or unborn child and that that information be highlighted in the informed consent document.

Dr. Somia asked the investigators to provide experimental data to explain (or to speculate about) the enhanced survival of NSCs in the presence of the human glioma cell line and to provide information on studies that indicate whether enhanced survival of NSCs is observed if the glioma in the animal model is of murine origin. Regarding *myc* expression, he wondered whether the expression of *myc* is downregulated only after differentiation and whether the apoptosis observed after NSC implantation is related to *myc* downregulation. Dr. Somia asked the investigators whether they had looked at the nature of the gene close to the integration site regarding whether they are upregulated or disrupted. He requested that the investigators expand on the immunological status of the NSCs and discuss the predicted outcome of the proposed trial if the NSCs give rise to a potent immune reaction.

One of Dr. Vile's primary concerns was the ability of these adoptively transferred NSCs to cause toxicity rather than or in addition to their purported antitumor effect. Another major concern was the possibility that one of the human NSC lines, HB1.F3-CD cells, would form new tumors; he suggested conducting nonhuman primate studies with the human cells to exclude the chance that these cells may become tumorigenic in the immune-privileged sites within which gliomas often exist. Dr. Vile suggested further elucidation of the mechanisms of the downregulation of endogenous *myc*, especially for the purpose of allaying public concern about the intentional implantation of stem cells with presumed proliferative capacity. The investigators stated that HB1.F3-CD cells are not viable after 1 week in the normal brain environment, and they propose to administer the prodrug 4 days after implantation of those cells; Dr. Vile wondered whether the timeframe for prodrug administration might be too late, since the HB1.F3-CD cells

will be beginning to lose viability, and he requested additional data regarding this timing issue. He asked whether attempts had been made by the investigators to identify factors secreted by HB1.F3-CD cells that may propagate tumor growth. Because it is difficult to be sure that no immunological AEs would occur with antigenically mismatched cells implanted into the research participants, Dr. Vile requested a review of the immunogenicity data with this particular cell line after culture and extensive selection for v-*myc* and CD expression.

Dr. Goldman commented that the area of a glioma is not immune privileged, since it is associated with local breakdown of the blood-brain barrier (with extravasation of both cells and protein), and therefore the investigators cannot assume that the NSC grafts will not be rejected. In addition, the clearance of the grafts by the end of week 3 (and likely within the first or second week) raises serious doubt as to the sustainability of the 5-FU effects. Dr. Goldman wondered whether, in the volume of the human brain, the implanted cells would live long enough to migrate out to potentially distant sites of glioma invasion in time to metabolize the prodrug and deliver 5-FU before rejection occurs; he asked whether the investigators had modeled this strategy in animals larger than mice. He noted that injecting v-*myc* immortalized cells runs the risk of replacing one tumor with another and asked whether the investigators had assessed the survival length of immunodeficient or immunosuppressed mice injected with HB1.F3-CD cells. Dr. Goldman also asked whether the investigators had assessed the clearance of HB1.F3-CD cells implanted into immunocompetent mice with and without 5-FU prodrug administration.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Dr. Vile asked whether any of the mouse studies had been done in the presence of dexamethasone.

- Dr. Weber noted that the terminology used to describe the researchers and the participants' doctors was confusing and requested that the investigators use "doctor" to mean the participant's physician and "investigator" to mean the person with whom the participant interacts during this clinical trial.

- Dr. Ertl suggested exclusion of potential participants with any brain inflammation.

- Dr. Albelda emphasized the importance of capturing as much other data as possible in addition to safety and feasibility data. Because persistence and trafficking of the cells are important pieces of information, he requested that the investigators conduct imaging in this trial similar to what was done in the animal trials. Anything that can be learned about gene transfer and where and how far the cells migrate will be critical.

- Dr. Williams asked about the efficiency of the kill of NSCs if they are proliferating rather than quiescent.

- Dr. Wei suggested that the investigators use matched controls because the research participants, by design, will be gravely ill.

D. Investigator Response

1. Written Responses to RAC Reviews

In response to a question about whether the NSCs would cause an adverse immune reaction, the investigators explained that the research participants will be treated with dexamethasone at the time of surgery to reduce inflammatory response, which will then be tapered during the next 10 to 14 days, and they will then remain on low doses for a few weeks. This regimen will act as an immunosuppressant for the first 10 to 14 days following NSC injection. The NSCs do not express class II human leukocyte antigen (HLA) and only low levels of HLA class I and are not expected to mount a significant immune

response in an allogeneic brain transplant, especially within the first 2 weeks, at which time the experimental regimen will be complete.

Although the investigators hope to demonstrate some therapeutic efficacy, this proposed pilot study is focused on demonstrating the safety and feasibility of injecting donor NSCs into the brain. The FDA advised the investigators to administer only one round of NSCs and one round of 5-fluorocytosine (5-FC) treatment. Once safety is demonstrated, subsequent Phase I/II studies would include determination of the therapeutic NSC and 5-FC doses and may include multiple rounds of NSC administration.

Regarding modeling the proposed strategy in animals larger than mice, the investigators stated that larger nonhuman animal studies are neither justified nor ethical, given that such studies would not provide a definitive answer as to how the NSCs will behave and what distance they will migrate to target tumor foci in the allogeneic human brain. However, the investigators are repeating their animal studies by prelabeling the NSCs with iron nanoparticles, which allows high-resolution, *in vivo*, real-time cell tracking. It is hoped that the data generated will result in IND approval for this method of cell tracking in the brain; adding iron nanoparticle labeling in subsequent Phase I/II studies will produce a more accurate assessment of the timing and extent of NSC migration and distribution in the human brain and provide additional relevant information for improving therapeutic efficacy.

The investigators explained that v-*myc* downregulation occurs constitutively and spontaneously and correlates with the typical quiescence of engrafted cells within 24 to 48 hours posttransplantation. During this quiescent state, the NSCs may differentiate into neurons. However, if no pathology is present in the brain, these quiescent NSCs undergo apoptosis. Based on the preclinical data, the NSCs continue to migrate and track down tumor cells for 2 to 5 days, so the investigators expect that the majority of NSCs will survive during this active tumor-tracking period. Furthermore, immunohistochemical data indicate that the anticancer attack by these NSCs can be launched once the 5-FC prodrug is administered.

In response to questions about the possibility of the HB1.F3-CD cells being protumorigenic, the investigators explained that, in numerous preclinical studies using NSCs to deliver therapeutic drugs, the tumor-bearing animals that received no NSCs died much earlier than the animals that received NSCs (but no prodrug). These data suggest that NSCs are unlikely to promote tumor growth but will deliver the 5-FU anticancer drug.

2. Responses to RAC Discussion Questions

In response to whether they have considered experiments with higher animal models, the investigators explained that such additional preclinical experiments would not be justified, considering the 3-month expected additional lifetime of the research participants. Such research would still represent results from a nonhuman animal, which would not provide the answers needed regarding human reactions.

Dr. Aboody agreed to conduct a smaller but similar preclinical study with dexamethasone, in response to several RAC members' concerns that the research participants will undergo concurrent dexamethasone treatment.

Noting that a standard legal representative is part of the standard consent form and thus must sign the informed consent document, Dr. Portnow explained that this clinical trial will include the services of a research study advocate (RSA). After the investigator meets with the participants, each participant will take a test to make sure they comprehend the objective of the study, what their participation involves, and what they may or may not gain from this study.

Dr. Aboody stated that the investigators have preliminary data indicating that the imaging agent does not affect the migration, viability, or toxicity of the NSCs. These tests will be repeated in a therapeutic trial with iron imaging.

Regarding the kill efficiency of the NSCs, Drs. Aboody and Danks responded that 99 percent of the NSCs die off when they are dividing.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Preclinical Issues

- There are questions and concerns about whether using the v-*myc* oncogene to create an immortal cell line could make the NSCs oncogenic. Although the preclinical studies performed to date alleviate the concern somewhat, such studies provide only short-term data and may not adequately predict the risk for human research participants. This risk should be discussed in the protocol as well as in the informed consent document. In addition, since research participants will have undergone radiation of their primary tumor prior to gene transfer, a preclinical model involving tumor irradiation may provide a better assessment of the oncogenic potential of the transplanted NSCs because radiation may alter the cellular environment and provide a different survival advantage for the NSCs. A preclinical study using an irradiated tumor should be considered.

