
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 1-2, 2009

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

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[Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <http://oba.od.nih.gov/oba/index.html>.]

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting¹**

December 1-2, 2009

The Recombinant DNA Advisory Committee (RAC) was convened for its 119th meeting at 9:00 a.m. on December 1, 2009, at the Natcher Conference Center, National Institutes of Health (NIH), Bethesda, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. until 5:45 p.m. on December 1 and from 8:30 a.m. until 3:00 p.m. on December 2. The following individuals were present for all or part of the December 2009 RAC meeting.

Committee Members

Jeffrey S. Bartlett, Nationwide Children's Hospital/The Ohio State University
Michael J. Buchmeier, University of California, Irvine (*via teleconference*)
Hildegund C.J. Ertl, The Wistar Institute/University of Pennsylvania
Hung Y. Fan, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University (*via teleconference*)
Yuman Fong, Memorial Sloan-Kettering Cancer Center
Jeffrey P. Kahn, University of Minnesota
Joseph A. Kanabrocki, The University of Chicago
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Margaret Mallino, University Park, Maryland (*via teleconference on Day 1, in person on Day 2*)
Anna C. Mastroianni, University of Washington School of Law
Bernard Roizman, The University of Chicago
Susan R. Ross, University of Pennsylvania
Scott E. Strome, University of Maryland
Lee-Jen Wei, Harvard University
David A. Williams, Children's Hospital Boston/Harvard Medical School (*via teleconference*)
James R. Yankaskas, The University of North Carolina at Chapel Hill
John A. Zaia, City of Hope

Office of Biotechnology Activities (OBA)

Jacqueline Corrigan-Curay, Office of the Director (OD), NIH

Ad Hoc Reviewers, Presenters, and Speakers

Harlan D. Caldwell, Ph.D., Rocky Mountain Laboratory (RML), NIH
Morton J. Cowan, M.D., UCSF Children's Hospital
W. Robb MacLellan, M.D., UCLA School of Medicine
Philippe Leboulch, M.D., University of Paris
Naomi Rosenberg, Ph.D., Tufts University
Michel Sadelain, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center
Nikunj Somia, Ph.D., University of Minnesota, Twin Cities
Nancy P. Hoe, RML, NIH
James M. Schmidt, M.D., M.S., Occupational Medical Service, NIH

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.

Nonvoting Agency Representatives

Daniel M. Takefman, U.S. Food and Drug Administration (FDA), U.S. Department of Health and Human Services (DHHS)

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou, Ph.D.
Laurie Lewallen
Maureen Montgomery
Gene Rosenthal, Ph.D.
Tom Shih, M.D., Ph.D.
Mona Siddiqui, M.D.

Attendees

There were 62 attendees at this 2-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. Call to Order and Opening Remarks

Dr. Federoff, RAC Chair, called the meeting to order at 9:00 a.m. on December 1, 2009. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 16, 2009 (74 FR 58965). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of five protocols, an update on proposed changes to the *NIH Guidelines*, an update on a trial for beta thalassemia and sickle cell disease using a lentiviral vector, a discussion of a potential symposium regarding insertional mutagenesis, and a Major Action on the introduction of tetracycline resistance into *Chlamydia trachomatis*.

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 related questions be addressed to the OBA committee management officer.

II. Minutes of the September 9, 2009, RAC Meeting

RAC Reviewers: Drs. Kanabrocki and Kirchhoff

Dr. Kanabrocki noted that the minutes document was well written and that it accurately reflected the deliberations of that meeting, with a few minor edits. Dr. Kirchhoff had offered a few suggested changes to clarify statements, and he stated that the revised minutes document adequately reflected what had transpired at the meeting.

A. Committee Motion 1

Approval of the September 2009 RAC meeting minutes was moved by Dr. Kirchhoff and seconded by Dr. Kanabrocki. The RAC voted unanimously by voice vote to approve the September 9, 2009, RAC meeting minutes.

III. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Federoff, Strome, Williams, Yankaskas, and Zaia
Presenter: Steven Rosenberg, M.D., Ph.D., National Cancer Institute (NCI), NIH

A. GTSAB Report

Dr. Federoff reported that, of the 17 protocol submissions received by OBA in the past 3 months, 11 protocols were not selected for public review at this RAC meeting and 1 selected for review was deferred to a subsequent RAC meeting at the request of the principal investigator (PI). Of the 11 protocols not selected for public review, 9 are oncology protocols, 1 is for peripheral artery disease, and 1 is for diabetic foot ulcers; vectors used are 6 plasmid, 2 adenovirus, 1 vaccinia virus, 1 retrovirus, and 1 ribonucleic acid (RNA) transfer.

Of trials that have initiated enrollment in the past 3 months, five protocols submitted M-I-C-1 responses to OBA, of which two had been reviewed by the RAC at a previous public meeting. Dr. Federoff provided highlights of responses to the RAC recommendations. These two protocols were:

- #0904-977, Direct CNS Administration of a Replication Deficient Adeno-Associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human CLN2 cDNA to Children with Late Infantile Neuronal Ceroid Lipofuscinosis (*reviewed June 2009*). Research participants will be monitored closely for febrile illness during the first 3 months of dosing. Procedural steps to improve the informed consent process, including the use of research study advocates who are not affiliated with this study or the Department of Genetic Medicine at Cornell Medical College, have been employed.
- #0904-981, A Phase I/II Trial Assessing the Safety and Efficacy of Bilateral Intraputamenal and Intranigral Administration of CERE-120 (Adeno-Associated Virus Serotype 2 [AAV2]-Neurturin [NTN]) in Subjects with Idiopathic Parkinson's Disease. The sponsor, Ceregene, carefully considered RAC recommendations regarding performing polymerase chain reaction (PCR) on autopsy tissue from the previous trial, employing an adaptive design, and extension of the protocol up to 24 months, and will proceed with the protocol as reviewed by the RAC. With respect to the time of followup, given the projected rate of accrual, at the time the last research participant reaches 15 months approximately 50 percent of the participants are expected to have completed 21 months of double-blind followup. Several changes to the informed consent document were made in response to RAC recommendations.

A total of 23 serious adverse events (SAEs) were reviewed by the GTSAB from 15 protocols, including initial and followup reports. Analysis of these events was completed and the GTSAB concluded that only one such event raised issues that needed public discussion. As a result, the GTSAB invited Dr. Rosenberg to provide an update on an SAE discussed in June 2009 regarding Protocol #0804-920, Phase I/II Study of Metastatic Cancer that Expresses HER-2 Using Lymphodepleting Conditioning Followed by Infusion of Anti-HER-2 Gene Engineered Lymphocytes.

B. Presentation by Dr. Rosenberg

Dr. Rosenberg discussed the advantages of cell transfer therapy, described the HER-2 clinical protocol, and discussed the history and clinical course of the first subject dosed on this protocol. The likely cause of the complication that resulted in the death of this research participant was recognition of the very low

levels of HER-2 on normal lung epithelium leading to cytokine release, pulmonary capillary leak, and hypotension.

Three broad categories of immunotherapy are currently in use to treat patients with cancer:

1. Nonspecific stimulation of the immune system. Interleukin-2 (IL-2) is an example. A 15 percent objective response rate in patients with melanoma and kidney cancer has been seen, but that response rate does not appear to occur in patients with other diseases who are treated with IL-2.
2. Cancer vaccines. Tens of thousands of patients have been treated with cancer vaccines, with objective response rates below 5 percent.
3. Cell transfer or adaptive immunotherapy. This is the most effective way to use the immune system to cause cancer regression. In this approach, T-cells that are reactive against the cancer are identified, propagated, and then used to treat that patient's malignancy.

Cell transfer was used in the current protocol. The investigators in this protocol have reviewed three sequential trials that used tumor-infiltrating lymphocytes (TILs). TILs are not gene modified; they are natural autologous cells resected from a patient's tumor. The cells, which are found to be reactive against the tumor, are grown to large numbers and then given back to the cancer patient. While these trials demonstrated that cell transfer therapy can be an effective treatment for patients with metastatic melanoma, the challenge is to extend the approach. Cells with antitumor reactivity can only be grown from melanoma patients about 16 percent of the time, patients who do not have easily resectable lesions cannot be treated, and 40 percent of patients do not generate cells with antitumor reactivity; these challenges led Dr. Rosenberg and his colleagues to explore the use of gene modified cells.

In other protocols, circulating peripheral blood lymphocytes were extracted, transduced with a T-cell receptor (in this case, a recognized melanoma antigen), grown, reinfused 2 weeks later, and followed by infusion of IL-2, which is the requisite growth factor of those cells. Initial results reported approximately 12 percent objective regressions; more recent results using more potent T-cells show an increase up to a 30 percent objective response rate. In some cases, full disease-free survival has resulted for 4 years to date, in research participants who had been refractory to other cancer treatments. There have been no treatment-related deaths in any of the patients treated by Dr. Rosenberg and colleagues.

The next step for these investigators was to explore using this approach for other malignancies. The investigators chose to use HER2 as a target for a T cell receptor because in addition to being over-expressed on many breast cancers it is also expressed on colorectal cancers and other kinds of tumors. HER2 was selected as a target because it is a member of the epidermal growth factor receptor family and because it has a ligand-induced interaction that results in activation of tyrosine kinase intracellular domains that mediate cell growth, differentiation, and survival. Herceptin® (trastuzumab) is a monoclonal antibody that binds to HER2; this drug is in common use for treating patients with breast cancer and other cancers. The investigators genetically modified a lymphocyte so that it carried a chimeric receptor consisting of the antibody domain of HER2 and the intracytoplasmic signaling of a T cell receptor. Safety was based on the history of trastuzumab being given to hundreds of thousands of women as a monoclonal antibody with minimal toxicity; with some cardiac toxicity only seen at very high doses.

The single research participant treated in this protocol was given 1×10^{10} cells, a dose used in previous protocols. The subject was a 39 year old female with metastatic colorectal cancer to the liver and lungs with a fairly typical history for patients who die of colorectal cancer. She had a sigmoid resection in October 2006 for cancer of the colon and had positive lymph nodes and synchronous liver metastases. She received various chemotherapies including a monoclonal antibody against vascular endothelial growth factor. She was considered an appropriate candidate for inclusion in this protocol.

After receiving the cyclophosphamide and fludarabine as per the protocol, she received a cell infusion of 1×10^{10} cells and immediately experienced respiratory distress. Within 15 minutes she was short of breath and was transferred to the intensive care unit. A portable chest x-ray showed early pulmonary edema,

which had not been seen in prior research participants. Progressive problems included hemodynamic instability, kidney shutdown, cardiac arrest (successfully resuscitated), and progressive multi-organ dysfunction; she died five days later, despite intensive support. Autopsy showed widespread metastatic disease in her lungs and liver, and widespread hemorrhage resulting from the resuscitations and the multi-organ problems. The investigators believe that very low levels of HER2 in the lung epithelium led to acute pulmonary toxicity from the cells and cytokine release.

One lesson learned is to start at a 100-fold lower dose; however, the investigators for this protocol do not plan to treat another research participant with this receptor. Of the 145 individuals who had a variety of receptors and were treated prior to this research participant, no deaths occurred, all recovered from the treatment, and the investigators had not anticipated this acute toxicity.

C. RAC and Public Discussion

Questions and comments posed by RAC members as well as public participants included:

- In response to several questions about the possible cause(s) of the death of this subject, Dr. Rosenberg noted that she was quite allergenic. While her measured tryptase level had doubled in the 4 hours after dosing, experts at the NIH with whom Dr. Rosenberg consulted did not believe that was compatible with an anaphylactic reaction.
- Dr. Federoff wondered whether any of the conditioning regimens or any other antecedent therapy could have upregulated the endogenous expression of this antigen selectively within the target organ, which seemed to have been causing her pulmonary compromise and which could have led to her death. While Dr. Rosenberg stated that there have been no reports of such a reaction, it remained a possible explanation.
- Regarding the question whether this subject had metastatic disease to the lungs that was appreciably different or in greater amount than the previous melanoma patients, Dr. Rosenberg responded that, while she had several lung metastases and several liver metastases, her disease burden was about average compared with the tumor burdens of other research participants treated by the investigators.
- Richard Junghans, M.D., Ph.D., from Roger Williams Medical Center, asked whether the investigators had looked at the bowel of the subject since graft-versus-host disease is commonly associated in its severe form with hemorrhagic enteritis. Dr. Rosenberg noted that the bowel was sampled at autopsy and some evidence of hemorrhage in the bowel mucosa was found, as was the case in most other organs. This protocol involved autologous cells, so conventional graft-versus-host disease is not a possibility. However, he acknowledged that, because this patient had been resuscitated three times and had been hypotensive, it was difficult to distinguish 5 days later the path of the cells from the impact of the disease itself.
- Stephen M. Gottschalk, M.D., from Texas Children's Hospital, asked about the starting dose. Dr. Rosenberg stated that the dilemma is to determine a reasonable starting dose that is safe but that has some chance of mediating an antitumor effect; there is only one opportunity to treat these patients that have been through all standard treatments. He noted that the lowest effective dose in mice was found to be 1×10^6 , so if he were to repeat this protocol he would choose a starting dose of 1×10^6 or 1×10^7 .

Dr. Federoff suggested that further discussion of this SAE be scheduled for the June 2010 RAC meeting, at which time several of the investigators will have been able to extract clinically useful information that could be applied investigationaly by other researchers. It was suggested that further discussions of the appropriate starting dose and the use of multiple intracytoplasmic signaling domains be examined.

IV. Discussion of Human Gene Transfer Protocol #0910-1005 entitled: A Safety and Efficacy Study in Subjects with Leber Congenital Amaurosis (LCA) Using Adeno-Associated Virus Vector To Deliver the Gene for Human RPE65 to the Retinal Pigment Epithelium (RPE) [AAV2-hRPE65v2-301]

Principal Investigators: Albert M. Maguire, M.D., Children's Hospital of Philadelphia, and Stephen Russell, M.D., University of Iowa
Additional Presenter: Jean Bennett, M.D., Ph.D., Children's Hospital of Philadelphia, and Kathryn High, M.D., Children's Hospital of Philadelphia
Sponsor: Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia
RAC Reviewers: Drs. Bartlett, Federoff, and Kahn

Drs. Ertl, Kirchhoff, Kodish, Ross, Strome, and Yankaskas recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Leber congenital amaurosis (LCA) is a severe early onset retinal degeneration with diagnosis usually made during the first few months of life in infants who present with severely impaired vision, abnormal eye movements (nystagmus) and abnormal electroretinograms (ERG) indicating decreased retinal function. There is an inevitable progression to total blindness in these individuals due to death of photoreceptor cells. This study will focus on the form of LCA, *LCA2*, caused by mutations in the gene encoding the 65 kDa retinal pigment epithelium (RPE)- specific protein, *RPE65*. Clinical diagnosis is made by function tests of the eye, which can be confirmed by molecular testing to verify the *RPE65* mutation.

Results in relevant small and large animal models using gene augmentation demonstrated restoration of vision in a stable fashion with a high level of safety leading to a Phase 1 clinical trial administering AAV2-hRPE65v2 vector into the subretinal space of twelve adults and children with *LCA2*. The Phase 1 dose escalation study demonstrated that adeno-associated virus (AAV)-mediated delivery of the *RPE65* transgene via subretinal injection, at doses of 1.5E10 vg, 4.8E10 vg, or 1.5E11 vg, was safe with respect to ocular and systemic toxicity in individuals as young as eight years of age. Additionally, the results of the Phase 1 safety trial provide functional and physiologic evidence that *in vivo* gene delivery of the *RPE65* transgene is an effective treatment for vision loss in *LCA2*. At all doses tested, the administration of AAV2-hRPE65v2 resulted in rapid onset of improved vision/retinal function, as evaluated by objective, physiological and subjective, psychophysical measures of vision, in adults and children (ages 8-44) with *LCA2*. The greatest improvement was observed in the youngest individuals, several of whom gained ambulatory vision.

The proposed study is a Phase 3, open-label study of gene transfer by subretinal administration of AAV2-hRPE65v2. Up to twelve subjects, three years of age or older at the time of vector administration will be recruited. As with the Phase 1 study, participants will be screened to confirm that sufficient viable retinal cells remain as the presence of as close to a full complement of cells as possible is most likely to result in therapeutic effect. The initial nine subjects will each receive a unilateral injection of 1.5E11vg AAV2-hRPE65v2 in a volume of 300 μ l. Following establishment of safety and efficacy in the first nine subjects, three additional subjects will be enrolled to evaluate the safety and efficacy of sequential, bilateral administration. These three subjects will receive 1.5E11vg AAV2-hRPE65v2 in a total subretinal volume of 300 μ l in each eye; the individual surgeries for each bilateral administration subject will be performed on separate days no more than one week apart.

