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RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 21, 2006

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

Contents

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

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

June 21, 2006

The Recombinant DNA Advisory Committee (RAC) was convened for its 104th meeting at 8:00 a.m. on June 21, 2006, at the National Institutes of Health (NIH), Building 31C, Conference Room 10, Bethesda, Maryland. Dr. Diane Wara (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 4:50 p.m. on June 21. The following individuals were present for all or part of the meeting:

Committee Members

Stephen Dewhurst, University of Rochester Medical Center
Howard J. Federoff, University of Rochester
Terry Kwan, TK Associates
Nicholas Muzyczka, University of Florida
Glen R. Nemerow, The Scripps Research Institute
Madison Powers, Georgetown University *(via teleconference)*
Naomi Rosenberg, Tufts University
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities
Richard G. Vile, Mayo Clinic College of Medicine *(via teleconference)*
Diane W. Wara, University of California, San Francisco
David J. Weber, The University of North Carolina at Chapel Hill

Office of Biotechnology Activities (OBA) Director/RAC Executive Secretary

Amy P. Patterson, Office of the Director (OD), NIH

***Ad Hoc* Reviewers and Speakers**

David H. Abramson, Memorial Sloan-Kettering Cancer Center *(via teleconference)*
Debuene Chang, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH
Karl Csaky, National Eye Institute (NEI), NIH
Raynard S. Kington, OD, NIH
Ake Lernmark, University of Washington *(via teleconference)*
Hugo W. Moser, Johns Hopkins University

Nonvoting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services (DHHS)
Stephanie L. Simek, U.S. Food and Drug Administration (FDA), DHHS
Daniel M. Takefman, FDA, DHHS

NIH Staff Members
Kelly Fennington, OD
Linda Gargiulo, OD
Mary Groesch, OD

[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.

Kathryn Harris, OD
Laurie Lewallen, OD
Maureen Montgomery, OD
Marina O'Reilly, OD
Gene Rosenthal, OD
Thomas Shih, OD
Frosso Voulgaropoulou, National Institute of Allergy and Infectious Diseases
Daniel G. Wright, NIDDK

Others

There were 62 attendees at this 1-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 these Minutes.

I. Call to Order and Opening Remarks/Dr. Wara

Dr. Wara, RAC Chair, called the meeting to order at 8:00 a.m. on June 21, 2006. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on June 2, 2006 (71 FR 32108). Issues discussed by the RAC at this meeting included public review and discussion of five protocols, a gene transfer safety assessment board report, and a presentation and discussion regarding biosafety considerations for research involving lentiviral vectors.

Dr. Patterson reminded the RAC members of the rules of conduct that apply to them as special Federal Government employees.

II. Certificates of Appreciation for RAC Member Service to the NIH

Presenter: Raynard S. Kington, M.D., Ph.D., Deputy Director, NIH

Noting that the RAC has been a template for other committees at the NIH and elsewhere, Dr. Kington thanked the three RAC members whose service was ending with this RAC meeting. Dr. Kington recognized and thanked Ms. Kwan with a certificate. Dr. Wara, who had served on the RAC for five years and as its chair for two years, received a certificate plus a set of bookends of NIH Building 1. She thanked all RAC members, FDA representatives, and the OBA staff, particularly Dr. Patterson. Dr. Powers, the third member completing his service to the RAC, participated in this RAC meeting via teleconference.

III. Minutes of the March 15, 2006, RAC Meeting/Drs. Somia and Weber

Dr. Somia noted that the March 2006 RAC minutes was an accurate representation of the meeting. Dr. Weber stated that the minutes summarized complex topics in a concise way.

A. Committee Motion 1

It was moved by Dr. Somia and seconded by Dr. Weber that the RAC approve the March 15, 2006, RAC meeting minutes. The vote was 10 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Discussion of Human Gene Transfer Protocol #0604-769: A Phase I, Randomized, Placebo-Controlled, Open-Label, Cross-Over Safety and Pharmacodynamic Study of BHT-3021 in Subjects with Recent Onset Type 1 Diabetes Mellitus

Principal Investigator:	Peter A. Gottlieb, M.D., University of Colorado at Denver and Health Sciences Center
Submitters:	Patricia Murphy, Bayhill Therapeutics, Inc., and Nanette Solvason, Ph.D., Bayhill Therapeutics, Inc.
Other Presenters:	Erica J. Evans, Ph.D., Bayhill Therapeutics, Inc.; Hideki Garren, M.D., Ph.D., Bayhill Therapeutics, Inc.; Barbara Hickingbottom, M.D., J.D., Bayhill Therapeutics, Inc.; and Frank H. Valone, M.D., Bayhill Therapeutics, Inc.
RAC Reviewers:	Drs. Dewhurst and Heslop *(written review only)* and Ms. Shapiro
Ad hoc Reviewer:	Dr. Ake Lernmark, University of Washington

A. Protocol Summary

Type 1 diabetes is an autoimmune disease in which an individual's immune system attacks normal proteins produced by the pancreas and causes damage to the beta cells in the pancreas that produce insulin. BHT-3021 is a plasmid expression vector encoding full-length human proinsulin protein under the control of the cytomegalovirus immediate-early promoter/enhancer. Preclinical studies conducted at Bayhill using the murine homolog of BHT-3021 demonstrate efficacy in preventing development of diabetes in the Non-obese Diabetic (NOD) mouse model. This effect appears to be long-lasting as hyperglycemic NOD mice treated for 8 weeks did not develop diabetes during an additional 16 weeks of follow-up. Moreover for many hyperglycemic NOD mice, treatment with mouse homolog of BHT-302 1 reversed hyperglycemia and restored normal blood glucose levels. This observation suggests that pancreatic damage can be reversed in patients if the autoimmune process can be stopped prior to complete destruction of residual beta-cell function. Mechanism of actions studies showed that the murine homolog of BHT-3021 decreased the immune response to autoantigens, particularly the response to insulin.

This is a multi-center, randomized, double-blind placebo-controlled phase 1 trial in patients with type 1 diabetes (TD1). Subjects with T1D will be screened for eligibility. Key eligibility criteria are T1D diagnosed within 3 years of randomization, residual pancreatic β-cell function measured by stimulated C peptide level and a positive test for anti-insulin antibodies. Two dose levels of BHT-3021 will be tested: 1 .O mg and 3.0 mg. A cohort of twelve Subjects will be treated at each dose level for a total of twenty-four subjects. Subjects will be randomized to BHT-3021 or placebo in a 3:l ratio. BHT-3021 or BHT-placebo are administered intramuscularly weekly for 12 weeks (Weeks 0 to 11). Four weeks after the last dose of study drug (Week 15) each Subject undergoes a complete evaluation for safety, pancreatic function and anti-insulin immune responses after which the Subject's treatment assignment is unblinded. Subjects who received BHT-3021 enter Long Term Follow-up Period during which they are monitored for delayed adverse events, pancreatic function and anti-insulin immune responses. Subjects who received BHT-placebo subsequently receive 12 doses of open label BHT-3021 at their cohort's dose level. These cross-over Subjects are fully evaluated four weeks after the last dose of study drug after which they enter the Long Term Follow-up Period. An independent Data Safety Monitoring Board (DSMB) will monitor patient safety and provide advice on trial operations.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included concerns about the immunization of relatively healthy prediabetic individuals with a vaccine that may have the potential to cause more rapid disease progression and to reduce the effectiveness of the main therapy, insulin, in research participants who do progress.