- Since almost all of the research participants are expected to be taking dexamethasone before, during, and after the gene transfer, a preclinical study involving dexamethasone should be considered. Such studies would help determine whether v-*myc* can be properly downregulated in the presence of systemic dexamethasone and whether the NSC tropism to the glioma persists.

- Formal stereological analysis with traditional volumetrics should be carried out to provide more exacting measurements of tumor regression.

- Additional studies that can provide more information on the level of transgene production of 5-FU should be considered (e.g., using quantitative PCR to determine the levels of messenger RNA [mRNA] produced by the CD transgene). Data on the level of 5-FU generated by the transduced NSCs would allow comparisons with established alternative treatments such as 5-FU-secreting microspheres.

- Data indicate that v-*myc* downregulation occurs constitutively and spontaneously and that it correlates with the typical quiescence of engrafted cells within 24 to 48 hours. Since preclinical data show that the NSCs survive for up to 15 days, it would be helpful to determine whether the expression of v-*myc* is involved in the cells' longer survival.

- In addition to consulting the Human Genome Database to determine whether there are any potential oncogenic insertion sites for the retroviral vectors, the Mouse Retrovirus Tagged Cancer Gene Database (http://rtcgd.abcc.ncifcrf.gov/) should be searched for syngeneic loci for potential oncogenic insertion sites in this mouse database.

- To gather additional preclinical data on efficacy, 5-FU kill curves should be examined with primary glioma cells as well as with glioma cell lines since the curves may be different in these two types of cells.

Clinical/Trial Design Issues

- For safety reasons, potential research participants with preexisting T cells having class I HLAs expressed by the transplanted NSCs should be excluded.

- Pretreatment PBMCs should be collected and stored. These cells will enable the toxicity of T-cell responses to be assessed if any SAE occurs.

- Because the NSCs will track to areas of inflammation, potential research participants with chronic or active viral infections of the CNS should be excluded.

- If possible, nanoparticle cell tracking imaging should be conducted to measure neural NSC migration.

- Since data on the level of transgene expression may be essential for determining whether to move forward with further clinical studies, measures should be developed to gather such data.

- The research participants enrolling in this trial have a predicted life expectancy of 3 to 6 months. It may be difficult to distinguish toxicity from the NSC transplant from complications stemming from their underlying disease. A control group in which participants would receive systemic 5-FU without the transplant would help differentiate the toxicities.

Ethical/Social/Legal Issues

The following changes to the informed consent document should be made:

- Revise the benefit section. As currently written (particularly the statement that "this study may or may not make your brain cancer better"), it implies a 50-50 chance of benefit. The investigators should clarify that the protocol is a Phase I safety trial and is not expected to have any direct benefits.

- Use the terms "study doctor," "researchers," and "your physician" consistently. The term "investigator" should be used when referring to the physician-scientist conducting the trial and "your doctor" when referring to the research participant's personal physician.

- Delete the reference to 15 years in the discussion of long-term followup. Since research participants have such a limited lifespan, it is inappropriate to reference long-term followup of that length.

G. Committee Motion 5

Dr. Vile orally summarized the RAC recommendations, which were refined and finalized after this RAC meeting as listed above in section F. These comments and concerns of the RAC were included in a letter to the investigators and the sponsor. It was moved by Dr. Albelda and seconded by Dr. Kodish that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 2 recusals.

X. Discussion of Human Gene Transfer Protocol #0710-880: A Phase I/IIA Study of the Safety and Efficacy of Neuroprogenitor Cells (SB623) in Patients with Stable Ischemic Stroke

Principal Investigators:	Douglas Kondziolka, M.D., University of Pittsburgh, and Lawrence Wechsler, M.D., University of Pittsburgh
Additional Presenter:	Casey C. Case, Ph.D., Sanbio Biotechnology Resource, Inc.
Sponsor:	Sanbio Biotechnology Resource, Inc.
RAC Reviewers:	Drs. Kodish, Shah, and Zaia
Ad hoc Reviewer:	Steven A. Goldman, M.D., Ph.D., University of Rochester *(via teleconference)*

Dr. Kahn recused himself from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Stroke is the third leading cause of death and the most common cause of permanent disability in the United States. Current therapy is limited to rehabilitation therapy to train patients in the management of the neurologic deficits. Three million stroke survivors live with various degrees of neurological impairment, with a high incidence of deficits in sensorimotor function, vision, language, and cognitive ability.

The protocol proposes to evaluate the safety and efficacy of using genetically modified neuroprogenitor cells (SB623) to restore motor function in patients who have had an ischemic stroke. SB623 cells are allogeneic adult human bone marrow stromal cells transiently transfected with a plasmid expressing the human Notch-1 intracellular domain (NICD), a gene involved in neuronal differentiation. The SB623 cells are to be stereotactically implanted into grey or white matter sites adjacent to the infarct region of the brain. MRI will allow precise targeted delivery of the cells to specific areas of the brain where tissue damage has contributed to loss of motor function. SB623 cells may reverse neurodegeneration after implantation by direct replacement of damaged or dead neurons as well as by secretion of growth factors or other substances that provide support for host neurons. Subjects are also to receive the immunosuppressive drug cyclosporine A to prevent cell rejection.

B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of using stem cells transfected with a plasmid expressing the Notch-1 transgene to attempt to regenerate neurons at the site of injury, whether the SB623 product could trigger unexpected biological responses, whether the gene could have oncogenic effects and therefore whether a suicide gene should be included in the protocol design, whether an immune reaction would worsen the stroke-injured tissue, the safety profile of cyclosporine A (CsA) when used as proposed, and whether specific provisions would be needed for informed consent given that prospective participants have neurological impairments that might affect their decisionmaking capacity.

Three RAC members and the *ad hoc* reviewer provided written reviews of this proposed Phase I/IIA trial.

Dr. Kodish asked for specific language about the role of the RSA, whether surrogate consent is acceptable for this study even though it appears that inclusion will be limited to participants who are competent, and whether participants would be reconsented just prior to the intracranial injection, which is shown as occurring 2 weeks after they sign the informed consent document. He also suggested three changes to the informed consent document, which he averred was clear and well written for the intended audience, that would clarify the extent of safety of this procedure, explain the three-cohort study design more appropriately, and reduce unwarranted therapeutic optimism.

Dr. Shah asked the investigators to discuss how necessary and effective cyclosporine is in preventing immune response to allogeneic cells injected into the brain as well as whether such immune response is common in the absence of cyclosporine. He requested additional details about the risks associated with craniotomy in this setting and about the time course of recovery of neurological function after a stroke to determine whether a 2-week observation period is long enough to ensure a stable baseline neurological deficit. Noting that the investigators propose to exclude potential participants with an abnormal electrocardiogram or chest x-ray, Dr. Shah asked for clarification of the type of abnormalities that would result in exclusion and, given this stroke population in which such abnormalities are likely to be common, why such abnormalities should constitute exclusion. He asked the investigators to clarify the durability of this transfection (with a Notch plasmid), how sustained efficacy would be ensured, and how consistency among the different lots of cells would be ensured.

Dr. Zaia asked the investigators to clarify whether all participants would receive aliquots of cells derived from a common lot of SB623 cells or whether multiple lots would be used in this trial; if multiple lots are proposed, he wondered how their characterization for relative potency and their quality would be ensured. Dr. Zaia expressed several concerns about the use of CsA, including its potential to induce hypertension,

nocturnal headaches, vasoconstriction, and inhibition of angiogenesis, and requested that the investigators provide supporting data that CsA is either essential for the desired therapeutic effect or that it reduces the risk of intracerebral inflammation. Additional concerns about the use of CsA included how easy it is to establish stable therapeutic levels of CsA without SAEs, what method would be used to minimize the use of concomitant medications that could affect CsA levels, and whether a run-in period would be useful to establish stable CsA levels prior to the experimental treatment. He requested that the risks of CsA and the need to be monitored for CsA levels—and for concomitant medications affecting CsA—should be explained in the informed consent document. Dr. Zaia noted that the benefit section of the informed consent document should more accurately indicate that benefit will not likely occur from participation in this trial.