The objectives of the study are to determine the safety, tolerability, and efficacy of subretinal administration of AAV2-hRPE65v2 to subjects with Leber congenital amaurosis due to *RPE65* mutations. Efficacy will be measured using pupillary light reflexes (PLR). The primary objective is to determine whether AAV2-hRPE65v2 improves light sensitivity as measured by pupillary light reflexes in adults and children, three years of age or older, as compared to the individual

subject's baseline visits, and the contralateral uninjected eye in the case of unilateral administration. The secondary objective of this study is to determine the safety and tolerability of AAV2-hRPE65v2 in the stated population. If safety and tolerability is demonstrated, further studies may include younger participants, ages six months to three years of age.

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included that, although there is clinical safety data in older children and adults, the proposal will test the administration of the highest tested dose of AAV2-hRPE65v2 in young children along with systemic corticosteroids. Of note is the lack of dose-response with respect to improvement in visual acuity. The risks versus benefits of proceeding with the highest dose in children as young as 3 years was deemed to need discussion; this highest dose was tested in only 3 of the 12 research participants, with the youngest being 11 years old.

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

Dr. Bartlett asked how foreign microorganisms are cleared from the eye, whether this clearance mechanism involves an inflammatory response, and whether the investigators could speculate on how vector might be cleared from the eye in the absence of either an inflammatory response or significant immune response. Because the rationale for the use of systemic corticosteroids is unclear, he asked the investigators to provide additional rationale for steroid use, especially in the proposed pediatric population. Dr. Bartlett requested that the investigators explain the rationale for increasing the dose of vector in this trial, in light of the fact that the Phase I trial showed no significant dose response. He asked for comment on the investigators' decision to increase dose in order to define a maximum tolerated dose (MTD) when a minimum useful dose had already been established, and he asked for comment on the extent to which reducing the age of the study population would significantly lessen phenotypic variation, especially with such a small population available for potential enrollment. He noted that initiation of a Phase III trial without previous definition of MED or MTD, and only a vague idea of target dose, is unusual. Dr. Bartlett also requested that the investigators comment on the potential for additional risk, via increased dose and the concomitant use of corticosteroids, versus the potential for increased scientific and/or clinical understanding that might be obtained from this study that would direct future clinical inquiry.

Dr. Federoff asked the investigators to further explain the choice of a Phase III trial designation and to provide additional detail regarding the statistical plan. He wondered whether hypothalamic-pituitary-adrenal (HPA) axis suppression could occur, perhaps as a function of age, at the proposed doses of corticosteroids and duration of administration. Regarding the nonhuman primate (NHP) experiments, he requested elaboration on the observed perivascular cuffing in two of the NHP eyes that received vector and comment as to whether the potential clinically observable findings might extrapolate to humans. Dr. Federoff asked the investigators to comment further on finding the vector in spleen and liver in preclinical studies, and what those findings might portend for the proposed clinical study. Noting that the investigators had selected a duration of greater than 1 week between injecting two eyes, he requested discussion about the implications with regard to immune responses.

Dr. Kahn stated that this protocol is commendable for its comprehensiveness and careful construction, including the informed consent process and documents. He asked for discussion of the rationale for requiring participants to abstain from unprotected sex for 1 year as stated in the protocol, the informed consent process, the informed consent document, and the response to Appendix M. Dr. Kahn requested that the investigators clarify whether the sponsor would pay the costs of longterm followup visits and, if yes, he suggested that this information be noted in the informed consent document and process.

C. RAC Discussion

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

- Dr. Wei asked whether any research participants in the Phase I/II trial were losing visual acuity instead of the stabilized, improved acuity reported to date.
- Dr. Wei asked how quickly this trial might accrue participants.
- Dr. Wei inquired as to when the investigators would decide whether participants' second eyes would be treated.
- Dr. Zaia asked about the risk of greater deterioration in the undosed eye because of the young age of the participants.
- Dr. Wei asked whether it would be possible to design an effective sham surgery to be delivered to the undosed eye.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding the rationale for the requirement that research participants abstain from unprotected sex for one year, the investigators explained that this limitation was retained from the Phase 1 study. Vector shedding studies from the earlier clinical trial participants determined there was very limited extraocular exposure to vector delivered to the subretinal space. Tear samples were positive in 6 of 12 subjects, at all dose levels tested, for up to 4 days from AAV2-hRPE65v2 administration. Blood samples were positive in two of twelve subjects, both in the high dose cohort, out to Day 3 post-vector administration; however, these positive samples were non-quantitative by Q-PCR detection method. Other AAV clinical studies, involving systemic vector administration at doses approximately 25 to more than 750 times higher than the total dose proposed here, have observed transient detection of vector sequences in the semen of male subjects. Fractionation studies have been performed and the vector is detectable only in the seminal fluid and not in the germ cells; this positive signal was cleared in all subjects by 16 weeks following vector administration. Despite these findings suggesting the risk of transmission is very low, the potential risks of inadvertent germline transmission of recombinant AAV vectors are not known. As such, the investigators of this study have employed a very conservative approach. In light of the variety of findings, the investigators have agreed that it would be reasonable to reduce this length of time to as little as 4 months, with concurrence of FDA reviewers and local ethics committees.

Removal of particulate foreign matter from the eye typically involves mechanical clearance and can also involve a local inflammatory response. Most substances will escape the eye through the trabecular meshwork or the conjunctival lymphatic vasculature; animal experiments have shown that foreign microorganisms were cleared by these natural defenses without significant inflammation. Larger inoculums cause a clinical infection with bacterial organisms, in which case the use of antimicrobial agents is required. With respect to subretinal AAV vector delivery, the investigators believe the majority of vector particles transduce the retinal pigment epithelial cells; limited vector could escape to the vitreous, where it would be expected to be cleared, or might cross the RPE cell barrier and gain access to the choroidal vasculature. Regardless of the fate of the AAV2 capsids, there have been no adverse events deemed related to AAV2-hRPE65v2 in the 12 Phase I clinical trial participants, no adverse events deemed related to the AAV2 vectors in the other two Phase I clinical trials, and no ill effects in one of the originally treated dogs, which has been followed since receiving a subretinal administration of AAV2-hRPE65 vector more than nine years ago.

The frequency of clinical use of systemic corticosteroids for eye surgery is inversely proportional to the age of patients. Children typically exhibit more inflammation following eye surgery than adults, and it is often more difficult to administer the standard postoperative topical steroid drops/ointment in children. The investigators believed they could safely lower the maximum dose of prednisone during the Phase I study because a research participant whose body weight corresponds to the 40 mg/day dose would likely be old enough to adjust to the topical steroids and would experience less of a postoperative inflammation reaction. There is no set age for discontinuing the use of systemic corticosteroids; the decision is often predicated on other complicating conditions that may indicate or contraindicate the use of prednisone. The maximum doses (40 mg/kg/day and 20 mg/kg/day) and duration (17 days) of prednisone proposed in this Phase III clinical trial, and previously used in the Phase I clinical trial, are used routinely to prevent postoperative complications in eye surgery, and are comparable to those used for other common

conditions, such as asthma and eczema. The investigators have not observed, and do not expect, longterm sequelae from the doses and regimens proposed in this protocol.

In animals in the preclinical canine and nonhuman primate studies, signs of an inflammatory response were seen approximately 3 months after vector administration. However, these animals did not receive systemic corticosteroids, were given higher doses per eye than the dose proposed in this Phase III clinical trial and previously tested in the Phase I clinical trial, and received AAV vectors encoding human RPE65 protein rather than species-specific RPE65 protein. No inflammation was observed in the Phase I clinical trial participants; however, these individuals all received either the same or a higher dose of systemic corticosteroids as proposed in the Phase III trial. In summary, an inflammatory response was observed in animals receiving vector but not in humans; this difference may be accounted for by the use of systemic corticosteroids in the humans.

Regarding vector dose, the investigators do not believe that the increase in total dose, as proposed for this Phase III trial, represents a significant increase in risk because no adverse events observed to date have been deemed related to AAV2-hRPE65v2. Nonclinical studies involving bilateral administration in dogs and nonhuman primates also support the safety of this dose and higher doses, with respect to both ocular and systemic toxicity. Recombinant AAV vectors have been delivered to hundreds of research participants, at doses several hundredfold greater than the proposed dose, using local, systemic, and central nervous system delivery, resulting in very few vector-related adverse events. The volume used for the Phase I high-dose cohort and proposed for the Phase III clinical trial targets a larger portion of the retina, twice that of the Phase I low and middle doses. Therefore, the selected dose-volume combination, previously tested as the high dose in the Phase I clinical trial, provides a greater likelihood of direct benefit to the participants even in the absence of any dose-response effect.

Two study groups are proposed for this Phase III clinical trial, with a primary objective of showing clinically significant symptomatic improvement in visual function. The first group (n=9) will receive unilateral administration of the experimental vector; comparisons will be made to the unmanipulated, contralateral control eye and to the individual participant's baseline retinal/visual function tests. The second group (n=3) will receive bilateral, sequential administration of the experimental vector in each eye; comparisons will be made to the individual participant's baseline retinal/visual function tests. This study design allows for direct comparison between the injected and uninjected eyes of the first nine participants, as well as to the baseline evaluation, in an effort to demonstrate efficacy convincingly. The second group may allow for the eventual approved product to be delivered to both eyes of LCA2 patients if bilateral administration is found to be safe; ideally, treatments for retinal degenerative conditions would involve one-time, simultaneous, bilateral administration of a therapeutic agent shortly after confirmed diagnosis. The investigators acknowledged that the potential exists for additional risk with bilateral administration for the latter three participants; however, this additional risk is balanced by the increased prospect for benefit, especially given the possibility of establishing binocular visual function.

Further studies to increase scientific and/or clinical understanding, such as studies in younger participants and studies exploring re-administration of earlier unilateral study participants, will be explored after completion of the Phase III study if the data is supportive. These studies might be conducted as postmarketing studies if the Phase III clinical trial supports submission of a Biologics License Application.

The Sponsor has been granted orphan-drug designation for AAV2-hRPE65v2 for treatment of LCA2. Due to the extreme rarity of this condition, with an overall prevalence of approximately 1:81,000 for all forms of LCA, and the fact that there is no treatment yet available for LCA, the investigators believe that this trial design approach will best serve the small patient population.

To assess efficacy for the nine participants with unilateral injections, improvement will constitute development of an afferent pupillary defect or an increased sensitivity to light as evidenced by higher amplitude of constriction and/or higher velocity of constriction as compared to the contralateral, uninjected eye. Improvement will be defined as amplitude/velocity of constriction at least 20 percent higher than that of the uninjected eye. Pupillary responses will be measured at baseline, Day 30, and Day 90, and the threshold light intensity at which the participant shows a response will be identified for each timepoint.

For the three participants with bilateral injections, improvement will be defined as an increased sensitivity to light as evidenced by higher velocity of constriction and/or higher amplitude of constriction as compared to baseline. A 20 percent increase in amplitude and/or velocity over baseline in each eye will be defined as a bilateral success.

An unexpected finding of the nonhuman primate toxicology study was that samples from spleen, and to a lesser extent liver, from the majority of animals were mildly positive for vector sequences. The strength of the signal was related to dose, with weak or no signal identified at the lower dose; these levels were several orders of magnitude less than those found in ocular tissue. The investigators speculated that test article sequences in the spleen and liver might be due to migration of immune cells, that could have engulfed vector or cells exposed to vector, to these organs. The animals showed no significant humoral antibody response or cell-mediated immunological responses to the test agent, further supporting the hypothesis that vector or transduced cells may have been engulfed by immune cells that subsequently migrated to the spleen and liver, rather than that vector spread to these organs by moving through the vascular system. Nonhuman primates in this study were not given systemic corticosteroids; the use of these lympholytic agents in human clinical trial participants might prevent these findings.

While it is possible that a limited, transient HPA axis suppression could occur, it is likely for a few days to few weeks at most. It is also possible that age may play a role in this phenomenon, though colleagues in the Endocrinology Division at CHOP are not aware of any data suggesting that young children are at more risk or have longer suppression than older children or adults. A longer duration of high dose prednisone could result in some degree of suppression for as much as a few months, but this is not expected with a seventeen day regimen. Clinical trial participants will be closely monitored in the post-operative period, and the investigators are prepared to adjust/extend the corticosteroid taper if clinically indicated.

Perivascular cuffing is a sign of inflammation and results from presence of inflammatory cell(s) on the periphery of a blood vessel. In two NHP eyes injected with AAV2-hRPE65v2, there was mild perivascular cuffing directed toward the vitreous and some inflammatory cells in the vitreous near the optic disc. Importantly, this finding was rare and detected in only a few isolated spots with microscopic analysis of tissue sections; perivascular cuffing was not observed by ophthalmoscopy. The finding of isolated foci of inflammatory cells is likely due to exposure to the test article and/or the surgical procedure to administer the vector to the subretinal space. Inflammatory cells were only present in focal spots whereas the test article exposed a much broader area of tissue. The findings are not adverse as they were asymptomatic, were only visible with histopathologic analysis, and did not cause significant reduction in retinal/visual function. Participants in the Phase 1 clinical trial injected with doses up to 1.5×10^{11} vg have shown no evidence of focal inflammation, and no loss of restored visual function, since the first high dose subject was injected more than nine months ago.

The investigators clarified that the surgical subretinal administrations of AAV2-hRPE65v2 for sequential, bilateral administration must occur on separate days within seven days of each other. This restriction was included in an effort to preclude possible immune responses, such as a humoral response which could prevent vector transduction in the contralateral eye or a cell-mediated cytotoxic response which could theoretically result in immune-mediated destruction of vector transduced cells.

2. Responses to RAC Discussion Questions

Dr. High reported that the investigators would be willing to reduce from 1 year to 4 months the requirement that participants use barrier contraception after dosing. The data for this timeframe is based on a rabbit model in which duration of vector positivity in seminal fluid was dose dependent as well as two clinical trials in which the longest duration of positivity in males, who had received vector infusion intravenously or into the hepatic artery at a dose 750 times higher than the proposed dose, was about 16 weeks. She acknowledged that there are no data to address introduction of AAV into the noncycling oocytes.

Noting that the investigators could inject some of the vector into either nonhuman primates or LCA affected dogs and could administer steroids at the same time, Dr. High opined that she would not advocate altering the protocol now at a time when all the participants seem to tolerate it well and the results are good. The steroid use planned for this protocol will produce a relatively mild level of immunosuppression.

Dr. Maguire explained that the oldest research participants in the Phase I/II trial did respond to the injections – they went from a light-perception-only vision to the ability to see hand motion or one or two letters on an eye chart. While the physiologic responses were impressive, not much changed for these individuals behaviorally and functionally. As more information is gathered in the younger age groups, the investigators found that near-normal responses occur in their visual function and behavior. In this pediatric disease, the investigators are trying to work with an age group in which it would be possible to restore near-normal function to avoid patients developing “blind behaviors” as well as maximizing other aspects of visual function such as binocularity. Focusing on younger research participants will result in the advantage of having more useful vision.

The investigators plan to conduct a substudy using functional magnetic resonance imaging to check for possible loss of synaptic connections as a result of vision loss. This will be in addition to the traditional subjective studies of visual function.

Dr. Bennett explained that visual acuity has started to decrease in the contralateral untreated eye of participants in the Phase I/II trial, which is likely due to continued disease progression in that eye. Acuity has not decreased in the participants who have had initial increases in visual acuity. In consultation with the FDA, the investigators have to treat the originally undosed eye as a postmarketing study.

Dr. Maguire stated that accrual to this Phase III trial would occur quickly, as the patient community is already aware of the pending status of this trial.

From a physiologic point of view, Dr. Maguire explained that this disease has a stereotypical rate of cell loss in the untreated condition – the percentage of remaining cells that are lost due to the disease is fairly constant. Regarding the risk to the other eye in terms of the possibility of developing amblyopia, he explained that amblyopia is treatable – a regimen of either atropine penalization or wearing a patch for a few hours a day would be sufficient to compensate for amblyopia in the untreated eye, were that to occur.

Regarding the possibility of sham surgery, Dr. Maguire noted that it would require general anesthesia, which is an increase over minimal risk. In addition, he noted that a good sham could not be fashioned because part of the procedure is putting an air bubble in the eye, and it would be unlikely that a person would not notice that they do not have an air bubble growing around their eye for a few days after the procedure.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC’s indepth review and public discussion:

Clinical/Trial Design Issues

- Research participants enrolled in the Phase I study had different gene mutations and were at varying stages in their disease. While it appears that the potential benefit of the intervention is greatest in young patients, it would be helpful to collect data that could shed further light on which patient population would most benefit from the intervention and the factors that would be predictive of a clinically meaningful response.