Dr. Dewhurst stated that the risk of anaphylaxis was his most serious concern and requested an update on the preclinical study to test the potential for anaphylaxis following repeated vaccine dosing. He suggested that the anaphylaxis study include a group receiving both vaccine and insulin as most research participants would be receiving both. In addition, procedures should be in place in case of anaphylactic reaction, and inclusion of this possibility should be in the informed consent document along with the possibility that participants may not longer respond to insulin injections. He asked the investigators to comment on the data suggesting a trend towards accelerated diabetic disease progression. He also asked whether the inhaled form of insulin would change either the immune response or the outcome and about the choice of the saline placebo rather than an irrelevant plasmid control.

Dr. Heslop requested discussion of the preclinical data that suggested potential risk for more rapid disease progression. She asked the investigators to comment on the predictive ability of their animal model, given the differences in diabetes between mice and humans. Dr. Heslop also requested an update on the investigators' vaccination study in multiple sclerosis (MS), particularly whether any participants' symptoms have worsened. She asked for additional details on the placebo to be used and whether the investigators plan to follow the research participants for longer than 4 years, and she asked the investigators to consider adding a request for autopsy to the informed consent document.

Ms. Shapiro asked the investigators to describe the procedures that would take place in the event that a research participant suffers an anaphylactic reaction following a DNA injection, noting that the possibility of anaphylaxis should be included in the "risk" section of the informed consent document. She requested that the investigators expand on the risk-benefit evaluations since the participants will be relatively healthy, the vaccine could cause more rapid disease progression, and the vaccine could reduce the effectiveness of mainline therapy for those participants who do progress to diabetes. To appropriately evaluate risks in relation to benefits in this protocol, Ms. Shapiro asked the investigators to provide details about what would happen if a participant were to develop a significantly diminished response to insulin as a result of this clinical trial.

Dr. Lernmark noted that the research participants with new onset type I diabetes are likely to have similar immunogenetic backgrounds which may influence their response to the vaccine. For instance, type I diabetic patients with the HLA-DR3 haplotype have a poor response to hepatitis B. He suggested that it would be useful to determine the immunogentic reaction to the plasmid used in the vector. Preclinical studies may be useful using B10 mice to determine immune reactions (e.g., pro-insulin antibodies, cell mediated responses, and biopsy of infection site) for comparison against H2 type.

C. RAC Discussion

During the meeting, the following additional questions and issues were raised:
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- Ms. Kwan asked the investigators to consider incorporating the model language for autopsy, which may be found in the informed consent guidance document on the OBA Web site (see http://www4.od.nih.gov/oba/rac/ic/appendix_m_iii_b_2_c.html). Regardless of how remote the possibility of death, autopsy information adds to the scientific knowledge database on gene transfer.

- Dr. Federoff wondered whether the investigators had considered other antigens related to proinsulin that may be inadvertently targeted in an immunization strategy, such as insulin-like growth factor 1 or others.

- Dr. Weber expressed concern about the nature of the cross-over design that the investigators propose to implement after 15 weeks: Rather than maintaining a control group, they propose to move participants from the control group to the active group at that time. He asked the investigators why they chose not to continue following the placebo group as a placebo group, and Dr. Weber wondered how they will know whether observed effects in the long-term safety studies are related to the intervention if a control group no longer exists.

- Dr. Weber asked the investigators about their safety criteria for moving to a higher dosing level and what role the data and safety monitoring board (DSMB) would play in assessing how the dose escalation would be implemented.

- Dr. Weber noted that the informed consent document states that, if there are adverse events (AEs) that relate to the study, participants will be reimbursed for all or part of the expenses incurred. He asked the investigators to define what "part" would be covered and suggested that the investigators make explicit the expense reimbursement plan for participants.

D. Investigator Response

The multiple sclerosis clinical trial involved the use of a plasmid with an identical backbone expressing human myelin basic protein (MBP). Dosing has been completed and all participants have entered follow-up. At all three doses, treatment related AEs were equal or lower than for placebo and there was no evidence of disease worsening by clinical or brain MRI measures.

The objective of the six-month non-human primate study was to determine the potential toxicity of repeat dosing of BHT-3021 when administered by weekly IM injection over 13 or 26 week period and to assess late onset of any toxicity or reversibility of toxicity after a recovery period. The study was ongoing, but to date no adverse clinical effects were observed. One male animal died due to severe hypoglycemia but the death was not considered to be due to the agent administration.

Anaphylaxis studies were performed in groups of young NOD mice that received treated with different doses of proinsulin II DNA or a positive control, the immunodominant β9-23 region of insulin, which induces fatal anaphylaxis. No signs of anaphylaxis were noted in any of the DNA treated mice at any dosing frequency or route of administration. While the mice in this study did not receive both plasmid and DNA, in other studies to date in which insulin was administered, no toxicity was associated with the combination.

In the event that an anaphylactic response should occur, the attending physician would render the necessary treatment in accordance with good medical practice and the event reported to the data safety monitoring board which would make decisions about dose escalation and the continuation of the study. Dr. Hickingbottom explained that the research participants will be required to remain in the clinic for several hours after dose administration.

Regarding the choice of a saline placebo, saline will be used to ensure blinding of treatment assignment without the potential risk of an immune response to any immunostimulatory sequences in the plasmid. Experiments were performed using null vectors without an effect on NOD disease course.

In response to Dr. Dewhurst's concern about two groups of male animals showing a change in the timed blood glucose concentration at Day 22, which might suggest a decrement in the animals' ability to respond to insulin, Dr. Evans explained that the clinical pathology reviewer at the toxicology lab indicated that, although this result was statistically significant, it was considered to be due to individual animal variation within the group of nonhuman control animals. The insulin level of the control animals on the day in question was much lower than usual. Because the glucose level is tested in a fasting state, the time of day or the hours between when the food was removed the night before might affect the blood glucose on any particular day.

In response to Dr. Lernmark's comments, Dr. Evans explained that the investigators have conducted a study in nonhuman primates (cynomolgus monkey) to look at immunological responses to plasmid while monitoring antibody production and injection site reactions to the plasmid. Although not yet available, these data will be part of the safety package.

Dr. Garren explained that, because the protocol will not provide costimulation, other antigens are not expected to be cross-stimulated. DNA vaccines are a poor immunogen, and the investigators have not

seen any evidence of cross-immunization in nonhuman animal studies, either in this proinsulin product or MBP plasmid.

Regarding the proposed crossover of control group participants after 15 weeks, Dr. Hickingbottom noted that the FDA also commented on concerns about this proposed structure. On the basis of those comments, the investigators have revised their proposal and will not cross participants over until 1 year after they have received their first dose of either placebo or the active drug. In addition, the investigators plan to follow these participants in a blinded fashion.

Dr. Valone clarified Bayhill Therapeutics' position about participant reimbursement—participants who are injured as a part of a Bayhill trial pay nothing. Some ambiguity may exist about who pays what—the participating hospital, the individual's insurance, or Bayhill Therapeutics. However, company policy is that participants should have no out-of-pocket expenses, and Bayhill even pays for parking, transportation, and any other cost that may be considered beyond normal medical care but is associated with a clinical trial.