Dr. Goldman noted the weak data in support of the premise that bone marrow stromal cells (BMSCs) can give rise to or become neurons or that Notch 1 intracellular domain (NICD) overexpression mediates neuronal differentiation from BMSCs; he requested that the investigators show more credible and rigorous data indicating that the cells are capable of neuronal differentiation *in vivo* as well as *in vitro*. He stated the need for longer term safety assessments in primates to allay concerns about the potential for tumorigenesis in long-surviving recipients of Notch-transduced mesenchymal cells. Dr. Goldman requested more detail regarding the use of CsA in long-term immunosuppression and suggested that the informed consent document should include more detail about the known risks of such long-term use as well as the relative lack of information about the effects of CsA use poststroke. The informed consent document should also include specific recognition of the theoretical risk of tumorigenesis by the implanted NICD-transduced BMSCs. Noting that cortical stroke is associated with a high incidence of poststroke seizure disorders, Dr. Goldman suggested that the investigators consider how the grafts might impact the probability or course of poststroke epileptogenesis and asked whether any data exist from their animal models regarding this issue.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Dr. Weber stated that much additional work is needed on the informed consent document.
- Dr. Ertl requested that the investigators exclude anyone who has preexisting T cells to major histocompatibility complex class 1 HLAs.
- Dr. Ertl asked the investigators to cryopreserve PBMCs before starting the trial and then look for CsA approximately 2 weeks after the final CsA administration.
- Dr. Kodish suggested that the investigators speak with the RSA about situations related to this protocol in which the RSA could assist with the consent process.
- Dr. Wei suggested the possible use of a factorial design with doses divided into two groups—one group of participants that receives CsA and one group that does not.
- Acknowledging that the consent process was scheduled for the appropriate time, directly after potential participants “pass” the inclusion and exclusion criteria, Dr. Kodish noted that a 2-week waiting time until the study procedure (burr-hole craniotomy) would be conducted. He encouraged the investigators to talk with the participants once again before the craniotomy to be sure they still want to proceed and to give participants another exit opportunity.
- Dr. Zaia asked why the investigators decided not to begin with the cell that they might be able to use in the future Phase II study.
- Dr. Zaia asked whether the cyclosporine could defeat the proposed experiment. He explained that, if angiogenesis is necessary to correct the defect that occurs in ischemic stroke, CsA is an

angiogenesis inhibitor. Therefore, it is possible that the investigators are subjecting their experiment to risk by using CsA.

- Dr. Dewhurst suggested that the investigators consider building into the proposal a method of following the cells by labeling them.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding their proposed use of CsA, the investigators explained that cyclosporine has been used in all the pharmacology and primate safety studies of SB623; thus, the favorable outcomes in the pharmacology studies indicate that CsA does not interfere with the effect of the cells. The risks of short-term use of CsA in the proposed population are not regarded as high. No adverse effects were seen in the 6-month primate study that combined CsA with the SB623 cells, indicating no apparent additive toxicity. The role of immunosuppression in cellular implantation for brain disorders remains unclear; however, immunosuppression typically has been used, and cyclosporine has been the most common agent. The effects of CsA on the investigators' cell product have not yet been examined *in vitro*. A rat stroke efficacy experiment was performed in cyclosporine-treated rats, clarifying that the drug does not destroy the beneficial effects of the cells.

The research participants for this proposed study will have a small (14-mm diameter) burr hole placed at surgery. The risks associated with a burr hole and stereotactic cannula placement are low, with an incidence of symptomatic intracerebral hemorrhage at approximately 1 percent. In a prior trial sponsored by the University of Pittsburgh and Stanford University, no research participant developed a hematoma; however, one participant did experience a chronic subdural hematoma at a later date, without neurological deficit. The risk of infection is expected to be less than 2 percent.

The investigators put forth several additional mechanistic hypotheses to explain the beneficial effect of the proposed cell therapy product, since cell replacement alone seemed insufficient to explain the results of the animal efficacy studies. They believe that trophic support of surviving host neurons makes a significant contribution to the biological effects observed, and the cells' neural progenitor-like properties may contribute to their efficacy. In addition, the investigators have found that NICD overexpression in the cells, using their plasmid and their transfection/selection method, creates progenitor cells that, upon stimulation with a cocktail of trophic factors, differentiate into cells that resemble neurons morphologically, stain positively for neuronal markers, express a number of neuronal-associated genes at the level of mRNA and protein, and cause dramatic improvement in movement disorders in a model of ischemic stroke.

Regarding the potential correlation of Notch misregulation with tumorigenesis, the investigators have completed a 1-year study of the athymic nude rat in which cells were implanted in their brains; 6-month histology showed nothing remarkable, and the 1-year histological evaluations are forthcoming. They have also tested the safety of their proposed cell product in cyclosporine-treated cynomolgus monkeys. The in-life phase of this 6-month study has been completed without incident, and the histology from the 1-month and 3-month groups showed nothing remarkable; the investigators are awaiting histological evaluations from the 6-month group.

In response to concerns about how the grafts might impact the probability or course of poststroke epileptogenesis, the investigators stated that they have not encountered any seizures in any of the animal studies. In the two safety studies, surgical intervention and cell implantation were done in the cortex as well as in the subcortical area, without event.

2. Responses to RAC Discussion Questions

In response to concerns about the use of CsA as an immunosuppressive agent, Dr. Kondziolka discussed the investigators' past experience in which 12 patients received a CsA dose for 2 months and 14 patients

received that same dose for 6 months. No lingering or long-term effects were observed in those 26 patients, who were evaluated for up to 2 years following CsA administration.

Noting a few gaps in the protocol regarding measurement of CsA, Dr. Kondziolka agreed to insert additional measurements of CsA levels. As currently written, the protocol includes one measurement at 2 weeks and one at 8 weeks, but Dr. Kondziolka proposed monthly measurements of CsA levels.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Preclinical Issues

- There are standard criteria for defining neuronal phenotype. Since SB623 cells have not been characterized as neuronal cells, one cannot infer that they will act as replacement cells until additional preclinical data are gathered and the cells are shown to be neuronal. The protocol should be modified to acknowledge these points.

- Given the possible tumorigenicity of NICD, it is critical to gather long-term animal data on these grafts, at least up to 6 months postimplantation. The data should include cell cycle indices, specific concentration of donor cells, and NICD gene expression.

- CsA is associated with a number of potential toxicities, including renal toxicity and increased risk of infection and hypertension. Although CsA is used in other neural cell transplant trials, given its side effects, the benefit of using CsA in this trial should be confirmed through additional rat studies. Unless such data are gathered, the protocol and the informed consent document should be revised to acknowledge that there are no preclinical models to support the use of CsA and that its use in this proposed trial is based on precedent derived from other early-phase clinical trials involving transplanted cells for neurological indications.

- If CsA is used, additional preclinical studies should be carried out to elucidate whether it has selective effects on BMSCs and, in particular, on those transduced with the NICD gene.

Clinical/Trial Design Issues

- If CsA is used, close monitoring of research participants, including CsA blood levels, will be critical to the trial's safety.

- If CsA is used, a factorial design should be considered in which some research participants do not receive the drug.

- The grafts may affect the probability or course of poststroke epileptogenesis, and this possibility should be discussed in the protocol and in the informed consent document.

- To provide additional data on the mechanism of action of the transplanted cells, consideration should be given to developing imaging methods to track the cells that are implanted.
- Since the treatment for chronic atrial fibrillation involves anticoagulation to prevent stroke and since these medications would have to be stopped for the research participant to participate, chronic atrial fibrillation should be an exclusion criterion. Patients with other ventricular rhythm

conditions may also need to be excluded. In addition, patients with pacemakers and/or defibrillators who are unable to undergo MRI imaging should also be excluded.

- Potential research participants who have preexisting T cells bearing class I HLAs expressed by the BMSCs transduced with the NICD gene should be excluded to help prevent an immune reaction against the implanted cells.

- Collecting and storing research participants' PBMCs prior to treatment would be prudent because, in an unexplained toxicity, those cells could help elucidate the role of T-cell-mediated immune reactions.