- Light sensitivity, as measured by the pupillary light reflex (PLR), is being used as the primary endpoint in this trial because it is an objective measure that can be used in young children as well as adults. While there is no evidence that age affects the magnitude of the PLR response, because it is the primary study endpoint, additional data should be gathered to demonstrate that the PLR response is not affected by the age.

Ethical/Social/Legal Issues

- Since the protocol may enroll participants with reproductive capacity, careful consideration should be given to the length of time participants should abstain from unprotected sex. The current recommendation of 1 year from dosing will significantly limit reproductive choices and may be longer than necessary, at least for male participants; data is not available for assessing the risk for female participants. Data from a study that administered an AAV vector systemically at doses 25 to 750 times greater than what will be used in this protocol detected the vector in the seminal fluid but it was cleared in all participants by 16 weeks. The optimum time period based on this data should be considered.

G. Committee Motion 2

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Bartlett and seconded by Dr. Buchmeier that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 6 recusals.

V. Discussion of Human Gene Transfer Protocol #0908-995 entitled: A Phase I Open-Label, Dose-Escalation Trial of JX-594 (Thymidine Kinase-Inactivated Vaccinia Virus Plus GM-CSF) Administered by Intratumoral Injection in Pediatric Patients with Unresectable Refractory Solid Tumors

Principal Investigator: Timothy Cripe, M.D., Cincinnati Children's Hospital Medical Center (CCHMC)
Additional Presenter: David H. Kim, M.D., Jennerex, Inc.
Sponsor: Jennerex, Inc.
RAC Reviewers: Drs. Buchmeier, Kodish, and Zaia

Dr. Williams recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

Oncolytic, replication-selective viruses hold promise as novel anti-cancer therapeutics that can destroy tumors by multiple mechanisms. These viruses are engineered to replicate and spread efficiently in cancer tissues but not normal tissues. Cancer cells can be destroyed by apoptosis, virus-induced cytopathic effects, induction of cancer-specific cytotoxic T-lymphocytes and induction of anti-tumoral and anti-vascular cytokines. Vaccinia viruses have several distinct advantages as oncolytic therapeutics. These include rapid cancer cell killing and spread; large capacity for therapeutic and/or inactivating transgene insertion; extensive human experience with the virus in vaccine recipients and in melanoma patients receiving intratumoral injections.

The thymidine kinase (TK)-inactivated vaccinia virus JX-594, expressing GM-CSF (granulocyte macrophage colony stimulation factor) and humanized Escherichia coli β -galactosidase, holds promise as a selective oncolytic virus for the treatment of cancer. The rationale for the construction of this virus is as follows. The TK gene was inactivated because TK- vaccinia viruses have been shown to replicate efficiently in cells with high nucleotide pools such as proliferating cells and cancer cells. TK- vaccinia viruses have also been hypothesized to be selective in tumor cells. GM-CSF was chosen because it was

the most potent stimulator of systemic anti-tumor immunity among several tested, probably due to its unique ability to promote differentiation of hematopoietic precursors into dendritic cells. These cells act as professional antigen-presenting cells and may take up and present tumor antigens released as the tumor cells are killed by the vaccinia virus. The GM-CSF gene is under the control of a synthetic early/late promoter that contains regulatory elements from early and late vaccinia promoters resulting in constitutive expression throughout the virus replication cycle. The β -galactosidase gene was inserted to facilitate localization of replicating virus in biopsy tissues.

JX-594 was well tolerated in a Phase I/II clinical trial of seven research participants with metastatic melanoma in which research participants received intratumoral injections twice a week for at least 6 weeks. Five of the seven participants experienced tumor shrinkage at a site of injection, including two complete responses. At time of entry into the trial, four participants had skin tumors that shrank after receiving JX-594 not by injection into the tumors but by dosing elsewhere in the body, suggesting that JX-594 has the potential to treat distant metastatic disease.

Safety and antitumoral effects have been demonstrated in research participants with multiple tumor types, including skin cancer, liver cancer, and lung cancer. Studies conducted in humans have demonstrated safety up to the 1×10^9 dose level, with all participants experiencing mild to moderate flu-like symptoms. Acute, mild-to-moderate hypotension was observed in some participants during the first 24 hours and acute, mild-to-moderate fever usually lasted 18 to 24 hours, with severity of fever and hypotension appearing to be dose-related. No significant organ toxicity was reported, although some changes in the blood were observed following dosing.

In the proposed Phase I clinical research study, pediatric patients with unresectable solid tumors that are refractory to standard therapy will be enrolled. This dose-ranging study is being conducted to determine the safest and most effective dose of JX-594 when delivered by intratumoral injection in pediatric participants. Approximately 12 to 15 research participants will be dosed for 4 to 6 weeks. Participants who are doing well on the regimen may receive as many as three additional dosings.

Participants will receive a single dose and will have radiographic response assessment conducted at week 3; doses will escalate between cohorts using a standard Phase I dose escalation design. Safety assessments – including blood testing, adverse event collection, and physical examinations – will be carried out at all study visits. Over time posttreatment, the amounts of virus levels in blood, urine, and throat as well as immune response will be assessed. Tumor responses and time-to-tumor-progression also will be assessed.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the safety of using a replication-competent vaccinia virus in children who are likely to be immunocompromised and have never been vaccinated against vaccinia (as were many of the adult research participants), even though clinical safety data exists for adults.

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

Dr. Buchmeier suggested that the protocol include a precise reference to the genetically modified strain of the Wyeth Dryvax® vaccinia, JX-594, being proposed for this study, along with a clear assessment based on experimental data of the pathogenic potential of JX-594. The protocol and the informed consent document cite statistics for the adverse responses to vaccination but do not make it clear that these numbers apply to Dryvax® and not to the JX-594 construct. Because no information is provided to allow an assessment of the immune status of the participants, he suggested that the status of potential participants' immune systems be assessed at the level of CD4 ratios prior to vaccinia administration, since severely depressed CD4/CD8 ratios would argue against participation in this trial. Dr. Buchmeier asked about the immediate and longterm consequences of the dose-limiting toxicity (DLT) reaction referred to as a possible complication, and queried the investigators as to whether sufficient time would be available to obtain the intravenous immunoglobulin and to initiate treatment. He suggested that viral

titers be monitored in blood during the period of active replication in order to monitor vaccinia spread outside the tumor. Dr. Buchmeier recommended providing an informed consent document written in language that could be understood by children, given that this study is proposed to include children as young as 2 years old.

Dr. Kodish asked whether the investigators intend to include an upper age limit in the eligibility criteria; if yes, such a statement should be included in the protocol description and, if no, the investigators should explain why not. He suggested that, if the biopsy will be part of routine clinical care for these children, the incremental risk without sufficient accompanying benefit should be communicated more clearly in the informed consent document. Noting that the informed consent document is quite lengthy, Dr. Kodish expressed concern that potential participants and their parents might suffer “consent fatigue.” However, the “Potential Benefits” section is appropriately brief and circumspect. He suggested that references to “you” throughout the informed consent document should be changed to “you/your child,” because the participants will be children. Specific concerns enumerated by Dr. Kodish included the need for clarification of terms, suggestions for language change surrounding the possibility of therapeutic misconception, and other rewording.

Dr. Zaia asked for clarification about whether a virus factor is the basis for JX-594’s selective replication in cancer cells – and requested comparative data to the parental Dryvax® strain for strain-specific growth in cancer cells – or whether the selective strategy is based on the nature of the tumor cell compared to normal cells as was suggested on page 27 of the protocol. He noted that children with relapsed or refractory solid tumors could likely be immunodeficient based on prior therapy, and therefore some measure of cellular immune function should be indicated in the inclusion criteria. In view of the concern for recognition of disseminated virus infection, Dr. Zaia requested an explanation of the exclusion of lymphopenia and transaminitis from DLT determination, suggesting that DLTs be defined as any AE of grade 3 for more than 7 days or of grade 4 for less than 7 days. In anticipation of some post-infection viremia, he asked for a description of assay sensitivity for quantitation of vaccinia in blood that could be used to define the course of virus infection and clearance. Because the anticipated AEs are based on the experience in adults treated for cancer and it is likely that a proportion of those individuals were vaccinated previously with vaccinia, he asked the investigators to state the distribution of AEs observed in adults based on age of birth before or after 1973; none of the children in this study will have residual vaccinia immunity.

Regarding the informed consent document, Dr. Zaia suggested that the investigators clarify the selectivity of JX-594, remove the precise enumeration of the experience in adults and note that experiences to date are based on use in adults that does not predict safety in children, include the risks associated with the possible failure of cidofovir treatment for virus dissemination, reword the benefits paragraph to state that benefit to “you (your child) is unlikely but the knowledge gained may be beneficial to others,” and rewrite the informed consent document in a style more appropriate for pediatric consents.

C. RAC Discussion

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

- Dr. Ertl suggested excluding children who are immunocompromised for genetic reasons or for infection, children shortly after they obtain their vaccination against measles virus, and children who have immunocompromised parents, siblings, or other household contacts, to avoid any risk that they may become infected in case higher titers than expected develop.
- Noting that the investigators had shown infection of the ovaries in female animals and in the testes in male animals, Dr. Ertl requested that the investigators discuss evidence as to whether this potential treatment would affect participants’ ability to bear or father children. She asked whether semen samples should be analyzed.
- Dr. Kodish wondered if the investigators should consider excluding participants with lymphomas.

- Dr. Roizman asked whether the investigators had looked at the possibility of hepatotoxicity in relation to JX-594.
- Dr. Kanabrocki suggested that the clinical staff be made aware of the exclusion criteria so they could choose to remove themselves from contact with these research participants when appropriate.
- Discussion arose as to whether the vaccinia titers should be measured in real time.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that the optional biopsy included in the protocol is not standard of care and is being considered to gain scientific knowledge about the ability of JX-594 to grow in pediatric-specific cancers. However, the sponsor believes that these data can be obtained from adult studies and that there is no strong rationale to believe that virus replication will differ in pediatric cancers as opposed to adult-specific cancers. Therefore, the sponsor removed the optional biopsy from the protocol and informed consent document.

The typical informed consent document used in CCHMC pediatric trials is 20 pages long; the informed consent document for this protocol is 24 pages long. At the suggestion of Dr. Kodish, the sponsor attempted to simplify the language and provided an updated copy to the RAC prior to this meeting. The sponsor noted that they were constrained by the language needed to fulfill regulatory requirements while properly explaining the study procedures and purpose to the parents and the research participants.

The sponsor redefined the DLT as any of the following treatment-related adverse events that would be evaluated and reported through the 4-week evaluation period: any Grade 4 toxicity or a Grade 3 toxicity persisting for longer than 7 days.

While JX-594 has been dosed in adult patients with liver enzymes of up to five times the upper limit of normal with good safety, the sponsor agreed that it is prudent in this first study in children to lower the cutoff to two and a half times the upper limit of normal liver enzymes.

The sponsor acknowledged the potential that JX-594 will have increased activity in research participants who, as in this protocol, have not been previously vaccinated or do not have significant antibody titers against vaccinia. JX-594 is a highly attenuated vaccinia strain and is based on the Wyeth vaccine strain that has been demonstrated to be safe in millions of children who were not previously vaccinated. The modifications to JX-594 make the virus more attenuated in normal cells and less likely to cause significant complications. While at present there is no specific data to show any difference in safety between individuals with a high vaccinia antibody titer and those with low antibody levels, it is possible that replication is more pronounced in individuals with a lack of vaccinia antibodies. Because of this possibility, the investigators will take a more cautious approach in this first pediatric study by using an initial starting dose in children that is significantly lower than the current adult dose. In addition, they will dose JX-594 via the intratumoral route of administration, which is believed to have less potential for systemic side effects and more direct exposure of the tumor to JX-594.

The sponsor agreed with all of the RAC's suggested changes to the language in the informed consent document and provided a revised document prior to this meeting. The sponsor also agreed to suggested clarifications within the protocol description and provided a revised protocol document prior to this meeting.

The inclusion criteria will be updated to define an upper age limit of 21 years. CD4 counts to be included in the inclusion criteria of the protocol

In response to the RAC request to clarify mechanism of selectivity, vaccinia virus has been shown to exhibit natural selectivity towards cancerous tissues relative to normal. In addition, JX-594 is genetically modified to inactivate TK which enhances the natural selectivity of vaccinia. Additional preclinical studies were conducted that also demonstrate the selectivity of TK-minus vaccinia and JX-594, and mechanisms behind in vivo selectivity. Selectivity has been demonstrated in studies showing preferential replication of JX-594 in tumor vs. normal cells in vitro, and the data supports TK-inactivation as a mechanism behind this selectivity. The in vitro work also suggests that defects in the interferon response pathway of cancer cells contribute to JX-594 selectivity. In addition, JX-594 selectivity was demonstrated using an assay in which primary tumor biopsies and companion normal tissues were infected ex vivo; tumor tissues were shown to be more susceptible to JX-594 infection than the normal tissue counterpart.

Quantitative polymerase chain reaction (Q-PCR) is used to measure JX-594 genomes in the blood due to its reproducibility and ability to detect product regardless of antibody and/or complement neutralization.

2. Responses to RAC Discussion Questions

The investigators agreed to exclude from participation any individuals with known severe immunodeficiencies. Dr. Cripe acknowledged that the investigators do not expect their potential participants to have perfect immune systems, in part due to having been treated with multiple cytotoxic chemotherapies.

Dr. Kirn explained that the investigators have conducted testicular exams, which to date have shown no abnormalities; if abnormalities are encountered in the future, semen samples would be analyzed. Dr. Cripe noted that most of the individuals enrolling in this trial would likely be sterile from their prior high-dose cytotoxic chemotherapy.

The investigators agreed to exclude from participation individuals with lymphomas, because the immune system is imbalanced even further in such patients.

With regard to hepatotoxicity, Dr. Kirn stated that, in humans with high doses of JX-594 injected directly into the liver and/or given intravenously, the investigators have not seen evidence of transaminitis that would be suggestive of clinically significant liver cell damage and hepatotoxicity.

Regarding clinical staff contact with the research participants, Dr. Cripe explained that the investigator brochure includes a lengthy biosafety section and instructions for participants, families, and caregivers about a variety of specifics. Training will be provided to staff members, who might come in contact with study participants.

E. Public Comment

Dr. Borrer noted that the informed consent document contained no description of “CT scan” or “ultrasound,” both of which are proposed to guide the needle into the tumor.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC’s indepth review and public discussion:

Clinical/Trial Design Issues

- Even though an attenuated vaccinia virus based on a vaccine strain is used to produce JX-594, there is still a risk that disseminated vaccinia could develop. The protocol addresses this safety issue by excluding patients with evidence of compromised immune systems, including individuals with human immunodeficiency virus (HIV), those who have used immune suppressive medications within 3 weeks of the first dosing, and those with low CD4+ T-cells counts. Two other exclusion criteria should be incorporated, namely patients with an underlying genetic

immunodeficiency disease and patients with lymphomas whose immune systems are likely to be more impaired than those with solid tumors. As a further safety measure, an appropriate waiting period should be instituted before administration of the vector if a child is vaccinated with a live vaccine, e.g., for measles, mumps, and rubella.

- The risk of disseminated vaccinia is also addressed through close monitoring of research participants. In addition, information on replication of the virus will be examined by analyzing vaccinia titers. However, there are no data on whether vaccinia titers or other laboratory tests could be predictive of early dissemination of vaccinia. It would be helpful to analyze the data gathered during this trial in an effort to identify laboratory tests that could act as an early marker of disseminated vaccinia or that could be used to quickly establish whether clinical symptoms are indicative of disseminated vaccinia.
- The simplified definition of a DLT is an improvement to the protocol.
- The protocol addresses the risks of inadvertent spread of vaccinia virus to immunocompromised close contacts of the participants by requiring alternative living arrangements for a period of at least 3 weeks following the last dose of the study medication. Clinical staff should be made aware of the risks and potential contraindications for vaccinia exposure both to them and any close contacts, especially individuals with compromised immune systems.
- Under the *NIH Guidelines*, vaccinia viruses other than monkeypox and restricted poxviruses – such as alastrim, smallpox, and whitepox – are classified as Risk Group 2 agents. Experiments with such agents generally require a containment level of Biosafety Level (BL) 2, even for attenuated vaccine strains. As always, however, the local institutional biosafety committee is required to do a thorough risk assessment for such constructs and to review the administration protocols.