E. Public Comment

Dr. Borror stated that the informed consent document must be accurate, particularly about the issue of participant compensation. She also requested that language discussing the use of birth control be clarified on page 5 in the section about the risk of gene changes to future children.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- The immune response induced by inhaled insulin can differ from the response induced by injected insulin. Since some research participants may be using inhaled insulin, it would be advisable to study its effects in the mouse studies.

- Immunogenetics may cause different immune responses to the vaccine. Therefore, it may be useful to explore the possibility that immune responses may differ in relation to H2 type by assessing pro-insulin antibodies, cell mediated immune responses to the plasmid, and histopathology at the injection site as related to the H2 type in the mouse model. Determining the HLA types of the research participants may also be helpful.

- The cross-over study should be designed to allow interpretation of long term safety results.

- The protocol "stopping rules" should be clarified prior to initiation of the study.

- The informed consent document should be modified in the following ways:
    - the placebo should be referred to as "buffered salt water;"
    - the reproductive risks of the protocol and recommendations for use of birth control should be clarified;
    - the discussion about the provision of medical treatment for research related injuries should be clarified; and
    - a request for autopsy should be added.

G. Committee Motion 2

It was moved by Dr. Dewhurst and seconded by Dr. Weber that the RAC recommendations, summarized orally by Dr. Wara, be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. Although not reminded specifically, RAC members were aware that they were voting on the issues raised, not on the specific wording of the recommendations. The vote was 10 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Discussion of Human Gene Transfer Protocol #0604-774: A Phase I, Multicenter Study Evaluating the Safety and Potential Activity of Three Escalating Doses of hMaxi-K Gene Transfer in Female Participants with Overactive Bladder Syndrome and Detrusor Overactivity: Double-Blind, Imbalanced, Placebo-Controlled Design within Three Sequential Active Treatment Groups

Principal Investigator:	Andrew McCullough, M.D., New York University School of Medicine
Submitter:	Arnold Melman, M.D., Albert Einstein College of Medicine
Sponsor:	Ion Channel Innovations, LLC
Other Presenters:	George J. Crist, Ph.D., Ion Channel Innovations, LLC, and Kelvin P. Davies, Ph.D., Ion Channel Innovations, LLC
RAC Reviewers:	Dr. Federoff, Ms. Kwan, and Dr. Nemerow
Ad hoc Reviewer:	Debuene Chang, M.D., NIDDK, NIH

A. Protocol Summary

Plasmid DNA encoding the gene hMaxi-K is proposed to be used for gene transfer through a urethral catheter in the bladder lumen for the indication of overactive bladder syndrome. More than 17 million people in the United States have overactive bladder (OAB), and 15 percent to 30 percent of people older than 60 years who live at home have incontinence. In addition, at least half of the 1.5 million Americans who reside in nursing homes are incontinent. However, despite $1 billion spent in the United States alone, the utility of oral therapy for OAB has been hampered because the currently available drugs have limited effectiveness, must be taken daily, and produce significant side effects such as dry mouth, dry eye, constipation, and problems with thinking.

A Phase I safety study for the indication of erectile dysfunction using the same vector (hMaxi-K plasmid DNA) was completed in February 2006. hMaxi-K is a potassium channel that is found in cell membranes, and the plasmid DNA is used to deliver it to the cells. There were no AEs related to the use of the study drug. A Phase I safety trial is now proposed in human female participants with OAB. The approach will involve a single instillation of 90 mL of PBS-20 percent sucrose hMaxi-K plasmid DNA solution through a urethral catheter placed into the empty bladder. It is expected that the expressed potassium channels in the smooth-muscle cells of the bladder wall will regulate the smooth-muscle cell spasm/contraction of the bladder by decreasing the activity of calcium channels and reducing the entry of calcium ion into the cell. Because the sustained influx of calcium ion is necessary to maintain smooth-muscle cell contraction, the cells will relax. Use of this gene transfer system causes those cells that contain the new gene to express increased amounts of the protein that forms the alpha- or pore-forming unit of the potassium channels and/or results in the formation of a more active potassium channel in the cell membrane. In either scenario, the end result is potentially enhanced relaxation of the smooth-muscle cells, overcoming the cell spasm/contraction, and thus correcting the OAB.

Preclinical animal studies completed using the vector indicate that OAB can be corrected following a single bladder administration. In this proposed safety trial, 39 human female research participants with OAB will be followed for six months after gene transfer. The participants will be monitored closely for possible local and systemic AEs and for potential efficacy of the product with the use of voiding diaries and specific bladder tests.

B. Written Reviews by RAC Members

Four RAC members voted for in-depth review and public discussion of this protocol. Key issues include the novel disease indication, the proposed otherwise healthy participants, and the use of the vector construct in a new setting.

Dr. Federoff asked for information about the known pathophysiology in the rat bladder obstruction model and human OAB in evaluating the ability of the rat bladder model to predict effects in humans. The

investigators were asked to explain the enhanced gene content in biceps muscle one week after instillation into the bladder and because a small amount of h*slo* DNA was detected in the rat lymph nodes, the investigators should examine whether rats have antibodies to the channel gene product and discuss whether this is a relevant concern for the clinical trial. He asked the investigators to discuss what is known about possible protein interactions in bladder smooth muscle and what effects might be anticipated on the basis of these interactions; this is particularly important because the h*slo* gene product can interact with many other proteins and could alter their normal function. The study design includes participants receiving a placebo, so the investigators should elaborate on any known placebo effect in OAB.

Ms. Kwan requested that the investigators provide a clear explanation of how the proposed strategy for OAB might be equal or superior to existing treatments. She wanted to know how frequently and for how long an individual would need to be "re-treated" if the gene transfer proved to be effective and safe. Ms. Kwan also requested a summary review of the results of Protocol #0204-528, the investigators' prior trial using this same product for erectile dysfunction, and requested an explanation of how those results inform and relate to the current proposal. She also asked for an explanation of the present or anticipated financial interests from the individuals and institutions participating in this protocol and those between the sponsor and the investigators. Ms. Kwan also asked how the investigators would ensure that participant recruitment and analysis of results would be conducted away from the influence of those who have such financial interests.

Dr. Nemerow's concerns centered on the fact that the proposed trial is a new use of the plasmid for a non-life-threatening syndrome and for which there are alternative treatments. He asked about the maximal duration of transgene expression in the rodent models, the turnover rate of the target smooth-muscle cells in the bladder, and whether the investigators anticipate that this approach in humans might require multiple plasmid instillations. Given that high-dose administration of the vector in the preclinical studies yielded some vector presence in cardiovascular tissue (including the aorta), Dr. Nemerow asked about the potential consequences of expression of this ion channel protein in endothelial cells and whether any cardiovascular AEs had been seen as a result of performing systemic administration of the vector in rodents. Because OAB is a new target for this plasmid vector, he suggested that the investigators consider long-term followup of research participants and that the informed consent document be amended to reflect this intention.

Dr. Chang's primary concern focused on the ability of rat bladders to model the human situation, particularly the applicability of the male rat model to women, who are proposed as the only research participants in this Phase I trial. She requested a detailed comparison of the female/male rat model of obstruction to findings of OAB and detrusor hyperactivity in women and men. She asked whether four weeks is an appropriate follow-up time to assess urinary function, and what plan is in place for participants who do not clear h*slo* from their urine and whether such individuals could contaminate their household or sexual partners. She also asked whether the dosage and volume calculations proposed for the clinical trial should be based on the rat data, given that no interim preclinical data are available. She suggested that there is a need for clarification of the financial interests of all parties involved in the proposed trial.