Ethical/Social/Legal Issues

The following changes should be made in the informed consent document:

- If CsA is used in the study, the investigators should add a discussion of (1) the known risks of CsA use, including the risks outlined in the FDA package insert (e.g., hypertension, renal toxicity and infection); (2) the uncertainties about its effects in the poststroke setting; (3) whether the FDA-approved package insert for CsA includes the use of CsA in this setting; and (4) a list of drugs that may interact with CsA.

- The investigators should discuss the risks of the surgical procedures (the halo and burr holes), the CT scan (e.g., radiation exposure), and other procedures.

- The investigators should discuss the main inclusion and exclusion criteria.

- To reaffirm the voluntary nature of the study for research participants who may have developed second thoughts about proceeding with the study between the time the consent form is signed and the surgery, it would be helpful to ask all research participants before the surgery begins to reconfirm their wishes to proceed with the study.

- The informed consent document should clarify whether the University of Pittsburgh's Human Subject Protection Advocate will be involved in this study and, if so, in what capacity. Given the complexity of the protocol, the involvement of an advocate could help enhance research participants' understanding.

G. Committee Motion 6

Dr. Federoff orally summarized the RAC recommendations, which were refined and finalized after this RAC meeting as listed above in section F. These comments and concerns of the RAC were included in a letter to the investigators and the sponsor. The vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XI. Biosafety Working Group Update on Synthetic Biology/Dr. Patterson

Dr. Patterson explained that the Department of Human Services through the NIH has been tasked with examining the biosafety implications of the emerging field of synthetic biology. This mandate is the result of the adoption by the U.S. Government of a recommendation by another NIH Advisory Panel, the National Science Advisory Board on Biosecurity (NSABB) with respect to synthetic genomics.

The overarching charge to the National Science Advisory Board for Biosecurity (NSABB) is to recommend strategies for the efficient and effective oversight of federally funded life sciences research to minimize the possibility that knowledge and technologies emanating from life sciences research will be misused to threaten public health or national security ("dual use" concern), along with consideration of both national

security concerns and the needs of the life sciences research community. The charge to the NSABB regarding synthetic biology is to identify potential biosecurity concerns raised by the synthesis of select agents by assessing the adequacy of the current regulatory and oversight framework and recommending potential strategies to address any biosecurity concerns.

The NSABB recommendations were considered through a trans-Federal policy coordination process led by the White House Homeland Security Council and the Office of Science and Technology Policy. The biosafety guidance acceptable to the U.S. Government was recommended, with the understanding that implementation would be through modification of the *NIH Guidelines* as appropriate. It was also recommended that the DHHS update and revise as appropriate the *NIH Guidelines* and the *Biosafety in Microbiological and Biomedical Laboratories* manual (*BMBL*) manual. In addition, guidance should be developed for investigators and laboratory workers that addresses the unique safety issues related to work with certain synthetic nucleic acids and offers practical and effective options for managing risks to personnel and risks to public health associated with such research.

The RAC Biosafety Working Group has been charged with clarifying the applicability of the *NIH Guidelines* to synthetic biology as well as to "traditional" recombinants and to develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic biology. The RAC Biosafety Working Group is considering how the *NIH Guidelines* applies to synthetic biology by determining to what degree this technology is currently covered and whether the scope of the *NIH Guidelines* needs to be modified to capture synthetic biology. The group is also developing draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic biology.

Once recommendations for revision of the *NIH Guidelines* have been made by the RAC Biosafety Working Group, draft work products will be reviewed and approved by the full RAC, the recommendations will be published in the *Federal Register*, and an opportunity will be provided for public comment. The recommendations ultimately will be conveyed to the NIH Director and DHHS leadership.

[At this point, Dr. Federoff resumed RAC Chair responsibilities.]

XII. Presentation on Use of Immunosuppression with AAV Vectors

Presenters: Katherine A. High, M.D., Howard Hughes Medical Institute/The Children's Hospital of Philadelphia, and Sander van Deventer, M.D., Ph.D., University of Amsterdam/Amsterdam Molecular Therapeutics BV (AMT)

A. Presentation by Dr. High

Dr. High discussed a Phase I/II trial of AAV-mediated liver-directed gene transfer for hemophilia B that was based on data that demonstrated long-term expression of the factor IX (F.IX) gene in the livers of experimental animals. In one participant that received the highest vector dose, F.IX levels were observed to increase from a baseline of 1 percent to 10 percent, but at four weeks post-administration began to decline back to baseline. Similar transaminitis was observed in another participant. At the same time, the research participant developed asymptomatic transaminitis with liver function tests peaking at five weeks and then returning to normal. Subsequent studies indicated that the transaminitis and loss of vector expression were due to immune-mediated destruction of the transduced hepatocytes. The vector expresses only two antigens, F.IX and AAV2 capsid proteins which, while initially present in the transduced hepatocytes, would not be synthesized and would be gradually degraded. Interferon-γ ELISPOT assays indicated a T-cell response to AAV capsid but not F.IX. Pentamer staining was used to identify CD8+ T cells specific to capsid peptides. The expansion and contraction of the T cell population paralleled the rise and fall of transaminases following gene transfer. The CD8+ cells were functional and can specifically lyse HLA matched peptide-loaded target cells.

One approach to the transaminitis problem is to use transient immunosuppression until the capsid peptides are cleared from the transduced cells. She noted that humans have primed memory T cells to

capsid and probably low frequency resting memory CD8 T cells; however, patients with hemophilia have peripheral tolerance to F.IX. Any immunosuppression regimen would need to manage the memory CD8 T cell response until capsid is cleared without breaking tolerance to F.IX. The safety of regimens was tested in non-human primates. Use of mycophenolate mofetil (MMF) and sirolimus did not promote the formation of neutralizing antibodies to F.IX. Based on the available animal and human data, sixteen weeks was estimated as the necessary duration of immunosuppression.

As a result of these findings the protocol was being amended to add an immunosuppressive regimen consisting of MMF and serolimus. In the resumed trial, Dr. High proposed that research participants would receive a lower dose of vector, and serolimus would be added at the time of infusion with MMF started seven days later. Immunosuppression would be continued for eight weeks at which time if F.IX levels were not detectable, immunosuppression would be stopped. If F.IX expression was detected, immunosuppression would continue for 16 weeks and tapered for the following four weeks.

Steps taken to ensure the safety of this regimen include the fact that two of the four members of the DSMB are transplant physicians (renal and cardiac) experienced in the use of anti-T-cell regimens. In addition and at the suggestion of the DSMB, at every site where research participants are managed during the 16-week period while on these agents, a physician experienced in the use of immunosuppressives will provide written documentation of assistance to the hemophilia physician in managing the immunosuppression regimen.

B. Presentation by Dr. van Deventer

Dr. van Deventer discussed a gene transfer clinical trial for lipoprotein lipase (LPL) deficiency. LPL is an enzyme normally synthesized in muscle and adipose tissue that breaks down triglycerides in chylomicron and very low density lipoprotein (VLDL) particles. LPL deficiency is a rare autosomal recessive disorder caused by mutations in the LPL gene and results in type I hypertriglyceridemia. The clinical phenotype includes hypertriglyceridemia, abdominal pain, and acute pancreatitis that is potentially lethal. Current treatment for LPL deficiency is ineffective.

The trials used an AAV1 vector expressing the LPLS447X transgene, a naturally occurring variant truncated at the C terminus by two amino acids which is associated with a beneficial lipid profile and decreased risk of coronary artery disease. A reduction in median triglyceride level was seen in subjects. Analysis of immune responses showed no antibodies observed against the LPL transgene product. However, antibodies were observed against the AAV-1 capsid in the research participants. In addition, capsid-specific T-cell responses were detectable in several research participants 12 weeks after gene transfer. A pattern similar to that seen in the hemophilia B trial was observed in one subject in the higher dose group. Following an initial decrease in triglycerides, there was a rebound in the level coinciding with an increase in creatinine phosphokinase (CPK) levels and the T cell response.