Ethical/Legal/Social Issues

- The RAC concurred with removal of the optional tumor biopsy to evaluate JX-594 protein expression and genome detection by Q-PCR following intratumoral injection because the research participants are children, the risks are not minimal, and there is no prospect of direct benefit from the procedure.
- As with other pediatric studies, it will be challenging to ensure the validity of each participant's consent. To enhance the participant's understanding of the study (and that therapeutic benefit is unlikely) and its risks and burdens, the consent and assent documents should be simplified and shortened.

G. Committee Motion 3

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Zaia and seconded by Dr. Yankaskas that the RAC approve these summarized recommendations. The vote was 19 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VI. Discussion of Human Gene Transfer Protocol #0910-1004 entitled: An Open Label Dose Escalation Study to Evaluate the Safety of a Single Escalating Dose of ACRX-100 Administered by Endomyocardial Injection to Cohorts of Adults with Ischemic Heart Failure

Principal Investigator: Douglas W. Losordo, M.D., Feinberg Cardiovascular Research Institute
Additional Presenters: Rahul Aras, Ph.D., Juventas Therapeutics, Inc; Joseph Pastore, Ph.D., Juventas Therapeutics, Inc.; Marc Penn, M.D., Ph.D., Cleveland Clinic
Sponsor: Juventas Therapeutics, Inc.
RAC Reviewers: Drs. Ertl and Flint

Ad hoc Reviewer: W. Robb MacLellan, M.D., University of California, Los Angeles

Dr. Kodish recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

One of the primary causes of heart failure is previous damage due to ischemic cardiovascular disease including myocardial infarction (MI). A majority of ischemic heart failure patients have systolic dysfunction or impaired cardiac pumping ability. Novel, effective heart-failure treatments are needed to improve quality of life and reduce the number of heart-failure hospitalizations as well as to provide clinical benefit and savings to the healthcare system. Unlike current treatments that focus on either alleviating symptoms or reducing cardiac workload, regenerative medicines have a high therapeutic potential for treating ischemic cardiac disease because they provide an opportunity to repair and retain function in degenerating organs.

Nonviral gene delivery – the application of naked plasmid DNA to express a therapeutic protein at a specific site – is a delivery method that has been tested clinically in ischemic patients for more than 15 years. A substantial body of preclinical and clinical literature has demonstrated that nonviral vector delivery of therapeutic genes is safe and effective. The safety profile of nonviral gene delivery also is attractive when compared to viral vector delivery because it does not produce a significant inflammatory response.

Juventas Therapeutics, Inc., has identified stromal cell-derived factor-1 (SDF-1), a naturally occurring protein that is produced rapidly in response to tissue injury. SDF-1 induction stimulates a number of protective anti-inflammatory pathways and is increased in the myocardium after a heart attack, but only lasts for a matter of days and therefore the protective response fades quickly. The short duration of SDF-1 action reduces the potential for tissue repair. Juventas has developed ACRX-100, a nonviral SDF-1 producing plasmid, for treatment of heart failure because it provides a potentially safe means through which to obtain longer, albeit transient (less than 15 days) therapeutic protein production in the heart, while allowing for the possibility of repeat administrations.

Juventas proposes to initiate a Phase I safety study of ascending doses of ACRX-100 in 16 research participants with ischemic cardiomyopathy. ACRX-100 will be delivered to the myocardium via direct catheter-guided injection from within the left ventricular chamber using the BioCardia Helix™ transendocardial delivery catheter system. Safety will be tracked at each dose by documenting all AEs, with the primary safety endpoint being the number of major cardiac events at 30 days. In each cohort, participants will receive an injection of ACRX-100. In all cohorts, dosing efficacy will be evaluated by measuring the impact on cardiac function using standard echocardiography measurements, cardiac perfusion via single photon emission computed tomography (SPECT) imaging, and improvement in New York Heart Association (NYHA) class.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the. Key issues included the novelty of this agent that has not been used in humans and the safety concerns raised by direct intramyocardial injection in research participants with impaired cardiac function, as plasmid vectors can induce robust immune responses and SDF-1 is a chemoattractant for Band T cells. The unusual dose-response relationship in the porcine studies, in which the highest dose failed to show clinical or histological evidence of efficacy, also was deemed worthy of discussion.

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

Regarding the porcine preclinical study, Dr. Ertl asked whether control animals were included in the study and what methods were employed to distinguish among inflammatory infiltrates caused by the procedure, the vector, and/or the transgene. The vector leaks to multiple sites, including the brain; therefore, Dr. Ertl requested that the investigators provide additional information on transfer of vector to the brain. She

asked if the investigators tested for an adaptive immune response to the transgene product, since the vector also could be detected in lymphatic tissues. Dr. Ertl requested the results of the clinical chemistry, which showed increases in creatine kinase, and for more detailed information about the animals that died following the procedure. Regarding informed consent issues, she noted that the word “therapy” should be replaced wherever it currently occurs in the protocol documents, to avoid therapeutic misconception. Considering the vector’s ability to induce innate and adaptive immune responses, Dr. Ertl suggested that research participants be monitored for both.

Dr. Flint expressed concern about the results of the preclinical study in a porcine model in which three doses of ACRX-100 were injected into the left ventricle; the proposed human dosing regimen is based on these results. Because of inconsistent reporting of persistence of ACRX-100 DNA post injection, she requested that the investigators comment on factors that might account for the large animal-to-animal variation in persistence of ACRX-100 DNA and the possible impact of such variation on research participants’ responses, and the apparent lack of correlations among the dose, the initial concentration of plasmid DNA at day 3 post injection, and the concentration of ACRX-100 DNA persisting at 30 or 90 days. Dr. Flint asked that the investigators describe how extra-cellular and intra-cellular concentrations of plasmid DNA were measured, and that they provide the data collected during these measurements. In addition, she requested discussion of the intracellular plasmid DNA concentrations achieved in the context of other data that might shed light on whether significant production of PDF-1 can be achieved. She noted that the high-dose group in the preclinical porcine study was without significant effect and asked that the investigators comment on factors that might account for that lack of effect.

Dr. Flint asked the investigators to clarify two aspects of the chemical protocol. Since the NYHA Class III participant population chosen for this study has a 1-year survival rate of 50 percent to 80 percent, the investigators should discuss how cardiac AEs related to the protocol would be distinguished from those occurring spontaneously. Noting that the investigators have stated that the serum levels of SDF-1 would be monitored in the research participants, she suggested that the protocol state clearly what the investigators expect to learn from such measurements.

Regarding the informed consent document, *ad hoc* reviewer Dr. MacLellan requested clarification of the statements within the “Compensation for Injury” section and of the proposed doses, which are listed differently on page 1 of the informed consent document compared with page 6 of the protocol description. Regarding the preclinical data, Dr. MacLellan asked why the plasmid without insert was not used as a control in the preclinical studies. He wondered whether SDF-1 recruited circulating cells in the pig model, whether these cells were carefully characterized, the impact of SDF-1 expression on myocardial fibrosis in the preclinical model, and how the potential impact of increased cardiac fibrosis would be assessed in the proposed clinical trial. Dr. MacLellan asked for a description of the background medical therapy for the pigs after MI and for the investigators to comment on the data that supports the addition of SDF-1 on top of usual care, as is proposed in this protocol. Regarding the protocol, Dr. MacLellan asked the investigators to comment on the expected study population of endstage ischemic cardiomyopathy, given the inclusion criteria in the protocol, versus that studied in preclinical models. He requested discussion of the data that supports the use of SDF-1 in endstage ischemic cardiomyopathy as well as how the primary mechanism – increased vasculogenesis – will help this population. In addition, he asked the investigators to discuss the complication rates associated with the use of the BioCardia Helix™ needle injection catheter in heart-failure patients.

C. RAC Discussion

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

- Dr. Strome expressed concern about the use of surrogates to indicate expression of SDF-1. He noted that the investigators have not shown that they have been able to express the protein of interest, which makes the data difficult to interpret.
- Dr. Strome asked whether the investigators have shown that the receptor binds to the relevant species model, noting that a single amino acid change can abrogate binding completely.

- Dr. Strome requested additional information about the drug-device combination that is basically a corkscrew inserted into the heart.
- Dr. Ertl asked why the highest dose in the pigs was not efficacious. She noted that efficacy was highest in the mid dose but the greatest plasmid expression for the longest time was seen in most of the animals receiving the highest dose. For some of the animals, the lowest dose was shown to be most efficacious, a confusing result.
- Dr. Federoff summarized RAC members' concerns about the lack of convincing data regarding the proposed starting dose in humans for this clinical trial.

D. Investigator Response

1. Written Responses to RAC Reviews

Previous studies have demonstrated that overexpression of SDF-1 in the heart by implanted cardiac fibroblasts increased the homing of CD117+ and CD34+ progenitor hematopoietic stem cells and angioblasts, respectively, to the myocardium. Although development of fibrosis is part of the normal healing process following MI, no increased fibrosis above normal healing in the heart was noted in any of these studies. Recruitment of circulating cells was not measured in the porcine study because the appropriate antibodies for pigs are not readily available; however, based on the previous studies, the sponsor believes it is reasonable to hypothesize that recruitment of circulating cells was a key mechanism driving cardiac benefit in the ACRX-100 low-dose and mid-dose animals. The investigators will indirectly measure cardiac fibrosis as a function of cardiac remodeling in participants treated with the SDF-1 plasmid. Previous studies and the investigators' preclinical results support the hypothesis that SDF-1 gene transfer will attenuate remodeling and increase heart function, which indirectly correlate with decreased fibrosis.

The justification for the addition of SDF-1 on top of usual care is that the mechanisms of action of SDF-1 might be additive to those of optimal pharmacological therapy. Based on recent meta-analyses, this would appear to be the case for cell therapy. In this clinical study, SDF-1 has the potential to increase vasculogenesis, a result that was demonstrated in the sponsor's rodent and porcine studies as well as by others. In contrast, angiotensin-converting enzyme inhibitors, beta blockers, and aldosterone antagonists do not elicit vasculogenesis or other regenerative properties. Therefore, SDF-1 might be synergistic with optimal medical therapy and could provide additional cardiac benefit to the heart failure patient.

Based on assessment of persistence and of transgene expression in the injected areas in the porcine study, the investigators concluded that plasmid uptake and expression occurred in viable, hibernating, and scar tissue, suggesting that this delivery strategy is sufficient for successful delivery of ACRX-100.

Regarding differences between the preclinical model and the potential clinical population, the investigators explained that the preclinical model is a less chronic (30 days post-MI) model of heart failure compared to the clinical population (more than 6 months removed from an MI), due to limitations resulting from the pigs' rapid growth rates and challenges related to obtaining quality echocardiograms as their size increases. However, the preclinical model has the fundamental characteristics of systolic ischemic heart failure, including reduced ejection fraction and significant left ventricular remodeling, similar to characteristics observed in the target human population. The research participants will be NYHA functional class III heart failure patients who are symptomatic upon mild exertion, have significant pathological remodeling, and have significant systolic dysfunction. Since the preclinical study demonstrated cardiac functional benefit in pigs with previous MI, systolic dysfunction, and pathological remodeling, the investigators believe it is reasonable to hypothesize that NYHA Class III patients with systolic dysfunction due to previous MI will benefit from SDF-1 treatment. The clinical study will not include NYHA Class IV patients, who are symptomatic at rest and considered endstage.

Clinical use of the BioCardia Helix™ catheter has been performed in 38 research participants in four clinical studies to date. A first study performed in 2001 showed no adverse effect on three participants.

In two cases in the second study, performed in 2004, the first two participants experienced AEs with pericardial effusion. Since this occurrence, instructions for use of the BioCardia Helix™ catheter were modified, physician training for the catheter has been modified extensively and completed, and there have been no observations of pericardial effusion in the latest 33 participants injected with the catheter. No other significant AEs related to the catheter have been reported.

Regarding the animal-to-animal large variation in persistence of ACRX-100 DNA, the investigators explained that plasmid uptake is concentrated in regions closest to the site of injection. It was hypothesized that tissue taken proximal to injection sites would show significantly greater plasmid presence when compared to tissue taken at a distance from the injection sites. Data from the porcine study indicated that adjacent tissue samples could vary by as much as 1×10^6 copies. In addition to variability resulting from plasmid distribution, it has been demonstrated that injections of a luciferase-expressing plasmid delivered by the BioCardia Helix™ catheter resulted in gene uptake and protein expression at varying levels for a given dose, and data from other studies using the BioCardia Helix™ catheter suggest that 10 percent of injection sites might demonstrate no plasmid expression at all. These studies suggest that, for a particular dose, patient-to-patient variability in the amount of SDF-1 protein expressed is likely. Both low-dose (7.5 mg) and mid-dose (30 mg) treated animals showed equivalent improvement in cardiac function. The investigators will use the identical injection strategy employed in the porcine study, which should provide a large therapeutic window that will offer a significant opportunity for participants to respond positively to the protocol.

For the low and mid doses, cardiac benefit compared to control was observed, consistent with previous reports describing the role of SDF-1 in repairing myocardium post-MI. At the highest dose tested, no benefit was observed. These results suggest that delivery of ACRX-100 could result in protein expression sufficient to enhance cardiac function and that there might be a threshold at which this benefit is lost. In addition to the functional benefit, biodistribution studies performed for the ACRX-100 treated animals showed SDF-1 transgene expression, and clinical pathology demonstrated increased SDF-1 serum levels relative to controls at days 3 and 7, with levels returning to baseline by day 30. These data support the conclusion that the functional benefits observed in ACRX-100 animals are the result of SDF-1 expression in the target tissue.

The low (7.5 mg) and mid (30 mg) doses may express SDF-1 at levels that promote increased stem cell homing, while the high dose expresses SDF-1 at higher levels that promote much less stem cell homing, thus explaining why the low and mid doses showed substantially more cardiac benefit and increased vasculogenesis compared to the high dose. Compared to control, the high dose did not demonstrate toxicity, did not show a reduction in cardiac function, and did show a slight increase in vasculogenesis.

Regarding distinguishing spontaneously occurring cardiac events from those related to the protocol, the investigators explained that anticipated AEs are categorized into procedure related and ACRX-100 (drug) related. As AEs occur in the study, the data safety monitoring committee will use the list of anticipated AEs as a guideline to determine to which category the AE belongs.

For the porcine study, serum SDF-1 levels were monitored as a potential safety marker to determine if ACRX-100 treatment resulted in elevated systemic, circulating SDF-1 levels. The investigators will monitor SDF-1 serum levels in this Phase I clinical trial to confirm that the results observed in pigs are conserved in humans. No significant differences in SDF-1 serum levels are anticipated during the Phase I clinical trial.

Observations from the relevant animal studies suggest a mild, transient inflammatory response to the procedure, and that neither the vector nor transgene promote an increased inflammatory response identifiable by histopathology.

Regarding possible protein expression in the brain, the investigators stated that ACRX-100 biodistribution testing was completed for the highest dose (100 mg) evaluated in the preclinical study. All product was cleared from non-cardiac tissue within 90 days following injection. The data suggest that product was cleared through the kidneys, which demonstrated the greatest level of plasmid presence at Day 3. For all

other organs, minimal ACRX-100 was present at Day 3 and, by Day 30, the product was essentially cleared from all organs. For the brain, at the highest dose tested in the porcine study, the greatest amount of plasmid identified was 640 copies in one animal; the majority of animals had no identifiable plasmid levels. The investigators believe it is unlikely that the transient expression of such low-level plasmid in the brain would result in clinically relevant protein expression.

Regarding possible immune response, the investigators stated that a complete blood count was performed on all animals in the porcine study at Day 3, Day 30, and Day 90 post-injection. No significant changes from baseline were seen at any dose for leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated), absolute reticulocytes, platelet count, or blood cell morphology. These results suggest that pigs receiving ACRX-100 had no adaptive immune response to the SDF-1 transgene. In addition, these results are consistent with the fact that human SDF-1 transgene expressed through ACRX-100 is highly homologous to porcine SDF-1, making it unlikely that an immune response would be generated against this highly conserved protein. In the proposed clinical trial, the SDF-1 transgene would be indistinguishable from endogenous SDF-1; therefore, the investigators believe that an adaptive immune response would be unlikely during the course of this clinical trial.

The death of animals on study following dosing was not considered to be a result of the administration of the test article. Mortality was expected due to the level of heart failure required by the study protocol. Repeated surgical procedures, blood collections, and echocardiographic procedures might have contributed to the mortality noted after dosing.

The sponsor clarified that they will not reimburse medical expenses associated with injuries related to the procedure if it is determined that the research participant has not followed the study doctor's instructions about the study, since many of the protocol instructions for participants are safety related.