C. RAC Discussion

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

- Dr. Wara requested additional explanation of why men were not to be included in the participant pool for this Phase I clinical trial.

- Ms. Kwan expressed concern that adding a placebo arm might produce results that appear to show efficacy but may not be powered enough to do so.

- Ms. Kwan expressed concern about the presence of the study drug in other tissues, despite the fact that it cleared in a very short time. She asked what potential negative consequences that

brief presence could cause. Ms. Kwan also suggested the addition of a lay language explanation in the informed consent document noting the possibility of negative consequences, even though the investigators believe that possibility to be remote.

- Dr. Nemerow suggested that it might be valuable to know the duration of transgene expression or plasmid in a nonhuman animal system (a knockout mouse is available). Dr. Melman responded that negotiations are under way with a researcher at the University of Vermont.

- Noting that he did not see any reference to participants refraining from sexual intercourse after instillation of the experimental agent, Dr. Weber suggested that such a recommendation be added to the informed consent document. Dr. Melman agreed to do so.

- Dr. Weber suggested the use of a more sensitive measure of urinary tract infection (UTI) because of the small risk that using the catheter would induce a UTI, which might alter how the plasmid affects the bladder. Dr. Melman agreed to do so.

D. Investigator Response

Dr. Melman noted that the biodistribution studies showed no evidence of increased cell proliferation, apoptosis, or scarring. The detection of the gene product in biceps was attributed to contamination and detection in the lymph node followed leakage related to the procedure.

Regarding possible interactions between the gene product and other proteins, the investigators were not aware of any reported interactions. Over-expression of *hSlo* had only the predicted effect and deletion of m*slo* in the mouse, results in detrusor overactivity.

Regarding Dr. Federoff's and Dr. Chang's questions about the relevance of the animal study results to humans, Dr. Crist noted that the investigators assume that the bladder fluctuations, which are quite pronounced and severe, that are observed in the animals between micturitions are the presumptive correlates of what people feel with those contractions *in vivo*. Two-thirds of OAB patients have demonstrable detrusor overactivity, and 80 percent of people who have detrusor overactivity have lower urinary tract symptoms. Given that information, the investigators believe that their preclinical animal studies represent a reasonable clinical correlate.

Acknowledging that the research participants might require additional administrations, Dr. Melman explained that this protocol is designed for single use and that the participants are told that it is a single-dose regimen. Based on the ED rat model, the gene was expressed for at least 6 months. The turnover rate of bladder smooth muscle cells is very low. It is expected that repeat dosing interval in humans would occur months apart.

Regarding Ms. Kwan's concerns about conflict of interest, Dr. Melman stated that there is no direct or indirect relationship between any of the investigators and Ion Channel Innovations, LLC. The clinical trial sites will be separate from the company, as will the principal investigators (PIs).

Based on drug studies, there is an approximately 30% placebo rate.

Dr. Melman explained that the investigators have never observed any evidence in men or in the male rats that the plasmid ever got into the testes, prostate, or seminal vesicles.

In response to concerns about biodistribution, Dr. Melman reiterated that the method of administration for the animal study might increase biodistribution compared with the proposed administration of the agent in humans. When the investigators gave weekly intravenous injections to the rats (1,000 micrograms directly into the bloodstream), they observed no changes in cardiac pressure or any other cardiac parameters.

Regarding the inclusion of only women in this proposal trial, Dr. Melman explained that due to the difference in bladder disease between the sexes, more meaningful results could be obtained if only one sex was studied. A Phase I trial for men with erectile dysfunction had already been conducted. They believe that this protocol, when used in men in the future, may be efficacious because the transfer may affect not only the bladder but also the smooth muscle, for which the current primary mode of therapy is to cause smooth-muscle relaxation with an alpha-blocker.

E. Public Comment

Dr. Borror noted that the first half of the informed consent document refers to the "study drug" and does not state that hMaxi-K is a gene-based drug until the section on possible risks. As a result, participants could read the informed consent document and not understand that they would be getting a DNA-based drug. She suggested that the OBA Web site has some wording to help with this issue.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Scientific/Medical/Study Design Issues:

- The preclinical data may not be entirely predictive of the product's safety or efficacy in human studies because a 2-week-long partial outflow obstruction in the female rat model is not analogous to OAB syndrome in women.

- It is important to determine the duration of transgene expression in the rat model because the information will help determine dosing in the clinical studies.

- Further explanation is needed of the rationale for the inclusion of a placebo arm and how it will contribute to the assessment of toxicity or safety.

- Given the limited number of participants to be enrolled, the protocol is unlikely to be able to draw valid conclusions about safety.

Ethical/Legal/Social Issues:

- The fact that the study involves human gene transfer should be discussed earlier in the informed consent document, and the gene and vector also should be described. Examples of ways to explain human gene transfer research may be found at www4.od.nih.gov/oba/rac/ic/appendix_m_iii_b_1.html.

- The facts that any effects of the gene transfer may be transitory and that additional doses may be needed should be discussed.

- Arrhythmia, which could arise should there be inadvertent transgene expression in cardiac myocardium, is a risk, albeit remote, that should be discussed.

- Study participants should be advised to avoid sexual intercourse for 24 hours following administration of the study agent.

- The need for and importance of participating in long-term followup studies should be discussed. The proposed followup for 18 months after completion of the 6-month active portion of the trial seems appropriate. See the FDA's draft "Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events" (http://www.fda.gov/Cber/gdlns/gtclin.htm).

G. Committee Motion 3

It was moved by Dr. Weber and seconded by Dr. Federoff that the RAC recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 10 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Albelda, Federoff, Heslop, and Wara

Dr. Federoff provided the full report for the period of January 18, 2006, through April 25, 2006. The OBA received 22 protocol submissions, of which 17 were not selected for public review at this RAC meeting. Of those 22 protocols, 17 were for cancer; 7 used adenoviruses, 4 used plasmids, 3 used pox viruses, 2 used retroviruses, and 1 used a measles viral vector.

During the reporting period, 198 amendments were received by the OBA, of which 36 were site or PI changes, 9 were design modifications, 24 were protocol status changes, 80 were annual reports, 9 were responses to Appendix M(1)C(1) of the *NIH Guidelines*, and 40 others represented amendments and notifications.

A total of 190 AEs were reported during this period. Of 148 "A" or "C" events, 9 were A1 events, which is defined as serious, possibly associated, and unexpected. The RAC is still awaiting data regarding a device that is relevant to one clinical trial.

A. RAC Discussion

Regarding the device, Ms. Kwan summarized an issue that came up at her institutional biosafety committee (IBC) meeting when AE information was not communicated between multiple clinical sites. She noted that, with multicenter trials, some of the investigators are not receiving information about AEs in a timely manner. Dr. Wara explained that this is a systemic issue, noting that, with human immunodeficiency virus (HIV) multicenter clinical trials, she is often aware of serious AEs that impact clinical care many months in advance of an official notice.

VII. Biosafety Considerations for Research Involving Lentiviral Vectors

Presenter: Dr. Dewhurst

As a representative of the working group, Dr. Dewhurst presented a draft RAC guidance document on biosafety considerations for research with lentiviral vectors. Additional members of the working group were LouAnn C. Burnett (Vanderbilt University), Dr. Rosenberg, and Dr. Somia. The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The OBA receives frequent questions about the appropriate containment for lentiviral vectors, particularly those derived from HIV type 1 (HIV-1). Because the *NIH Guidelines* do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for IBCs and investigators on how to conduct a risk assessment of lentiviral vector research. Dr. Dewhurst summarized the findings and recommendations offered at the March 2006 RAC meeting.