The clinical trial initiated in Quebec, Canada, with AAV vector is a dose-escalating trial that has enrolled 14 participants with LPL deficiency. To date, two participants have been dosed without immune suppression, and four participants have been dosed with the cyclosporine-CellCept® combination. The safety data of these participants have been reviewed by the investigators, Health Canada, and the safety monitoring board, and the investigators have been given clearance to proceed to the next dose. The longest followup is 12 weeks for the combination of immune suppressive drugs and AAV, and no AEs have been observed. The combination of CellCept® + cyclosporine has been used in thousands of renal transplant patients, with excellent long-term followup.

In addition to the human trials, the investigators have initiated a long-term toxicity and biodistribution study in mice, which so far is not showing any toxicities; the biodistribution data will be available at a later date.

Intermediate conclusions from these studies are that muscle-targeted AAV-1 administration may lead to generation of CTLs that seem to be related to a loss of transgene expression, CTLs can be induced in

patients who test negative for both AAV-1 antibody and CTL, CTL epitopes are present in a wide range of AAV serotype capsids, and some individuals in the population have a low frequency of CTLs.

C. RAC Discussion

Dr. van Deventer explained that he and his colleagues have conducted muscle biopsies in all participants at 26 weeks, which show continued expression of therapeutic gene in the muscle that was injected.

Dr. High noted that the human data from these trials will be considerably more useful than what the investigators have been able to ascertain from tissue culture and animal studies, due to the inability to make an animal model that replicates the events in humans.

XIII. Discussion of Human Gene Transfer Protocol #0707-864: An Open-Label Dose-Escalation Study of a Self-Complementary Adeno-Associated Viral Vector (scAAV-2/-8-LPl-hFIXco) for Gene Therapy of Hemophilia B

Principal Investigator:	Arthur W. Nienhuis, M.D., St. Jude Children's Research Hospital
Additional Presenters:	Andrew M. Davidoff, M.D., St. Jude Children's Research Hospital, and Amit C. Nathwani, M.D., Ph.D., University College London (England)
RAC Reviewers:	Dr. Dewhurst, Dr. Ertl, Ms. Shapiro, and Dr. Strome *(via Dr. Federoff)*
Ad hoc Reviewer:	Barry J. Byrne, M.D., Ph.D., University of Florida

Drs. Bartlett and Kahn recused themselves from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

In this open label, dose-escalation, phase I/II study, a single dose of the novel self complementary AAV vector, scAAV2/8-LPl -hFIXco, will be administered into a peripheral vein of adult patients with severe Hemophilia B. Hemophilia B (HB) is an X-linked recessive bleeding disorder that results from a defect in the Factor IX (FIX) gene which encodes a serine protease critical for appropriate fibrin clot formation. Clinically the disease is characterized by frequent spontaneous bleeding, most commonly into such sites as joints and soft tissues, but which can also occur in the brain and potentially be life threatening. Studies in murine models and rhesus macaques have shown that scAAV2/8-LP 1 -hFIXco vector achieved therapeutic levels of FIX at relatively low doses compared to vectors evaluated in previous clinical trials. This study differs from previous HB clinical trials with AAV vectors in three important aspects. Firstly, an AAV8 pseudotyped vector will be used instead of AAV2, primarily because of the substantially lower prevalence of pre-existing immunity to this AAV serotype in humans. The second difference relates to the use of a vector containing a self-complementary genome which, because of its ability to rapidly form stable, transcriptionally active, double stranded linear molecules in target tissues, offers the unique opportunity to mediate efficient therapeutic gene transfer potentially at a low dose of vector. Finally, because the biodistribution of vector predominantly to the liver is the same regardless of the intravascular route of administration, scAAV particles will be administered via a peripheral vein. Two dose levels, 1.5~10" and 4.5~10" vector genomes per kilogram of lean body weight, will be tested. The primary objective of the study is to assess the safety of systemic administration of this vector while the secondary objectives are 1) to determine the dose of vector particles required to achieve stable expression of hFIX at or above *5%* normal; 2) to describe the immune responses to the hFIX transgene product and AAV capsid proteins and 3) to access viral shedding into various body fluids. Recruitment will be limited to adults (greater than or equal to 18 years of age) with a confirmed diagnosis of HB resulting from a missense mutation in the hFIX gene. Patients will be observed for 42 days following vector administration before enrollment of the next patient. Potential side effects include the development of immune hepatitis with destruction of transduced cells which has been observed in a previous trial with a rAAV2 vector. Despite the much less frequent natural infection of humans with serotype AAV8, immunosuppression may be initiated if signs of immune hepatitis develop because of serotype cross reactive, cytotoxic T-lymphocytes.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novel features of the vector construct, the high starting dose, immune response to the AAV capsid experienced by participants in a prior hemophilia protocol, and the proposed strategy to deal with the risk of transaminitis given the risks of immunosuppressive drugs.

Four RAC members and the *ad hoc* reviewer provided written reviews of this proposed Phase I/II trial.

Dr. Dewhurst explained that this protocol is being reviewed in an atmosphere of heightened sensitivity with respect to AAV vectors, and he urged the investigators to take a conservative approach and to carefully reconsider all aspects of their proposed study with a view to maximizing participant safety. Dr. Dewhurst asked about the tropism of AAV-2/-8 vectors for liver in mice and in macaques, the availability of data on integration of self-complementary AAV vectors in primates or mice, and the prevalence of neutralizing antibodies to AAV-8 in humans, specifically the target population for the trial. He asked about the apparently non-linear relationship between vector dose and FIX expression, particularly why human FIX expression levels in the low-dose macaques fell so dramatically while remaining stable in the higher dose animals, and for an update on the ongoing studies in macaques that are being used to determine the final dose level for the human studies. Regarding the proposed, extended immunosuppressive therapy, he asked about the effectiveness in preventing immune attack on AAV-transduced cells *in vivo*, particularly hepatocytes. Noting that initiation of immunosuppressive therapy in participants will be done on a case-by-case basis, following advice from the DSMB, Dr. Dewhurst asked the investigators to explain what criteria the DSMB might be expected to apply in determining the point at which elevated liver function tests require immunosuppressive intervention, how often the DSMB meets and how quickly it could be convened, and what would be the enrollment/continuation strategy if two or more participants were determined to require immunosuppressive therapy. Regarding the risks section of the informed consent document, Dr. Dewhurst advised the investigators to incorporate information about the recent death in the Targeted Genetics Corporation RA trial.

Dr. Ertl expressed concern about the starting dose, suggesting that the investigators consider starting with a more conservative dose of about $2x10^{10}$ vector genomes per kilogram because the novel proposed vector may be up to a hundredfold more effective than the AAV-2 vectors used in previous Phase I trials. Regarding the secondary endpoint of efficacy to be assessed during week 6, she suggested that a longer timespan would allow determination that the vector-transduced cells persist and continue to transcribe the transgene product. The investigators propose to collect PBMCs for T-cell analysis before gene transfer and at weeks 4, 8, and 12, but Dr. Ertl stated that the accelerated uncoating of AAV-8 vectors could cause an accelerated T-cell response with peak T-cell responses expected earlier, so she encouraged the investigators to include an earlier timepoint. She asked the investigators whether animal models have been used to address the issue of vectors that more rapidly express the transgene product, which may favor the induction of transgene product-specific antibody and T-cell responses. In addition, Dr. Ertl advised the investigators to develop procedures to ensure that participants who are treated with immunosuppression—as well as their primary caregivers—are made fully aware of the potential risks of immunosuppression.

Ms. Shapiro focused her review on ethical and legal concerns and the informed consent documents and noted the most serious concern relates to participant safety and risk-benefit ratio, along with the investigators' proposal to initiate immunosuppressive therapy if a participant develops signs of immune-mediated hepatitis. She requested additional information about the DSMB to evaluate the safety risks of this proposed trial. In addition, Ms. Shapiro offered many specific suggestions for enhancements and corrections to the informed consent statements in the Study Stage 1 (eligibility screening evaluations) and Study Stage 2 (vector infusion and followup). These suggested changes related to therapeutic misconception, clarity, and more precise explanations.

Dr. Strome asked the investigators to discuss what new knowledge supports the idea that AAV-8 will be safer and will induce less hepatic toxicity than AAV-2. He observed that the proposed immunosuppressive regimen includes tacrolimus and MMF, both of which render individuals vulnerable to

life-threatening infectious complications and, if used for prolonged periods, a heightened risk of malignancy. Dr. Strome therefore asked what experience the investigators have with treating and monitoring patients with immunosuppression, what the criteria would be for stopping the drugs, how the drugs would be dosed, and what the endpoint of treatment would be.