2. Responses to RAC Discussion Questions

The investigators do not believe that SDF-1 will increase arrhythmias, and there is some chance that it might decrease arrhythmias. Dr. Penn opined that implantable cardioverter defibrillators should be present when studying a heart failure population and injecting material into the myocardium.

Dr. Pastore described the experience with the BioCardia Helix™ transendocardial catheter delivery system. Trials have been conducted with research participants with chronic ischemia or heart failure, and the most recent trials are being performed on a similar population to the one proposed in this trial, symptomatic heart failure patients.

In response to a query about the possibility of using placebo controls in this trial, Dr. Penn explained that true placebo-controlled trials in cardiology that include injecting the heart would require saline injection to truly randomize research participants, because some benefit associated with merely injecting cells of any kind into the heart.

Dr. Penn responded to RAC member concerns about the confusing dosing results from the preclinical studies in which the lowest and mid level doses, rather than the highest dose, were efficacious in the pig model. Although the highest dose did not produce the desired results, it also did no harm. He stated that the investigators believe that intrinsic stem-cell trafficking occurs, which is important to the heart for repair and/or sustenance. Overexpressing SDF-1 might shut down that intrinsic trafficking; a large amount of SDF-1 has been shown to block cell migration. The investigators are currently working on preclinical data to support this theory, focusing on understanding the biology.

The investigators chose the proposed human trial doses by starting much lower than what was given in the pig model.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- While the human SDF-1 protein has a highly conserved sequence across species, there are some differences, in particular between human SDF-1 and porcine SDF-1. As such, preclinical studies should be conducted to determine whether human SDF-1 binds the porcine receptor, as this may help define the optimal dose of SDF-1 to be used in humans. Furthermore, most of the preclinical studies have tested for the presence of the vector rather than for SDF-1 expression. Before initiating a clinical trial, it would be useful to have data establishing SDF-1 expression and how it correlates with any histological and clinical changes.
- Given the counterintuitive preclinical finding of an inverse relationship between a high dose and both efficacy and vasculogenesis, it will be imperative to elucidate the biological basis of this effect as ACRX-100 is further studied and developed.

Clinical/Trial Design Issues

- As implantation of an automatic implanted cardioverter defibrillator is indicated in research participants with left ventricular ejection fractions equal to or below 35 percent, this participant population will have a high incidence of cardiac events, including arrhythmias and hospitalizations, due to complications from heart failure. Determining the causality of adverse events will be challenging. Currently, a total of 16 participants are to be enrolled in the protocol with the primary safety endpoint of major adverse cardiac events at 1 month post-injection and a 1-year followup. The enrollment level and the length of followup should be reevaluated to ensure appropriateness, given the nature of the participants' underlying disease.
- Peripheral blood mononuclear cells should be collected and stored for future immunological studies as needed, and the informed consent document should be augmented accordingly.

Ethical/Social/Legal Issues

- The sponsor is planning to cover the cost of medical care in the event of a research injury only if the participant has followed the protocol. Given that the protocol is a Phase I safety study and there are no prospects of direct benefits to participants, the sponsor should be willing to cover the cost of medical care for all participants who experience a research related injury, not just those who have been compliant.

G. Committee Motion 4

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Yankaskas and seconded by Dr. Kirchhoff that the RAC approve these summarized recommendations. The vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VII. Update on Proposed Changes to the *NIH Guidelines*

Presenter: Dr. Corrigan-Curay and Dr. Jambou

A. Presentation

Dr. Corrigan-Curay explained that revisions to the *NIH Guidelines* are being considered to expand its scope to cover research with synthetic nucleic acids and to change two parts of Section III regarding transfer of drug resistance to microorganisms and regarding tissue culture experiments involving less than two-thirds of the genome of any eukaryotic virus. She reviewed the current biosafety guidance.

Current biosafety guidance is provided by the *NIH Guidelines* and the *Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL)*. However, the *NIH Guidelines* only covers synthetic DNA when joined by recombinant methods; it does not cover synthetic DNA synthesized *de novo* nor does it cover synthesized RNA viruses. The *BMBL* is agent-specific and references the *NIH Guidelines* with respect to recombinant molecules, including synthetic recombinant molecules.

The charge to the RAC was to consider the application of the *NIH Guidelines* to experiments with synthetic nucleic acids – to what degree this technology is covered and whether the scope needs to be modified to capture synthetic biology research – and to develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic nucleic acids. To date, proposed revisions have been developed by the RAC Biosafety Working Group (BWG) and were approved by the full RAC in March 2008; a proposal was published in the Federal Register (FR) in March 2009 with opportunity for public comment. The public comments have been posted on the OBA Web site (<http://oba.od.nih.gov/oba/index.html>). A stakeholders meeting was convened with experts to discuss the public comments on June 23, 2009, and the BWG met in October 2009 to consider public comments and propose revisions to certain sections of language proposed in the FR notice.

Taking into consideration the public comments, the BWG recommends some revisions to the proposed language for the following sections of the *NIH Guidelines*:

- Section I-A: Purpose
- Section I-B: Definition
- Section III-F: Exempt experiments
- Section III-A-1-a: Experiments involving introduction of drug resistance into microorganisms (These experiments are considered Major Actions which require approval of the NIH Director.)

It was proposed that the definition of recombinant DNA molecules be extended to nucleic acids and a definition was added for synthetic nucleic acids that are chemically synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acids. The RAC members discussed the proposed exemptions for both basic and clinical research with certain synthetic nucleic acids. They also discussed the revised changes to section III-A-1-a to clarify the need for consideration of drug use by certain populations and the process by which an IBC could consult with OBA regarding specific experiments.

Dr. Jambou presented the proposed recommended changes to Section III-E of the *NIH Guidelines*, which deal with tissue culture experiments involving no more than two-thirds of the genome of any eukaryotic virus. Such experiments are initiated concurrently with IBC registration, so they are considered relatively low risk and can be conducted under BL1 containment. The proposed revisions to Section III-E-1 would apply to experiments involving no more than half of the genome of any Risk Group (RG) 3 or RG4 eukaryotic virus or those with complete deletions of certain essential viral genes.

B. RAC Discussion

The RAC discussed the proposed wording including:

- In the case of an oligonucleotide that could render an endogenous virus more pathogenic, Dr. Flint suggested that the definition remain as proposed but that if such an event occurred, OBA should refer it to the RAC for review.

- Dr. Corrigan-Curay reminded the RAC members that an IBC, under the *NIH Guidelines*, could refer a proposal to OBA for RAC review if any questions or concerns arose. It was agreed that the FR notice would state that an IBC can refer a protocol to OBA and can ask for further consultation with the RAC as deemed necessary.
- Regarding Section III-A-1-a, which addresses the need for the IBC and the investigator to make their own assessment of an experiment and recommends that they consult the RAC for advice, Dr. Federoff asked for clarification because the risk calculus includes more than the drug – it includes the other drugs that are used to treat the infectious organism. This point is particularly important for international use and the community health perspective regarding the availability of first-line antibiotics and cost.

C. Committee Motion 5

It was moved by Dr. Buchmeier that the RAC approve these proposed changes to the *NIH Guidelines*. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VIII. Day 1 Adjournment/Dr. Federoff

Dr. Federoff adjourned Day 1 of the December 2009 RAC meeting at 5:45 p.m. on December 1, 2009.

IX. Day 2 Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff, RAC Chair, called Day 2 of the December 2009 RAC meeting to order at 8:30 a.m. on December 2, 2009.

X. Update on Trial for Beta Thalassemia and Sickle Cell Disease Using a Lentiviral Vector: Observation of a Clonal Population of Cells in a Subject

Presenters: Philippe Leboulch, M.D, University of Paris, and Michel Sadelain, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center

A. Presentation by Dr. Leboulch

Dr. Leboulch reviewed a French clinical trial that had been reported recently to the AFSSAPS (the French government's health safety agency). The first thalassemia gene transfer subject was transplanted (in June 2007) without injection of backup cells under the regulatory authority of the AFSSAPS. Autologous CD34+ cells were transduced with a HIV-1 derived lentiviral vector that is self-inactivating (SIN), includes insulator elements from the chicken HS4 globin locus, and expresses the human β -globin gene.

One year posttransplant, conversion to transfusion independence had occurred but there was also evidence of partial clonal dominance (at less than 10 percent) with activation of the HMGA2 gene. No adverse events (AEs) have been observed but voluntary disclosure and formal presentation regarding the clonal dominance was made to the AFSSAPS in April 2009. The investigators initiated a voluntary temporary hold while further evaluating hematopoietic homeostasis and evolution of the clone and, after more than 6 months of further observation and evaluation and after formal presentation to the AFSSAPS in November 2009, the AFSSAPS Gene Therapy Advisory Committee voted unanimously to resume accrual of research participants in this clinical trial.

The investigators observed an approximately three-fold decrease in the count of erythroblasts between the marrow and the blood and an approximately tenfold increase in the lifespan of circulating red blood cells. Hematopoietic homeostasis was maintained, including normal blood and bone marrow cytology,

normal cytofluorometry analysis for many antigens, normal karyotype, no trisomy-8, cytokine independence, normal LTC-IC counts, and asymptotic stabilization of the clone-relative dominance.

Dr. Leboulch summarized the integration site analysis of the dominant clone. The vector had inserted into the third intron of the high mobility group AT hook 2 (HMGA2) gene causing aberrant splicing to a cryptic site within the vector insulator sequence. The truncated HMGA2 transcript lacked binding sequences for Let-7 microRNA (miRNA). The binding of the miRNA is involved with down-regulation of HMGA2 mRNA. HMGA2 expression is largely restricted to embryonic and stem cells. Activation of HMGA2 has been correlated with both benignity and malignancy.

The rationale for continuing participant accrual includes the severity of the disease, current proof that the vector expresses amounts of globin that can lead to transfusion independence, an absence of clinical adverse events, and the necessity to evaluate further benefit/risk ratios in a larger cohort. For these reasons, the advisory committee of the AFSSAPS voted unanimously to allow continuation of this trial.

B. Presentation by Dr. Sadelain

Dr. Sadelain provided an update of Protocol #0704-852, "A Phase I Open-Label Clinical Trial for the Treatment of β -Thalassemia Major with Autologous CD34+ Hematopoietic Progenitor Cells Transduced with ThalagenTM, a Lentiviral Vector Encoding the Normal Human β -Globin Gene," which was reviewed publicly at the June 2007 RAC meeting.

Ten years ago, Dr. Sadelain and colleagues reported that it was possible to treat β -thalassemia in mice using a retroviral vector that encodes the human β -globin gene, which is driven by its own promoter and key elements chosen from the locus control region, a powerful enhancer and chromatin structure determinant that regulates the expression of all globin genes in mice and humans. This approach has shown efficacy in animal models – correcting anemia and preventing secondary organ damage – and has shown longterm expression, peripheral selective advantage, and vector-copy-number-dependent expression.

Dr. Sadelain highlighted the differences between his trial and that of Dr. Leboulch, including the use of the normal human β -globin gene rather than a mutated version, different vectors, and different cell-collecting techniques. Following discussion with the RAC in 2007, Dr. Sadelain and colleagues decided to enroll participants who were at least 15 years old and who lacked a matched donor. One significant difference is that Dr. Sadelain's trial proposed the use of reduced-intensity busulfan conditioning while Dr. Leboulch's trial proposed the use of myeloablative conditioning.

Since the RAC review in June 2007, the investigators have been preparing to open this trial by verifying that they can safely and efficiently collect a target number of about 6 million CD34 cells/kg from the research participants and that those cells can be transduced at an appropriate level. The first three participants have been enrolled and all three have yielded cell numbers in excess of the range required for this protocol; this procedure was well tolerated without any SAEs. Cell transduction is proceeding and the cells have been purified and frozen under GMP conditions, and could be made available for use in a future trial if the participants are so interested. During the past 18 months, the investigators also have been focusing on a study to improve production of these vectors, with the goal of being able to produce one lot for an entire clinical trial.

Regarding safety, Dr. Sadelain reiterated that this approach is privileged in the context of sickle cell disease and thalassemia because gene expression is being targeted to the erythroid compartment using vectors that do not express in stem cells or other lineages. Soon after activation of the transgene in erythroblasts, the nucleus is excreted – a natural safety mechanism. Expression is highly specific.

Dr. Sadelain and colleagues followed a cohort of 300 mice, transduced with lentiviral vectors harboring the β -globin gene, for 12 to 14 months to look for leukemias or other SAEs; none have been found. autopsies were performed on 80 of these mice chosen at random and no hematological malignancies were found. In view of Dr. LeBoulch's finding, Dr. Sadelain and colleagues tried to find evidence of clonal

expansion in these mice, using archived DNA and peripheral blood; no evidence of clonal expansion has been found to date, although the study is ongoing. The net result of this study is that no transient clonal expansion has been documented at this point in this cohort of mice that were followed for 1 year.

C. RAC Discussion

Questions and comments posed by RAC members included:

- Dr. Strome asked whether the expression of HMGA2 was changed by the insertional mutagenesis. Dr. LeBoulch responded that expression was increased 10,000 fold in the erythroid lineage, but not detectable in the granulocyte-monocyte lineage due to the erythroid specificity of the locus control region. The amplification occurs in earlier progenitor cells possibly due to either transcription from the endogenous HMGA2 promoter with the lack of negative regulation by LET7 miRNA or lineage priming in which the tissue specific enhancer leaks in some stem cells and is later shut off.
- Dr. Williams stated that overwhelming data indicates no untoward effect of integration in this gene in a human system. Coupled with the result of a subject who has gone from being transfusion dependent – with the longterm iron overload consequences that are eventually fatal – to being transfusion independent, the risk/benefit ratio seems tipped in favor of potential benefit.
- Noting that preclinical modeling is often thought to be predictive of what to expect clinically, Dr. Federoff reiterated that everything that has been presented would suggest that is not the case here. He wondered whether concepts gleaned from the small numbers of human research participants could be retested in another animal model that might then become more predictive of subsequent clinical outcomes.

Dr. Sadelain responded that he and his colleagues rely extensively on the mouse models and do not plan to change that approach, although many unpredictable discrepancies are slowly accumulating over time with regard to mouse-model predictivity. Dr. LeBoulch noted that a mouse is about 3,000-fold smaller than a human, that the lifespan of a mouse is much shorter, and that thalassemic or sickle-cell marrow in mice has a different function and different dynamics. Dr. Sadelain added that he and his colleagues attempted to study these vectors in rhesus macaques, although they were aware of the innate resistance of rhesus macaques to HIV1-derived lentiviral vectors; the resulting data were not informative. Possibly the pigtail macaque may offer a better model.

XI. Discussion of Human Gene Transfer Protocol #0910-1006 entitled: Treatment of Subjects with Adenosine Deaminase (ADA) Deficient Severe Combined Immunodeficiency (SCID) with Autologous Bone Marrow CD34+ Stem/Progenitor Cells After Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector

Principal Investigator: Donald Kohn, M.D., University of California, Los Angeles (UCLA)
Additional Presenters: Fabio Candotti, M.D., National Human Genome Research Institute (PI at NIH); Kenneth Cornetta, M.D., Indiana University-Purdue University Indianapolis; Alan Ikeda, M.D., UCLA (PI at UCLA); Kit Shaw, Ph.D., UCLA
RAC Reviewers: Drs. Fan and Kodish
Ad hoc Reviewer: Morton Cowan, M.D., University of California, San Francisco

Dr. Williams recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

ADA-SCID is an inherited disorder in which patients have profoundly defective immunity with essentially no T-lymphocyte function and minimal-to-absent B-cell function. SCID patients typically present for

medical attention between 3 months and 9 months of age with multiple, recurrent, severe infections with common childhood pathogens or opportunistic organisms. Related findings often include failure to thrive along with wasting, persistent diarrhea, and delayed growth and dentition. Treatment options include bone marrow transplantation (BMT) and ADA enzyme replacement therapy.

The protocol proposes a clinical trial of gene transfer for patients with ADA-deficient SCID using a lentiviral vector (EFS-ADA). The central hypothesis of the proposal is: *ADA gene transfer using lentiviral vectors may be more effective and safer than using γ -retroviral vectors*. Lentiviral vectors may transduce human hematopoietic stem-progenitor cells (HSPC) more effectively than γ -retroviral vectors and may do so with a shorter time of *ex vivo* culture for transduction (e.g. 2 vs. 5 days for lentiviral vs. γ -retroviral vectors), which may better preserve the engraftment capacity of the HSPC. Additionally, high-titer lentiviral vectors can be made in which the strong transcriptional enhancers are deleted from the long terminal repeat (LTR) and using a cellular promoter lacking strong enhancers to drive expression of the passenger gene. This new design of lentiviral vector has been shown to have significantly lower risks for causing insertional oncogenesis in experimental models.