The major risks to be considered for research with HIV-1-based lentiviral vectors are the potential for generation of replication-competent lentivirus (RCL) and the potential for oncogenesis. These risks can be mitigated by the nature of the vector system and its safety features or exacerbated by the nature of the transgene insert encoded by the vector. In conducting a risk assessment, considerations should include the nature of the vector system and the potential for regeneration of replication competent virus (RCV),

the nature of the transgene insert, vector titer and total amount, the inherent biological containment of the animal host, and results of RCL testing.

Either BL2 or enhanced BL2 containment is often appropriate for research involving the use of advanced lentivirus vector systems (i.e., vector and packaging functions separated onto four or more plasmids, use of a heterologous envelope, additional safety features). Enhanced BSL-2 containment may be a locally defined term but would include attention to sharps and likely attention to personal protective equipment that would reduce the risk of mucosal exposure. In some settings, that equipment would include wearing a full face shield. Particular caution should be exercised regarding aerosol in the context of centrifugation.

RCL testing should be encouraged in order to inform and advance the field of lentiviral vector technology. However, RCL testing requires significant expertise with the appropriate assays and such expertise may not be available in laboratories that do not work regularly with infectious lentiviruses. In such laboratories, the use of positive control may increase risk relative to the use of the test material. IBCs may make a containment assignment without requiring RCL testing by undertaking a risk assessment that considers the nature of the specific vector system being used and experience with that system.

Regarding animal studies, the working group focused mostly on mice because they are so widely used. Wild-type mice cannot support replication of infectious HIV-1 because of multiple blocks to viral replication. The animal then can be viewed as a containment system in a sense—the virus is in the animal and can no longer replicate—so that housing the animal is a different containment consideration from initial inoculation. In general, the initial delivery of the vector should be performed under BL2-N or enhanced BL2-N containment for animals, so as to minimize the risk of autoinoculation by the investigator. After the animal has been inoculated, the site of inoculation has been cleansed thoroughly, and the bedding has been changed, then it may be appropriate to reduce the containment from BL2-N to BL1-N within a few days. Use of lentiviral vectors in animals engrafted with human cells or animal hosts permissive for lentivirus replication requires a higher level of containment.

Other nonhuman lentiviruses are also used as vectors, of which the most widely used is probably the feline immunodeficiency virus (FIV). The *NIH Guidelines* recommend a containment level appropriate for risk group 1 agents for certain animal etiologic agents not associated with disease in healthy human adults. However, BL2 containment may be required for vectors with a heterologous envelope that confers the potential to transduce human cells efficiently. Because FIV does not replicate in mice, BL1-N containment may be acceptable for housing and husbandry.

Dr. Dewhurst demonstrated the page on the OBA web site which would contain the guidance and additional information.

A. RAC Discussion

The RAC discussed the possibility of adding a table, algorithm or scenarios to the guidance to provide clarification and then discussing the guidance again at the Sept. meeting.

VIII. FDA Representative Replacement

Dr. Simek introduced Daniel M. Takefman, Ph.D., Acting Chief of the Gene Therapy Branch of the Office of Cellular, Tissue, and Gene Therapies, at the FDA's Center for Biologics Evaluation and Research. Dr. Takefman took Dr. Simek's place at the remainder of this RAC meeting and will be Dr. Simek's permanent replacement at future RAC meetings.

IX. Discussion of Human Gene Transfer Protocol #0602-758: Lentiviral-Mediated, Hematopoietic-Directed Gene Therapy for Mucopolysaccharidosis Type VII

Principal Investigator: Mark S. Sands, Ph.D., Washington University in St. Louis

Submitter:	Mark S. Sands, Ph.D., Washington University in St. Louis
Additional Investigators:	Rick Martin, M.D., Washington University in St. Louis; Shalini Shenoy, M.D., Washington University in St. Louis; and Gerhard Bauer, Washington University in St. Louis
RAC Reviewers:	Dr. Federoff, Ms. Shapiro, and Dr. Somia
Ad hoc Reviewer:	Hugo W. Moser, M.D., Johns Hopkins University

A. Protocol Summary

Lysosomal storage diseases (LSD) represent a large group (>40 distinct diseases) of inherited metabolic disorders usually caused by a deficiency in a single lysosomal enzyme The lack of one of these enzymes leads to the progressive intralysosomal accumulation of undegraded material in many cells of the body. Consequently, these diseases affect many organ systems and have a broad spectrum of clinical signs, including hepatosplenomegally, auditory defects, visual deficits, cardiac anomalies, skeletal dysplasia and cognitive impairment. For the vast majority of these diseases no conventional therapy currently exists. However, the principle of "cross-correction" provides the basis for the development of effective therapies for this class of disease. "Cross-correction" refers to the ability of lysosomal enzymes to be secreted from one cell and subsequently endocytosed by cells at a distance. It has been shown *in vitro* and *in vivo* that small amounts of endocytosed enzyme can reduce or eliminate lysosomal storage material in affected cells. This phenomenon has been exploited clinically to treat these diseases using bone marrow transplantation (BMT) Bone marrow-derived cells from a normal donor are able to reconstitute the hematopoietic system of an affected patient and supply the deficient enzyme to many tissues. Partial correction of the disease has been demonstrated clinically in several lysosmal storage diseases. However, BMT has limitations, including the lack of suitable donors and the harsh conditioning regimens required for engraftment Another severe consequence of allogeneic BMT is graft vs. host disease which can have a mortality rate as high as 50%. Preclinical experiments performed with syngeneic BMT, where graft vs host is eliminated, have demonstrated increased efficacy compared to allogeneic BMT. A hematopoietic-directed gene therapy approach using autologous hematopoietic progenitor cells (HPCs) would eliminate the need to identify a suitable donor and eliminate graft vs. host disease. In addition, we now have the ability to over-express the therapeutic protein in the hematopoietic compartment thereby potentially increasing the efficacy compared to normal donor bone marrow cells. Pre-clinical data in animal models of lysosomal storage disease support this conclusion. The protocol proposes to perform a lentiviral-mediated, HPC-directed gene therapy clinical trial in participants with the lysosomal storage disease mucopolysaccharidosis type VII. MPS VII is a lysosomal storage disease caused by a deficiency in P-glucuronidase activity. Glucuronidase deficiency results in the progressive accumulation of glycosaminoglycans in many cell types and leads to severe skeletal dysplasia, cognitive deficits, auditory defects, visual impairment and hepatosplenomegally. There currently is no conventional therapy for this inherited metabolic disorder. However, there is pre-clinical data demonstrating the efficacy of syngeneic BMT and HPC-directed gene therapy in the mouse model of MPS VII. In addition, a recent study has shown that human MPS VII HPCs can engraft a xenotransplantation model of MPS VII and express therapeutic levels of enzyme following *ex vivo* transduction with a lentiviral vector.

B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of this protocol. Key issues included that the vector construct has never been used in humans, no lentiviral vector has been used in humans who are HIV negative, gene transfer has never been tested in this disease, insertional mutagenesis is a risk of this protocol, and the investigators propose to enroll participants as young as two months old.