Regarding the trial design, Dr. Byrne requested further details on the composition and management of the proposed DSMB. Dr. Byrne suggested that the investigators demonstrate the presence of endogenous FIX antigen in participants' plasma to confirm that a participant with a known missense mutation is able to generate sufficient FIX to be detectable by Western blot. He also suggested that the investigators perform a quantitative serum assay for anti-AAV-8 capsid protein. Dr. Byrne suggested that the investigators remove immune suppression from the protocol as a planned study event unless there exists strong preclinical evidence that this approach could lead to clinical benefit.

C. RAC Discussion

During the meeting, the following additional questions, concerns, and issues were raised:

- Ms. Shapiro asked for further information about the at-the-bedside consultants.

- Dr. Albelda expressed his agreement with the plan to begin the protocol without administering immunosuppressants, which should be used only when they are needed.

- Within the informed consent document, Dr. Weber suggested that the investigators change the term "doctor" to "investigator," since its use is not describing a patient-doctor relationship. "Doctor" should be used when referring to the participant's own physician.

- Noting that some of the potential participants will have read about the Targeted Genetics Corporation study, Dr. Dewhurst suggested that it might be wise for the investigators to include a statement not only acknowledging that SAE but also stating that the best scientific evidence is that the vector was not the cause of that outcome. He stated that it might be best to leave to the investigators the decision as to whether and in what form to include this information. Dr. Byrne described another possible method for handling this information: List it in a section of the informed consent document titled "Anything Else You Should Know" and then describe what is known; in this way, the potential participant is actively involved in that discussion and is provided with the available information.

- Dr. Dewhurst asked about the strategy for dealing with a participant who develops transaminitis and is also positive for hepatitis C virus (HCV).

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that, because it has not been possible to generate an animal model of CD8+ T-cell responses to AAV-transduced cells that recapitulates the findings in humans, it is not possible to assess the efficacy of an immunosuppressive regimen in an animal model. However, the safety of such a regimen has been assessed in nonhuman primates in two studies by other investigators. Clinical experience with coadministration of short-term immunosuppression with an AAV vector is currently limited to an ongoing trial of intramuscular injection of an AAV-1 vector expressing lipoprotein lipase.

Regarding the criteria for initiating immunosuppression, the investigators stated that they have not attempted to establish clinical criteria for doing so and will rely on the collective clinical judgment of the DSMB since it is difficult to envision every circumstance and factor that would influence initiation of immunosuppression in individual research participants. Drug treatment may be initiated if there is a real risk of significant hepatitis as indicated by either a rapid, sequential increase in transaminases or if there is a progressive increase in transaminases beyond the 2- to 6-week period observed in the prior clinical

trial, which might suggest impending, significant chronic damage. The investigators will search diligently for other potential causes of transaminitis in individual participants to avoid unwarranted initiation of immunosuppression. The optimal immunosuppressive regimen will be determined with guidance from experts in immunology, transplantation, and hepatology. In addition, the investigators anticipate that a physician with experience in transplantation and immunosuppression will be available to provide drug management assistance to the hematologist responsible for the participant.

In response to questions about the DSMB, the investigators explained that the DSMB will meet via telephone at least quarterly. Each of the individual members has committed to be available by telephone on an emergency basis to consider individual research participants as necessary. The DSMB includes members with experience in immunosuppressive therapy from each of the institutions so that contact with individual participants will be possible.

If two or more participants are determined to require immunosuppressive therapy, the investigators plan to terminate enrollment in this trial and consider amendment of the protocol to include immunosuppression.

Regarding the starting dose in this clinical trial, the investigators stated that they are conducting ongoing dose-finding studies in nonhuman primates (rhesus macaques) to determine whether lower doses are appropriate and the results will guide their actual proposed initial dose submitted for FDA review and potential approval.

The primary endpoint of this trial is safety. Therefore, based on the experience in the AAV-2 trial that any liver toxicity will be evident by 6 weeks, the investigators propose to continue to enroll research participants in the absence of liver toxicity at that timepoint. As an additional safety feature, any concerns that arise in the evaluation of individual participants will be discussed with the DSMB before another participant is given the vector.

The investigators agreed to increase the number of timepoints for collection of PBMCs for T-cell analysis so that samples are collected at weekly intervals for the first 6 weeks.

It is not yet known whether AAV-8 in humans will be safer and will induce less hepatoxicity than AAV-2. Serotype cross-reactivity with a memory T-cell response does not occur in rhesus macaques. Although Dr. High has generated significant data indicating that cross-reactivity may occur in humans, the matter can be resolved only by a carefully conducted clinical trial.

Regarding the death of a participant in the Targeted Genetics Corporation trial, the investigators believe that that tragedy should not influence the assessment of other clinical trials of recombinant AAV vectors unless evidence emerges that the vector was implicated pathogenically in the unfortunate death of the trial participant. To further ensure a safe design for this trial, the investigators will extend the period of time that a given participant will be monitored to 6 weeks before an additional participant is enrolled. In addition, they will review the outcome for each participant with the DSMB prior to enrolling the next participant to ensure careful regulatory oversight.

The investigators agreed to perform a Western blot on individual participants to verify the presence of detectable FIX. ELISA assays are routinely performed to quantitate antibodies to AAV-8 capsid protein, and the investigators will monitor participants postinfusion to determine titer serially. In addition to the *in vitro* neutralizing assay, the investigators also will screen potential participants for neutralizing antibodies by an *in vivo* assay in immunodeficient mice, which receive human plasma followed by injection of recombinant AAV vector.

The investigators plan to exclude individuals who have an active infection with hepatitis B virus (HBV) or HCV or those recently exposed to HBV and HCV who are on antiretroviral therapy.

In rhesus macaques, no evidence of inflammation was observed by elevations in transaminases or IL-6 following intravenous infusion of AAV-8.

2. Responses to RAC Discussion Questions

Dr. Nienhuis explained that the at-the-bedside consultants will be on the clinical staff of each clinical center. They will act as clinical consultants, will be independent of the study, and will have an opportunity to make independent judgments about informed consent issues.

Regarding participants who develop transaminitis along with HCV, Dr. Nienhuis stated that PCR analysis will be conducted to determine whether the vector genome was present, which would potentially contribute to viral reactivation. Appropriate antiviral regimens will be considered. Dr. High confirmed that HCV patients are treated with immunosuppressants if they undergo organ transplantation, so immunosuppressants can be used, although the investigators would prefer not to do so.

Dr. Nienhuis explained the purification process to be used—a group separation followed by an ion exchange column followed by concentration in a second group separation. The investigators do not plan to use a density gradient.

E. Public Comment

Dr. Takefman asked for further clarification about the purification process to be used.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

Clinical/Trial Design Issues

- The novel vector to be used in the protocol, a self complementary AAV8 pseudotyped vector, is designed to bring about more rapid gene expression. Given that the kinetics of the vector are different from vectors used in prior studies, the optimal timing for the collection of peripheral blood mononuclear cell samples for T cell analyses may need to be established to ensure that immune responses can be detected.

- Subjects are to receive immunosuppression if they experience a transaminitis that is determined to be possibly immune mediated. The decision to begin immunosuppressive therapy should not be automatic. Instead, it should be based on a risk-benefit analysis that considers, among other factors, the potential effects of the specific drug or drugs to be administered on the subject's underlying clinical conditions (e.g., viral reactivation).

Ethical/Social/Legal Issues

- The informed consent document should be revised as follows:

    - To differentiate the roles of the research participant's physician and the investigator, do not refer to the investigator as "doctor" or "physician."

    - Specify the length of time that research participants are required to use birth control.
    - In the consent for Phase I, add a discussion of the risks of Phase II so that, in deciding whether to participate, prospective research participants can assess the risks of both phases.

    - Include a reference to the recent death of a research participant in a gene transfer trial for RA. Although the role of the vector in contributing to the research participant's death has not been established, it should be mentioned because an AAV is being used in this study.

- Clarify the potential conflict of interest from patent-related income to help prospective research participants understand and assess the nature of the conflict.