Eligible enrolled subjects will be withdrawn from polyethylene glycol-conjugated ADA (PEG-ADA) enzyme replacement therapy (if receiving it) and then will have their bone marrow harvested. CD34+ HSPC will be isolated from the bone marrow and transduced *ex vivo* using the EFS-ADA lentiviral vector. Subjects will be treated with a non-myeloablative dose of busulfan chemotherapy (4 mg/kg) and then will receive the transduced autologous CD34+ cells by intravenous infusion (if they meet release criteria). Subjects will be followed for end-points of safety and efficacy. The results will be compared to those in the prior clinical trials using γ -retroviral vectors in terms of the level of gene-containing leukocytes produced and the time-course and extent of immune reconstitution.

The primary objective of this proposed study is to examine the safety of autologous BMT for ADA-deficient SCID using a lentiviral vector to transduce CD34+ cells with a normal human ADA complementary deoxyribonucleic acid (cDNA), with transplantation after nonmyeloablative conditioning with busulfan and withholding of polyethylene-glycol-modified ADA (PEG-ADA) enzyme replacement therapy. Secondary objectives are to determine the frequencies of peripheral blood leukocytes containing the transferred ADA cDNA using real-time PCR, to assess ADA gene expression by measuring ADA enzymatic activity in peripheral blood leukocytes, and to examine the effects of ADA gene expression on immune function.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novel use of a lentiviral vector in a pediatric population with ADA-SCID. In two prior X-SCID gene transfer studies using γ -retroviral vectors, five research participants developed T-cell proliferative disorders due to insertional mutagenesis at or near oncogenes; one of the research participants subsequently died. No such cases have been seen in previous ADA-SCID trials using a γ -retroviral vector and some evidence suggests that a SIN lentiviral vector might pose less risk of insertional mutagenesis compared to the γ -retroviral vectors used in the X-SCID trials. However, the recent report from a French β -thalassemia trial of a clonal dominance due to insertional mutagenesis by a mechanism other than enhancer-mediated gene activation warranted further discussion of the risk/benefit of using the SIN lentiviral vector.

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

Dr. Fan asked the investigators to provide information on the total number of ADA-SCID gene transfer research participants, out of the total number of participants studied (all trials including their own), for which monoclonal expansion of vectored cells has been observed. Because lentiviral vectors have been reported to induce leukemias in mice, he requested discussion of what is known about the mechanisms of oncogenesis for these tumors and whether insertion within coding sequences of genes has been observed. In addition to the protocol stopping rules already enumerated, the investigators also should

consider placing the trial on hold if more than one participant develops a monoclonal outgrowth of transduced cells; Dr. Fan reasoned that a monoclonal outgrowth in even one participant would be cause for concern if the insertion/activation were into a gene known to contribute to oncogenesis. Noting that one of the advantages of lentiviral vectors is that they more efficiently (compared to γ -retroviral vectors) transduce the CD34+ CD38- hematopoietic stem cells that have higher reconstitution potential than total CD34+ cells, he queried whether the investigators considered using CD34+ CD38- cells in this trial.

Dr. Kodish asked why the tempo of accrual is expected to be relatively slow when an existing pool of 150 potential candidates exists and projected sample size for this study is only 10. Noting that the protocol is clear and well written, Dr. Kodish focused his review on questions related to currently available (but expensive) PEG-ADA therapy. He noted that the availability of an additional non-stem-cell-transplantation-based alternative therapy distinguishes the ethical considerations around research in individuals with ADA-deficient SCID from those with X-SCID. The “withholding” of PEG-ADA requires analysis, consideration, and discussion and Dr. Kodish suggested that, under section 2.5 of the protocol, the investigators should consider listing “withholding of PEG-ADA” as a potential risk of study entry and should provide justification and clarification of this point. The discontinuation of PEG-ADA to qualify for entry could be interpreted as depriving children of standard safe and effective therapy in order to conduct this experiment, and he noted that questions surrounding the discontinuation may be somewhat analogous to the ethical controversy around “wash out” study designs in research participants with mental illness – and are among the thorniest concerns in research ethics. Dr. Kodish requested explanation of one of the eligibility requirements and clarification of the child participants’ assent process. He noted that the length of the informed consent document is appropriate given the complexity and magnitude of the decision to participate in this trial, and that the description of alternatives contained therein is especially strong. Dr. Kodish suggested several specific changes and enhancements to the informed consent document. In particular, two additional bone marrow exams raise important ethical and regulatory questions as to whether these procedures are being conducted purely for research purposes; he noted that if they are purely research driven, institutional review boards should consider whether they meet criteria for minor increase over minimal risk.

Ad hoc reviewer Dr. Cowan expressed concern regarding the administration of busulfan at a time when it is still possible that the gene-transduced cell product might not meet the release criteria for infusion, which might result in the backup marrow cells being infused instead. To avoid that risk, he queried whether there is some way research participants could have their cells cleared for infusion prior to receiving busulfan. He asked for discussion of the likelihood that the cells would not meet the release criteria. He suggested that if a participant receives busulfan and for any reason is removed from the study, the participant should remain in the study for long-term follow-up of the effects of busulfan, and this arrangement should be mentioned in the protocol and the informed consent document. Dr. Cowan also expressed the concern that non-engraftment for 6 weeks could put the recipient at high risk for infection; to address this concern he requested that the investigators discuss whether data suggest that cells might not be seen at 4 weeks but will be seen at 6 weeks and whether the backup cells could be infused at 4 weeks. In addition, he noted that waiting until 6 months post therapy with no detectable ADA activity before starting PEG-ADA might be too long, creating an increased risk for opportunistic infections during this time without PEG-ADA or gene-transduced cells; he requested that the investigators discuss cases in which transduced cells and ADA activity have not been detected at 3 months but have been detected at 6 months. Dr. Cowan asked questions about the protocol that included additional data regarding rescue of the immune system, immune function testing, evidence of enrichment for gene-corrected cells, inclusion criteria, and several busulfan-related queries. Noting that the informed consent document is well written, Dr. Cowan expressed concern that the “Introduction” and “Purpose” sections might be too complex for the average layperson. He also offered several suggestions for improving the wording, clarifying concepts described, and providing sufficient information about the risks of busulfan.

C. RAC Discussion

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

- Dr. Wei asked about the protocol’s “futility rule” stopping point.

- Dr. Ertl asked about monitoring of oligoclonality and monoclonality and the definition of monoclonal outgrowth. Dr. Takefman responded that the FDA provides guidance on long-term follow-up that specifies requirements when using hematopoietic stem cells for a target with retroviral or lentiviral vectors. Investigators must monitor for monoclonal outgrowths using a sensitive assay. In addition, any monoclonal outgrowth must be reported in an expedited manner; the investigators must monitor for persistence of the monoclonality and the amount of clonal expansion, and then must sequence the integration site to determine whether it is within a locus known to have oncogenic activity. To date the FDA has not had any reports of monoclonal outgrowth, but Dr. Takefman noted that cases of outgrowth in Germany, France, and the United Kingdom have been reported.
- Regarding the potential use of busulfan if the final cell product does not pass release testing, Dr. Zaia asked whether the investigators could use previously frozen cells that could pass the release test, thus avoiding the use of busulfan.
- Dr. Federoff asked about the rationale for allowing inclusion in this protocol of children with dermatofibrosarcoma protuberans (DFSP).

D. Investigator Response

1. Written Responses to RAC Reviews

No cases of monoclonal expansion have been observed in any of the more than 25 ADA patients dosed in Europe or the United States. The stable low level oligoclonal marking reported six years ago in one subject receiving an ADA retroviral into cord blood CD34+ cells followed over 10 years after ADA gene transfer was not a monoclonal expansion, but the likely consequence of low engraftment of gene corrected progenitors. The investigators agreed that monoclonal expansion might need to be added as a stopping endpoint; however, it will be necessary to develop a definition of monoclonal outgrowth that distinguishes benign low-level transduction from outgrowth that heralds a potential premyelodysplastic or preleukemic conversion. They will develop such a definition.

While the CD38 (-) sub-fraction of CD34+ cells is enriched further for reconstituting hematopoietic stem cell activity, compared to the bulk CD34+ cell population, CD34+/CD38(-) cells are present within the target CD34+ population and isolating them does not increase their frequency or their transduction efficiency. In fact, performing some manipulation to isolate the CD38(-) cells from within the CD34+ cell population would likely lead to loss of cells. Additionally, there is no clinically-approved method for isolating CD38(-) cells and doing so would entail the development of a GMP-grade monoclonal antibody to CD38 and the development of an immune-affinity device or GMP-suitable FACS sorting method.

The pace of accrual is expected to be relatively slow, even though an existing pool of 150 potential candidates exists and the projected sample size for this study is only 10. The investigators base their projected accrual rate on prior accrual rates of two to four participants per year.

Withholding PEG-ADA is part of the protocol because its use might blunt the efficacy of gene transfer. A theoretical underpinning of the application of stem cell gene transfer for ADA-SCID is that the effects of low-moderate levels of engraftment of gene-corrected HSC will be amplified to a therapeutic level due to a putative “selective advantage” of gene-corrected lymphocytes and their progenitors. This selective advantage would arise because the genetically uncorrected lymphoid cells either fail to develop or have decreased survival. The blunting of ADA gene-corrected lymphoid cells by ongoing PEG-ADA has been shown in several settings, and the success of the trial from Milan, Italy, in which participants did not receive PEG-ADA, supports this contention.

Withholding PEG-ADA therapy represents a risk of participating in this study, and the investigators agreed to add text in the protocol to describe and clarify this risk.

The direct implications of stopping PEG-ADA therapy are that in the weeks and months following PEG-ADA withdrawal, the patient's lymphocytes that developed during the drug administration would disappear. PEG-ADA withdrawal does not have manifest clinical consequences. However, during the time needed for gene-corrected lymphocytes to develop, individuals would be progressively more at risk of infection, and isolation and prophylactic use of antibiotics, antivirals, antifungals, and intravenous immune globulins will need to be used to reduce such risk.

Children between 7-12 years old will be given a separate assent form, and Appendix MII-C will be amended to state that children 7-12 years old will be given an assent form.

Followup bone marrow aspirations are within the standard of what patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) may encounter and thus were not considered to be above minimal risk for patients undergoing autologous HSCT with gene correction. These aspirations might be important for assessing late problems, like loss of ADA-expressing cells and cytopenias.

Multiple possibilities explain why the final cell product would fail to meet release criteria, including in-process microbial contamination, endotoxin above the cutoff value, or physical loss; it is difficult to estimate the likelihood of these events. Backup marrow will be cryopreserved for each participant, and the backup marrow will be administered if the gene-modified graft is not available. The incalculable risks for not having a transplant graft are also present in clinical trials using autologous and allogeneic HSCT, where a living donor or cryopreserved cord blood unit could unexpectedly become unavailable due to illness or accidents (and no backup exists) after myeloablative conditioning has been administered. To date, the clinical gene transfer laboratories supervised by Dr. Kohn have prepared retroviral vector-transduced CD34+ cells for 25 participants in six clinical trials between 1993 and 2009, and all cell products have met release criteria and have been administered without complication.

It is not logistically possible to give the busulfan after the final cell product is formulated and tested for release criteria, such as endotoxin assay, as the final cell product consists of CD34+ cells in Plasmalyte, an glucose/electrolyte solution without the cytokines and extracellular matrix proteins present during culture which prevent hematopoietic stem cell differentiation and death. The final cell product is administered immediately after it passes release criteria because of its presumed fragility out of culture or out of the body. In contrast, busulfan is typically given as a 2 hour intravenous infusion followed with at least a 24 hour "wash-out" period to allow the busulfan to be cleared before infusion of hematopoietic stem cell grafts which could be damaged by exposure to busulfan. Thus, giving the busulfan only after the final cell product meets release criteria would require at least 26 hours of holding the final cell product under conditions that would damage the graft, and cryopreservation of the transduced stem cells may compromise their viability.

The FDA-mandated 15-year followup is for research participants who have received gene-modified cells, due to the unique nature of the possible complications. Individuals with ADA-deficient SCID will continue with the medical care that is standard for primary immune-deficient patients and for patients post-HSCT, which includes clinical examinations and blood tests.

From the myelosuppression data collected on their clinical trial participants to date, the investigators have noted variability in the time at which participants reach their neutrophilic nadir. Because some participants do not reach nadir until day 30, the investigators have defined non-engraftment at 6 weeks rather than at 4 weeks. Unnecessary administration of the backup cells, which might occur if administered at 4 weeks, carries small risks from the infusion but could blunt the effects of gene transfer.

In all participants treated to date, the investigators have been able to detect ADA enzyme activity at 3 months. Because of the variability in production of ADA enzyme among participants, the investigators have decided that 6 months would be the appropriate timepoint at which to assess whether gene expression is sufficient.

In the ADA gene knockout mouse, a low-level (1 percent to 3 percent) graft of normal or gene-corrected marrow leads to moderately strong reconstitution of immune function, primarily from ADA-deficient

lymphocytes, presumably by a cross-correction mechanism in which the ADA-replete cells are sufficient to achieve systemic detoxification of adenine nucleotides. The investigators posited that cross correction might play a role in immune recovery in human research participants as well.

The busulfan is intended to augment stem cell engraftment, which would manifest as the initial level of peripheral blood mononuclear cell ADA enzyme activity and by the level of long-term stem cell engraftment, allowing production of ADA-expressing myeloid cells to treat this systemic metabolic disease.

No data directly implicate busulfan in neurodevelopmental problems post-HSCT. While neurocognitive problems are seen in many children who survive HSCT, it remains difficult to isolate a single variable because many have had extensive pre-HSCT chemotherapy for malignancies; receive full dose busulfan along with cyclophosphamide; might have had respiratory, metabolic, nutritional, and infectious complications; and/or might have underlying genetic conditions that could lead to central nervous system problems. Nevertheless, the investigators agreed to add a statement about this hypothetical concern to the informed consent document.

During the initial referral period, the investigators will request that each participant's home physician agree to assist with the performance of this research by conducting blood draws and by submitting relevant forms. Assistance and materials will be provided, and these physicians will be provided with copies of the protocol, the informed consent document, and the investigators' brochure; after reading these documents, they will be asked to sign a form that avers they have been trained in relevant topics such as compliant trial conduct and SAE recognition and reporting. It would be impractical and would add risks to the participants if they were required to travel to one of the two study sites for each of the serial study timepoints.

2. Responses to RAC Discussion Questions

Dr. Kohn explained that the protocol includes a futility rule – if no marking occurs in three participants, and no benefit accrues, and all three participants are placed back on PEG-ADA, this result would be considered an SAE and the trial would be stopped. The data safety monitoring board for this trial has not yet been constituted.

Regarding the suggestion to use frozen cells, Dr. Kohn expressed concern that freezing cells at the end of transduction would be problematic and that frozen cells would have to be thawed, formulated, and tested as a final cell product before the research participant could receive them. Preservation of stem cell activity by freezing has not been studied adequately and, although frozen cord blood is not tested for endotoxin after freezing and before infusion, Dr. Kohn did not know if that same approach would be allowed with a manipulated cell product. Dr. Takefman stated that additional testing would be required only if the investigators washed the cells and planned to introduce endotoxin during the washing step.

Dr. Candotti explained that children with DFSP are being included in this protocol because it is a rare malignancy that does not metastasize and does not affect the lifespan of the patient. DFSP tumors are not treated with chemotherapy so having this disease would not change the clinical status of the research participant for this trial. Eight of 14 ADA-SCID patients seen by the investigators during the past 2 years have had DFSP lesions; the investigators currently are testing several theories as to why this is occurring. Dr. Candotti stated that the presence of these tumors would not alter data interpretation from this trial.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Clinical/Trial Design Issues

- While no cases of insertional mutagenesis have occurred in studies using lentiviral vectors, it is critical for the protocol to monitor for this potentially serious event. Monitoring clonal expansion of cells is important. A stopping rule should be considered in the event that clonal expansion is detected with vector integration in the area of a gene known to contribute to oncogenesis.
- DFSP is a dermatologic malignancy. Since the natural history of the condition is not well understood in patients with ADA-SCID, the analysis of adverse events in research participants with DFSP will be complicated. Detecting insertional oncogenesis could be particularly challenging. As such, it may be prudent to make DFSP an exclusion criterion.