Dr. Federoff asked whether any individuals with MPS VII have received a cytoreductive dose of melphalan similar to that being proposed, and if so, what has been the long-term result. He also asked whether sufficient numbers of bone marrow cells could be harvested from participants who are two months of age. He suggested that ultrasound be considered as one of the clinical measures and asked what outcome measure would be predictive of enzyme correction in the central nervous system. He also suggested that T-cell responses be measured in addition to measuring antibody responses to beta-

glucuronidase gene (GUSB). He asked about the potential for lentiviral vector integration to create a cell type that may result in clonal expansion and whether there are deleterious effects due to expressing very high levels of the GUSB.

Ms. Shapiro expressed concern about whether the small number of research participants proposed for enrollment (three) would be sufficient to allow conclusions to be drawn about the safety of the intervention. She reminded the investigators that the serious risks, some of which may have lifelong impact, require careful consideration of assent and consent procedures. With respect to the informed consent document, Ms. Shapiro noted some confusion about whether the document is written for the parent of a participant or for the participant. Other informed consent document issues she noted included confusing discussions of the severe combined immunodeficiency disease (SCID) adverse events and a trial using a lentivirus to transduce adult mature blood cells, wording that suggested infringement of a participant's right to withdraw, and whether the signature of both parents of a prospective child participant would be required.

Dr. Somia asked about the experience in the lysomal storage field of immune reactions against the correcting protein and, if a reaction to GUSB should occur, whether the reaction would exclude participants from future trials or make enzyme replacement therapy ineffective should it become available. Regarding CNS correction, he asked what improvement was observed in animal models and how early in the disease course did transplantation need to occur for improvement. He asked whether the MND promoter had been selected for use in the clinical vector, and asked for comment on the choice of a retroviral LTR promoter in regard to the potential for insertional mutagenesis. He suggested that preclinical studies could help identify promoters that would minimize transactivation of cellular genes and address immune reactions to the transgene product in large animal models and adult animals with mature immune systems.

Dr. Moser noted that, as a proof of principle, this trial could have applicability to therapies for related disorders that are more common. He recommended that possible neurological therapeutic efficacy be evaluated by the use of noninvasive imaging studies such as magnetic resonance imaging (MRI) and MR spectroscopy. He suggested that a neurologist evaluate potential participants to ascertain more precise exclusion criteria. Because there are at least three phenotypes of MPS VII, which vary greatly in terms of prognosis and do not correlate exactly with the amount of enzyme activity, Dr. Moser suggested assembling a more complete compilation of the historical data of disease progression which would be useful in attempting to predict the clinical course of research participants and thus be able to evaluate any therapeutic efficacy.

C. RAC Discussion

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

- Dr. Weber asked whether an assent process would be used or whether the investigators would say that assent is not necessary because MPS VII is a potentially fatal disease. He also wondered whether the assent forms would be written at the appropriate language level for the child to whom it is directed.

- Dr. Wara asked the investigators about the number of potential research participants, given that this disease is so rare.

D. Investigator Response

Dr. Sands stated that LSDs produce profound systemic disease and that, in at least 75 percent of LSD patients, there is profound CNS disease as well. To date, all the data that have been generated preclinically and clinically indicate that, if the hematopoietic system is targeted successfully, the best result would be to stabilize the cognitive function. If a very young patient with few cognitive deficits is treated, that patient may be able to develop better; if the patient already has cognitive deficits, it may be

possible to arrest the cognitive decline at that point. It may be necessary to treat the CNS and systemic diseases independently.

Regarding the myeloreductive conditioning regimen, the single MPS VII patient who had undergone a bone marrow transplantation had received a more aggressive myeloablative regimen as had seven MPS I patients with no regimen related toxicities. In the mouse model, the MPS VII mice engraft to identical levels as normal litter mates.

Sufficient numbers of cells should be obtainable from a bone harvest regardless of age, provided general anesthesia is feasible.

Dr. Sands explained the investigators chose the MND promoter because it results in a high level of transgene expression. However, the use of a strong retroviral promoter does increase the risk of transactivation of neighboring genes. In contrast to the X-SCID trial, lentiviral vectors may have an improved safety profile compared to retroviral vectors, the *ex vivo* transduction protocol will not stimulate the transduced cells to divide, and the GUSB expressing cells are not expected to have a selective advantage.

No clinical or biochemical abnormalities have been noted in transgenic mice that overexpress GUSB 10-20 fold. MPS VII mice injected at two months of age with the lentiviral vector bearing the MND LTR that expresses 20-40 fold higher than normal levels of GUSB have been observed out to eight months with no signs of adverse events.

Regarding parental consent and participant asset, Dr. Shenoy explained that Washington University considers parental consent adequate if the one parent who has custody of the child signs the consent form. If there is joint custody of the child, the investigators would make an effort to get both parents to sign the consent form. If the child is older than 6 years of age, the investigators are required to describe the study to the child and obtain assent.

Dr. Shenoy explained further that the Washington University guidelines state that the form for the child should be understandable at the sixth-grade level. The assent process consists of sitting down with the participant, if he or she is capable of understanding the intervention, and describing the contents of the consent form, then having the participant sign the assent section of the consent form. Dr. Sands explained that the patients' levels of cognitive deficit can be mild to profound, but he believed it unlikely that any of the children to be enrolled in this trial would be able to comprehend much of the informed consent document, written at any level, because the cognitive deficits resulting from this disease are significant.

Regarding the number of available research participants, Dr. Sands noted that there are 5 known patients in the United States, 3 in Brazil, 2 in Spain, and 1 in Japan, for a total of 11 patients identified to date.

E. Public Comment

Dr. Borror explained that, under DHHS regulations, research participants must be told that they are free to withdraw from a clinical trial at any time and that the investigators cannot require any participants to do any sort of followup; however, use of the terms "encourage" or even "strongly encourage" in relation to follow-up is acceptable.

Dr. Borror also explained that, under DHHS regulations, two categories of research require the parental permission of both parents, and two other categories allow parental permission from just one parent; she noted that it was not clear to her into which category this research falls. The categories of research that require parental permission from only one parent do not involve greater than minimal risk and/or involve greater than minimal risk but present the prospect of direct benefit to individual participants.

Dr. Borror cautioned the investigators to avoid the use of the words "treatment" and "therapy" in the informed consent document. She suggested using "investigational product," "study product," or "study drug."

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- Although the protocol is designed primarily to assess the safety of the gene transfer product, data from other studies suggest that some neurological benefit may be seen. As such, it would make sense to add neuroimaging studies (e.g., using MRI or MR spectroscopy) to the protocol to measure neurological status and determine whether any improvement in neurologic function can be detected.

- The vector being used, a lentivirus, is capable of integrating into the genome and, thereby, poses a higher risk of triggering a malignant transformation. As such, the protocol should include a plan for long-term followup of participants. See the FDA's draft "Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events" (http://www.fda.gov/Cber/gdlns/gtclin.htm).

- Since only three participants are to be enrolled, extra care should be taken in the interpretation of any AEs those participants may experience and in drawing any general conclusions about the safety of the gene transfer product.

- Since it is possible that antibodies against the GUSB gene expressed by the vector could develop in participants lacking GUSB protein expression, prospective participants should be tested and excluded from the study if they are found to be completely deficient in GUSB activity.

- On the basis of historical data, at least three phenotypes of MPS VII have been identified. Determining each participant's phenotype may provide useful information in helping predict that participant's clinical course and outcome assessment.