G. Committee Motion 7

Dr. Federoff summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. These comments and concerns of the RAC were included in a letter to the investigator and the sponsor. It was moved by Dr. Federoff and seconded by Ms. Shapiro that the RAC approve these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 2 recusals.

XIV. Day 2 Adjournment

Dr. Federoff adjourned Day 2 of the December 2007 RAC meeting at 5:15 p.m. on December 4, 2007.

XV. Day 3 Call to Order and Opening Remarks/Dr. Dewhurst, Acting Chair, NIH RAC

Dr. Dewhurst, temporary RAC chair substituting for Dr. Federoff, opened Day 3 of the December 2007 RAC meeting at 8:30 a.m. on December 5, 2007.

XVI. Followup on the Consideration of a Proposed Major Action Under *Section III-A-1* of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*: Deliberate Transfer of Chloramphenicol Resistance to *Rickettsia conorii* and *Rickettsia typhi*

Presenter:	Dr. Corrigan-Curay
Ad hoc experts:	David W. Hackstadt, Ph.D., NIAID, NIH; Didier Raoult, M.D., Ph.D., World Health Organization; and Daniel J. Sexton, M.D., Duke University
Discussants:	Abdu Azad, Ph.D., University of Maryland, and David H. Walker, M.D., The University of Texas Medical Branch at Galveston

Dr. Corrigan-Curay revisited the Major Action considered at the September 2007 RAC meeting. She discussed why Major Actions are needed and the OBA's outreach regarding Major Actions, reviewed the September 2007 discussion, discussed a Major Action analytical framework, and presented the conclusions of the relevant deliberations of the RAC's Biosafety Working Group.

Under *Section III-A* of the *NIH Guidelines*, a Major Action is required for experiments involving the transfer of a drug-resistance trait to a microorganism if the transfer could compromise the treatment of disease. After a 15-year hiatus, the OBA has put forth for RAC review two Major Actions within 1 year. OBA has seen an increase in inquiries about whether an experiment is a Major Action. In response, frequently asked questions will be available on the OBA Web site, and outreach is being conducted to investigators and institutional biosafety committees (IBCs) using the OBA listserv and presentations at scientific meetings.

The initial RAC recommendations from its September 2007 meeting were, based on the available data, a decision to defer to expert opinion on the availability of alternative antibiotics and, at the request of the PIs, *Rickettsia typhi (R. typhi)* and *Rickettsia conorii* (*R. conorii)* were considered together. In September 2007, the RAC recommended that experiments be permitted to proceed using both organisms at biosafety level 3 (BL-3), with additional stipulations. The RAC noted that its assessment at that time was based on the opinions of infectious disease experts in *Rickettsia* that antibiotics for *R. typhi* other than chloramphenicol are suitable—and perhaps preferable—second-line agents after doxycycline.

After presenting the perspectives of several international experts and experts at the Centers for Disease Control and Prevention (CDC), and with conflicting expert opinion, Dr. Corrigan-Curay reported that the Biosafety Working Group's recommendation was that the RAC should reconsider its conclusions of September 2007. This recommendation resulted from a teleconference held on October 5, 2007, to review the published evidence on antibiotics for *R. typhi*. The consensus was that, given the lack of controlled clinical trials, there were insufficient data to conclude that there are alternative second-line antibiotics for *R. typhi* in lieu of chloramphenicol.

The letter from the CDC stated that "tetracycline-class antibiotics, especially doxycycline, are currently considered the drugs of choice for treatment of all rickettsial infections, including murine typhus, and Mediterranean spotted fever." The CDC admitted that there are few clinical circumstances in which chloramphenicol might be recommended. It was the CDC's scientific opinion that there may be other selectable markers, for example rifampin and erythromycin, for producing genetic transformation of *Rickettsia*. Until the evaluations of these alternative markers are conducted and are shown conclusively to be inadequate methodologically, the CDC letter stated that it was prudent to exclude the use of chloramphenicol as a marker for *Rickettsia*.

On the basis of the Biosafety Working Group's assessment of *R. typhi* and because both experiments were to be considered together, it was recommended that the RAC reconsider both experiments in light of the better understanding of the literature and to incorporate the CDC expert opinion into the RAC's assessment.

Dr. Corrigan-Curay reviewed the framework for a Major Action. Because of the potential public health impact of an experiment in which one of the therapeutic antibiotics is being removed, in-depth review is warranted, along with expert consultation and public discussion. The review process for these proposals includes RAC Biosafety Working Group review, consultation with experts, discussion in a public RAC meeting, final RAC recommendations, and review and decision by the NIH Director. The purpose of the framework presented is to provide an evaluation of the potential public health impact of these types of experiments, assist investigators in their preparation, and provide transparency and consistency in the evaluation process. The framework consists of an assessment of the scientific and public health benefits, the evidence on the availability of alternative markers, the clinical utility of the drug proposed to be used as a resistance marker, any potential risk to the public and to laboratory workers, and proposed mitigation strategies. Throughout this process, expert opinion is consulted.

A. Synopsis of Expert Opinions

Dr. Raoult stated that, although uncommon in the United States, *R. typhi* is common in Southeast Asia and India. He noted that for many years all the relevant experts have been recommending doxycycline first, followed by chloramphenicol as a second-line treatment. It is known that quinolones work well against *R. conorii*; however, what works against murine typhus is currently unknown, and there is an anecdotal report of a closely related organism showing that quinolones might not work at all in human beings. In addition, chloramphenicol is still widely used outside the U.S. as a first-line therapy during fever. Based on the existence of only two compounds that could be used effectively in treating *R. typhi* (doxycycline and chloramphenicol), Dr. Raoult stated his belief that alternative ways of manipulating *Rickettsia* should be found instead of making them resistant to chloramphenicol. He also indicated that, within 2 or 3 years, a sufficient number of patients with *R. typhi* will have been treated with macrolide antibiotics to determine whether macrolides will become standard second-line treatment after doxycycline; however, currently that is not the case.

Dr. Sexton agreed that the spotted fever group is fundamentally different from the typhus group, both clinically and *in vitro*. However, chloramphenicol is not used in modern practice, and therefore, losing chloramphenicol as an option would likely not result in significant public or clinical detriment. Doxycycline is used 99 percent of the time, including in pregnant women and children, and the need to use chloramphenicol, at least in the developed world, is minimal if at all. Dr. Sexton agreed, however, that the RAC should consider *R. conorii* and *R. typhi* separately. In the United States, *R. typhi* is generally associated with relatively mild infections and is almost always treated with doxycycline, and

chloramphenicol is the recommended secondary treatment for *R. typhi* even in the United States, even though supporting data may be relatively weak. However, epidemic *R. typhi*, which occurs in the developing world, is a much more serious illness, and chloramphenicol is an important part of the therapeutic management of that disease.

Dr. Hackstadt noted the existence of a mariner-based transposon with a rifampin selectable marker that, in certain strains of *Rickettsia*, has been an effective selectable marker. He agreed that some difficulties with rifampin exist, but chloramphenicol is not a validated selectable marker either. Dr. Walker stated that the risk of escape from the laboratory is approximately zero, because these organisms do not spread from person to person; even if a laboratory worker were to become infected, that infection would be treated at that point and therefore prevented from spreading.

Dr. Walker disputed the CDC's assertion that vaccines are available and are effective. In addition, he did not believe rifampin or erythromycin could be used as selection markers; that the use of rifampin as a selection marker of resistance is not effective because of rapid spontaneous selection of resistance, and erythromycin has not proved to be a reliable method for genetic manipulation of *Rickettsia*. In addition, the mariner-based transposon is a random gene knockout method, whereas Drs. Walker and Azad seek to conduct site-directed mutagenesis. Dr. Walker quoted from an article on *R. typhi* published in *Antimicrobial Chemotherapy* by Drs. Maurin and Raoult that states, "Chloramphenicol displays only poor *Rickettsia* static activity and should not be considered a useful alternative, especially when antibiotic administration has been delayed or in severely ill patients."