Ethical/Social/Legal:

- The informed consent document should be revised as follows:
 - As currently written, the document suggests that infection is an unlikely side effect based on data from 1,700 research participants who received retroviral vectors. It is important to make clear that fewer than 100 participants have received lentiviral vectors.
 - In the discussion of the risks associated with busulfan conditioning, the document should clarify that if the gene-transduced cells cannot be administered (e.g., because the cells do not meet the release criteria), the risk will be assumed without potential benefit.
 - The parenthetical in the last paragraph of the “Alternatives to Participation” section suggests that gene transfer and transplantation are the only alternatives for ADA-SCID. Even if the intent was to cover only those approaches most likely to restore bone marrow function, reference should be made to PEG-ADA therapy as well.
- While the assent document is clearly written, for the most part, two points should be clarified:
 - The statement about the length of the hospital stay should be revised. As currently written, it may raise the participants’ expectations that their parents will be able to stay with them for the duration of the stay.
 - The section explaining the need for a bone marrow aspirate notes that it may be necessary “to return to the hospital” if insufficient cells are obtained. If the return to the hospital is primarily to obtain a second bone marrow aspirate, this should be stated.

G. Committee Motion 6

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Ertl that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XII. Discussion on Potential Symposium Regarding Insertional Mutagenesis

Moderators/Organizers: Dr. Fan; Naomi Rosenberg, Ph.D., Tufts University; Nikunj Somia, Ph.D., University of Minnesota

A. Presentation by the Organizers

Dr. Fan explained that the recent safety modifications to retroviral and lentiviral vectors were designed to decrease the frequency of enhancer-mediated activation of cellular genes. However, the lentiviral vector insertion in the French β -thalassemia trial dysregulated gene expression by an alternative mechanism – aberrant splicing resulting in expression of a truncated mRNA missing microRNA binding sequences involved in downregulation.

Because of advances in the understanding of retroviral and lentiviral vectors and new protocols using modified lentiviral and retroviral vectors in hematopoietic cells, this *ad hoc* working group of the RAC met to discuss whether RAC review of such protocols would be enhanced by a discussion of the current data in the context of a safety symposium. The general question for RAC discussion at this meeting was whether sufficient issues and new data warrant the organization of a safety symposium regarding insertional mutagenesis by retroviral or lentiviral vectors for human gene transfer. Possible symposium questions that had been discussed in the working group and posed to the RAC were:

- While there have been numerous studies of the integration patterns of retroviruses and lentiviruses, what data exist regarding clonal expansion and/or oncogenesis with lentiviral vectors?
- How useful are the available *in vitro* and animal models in predicting events in human gene transfer? If useful animal models do not exist, could they be developed?
- What alternative mechanisms of insertional oncogenesis should be considered?
- How might the different integration patterns for retroviral vs. lentiviral vectors affect the risks of insertional mutagenesis by different mechanisms (e.g., is there higher risk of gene disruption/truncation by lentiviral vectors inserting more frequently into transcription units)?
- What types of preclinical models should be used to detect such events?
- How could retroviral or lentiviral vectors be designed with additional safety modifications to address the alternative mechanisms of insertional mutagenesis?
- What can be learned from the retroviral vectors designed to induce insertional mutagenesis to screen for genes involved in cancer (i.e., how not to design a gene transfer vector)?

Dr. Rosenberg noted that an underlying basis for discussion of this potential symposium has been the singular focus in the vector community on enhancer-mediated activation and its role in oncogenesis as an adverse event following the introduction of these vectors. However, from the retroviral literature it is known that enhancer-mediated activation is only one mechanism by which oncogenesis or permanent alterations to gene function can result from viral integration. The organizers believe that these other mechanisms have not been thoroughly considered.

Dr. Somia added that the symposium would consider what constitutes outgrowth and what percentage of cells would need to have a single integrant before concerns would arise. Invitees might include people who are doing deep sequencing, or the LAM-PCR, who are investigating the contribution of a given integrant to the whole population of transduced cells, or who have designed mutagenizing vectors and then have inserted elements to pick out cancers in cells.

B. RAC Discussion

Questions and comments posed by RAC members included:

- Dr. Federoff asked whether such a symposium would focus on science translating to clinical practice, and therefore translational and clinical investigators would be invited to participate. Dr. Fan replied affirmatively.

- Dr. Federoff queried to as whether risk/benefit issues (especially regarding vulnerable populations) would be important to include.
- Dr. Federoff wondered whether such a symposium would be helpful to the FDA in the context of how regulatory decisions are made. Dr. Takefman agreed that such a symposium would be helpful to the FDA, stating that one potential challenge in the upcoming years will be investigators turning to newer vector designs aimed at improving safety as well as investigators proposing to conduct trials in less severe disease conditions without the animal data to support the design feature.
- Dr. Williams noted the ongoing issue of the lack of consensus of murine models for predicting genotoxicity in human trials, so that a major discussion topic for this symposium could be how to think about using animal models in a way that would be more predictive.
- Rachel Salzman, from the Stop ALD Foundation, suggested that the symposium should include clarification of terminology and nomenclature.
- Dr. Wei suggested that the symposium include discussion of productive data mining that could be undertaken from the studies that have already been conducted.
- Dr. Strome suggested that the symposium include discussion about determining insertional mutagenesis; he noted that the technologies have changed and it is important to consider how to define common insertion sites and standard techniques.
- Dr. Federoff suggested that symposia such as these could be the basis for a monograph, a series of publications, or a dedicated journal volume. Doing so would contribute to the field and help to define better what the RAC does.

Dr. Federoff summarized the discussion, clarifying that this symposium would not be a consensus-reaching meeting. One of the outcomes could be to develop a working group with a goal of discussing in depth about how to proceed with animal models, acknowledging that every clinical indication is different. The RAC members showed much interest in a symposium, with a framework that should be different from the investigator-initiated “stem cell clonality and insertional mutagenesis” meeting held every 18 months. The next step will be for the RAC review an agenda proposed by the organizers. The organizers were asked to reach out to the community to ensure that the draft agenda fits their expectations and needs.

It was agreed that discussion of this potential symposium would continue at an up-coming 2010 RAC meeting.

C. Additional Related RAC Discussion

Dr. Fong asked whether a database of shedding in humans exists for all vectors or whether autopsy data exists that shows distribution of a viral vector in tissues, either of which could guide how new trials move forward. Dr. Corrigan-Curay responded that no such database exists but the OBA database can be searched for a specific autopsy; she indicated that very few SAEs include autopsy data, which she attributed to the nature of obtaining autopsies. Dr. Takefman noted that the FDA is collaborating with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in writing a harmonized guideline on shedding.

XIII. Discussion of Human Gene Transfer Protocol #0910-1002 entitled: A Multiple-Site, Phase II, Safety and Efficacy Trial of a Recombinant Adeno-Associated Virus Vector Expressing Alpha 1 Antitrypsin (rAAV1-CB-hAAT) in Patients with Alpha 1 Antitrypsin Deficiency

Principal Investigators: Terence R. Flotte, M.D., University of Massachusetts Medical School; Noel G. McElvaney, M.D., Royal College of Surgeons; Robert A. Sandhaus, M.D., National Jewish Medical and Research Center; and Bruce Trapnell, M.D., Cincinnati Children's Hospital Medical Center

Additional Presenters: Jeffrey D. Chulay, M.D., AGTC

Sponsor: Applied Genetic Technologies Corporation

RAC Reviewers: Drs. Bartlett, Ertl, and Yankaskas

Dr. Williams recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

The primary objective of the proposed Phase 2 clinical trial is to evaluate the safety and efficacy of a serotype 1 recombinant adeno-associated virus vector expressing alpha-1 antitrypsin (rAAV1-CB-hAAT) administered by intramuscular (IM) injection in patients with alpha-1 antitrypsin deficiency.

Alpha-1 antitrypsin deficiency is an inherited, genetic condition characterized by reduced serum levels of alpha-1 antitrypsin (AAT). The only approved treatment of AAT deficiency is protein augmentation therapy, which has been shown to achieve nadir serum AAT concentrations above 11 μ M, but is not ideal due to high cost, the need for weekly intravenous infusions, and limited availability due to the finite numbers of human plasma donors. Gene transfer with an adeno-associated virus (AAV) vector provides an attractive alternative to protein-based treatment of AAT deficiency.

A previous Phase 1 clinical trial with rAAV1-CB-hAAT in nine AAT-deficient subjects enrolled sequentially in cohorts of three each at doses of 6.9×10^{12} vector genome particles (vg) per patient ($\sim 1 \times 10^{11}$ vg/kg), 2.2×10^{13} vg per patient ($\sim 3 \times 10^{11}$ vg/kg) and 6.0×10^{13} vg per patient ($\sim 1 \times 10^{12}$ vg/kg). Vector administration was well tolerated, with only mild local reactions and one unrelated serious adverse event (bacterial epididymitis). There were no changes in hematology or clinical chemistry parameters. Expression of normal (M-specific) AAT above baseline was observed in all six subjects in cohorts 2 and 3, and was sustained at >30 nM for at least 1 year in the highest dosage level cohort.

The proposed clinical trial is a non-randomized, open-label, multi-center, sequential, three-arm, Phase 2 clinical trial evaluating the safety and efficacy of administration of a rAAV1-CB-hAAT vector administered by IM injection. Three cohorts of three subjects each will receive rAAV1-CB-hAAT on a single occasion at dosage levels of 6×10^{11} vg/kg, 1.9×10^{12} vg/kg or 6×10^{12} vg/kg. Subjects in cohort 1 will receive 10 IM injections, subjects in cohort 2 will receive 32 IM injections and subjects in cohort 3 will receive 100 IM injections. Each injection will be given in a volume of 1.35 mL, at the appropriate vector concentration to achieve the desired total vector dose. The three cohorts will be enrolled sequentially, beginning with Group 1, and will proceed to subsequent groups only after review of safety data by a Data and Safety Monitoring Board (DSMB). After review of safety data from Group 1, participants will be enrolled in Group 2. After review of safety data from Group 2, participants will be enrolled in Group 3.

Safety will be monitored by evaluation of adverse events, hematology and clinical chemistry parameters, histological examination of muscle biopsies, and measurement of serum antibodies to AAT. Efficacy will be measured by evaluation of serum concentrations of M-specific AAT and total AAT, and serum AAT phenotype determined on isoelectric focusing gels. Additional information to be collected will include presence of the vector in blood or semen, changes in serum anti-AAV antibody titers, and changes in T cell responses to AAV and AAT.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Key issues included potential safety concerns regarding the changes in the vector and dosing from those tested in the Phase I trial. The increase in the dose of this potentially more potent vector might lead to a more robust immune response to either the vector or to the alpha-1 antitrypsin produced, which could render a research participant less amenable to the current treatment for this disease. In addition, while IM injection was well tolerated in the initial study, participants in the highest dose cohort in this study will be given 100 injections compared to the 9 injections given to participants in the highest dose cohort of the Phase I trial. This increase in injection number raises new safety issues and should be balanced against the potential benefits in this Phase II trial, given the subtherapeutic levels of transgene production in the Phase I trial.

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

Dr. Bartlett asked the investigators to speculate on reasons for the observation that none of the research participants in the Phase I trial developed antibody responses to AAT. While this was consistent with clinical experience with AAT augmentation therapy, primates dosed with rAAV1-CB-hAAT vector had a clear antibody response with sub-therapeutic serum AAT concentrations. Mice, however, did not mount an antibody response. He wondered whether protein immunization studies had been conducted to demonstrate effective primate responses and lack of a murine response to human AAT. Some prior evidence/speculation supports this concern that the higher expression levels seen with the HSV-produced vector might overwhelm secretory pathways in transduced muscle, leading to the production of misfolded or aggregated protein product and anti-AAT immune responses. Dr. Bartlett requested that the investigators comment on the possibility that production of even a small amount of misfolded enzyme in transduced muscle might lead to an immune response, which could cross-react with properly folded protein or could break tolerance to exogenously administered protein. Along those lines, he asked whether any studies had been conducted to relate serum AAT concentrations to AAT activity and whether any evidence exists of anti-AAT responses to misfolded enzyme in AAT-deficient patients. Noting that the investigators make numerous comparisons to a previous clinical trial with rAAV2-F.IX, in which vector was given IM at up to 90 0.5 ml injections, Dr. Bartlett pointed out that the spacing of vector injection sites in the previous trial is the same as the investigators propose here – 1 cm apart in a grid pattern. However, he observed that volume was considerably less (0.5 ml in that trial compared to 1.35 ml proposed for this trial) and injections in the previous trial were done under ultrasound guidance to minimize the risk of injection into a large blood vessel. Dr. Bartlett asked the investigators to comment on how the larger injection volume per muscle area might influence injection site reactions and why they are not similarly concerned about injection into larger blood vessels.

Dr. Ertl asked for an explanation of the fluctuating AAT levels in one of the high-dose participants in the Phase I trial; that participant first showed an AAT increase, then a decrease, and then an increase that appeared to be sustained. Noting that the doses proposed for this trial appear to be considerably below therapeutic levels (even though AAT is likely to be detected), she asked for clarification as to why this trial is proposed as Phase II and for data based on identical dosing and regimens in cynomolgus monkeys so as to be able to assess whether the trial might result in levels of AAT expression that are at or close to therapeutic levels. Dr. Ertl requested explanation of why this trial has an endpoint of efficacy but also hypothesizes that no efficacy will occur, and she requested to see the toxicology results from the bridging study. She noted that the investigators propose to use an enzyme-linked immunospot (ELISpot) assay to analyze peripheral blood mononuclear cells for T-cell responses to the vector or the transgene product. Instead or in addition, she encouraged the investigators to use an intracellular cytokine staining (ICS) assay; ICS is as sensitive as ELISpot, allows distinguishing between CD4 and CD8 T cells, and provides key information regarding T-cell functionality, which might provide clues to the potential role of AAV-specific T cells in AAV-mediated gene transfer to muscle.

Dr. Yankaskas asked the investigators to explain how they will distinguish local and systemic side effects due to the injected volumes from those due to the viral genome number, given that the planned injection volumes and viral genome numbers are significantly larger than those used in previous human studies. Noting that the nonclinical rAAV1-CB-hAAT studies in mice and rabbits found dose-dependent injection

site inflammation and vector biodistribution in both species, he requested a description of the detailed results, including the organs examined in each species and the time course of the studies. He also requested the toxicology results of the ongoing rAAV1-CB-hAAT in C57BL/6 mice. Dr. Yankaskas asked what would be done if immune responses to AAT protein were detected in this study and whether the effectiveness and sensitivity of the planned muscle biopsy studies – vector genome quantification and antibody stains – have been established in human muscle biopsy tissues.

C. RAC Discussion

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

- Dr. Wei asked about the timepoint for measuring serum AAT.
- Dr. Roizman asked whether the investigators plan to monitor for the presence of HSV DNA proteins and whether they expect to see antibody to the experimental products.
- Noting that the frequency of seropositivity to HSV1 is approximately 80 percent in adults and assuming HSV will be a contaminant in rAAV1-CB-hAAT, Dr. Federoff suggested that the investigators determine whether immune responses to HSV are elicited in any of the preclinical studies currently being conducted.
- Dr. Kahn asked about the purpose of collecting semen samples at five timepoints – once before administration and four times thereafter. He suggested that the informed consent document clarify the purpose for which semen specimens would be collected. If the purpose is to look for vector in the semen samples, Dr. Kahn suggested that the investigators inform participants that they might be at risk of shedding virus in their semen. In addition, he suggested that the investigators disclose what would be done with the semen samples, what they would be tested for, and ultimately what would happen to the samples.

D. Investigator Response

1. Written Responses to RAC Reviews

One research participant in the Phase I trial first showed an increase, then a decrease, and then an increase in serum AAT levels that then appeared to be sustained. The investigators acknowledged that they do not have a definitive explanation for the fluctuation of serum AAT levels observed in this individual. The fluctuations within the first few months are within twofold of the background level and the investigators posited that those fluctuations might be related to the precision of the assay.

The investigators plan to perform ELISpot assays as specified in the protocol. If antigen-specific T cells are present at high enough frequencies, they agreed to consider performing intracellular cytokine staining (ICS) assays, as recommended by Dr. Ertl, to distinguish between CD4 and CD8 T cells and to evaluate T-cell functionality. ICS assays were used in the Phase I study.

An immune response to human AAT has been observed in every nonhuman species in which it has been evaluated, including mice, rabbits, baboons, and cynomolgus macaques. Because the sequence differences between human and macaque AAT is at least as great as the difference between human and baboon AAT, the investigators stated that they were not surprised that expression of human AAT in cynomolgus macaques induced an anti-hAAT antibody response. They stated that they know of no data to suggest that there is an adjuvant effect of HSV-produced recombinant AAV vectors. Anti-AAV responses were not measured in the studies comparing HSV-produced and transfection-produced rAAV1-CB-hAAT.