- The informed consent document should be revised. Terms and statements that could mislead prospective participants about the benefits of study participation (e.g., by referring to the experimental intervention as a "treatment") should be deleted; the terms "experimental agent" or "study agent" should be used instead. In addition, the long-term followup plan and its purpose and benefit to participants should be described.

- The discussion in the consent document about the provision of medical treatment for research-related injuries is confusing and should be clarified.

G. Committee Motion 4

It was moved by Dr. Federoff and seconded by Dr. Weber that the RAC recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 10 in favor, 0 opposed, 0 abstentions, and 0 recusals.

X. Discussion of Human Gene Transfer Protocol #0604-767: AdV/RSV-*tk* Followed by Valganciclovir for Treatment of Patients with Retinoblastoma Complicated by Vitreous Seeds

and

Discussion of Human Gene Transfer Protocol #0604-768: Pediatric Phase I Study of AdV/RSV-*tk* Followed by Valganciclovir for Treatment of Patients with Retinoblastoma

Principal Investigator:	Richard L. Hurwitz, M.D., Baylor College of Medicine/Texas Children's Hospital
Submitter:	Richard L. Hurwitz, M.D., Baylor College of Medicine/Texas Children's Hospital
Additional Presenter:	Patricia Chévez-Barrios, M.D., The Methodist Hospital Physician Organization
RAC Reviewers:	Drs. Powers *(via teleconference)*, Vile, and Wara
Ad hoc Reviewers:	David H. Abramson, M.D., Memorial Sloan-Kettering Cancer Center *(via teleconference)*, and Karl Csaky, M.D., Ph.D., NEI, NIH *(no written review)*

Drs. Abramson, Powers, and Vile participated in this discussion via telephone. Dr. Nemerow recused himself from initial and public discussion of these proposals due to conflict of interest.

A. Protocol Summary

Retinoblastoma is the most common primary malignant tumor of children and usually occurs in children younger than 3 years old. The current standard treatment for nonmetastatic retinoblastoma is enucleation. Although this results in a high rate of survival, enucleation results in blindness. Recently, attention has turned to finding alternative therapies that not only will result in a high cure rate but also will allow salvage of the affected eye. Occasionally, a child presents with a small tumor that can be eradicated with cryotherapy or laser photocoagulation while still preserving the eye and useful vision. Unfortunately, most children present with tumors that are too large for these types of therapies. In an attempt to shrink a large tumor to a size that can be managed by these local therapies, clinical investigators have begun trials using systemic chemotherapy and/or radiation therapy instead of enucleation. Although preliminary studies have shown promise, these therapies have significant side effects, including an increased rate of second malignancies. Because patients with retinoblastoma have a significant second malignancy potential, an alternative therapy without systemic toxicity is desirable.

These investigations will examine the safety and efficacy of using an adenoviral vector to deliver the herpes thymidine kinase gene (AdV/RSV-*tk*) directly into the tumor (767) or to the vitreous seeds (768) followed by oral administration of valganciclovir for the treatment of patients with retinoblastoma. The protocols allow for re-treatment of participants who have previously been treated with AdV/RSV-*tk*.

In a Phase I study previously undertaken using a gene transfer using AdV/RSV-*tk* in children with vitreous seeds associated with retinoblastoma, none of the research participants developed gene transfer-related toxicities that were indications for enucleation, and no dose-limiting toxicities were observed. The goal of these studies is to expand on knowledge gained from the Phase I study to further evaluate the safety and efficacy of this treatment in patients with retinoblastoma with or without vitreous seeds.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Key issues included the fact that these protocols will involve children for whom an alternative treatment (enucleation) has a high survival rate.

Dr. Powers expressed particular concern that both protocols propose to involve children for whom an alternative treatment—enucleation—has a high survival rate. He asked the investigators to discuss their rationale for proceeding with a gene transfer study in light of the success rates associated with the standard therapy. In addition, Dr. Powers requested that the investigators address the criteria for the list of risks associated with the various elements of the protocols and suggested that some of the additional risks raised by RAC reviewers might need to be disclosed to participants and their guardians.

Dr. Vile expressed concern regarding the proposed dose of valganciclovir. He also asked what evidence exists that injecting into the tumor increases the chances of metastasis to other areas and whether there

is any clear indication of whether the inflammation (banding) is an antiviral response, an antitumor response, or the result of cell killing. Dr. Vile suggested that the investigators more expansively address the risks and benefits of leaving the tumor in place during the course of the proposed clinical trials.

Dr. Wara asked about the prior Phase I study results, including the number of participants in the prior trial who received the dose selected for the current study, what the response rate was, and how many participants in the prior study were able to avoid enucleation. She also asked whether ocular inflammation had any impact on visual acuity and whether the metastatic disease that resulted in the death of a participant in the prior study was the result of an aggressive tumor rather than delay to enucleation imposed by the protocol. She asked the investigators to explain the requests for single subject exemptions in the previous trial and the outcomes. Regarding clinical trial design, she requested the indications for a participant to go off-study and the indications for enucleation, enumeration of the stopping rules for both protocols, the strategies other than enucleation that could be used to determine the effect of gene transfer on the vitreal particles and how sensitive these approaches are. She recommended that the informed consent document include language describing the risks of delayed chemo/radiation treatment or enucleation and the death of the participant in the phase I trial.

Dr. Abramson noted a number of misstatements and outdated references in the protocol that suggested the investigators may have greater familiarity with gene transfer than retinoblastoma. Dr. Abramson expressed concerns regarding the techniques to be used in the protocol. He discussed well-documented examples of patient deaths related to tumors exiting the eye following the introduction of a needle into the eye. Dr. Abramson expressed significant reservations as to whether the procedure proposed could actually be done—injecting into a soft eye creates difficulty in getting the injection where it is supposed to be, and if the eye is firm, there is concern about liquid getting out. Even under an operating room microscope, the proposed procedure would be very difficult to perform, and Dr. Abramson expressed concern that it is considered on the borderline of acceptable medical practice. Noting some published cases in which introduction of needles into the eyes of children with retinoblastoma have *not* resulted in spread and death, Dr. Abramson cautioned that the wording in this proposal does not reflect the sense of fear that pervades the ophthalmic community on this subject. He noted that the investigators propose, after the two injections, to use a cryoprobe to freeze the site of needle entrance; however, Dr. Abramson doubted that a child's eye could tolerate the likely significant corneal destruction, scarring, astigmatism, iritis, and pain that would occur as a result of the proposed four different sessions. He was particularly concerned with the way the earlier Phase I trial was presented, especially regarding the inflammation that occurred. The inflammation, vitreous condensation, and banding would have affected vision and made evaluation of effectiveness nearly impossible.

Noting that the goal of treatment is to preserve the eye and thus preserve vision, Dr. Csaky asked the investigators to predict their goal for these protocols on the basis of patients they have treated and the possibility of inflammation occurring as an AE. He also wondered what evidence might exist to suggest that the intense inflammation observed might be a risk factor for metastasis.

C. RAC Discussion

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

- Ms. Kwan stated that the investigators had misstated the role of the RAC in an article concerning the first protocol, in the November 1, 2005, *Journal of Clinical Oncology*. In two separate sections, the statement is made that the study was approved by the Recombinant DNA Advisory Committee. She emphatically reiterated that the RAC has no approval authority, and "approval" by the RAC should never be stated in a document, in an informed consent, or elsewhere.