Dr. Azad disagreed with Dr. Hackstadt's statement about other means of genetic manipulation of *Rickettsia*. In work with rifampin and erythromycin, the point mutation occurs so frequently that it would delay Dr. Walker's and Dr. Azad's work up to 10 years compared with what researchers could do with chloramphenicol in a short period of time. To date, colleagues have shown that chloramphenicol transformation works well and can be used effectively in other nonpathogenic *Rickettsia*.

Dr. Walker explained why he and Dr. Azad want to conduct simultaneous experiments with both *R. conorii* and *R. typhi*: These are the two best animal models in which to test virulence—one mouse model of dose-dependent mortality that resembles Rocky Mountain spotted fever for *R. conorii* and another mouse model of epidemic mouse-borne typhus for *R. typhi*. Their intention is to prove the related scientific principles by doing one experiment and confirming the results by conducting a complementary experiment, using both of these mouse models to achieve the scientific proof they seek.

Regarding the BL-3 laboratory, Dr. Walker noted that, since moving into his current facility 12 years ago, more than 50 certified trained scientists have worked with *Rickettsia* without a single laboratory infection. No one who has antibiotic sensitivity to tetracyclines or is pregnant will be exposed to these bacteria. It has been reported that a single dose of doxycycline is therapeutic for murine typhus, so even if a person is allergic to doxycycline, that allergy will not be known until after the person has been treated and cured. In the 34 years Dr. Walker has worked with *Rickettsia* in BL-3 labs, human-to-human transmission has not occurred, and *Rickettsia* has not escaped; with the improved procedures currently in place, *Rickettsia* will not escape.

Dr. Walker pointed out that a recommendation by the RAC to separate the two experiments and to wait to approve work on *R. typhi* until a proof-of-concept study has been completed would deny his esteemed colleague Dr. Azad, one of the top scientists in the field of rickettsiology, the opportunity to conduct his research.

B. RAC Discussion

Extensive discussion occurred among RAC members and with the two affected investigators, Drs. Azad and Walker.

Dr. Dewhurst reported that Dr. Wood provided the RAC with written comments indicating that he believes chloramphenicol to be a far superior selectable marker to rifampin or erythromycin, based on Dr. Wood's reading of the literature and on his experience.

Noting that chloramphenicol is still used as a second-line drug in these diseases, Dr. Williams stated that public health issues should be paramount. Several RAC members agreed with Dr. Williams' assessment. Dr. Grant added that the decision to separate the two types of *Rickettsia* is essential and that she would be comfortable with going ahead with *R. conorii* but not with *R. typhi*.

Dr. Kirchhoff enumerated the key issues, which included containment, alternative antibiotics, and the unlikely possibility of a public health problem related to acquiring resistance to multiple antibiotics.

Dr. Corrigan-Curay clarified the ability of the NIH to restrict where *Rickettsia* could be transferred. However, although the NIH can set containment rules and rules about how experiments can be conducted, it is not clear under what authority within the *NIH Guidelines* transfer rules could be enforced. Although the NIH can make recommendations, those recommendations likely would have to be carried out at the local level through IBCs.

Dr. Rosenberg asked about the rationale for starting with both organisms simultaneously.

Even if a break in security occurs, Dr. Williams noted that the chance of a public health issue occurring seems, based on the expert testimony, to be quite low. Low probability of occurrence multiplied by low probability of a public health problem results in an extremely low probability that a real problem would occur.

Dr. Dewhurst stated that, if the intended scenario that Dr. Azad has already outlined for *R. typhi* proves to be effective for *R. conorii*—if it is possible to select agents effectively *in vitro* using greatly subtherapeutic levels of chloramphenicol—then he would reconsider the experimental proscription. Several other RAC members agreed that data produced from the *R. conorii* experiment would constitute proof of concept such that research could proceed with with *R. typhi*.

C. Stipulations from the September 2007 RAC Meeting

Dr. Corrigan-Curay stated that the stipulations for approval from the September 2007 RAC meeting would still apply, as would the agreement that the authority to create chloramphenicol-resistant *Rickettsia* be limited to Drs. Walker and Azad. The wording is as follows:

Stipulations:

- The discussion and development of recommendations put forward are founded on the opinions of infectious disease experts in *Rickettsia* that antibiotics other than chloramphenicol are suitable and preferable as second-line treatments after doxycycline.

- With regard to the containment of experiments in the laboratories of Drs. Walker and Azad, all research should use BL-3 practices. Access should be restricted to well-trained personnel, and a standard initial and ongoing training procedure should ensure that these personnel are properly trained.

- Backup power for the BL-3 facility should be used to ensure that security remains in place.

- Genetic barcoding should be employed, as described by Dr. Azad.

Health surveillance program:

- Baseline rickettsial titers should be taken from all laboratory workers, and baseline blood samples should be taken from all laboratory workers and then stored.

- Individuals who are allergic to doxycycline should be excluded from working on this research.

- A medical card to be carried by all laboratory workers would identify the organism to which the lab worker has been exposed, list the key personnel responsible for diagnosis and treatment, and list the CDC telephone number and a 24-hour contact number for the PI.

- A detailed standard operating procedure in case of lab exposure should be outlined, including key personnel who are tasked with diagnosing and treating such exposure and the steps an exposed lab worker should follow when key personnel are not onsite.

The authority to create chloramphenicol-resistant *Rickettsia* is limited to Drs. Walker and Azad. Any work with these organisms must be carried out at the BL-3 level, with the same stipulations outlined above, and must utilize the security measures currently used in these investigators' laboratories should these organisms be transferred to another investigator.

D. Committee Motion 8

In two separate votes, phrased slightly differently, the RAC members voted 6 to 5 (preliminary vote) and then 6 to 5 (final vote, moved by Dr. Kirchhoff and seconded by Dr. Weber) for the RAC to see the data resulting from the research with *R. conorii* before approving the same experiments with *R. typhi*. This vote will be transmitted in a letter to Dr. Elias Zerhouni, NIH Director, as the recommendation of the RAC; Dr. Zerhouni will make the final decision.

XVII. Closing Remarks and Adjournment/Dr. Dewhurst

Dr. Dewhurst thanked the RAC members and the OBA staff and adjourned the meeting at 10:45 a.m. on December 5, 2007.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, actions are not considered final until approved by the NIH Director.]

_____
Jacqueline Corrigan-Curay, J.D., M.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

These Minutes will be formally considered by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

_____
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Attachment I
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Attachment III
Abbreviations and Acronyms

5-FC	5-fluorocytosine
5-FU	5-fluorouracil
AAV	adeno-associated virus
AAV-2	adeno-associated virus serotype 2
Ad	adenoviral, adenovirus
AE	adverse event
BMBL	*Biosafety in Microbiological and Biomedical Laboratories* manual
BMSC	bone marrow stromal cell
BSL-3	biosafety level 3
CDC	U.S. Centers for Disease Control and Prevention
CNS	central nervous system
CsA	cyclosporine A
CTL	cytotoxic T lymphocyte
DBS	deep brain stimulation
DC	dendritic cell
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
FDA	Food and Drug Administration, DHHS
FIX	Factor IX
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
GFP	green fluorescent protein
GTSAB	Gene Transfer Safety Assessment Board
hIL-12	human interleukin-12
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HSV	herpes simplex virus
HSV-1	herpes simplex virus type 1
LPL	lipoprotein lipase
MMF	mycophenolate mofetil
MRI	magnetic resonance imaging
MTD	maximal tolerable dose
NHLBI	National Heart, Lung, and Blood Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
NICD	Notch-1 intracellular domain
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NSABB	National Science Advisory Board for Biosecurity
NSC	neural stem cell
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PAF	platelet activating factor
PBMC	peripheral blood mononuclear cell
PD	Parkinson's disease
PET	positron emission tomography
PI	principal investigator
RA	rheumatoid arthritis
rAAV	recombinant adeno-associated virus

RAC	Recombinant DNA Advisory Committee
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RSA	research subject advocate
RTS	RheoSwitch® Therapeutic System
R. typhi	*Rickettsia typhi*
SAE	serious adverse event
scAAV-2/-8-LPI-hFIXco	self-complementary adeno-associated viral vector
STN	subthalamic nucleus
TNF	tumor necrosis factor
UPDRS	Unified Parkinson's Disease Rating Scale
WPRE	woodchuck hepatitis posttranscriptional regulatory element