There is no evidence for misfolding of hAAT secreted from muscle. Although muscle is not normally considered as a source of secreted proteins, skeletal muscle is the primary source of plasma gelsolin, an actin-binding protein present in serum at >200 µg/mL. Muscle-secreted hAAT binds to human neutrophil

elastase (hNE) in a dose-dependent manner, with the same optimal molar ratio (1:1) as does authentic, liver-derived human serum AAT, and both muscle- and liver-expressed hAAT interact with hNE and form a similar complex as measured by SDS-PAGE, indicating that murine muscle-secreted hAAT is functional.

Regarding studies that have been conducted to relate serum AAT concentrations to AAT activity, the investigators stated that serial dilutions of serum from mice that had received an IM injection of rAAV1-CB-hAAT showed serum concentration-dependent inhibition of elastase activity. In addition, mouse serum containing muscle-secreted hAAT at a concentration of 2.25 µg/mL had elastase inhibitory activity that was essentially the same as the elastase inhibitory activity of normal mouse serum to which authentic hAAT (purified from normal human plasma) was added at the same concentration.

The investigators report no evidence of anti-AAT responses to misfolded enzyme in AAT-deficient patients.

The investigators explained that the difference in injection volume proposed for this trial compared to the volume used in the Phase I clinical trial (1.35 mL vs. 1.1 mL) is not great and is not anticipated to have an important impact on injection site reactions, but any reactions will be evaluated during the trial. The decision not to use ultrasound guidance during injection in this Phase II trial was based on experience from the Phase I trial, in which ultrasound guidance did not appear to offer an advantage over the usual clinical practice of pulling back on the syringe plunger to confirm that the needle is not in a blood vessel before injecting the contents of the syringe into the muscle.

To distinguish between AEs related to the injected volumes and those related to the vector genome number, the investigators either could maintain a constant injection number and volume and vary the vector concentration or could include a placebo control group. The investigators used the first of these approaches in the Phase I trial. If dose-limiting toxicity is observed in this Phase II study and if it is deemed important to distinguish between the two dose-limiting toxicity possibilities, the investigators agreed to consider conducting a subsequent study with a placebo group.

The nonclinical rAAV1-CB-hAAT studies in mice and rabbits found dose-dependent injection site inflammation and vector biodistribution in both species. Regarding mice, the injections were well tolerated in all animals, there were no significant differences in hematology or clinical chemistry values between vector-treated and control animals, and no gross pathology findings were related to the test article. The only finding believed to be vector- and dose-related was mild, focal, chronic inflammation at the site of injection within the skeletal muscle; this inflammation was not associated with obvious clinical detriment to the animals and no significant or consistent changes were seen in any other organs. Vector DNA was present in most organs tested in a dose- and time-dependent manner, with the highest concentrations detected in blood and skeletal muscle. Lower concentrations were detected in liver, spleen, heart, kidney, and lung, and vector DNA was detected only at the highest dosage level tested in jejunum, pancreas, gonads, brain, and spinal cord. Vector DNA concentrations decreased over time in most organs except skeletal muscle, where there was little change between Day 21 and Day 90 after injection.

Regarding rabbits, the injections were well tolerated in all animals. The only significant finding from the clinical chemistry panel was an elevated creatine kinase level during the first 24 hours after injection in both vector-treated and control animals. No gross pathology findings were related to the test article. The only test article-related histopathological findings that were believed to be treatment-related were minimal to mild chronic inflammation at the site of injection within the skeletal muscle in vector-injected animals at all dosage levels compared to the vehicle controls. The inflammation was not associated with obvious clinical detriment to the animals and no significant or consistent changes were seen in any other organs examined. In addition, AAT staining confirmed target cell transduction with transgene expression. Vector DNA was present in blood, liver, gonads, and semen in a dose- and time-dependent manner. High levels of vector DNA in blood on Day 1 declined rapidly, and levels of vector DNA in blood and liver declined slowly after Day 21 and remained positive through Day 90. Vector DNA was detected in gonads beyond Day 21 only at the highest dosage level, and low levels of vector DNA were detected in semen only

during the first week after injection and not thereafter. No vector DNA was detected in the brain of any animal.

If immune responses are detected, the investigators explained that they would attempt to correlate these responses with the magnitude and duration of immunoglobulin M-specific AAT concentration and the magnitude and characterization of any inflammatory response in muscle biopsies, and they would evaluate the ability of serum from such participants to inhibit the activity of AAT *in vitro*. However, they reiterated their belief that immune responses to AAT are unlikely to develop in this study.

2. Responses to RAC Discussion Questions

Regarding the timepoint for measuring efficacy, Dr. Chulay explained that participants' AAT levels would be measured serially until day 365, and efficacy would be defined as achieving a level of 11 μ M of AAT on an ongoing basis. AAT levels would be measured every 2 weeks for the first 3 months, and then every 3 months through month 12; participants who have sustained expression through month 12 would be monitored yearly for an additional 4 years, for a total of 5 years of followup. When 12-month data are obtained for all participants, the investigators will complete a clinical study report and submit it to the FDA.

The purpose of semen sample collection is to establish whether or not there is the potential for inadvertent vertical transmission of vector DNA.

The investigators agreed to be explicit, in the informed consent document, about the use of the semen samples and the possible risk of virus shedding in their semen.

Dr. Chulay stated that the investigators had not looked for antibodies to HSV in animals that have been injected with it, but agreed to consider doing so.

Dr. Flotte agreed to determine whether immune responses to HSV are elicited, using the already-collected serum from the animals that received higher and proportional doses. These data would be useful since these animals probably would not have background-level responses to the HSV1 product.

E. Public Comment

Susan Ferro, of AlphaNet, Inc., described her family's experience with AAT deficiency; her mother, her twin brothers, and she are afflicted. She talked about the current treatment for this genetic problem and the positive potential for the AAT-deficiency community if the proposed gene transfer trial were successful. She expressed personal willingness to submit to 100 injections, as proposed in this trial, because doing so might help others in her family who have inherited AAT deficiency.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- Since HSV is being used to generate a more potent vector, it is important to understand the risks of transmission of HSV DNA or proteins that might lead to an immune response upon vector application. As such, serum samples collected in the preclinical studies should be reexamined to determine whether either HSV DNA or HSV proteins were transferred and whether antibodies to HSV developed.

Clinical/Trial Design Issues

- In the Phase I protocol, a T-cell response to AAV capsid was observed, but there was no evidence that this response interfered with sustained transgene expression. Including an assay

to analyze for T-cell responses against the vector and the transgene product should help elucidate the vector responses. However, rather than an ELISpot assay, which was used in the Phase I study and for which there were variations in the positive control, it might be better to use another assay. For example, ICS is as sensitive as an ELISpot assay, but also can distinguish between CD4⁺ and CD8⁺ T cells and could provide key information of T-cell functionality. Such data may provide important information as to the potential role of AAV-specific T cells on AAV-mediated gene transfer to muscle.

- In addition to reexamining the preclinical serum samples for evidence of HSV infection, an assay should be added to the protocol to monitor participants for an immune response to HSV, both antibody and T-cell response.

Ethical/Legal/Social Issues

- The informed consent document should clarify the risk of germline transmission. Although the consent refers to the need to collect five semen samples throughout the study, insufficient information is provided to explain why the samples are being collected, how they will be used, how long they will be retained, and whether any additional studies will be performed. The informed consent document also should state that participants will be notified immediately of a positive test result in any participant.

G. Committee Motion 7

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Zaia and seconded by Dr. Ertl that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XIV. Major Action: Introduction of Tetracycline Resistance into *Chlamydia trachomatis*

Presenters: Harlan D. Caldwell, Ph.D., Rocky Mountain Laboratory (RML), NIH; Dr. Kirchhoff; Nancy P. Hoe, RML, NIH; James M. Schmidt, M.D., M.S., Occupational Medical Service, NIH

A. Introduction by Dr. Kirchhoff

Dr. Kirchhoff reviewed the characteristics of and process for a Major Action under the *NIH Guidelines*. Creation of a novel microorganism that is resistant to a therapeutic drug raises potential public health issues and requires RAC review and NIH Director approval. Public review and RAC/NIH Director approval of this type of experiment occurred in 2007 for the transfer of tetracycline resistance (tet^R) to *Chlamydia trachomatis* and in 2008 for the transfer of chloramphenicol resistance to *Rickettsia conorii*.

He discussed introducing tet^R into *C. trachomatis* and the NIH requirements for such work. Compared to currently approved research, the differences proposed by Dr. Caldwell are:

- Probe sonication would be used rather than cup sonication with respiratory protection.
- Laboratory staff with known allergy or sensitivity to azithromycin would not be required to be excluded from this research.
- Medical cards would include a 24-hour NIH OMS number but not a CDC contact phone number for reporting exposure or infection.

Chlamydia trachomatis is an obligate intracellular pathogen with significant public health impact. It is a major cause of sexually transmitted disease worldwide, leading to cervicitis, urethritis, pelvic inflammatory disease and consequent infertility, epididymitis, lymphogranuloma venereum (LGV) (a genital ulcer with adenopathy syndrome), and proctitis. Ocular strains of this pathogen are the major cause of preventable blindness outside of the United States and tetracycline cream is often used to treat disease caused by

this pathogen. It was for this reason that in its previous review of the introduction of tetracycline resistance into *C. trachomatis* that the NIH Director adopted the RAC recommendation prohibiting work with *C. trachomatis* serovars A, B, or C to be conducted in the same laboratory in which tet^R is being introduced in the *C. trachomatis* serovars that cause genital disease, to avoid cross contamination and introduction of the tet^R gene into the serovars that cause ocular disease.

Dr. Caldwell proposed to work with strain L2(25667R), an L serovar, which is associated with an invasive form of disease, LGV. Currently available antibiotics for LGV include doxycycline, erythromycin and more recently azithromycin, a macrolide antibiotic related to erythromycin. Genital disease caused by other serovars of Chlamydia can be treated with these antibiotics as well as the fluoroquinolones. No work with *C. trachomatis* serovars A, B, or C shall be conducted in the same laboratory in which tet^R is being introduced in the *C. trachomatis* serovars that cause genital disease, to avoid cross contamination and introduction of the tet^R gene into the serovars that cause ocular disease.

The NIH Director approved transfer of tet^R in nonocular strains of *C. trachomatis* in genital strains at BL2 containment with BL3 practices, for Daniel Rockey, Ph.D., and Walter E. Stamm, M.D., only; other investigators who wish to conduct similar experimental work must have their proposed work reviewed by the RAC and approved by the NIH Director.

B. Presentation by Dr. Caldwell

Dr. Caldwell discussed major chlamydial diseases of humans, the chlamydial developmental cycle, control of chlamydial diseases, vaccine challenges, obstacles in the development of a chlamydial genetic system, why a LVG is being selected as a tet^R transformation target, proposed tet^R constructs, and the primary differences between his proposed experiments and the related previously approved studies.

C. Presentation by Ms. Hoe

As the RML biosafety officer, Ms. Hoe discussed risk mitigation issues proposed to accompany these proposed experiments, including dedication of a BL2 laboratory with an electronic card reader for entry, practices and procedures, and planned modifications based on RAC recommendations that include replacing probe sonication with cup sonication and modifying exposure reporting to be consistent with the Medical Services Program for after-hours exposures.

All workers would be trained on the standard procedures submitted in the RML proposal, and all work with the infectious material would be performed in a biosafety cabinet with the workers wearing lab coats, gloves, and an N100 respirator. Work with the ocular serovars would not be performed at the same time as experiments involving tet^R. All potential and confirmed tet^R strains would be stored in the restricted access laboratory, and intra-facility transfers of these strains would be coordinated and approved through the biosafety officer.

D. Presentation by Dr. Schmidt

Dr. Schmidt discussed the request to preclude individuals from working on this research if they have a history of allergy or sensitivity to azithromycin. He reported having checked with four infectious disease specialists, all of who agreed that a broader spectrum of antibiotics – four quinolones or other macrolide antibiotics – could be used.² Only two people will be working on this proposed research and neither of them has a problem with azithromycin and would not be precluded from participating in the research. In general, he noted that there is no way to test for “sensitivity” to azithromycin other than by self-report, but he agreed to defer to the RAC on this issue.

² Please see the minutes of the March 10-11, 2010 meeting of the RAC for clarification of this statement.

E. RAC Discussion

Regarding laboratory workers who might be allergic or sensitive to azithromycin, Dr. Ertl suggested advising them that their allergies would deprive them of two options – tetracycline to which is the altered organism is now resistant) and the drug to which they are allergic – but that others are available. They can then decide whether or not to work on that experiment.

Dr. Fong expressed concern about what would happen once the tet^R strain was transferred to another institution, and asked who would assure that these same biosafety procedures were followed.

Dr. Corrigan-Curay explained that the Major Action covers creation – but not transfer – of the strain. The NIH places primary responsibility on the IBC of the institution where a strain is created to communicate with the institution to which the strain would be transferred, to make sure that the related responsibilities are understood. All IBCs should follow the standard procedures as specified in the *NIH Guidelines*. Communication would be IBC to IBC; transfer would be covered by the NIH material transfer agreement and possibly other mechanisms.

F. Committee Motion 8

Dr. Roizman moved that this Major Action request be transmitted to the NIH Director, noting the approval of the RAC. The motion was seconded by Dr. Ertl. The vote was 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XV. Closing Remarks and Adjournment/Dr. Federoff

Dr. Federoff thanked the RAC members and the OBA staff and adjourned Day 2 of the December 2009 RAC meeting at 3:00 p.m. on December 2, 2009.

[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: RAC Roster Recombinant DNA Advisory Committee

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Adriana Byrnes, NCI
Harlan Caldwell, DHHS/NIH
Jeffrey Chulay, Applied Genetic Technologies Corporation
Fabio Condotti, NHGRI
Tim Cripe, Cincinnati Children's Hospital
Boro Dropulic, Lentigen
Susan Feno, AphaNet, Inc.
Lisa Ferstenberg, Accelovance
Terence Flotte, University of Massachusetts
Linda Griffith, NIAID
Nancy Hoe, DOHS/NIH
Margaret Humphries, University of Massachusetts Medical School
Syed Hussain, CBER/FDA
S. Karl, FDA
Jocelyn Kaiser, *Science* Magazine
Scott Kennedy, Solving Kids' Cancer
Nick Leschly, Third Rock Ventures
Douglas Losordo, Northwestern Memorial Hospital
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Kit Shaw, UCLA
Bruce Trapnell, Cincinnati Children's Hospital
Amanda Trevisan, Accelovance
Carolyn Wilson, FDA/CBER
Hatem Zayed, Lentigen

Attachment III Abbreviations and Acronyms

AAT	alpha-1 antitrypsin
AAV2	Adeno-Associated Virus Serotype 2
ADA	Adenosine Deaminase
BMBL	<i>Biosafety in Microbiological and Biomedical Laboratories Manual</i>
BMT	bone marrow transplantation
BL	biosafety level
BWG	RAC Biosafety Working Group
CCHMC	Cincinnati Children's Hospital Medical Center
cDNA	complementary deoxyribonucleic acid
DFSP	dermatofibrosarcoma protuberans
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ELISpot	enzyme-linked immunospot
FDA	Food and Drug Administration, DHHS
FR	<i>Federal Register</i>
GM-CSF	granulocyte-macrophage colony stimulation factor
GTSAB	Gene Transfer Safety Assessment Board
HIV	human immunodeficiency virus
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
IBC	institutional biosafety committee
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IL-2	interleukin-2
IM	intramuscular
LCA	Leber Congenital Amaurosis
MED	minimum effective dose
MI	myocardial infarction
MTD	maximum tolerated dose
NCI	National Cancer Institute, NIH
NHP	nonhuman primate
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NTN	neurturin
NYHA	New York Heart Association
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
PI	principal investigator
PLR	pupillary light reflex
rAAV1-CB-hAAT	recombinant adeno-associated virus vector expressing alpha-1 antitrypsin
RAC	Recombinant DNA Advisory Committee
RML	Rocky Mountain Laboratory, NIH
RNA	ribonucleic acid
RPE	retinal pigment epithelium
SAEs	serious adverse events
SCID	Severe Combined Immunodeficiency
SDF-1	stromal cell-derived factor-1
SPECT	single photon emission computed tomography

tet^R
TILs
UCLA

tetracycline resistance
tumor-infiltrating lymphocytes
University of California, Los Angeles