- Noting that the significant risk relates to the surgical approach and the propensity of that approach during injection and immediately thereafter to create an opportunity for tumor cell seeding at sites other than at the site of the injection, Dr. Federoff asked whether a model exists in which that risk can be rigorously addressed.

- Dr. Csaky noted the potentially devastating complications associated with removal of aqueous fluid by making holes in eyes that will never seal. He stated that, even if any treatment ultimately does not change the risk of enucleation but changes the risk of metastasis from .0001 to .001, that small change is unacceptable.

D. Investigator Response

Dr. Hurwitz explained how the investigators have dealt with the single patient exemptions that needed to be requested in a previous study; these exemption requests were grouped into the categories of participants who had a response and participants who could not adhere to the injection schedule for a variety of reasons. After consultation with the FDA, the investigators have built into the current proposed protocol a retreatment arm for both of the situations that arose in the trial. As a result, they do not expect to have any single patient exemptions in this trial.

In the previous trial, all the participants proceeded to standard care after gene transfer, but all required enucleation because of progression of primary tumors resistant to standard care.

In response to Dr. Csaky's question regarding preservation of vision, Dr. Hurwitz responded that vision of participants improved as the vitreous banding decreased. The ocular inflammation or "banding" impaired vision due to interference with light striking the retina. Visual acuity improved as the banding improved over time resulting with two participants with better than baseline vision, one with stable vision, and five participants whose vision did not return to baseline levels. One participant retained her eye for 3.5 years with 20/30 vision until progression of the primary tumor required enucleation.

In response to several RAC members' concerns about whether injecting into a tumor in the eye would increase the risk of metastasis, Dr. Hurwitz explained that the investigators would not biopsy the tumor—only inject into the tumor, not pulling any material out of the tumor, thus reducing the risk of metastasis. In addition, he noted that a paper from a Swedish research group was published in the 1960s about the feasibility of intravitreous injections for retinoblastoma, and recently in Japan, intravitreous injection of chemotherapy has become the standard of care for patients with vitreous seeds. The Japanese ophthalmologists use a transscleral approach, which is considered riskier than the transcorneal approach proposed for this clinical trial. More than 100 patients have been injected in Japan, and there have been no reported cases of metastatic disease.

Regarding the research participant who died of metastatic disease in the phase I trial, histopathological examination indicated that the metastatic disease most likely was the result of an aggressive tumor that invaded through a weakened scleral site that resulted from previous radiation and anatomical disposition.

The research participants in the study will have failed chemo/radiation therapy. Participants whose vitreal seeds resolved would go off study for standard therapy while those with progressive disease would go off study for enucleation. Other reasons for going off study would include a dose-limiting toxicity or the request of the participant.

Regarding the stated end points for these two proposed protocols, Dr. Hurwitz stated that the end point is not to *reduce* the number of vitreous seeds but rather, over a long period of time, to *eliminate* them. In the standard sections in the protocols about partial responses, he agreed that wording might need to be changed because partial response is not an end point in the vitreous seed protocol. The end point is complete response, defined as elimination of vitreous seeds or stable calcified seeds over time; current therapies are not able to accomplish that end point. One month after the first injection, the investigators will look for the continued presence of vitreous seeds; assuming vitreous seeds remain, they would give another injection. As long as there is not progressive disease, the investigators will continue injecting up to four times.

Dr. Hurwitz reiterated that, for the vast majority of unilateral cases, this potential therapy is not appropriate. When an individual has unilateral disease with advanced tumor vitreous seeds, the eye is

enucleated. The investigators working on this protocol are proposing to enroll only research participants with bilateral disease.

E. Public Comment

Dr. Borror commented that the informed consent document referred to experimental intervention as "treatment" and used the words "treat" and "therapy," all of which should be changed to reflect the experimental nature of this protocol and gene transfer in general.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

The RAC concluded by unanimous vote that the protocols should not move forward as currently conceptualized. If the investigators wish to advance this experimental approach for the treatment of retinoblastoma, the RAC recommended consideration of the following concerns as well as the more specific points outlined below: (1) There is an effective standard of care for children with retinoblastoma; (2) the vector administration procedure involves a surgical approach that could seed the tumor cell and cause metastatic disease; and (3) the protocols were replete with factual errors and proposed no quantitative measures of the participants' clinical response to gene transfer.

Other Issues:

- The selected dose of oral valganciclovir may be inadequate. Pharmacokinetic data from other pediatric clinical studies (non-gene transfer studies) of oral valganciclovir should be used to guide dosing decisions in these studies.

- Because ocular inflammation or "banding" may have an impact on visual acuity, the pathogenesis of "vitreal banding" following vector injection should continue to be investigated to determine whether it is due to antiviral or antitumor effects or is a response to cell killing.

- Injecting a needle through the cornea or iris may cause ocular toxicities, including inflammation along the needle track, and lead to significant decreased vision. These risks should be addressed in the study design.

- "Partial response" is defined in Protocol #0604-767 as a reduction in vitreous tumor seeds. However, because the percentage decrease in vitreal seeds (e.g., either 25 percent or 50 percent) cannot be scientifically quantified, a better measurement technique should be developed. Alternatively, rather than employing a partial-response measure, total tumor regression after one or two injections should be used as the study end point.

- An independent pathologist should be involved in confirming whether the treated eyes show no active retinoblastoma.

- The overriding safety concern of the protocols—the risk of tumor cell seeding and metastatic disease —was inadequately described in the informed consent document. The informed consent document also should avoid using the terms "treatment" or "therapy" because they can mislead participants about the potential benefits of participation. For more information, please refer to the *NIH Guidance on Informed Consent for Gene Transfer Research* <http://www4.od.nih.gov/oba/rac/ic/>.

If the investigators plan to continue pursuing the approaches outlined in these protocols, the RAC requested that they resubmit the revised protocols to the RAC for another round of review before proceeding with any other steps. It was reiterated that the purpose of RAC review is to assess and make

public recommendations about the scientific and ethical issues associated with clinical gene transfer research; the RAC's purpose is not to approve human gene transfer protocols.

Committee Motion 5

It was moved by Dr. Federoff and seconded by Dr. Rosenberg that the RAC statement be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 11 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XI. Closing Remarks and Adjournment/Dr. Wara

Dr. Wara thanked the participants and adjourned the meeting at 4:50 p.m. on June 21, 2006.

[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.]

_____
Amy P. Patterson, M.D.
RAC Executive Secretary/OBA Director

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and 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: _____ _____
Diane W. Wara, M.D.
Chair

Attachment I
Recombinant DNA Advisory Committee Roster

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Attachment III
Abbreviations and Acronyms

AE	adverse event
BMT	bone marrow transplantation
BSL	biosafety level
CNS	central nervous system
DHHS	U.S. Department of Health and Human Services
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
FDA	U.S. Food and Drug Administration
FIV	feline immunodeficiency virus
GUSB	beta-glucuronidase gene
GVHD	graft-versus-host disease
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HLA	human leukocyte antigen
IBC	institutional biosafety committee
LSD	lysosomal storage disease
MPS VII	mucopolysaccharidosis type VII
MRI	magnetic resonance imaging
MS	multiple sclerosis
NEI	National Eye Institute, NIH
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OAB	overactive bladder
OBA	NIH Office of Biotechnology Activities
OD	NIH Office of the Director
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentivirus
RCV	replication-competent virus
SCID	severe combined immunodeficiency disease
UTI	urinary tract infection