RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 15-16, 2005

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 <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 15-16, 2005

The Recombinant DNA Advisory Committee (RAC) was convened for its 100th meeting at 8:30 a.m. on June 15, 2005, at the Bethesda Marriott Hotel, 5151 Pooks Hill Road, Bethesda, MD. Dr. Diane Wara (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 4:45 p.m. on June 15 and from 8:00 a.m. until 12:30 p.m. on June 16. The following individuals were present for all or part of the meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania Medical Center
W. Emmett Barkley, Howard Hughes Medical Institute
Martha C. Bohn, Northwestern University
Neal A. DeLuca, University of Pittsburgh
David L. DeMets, University of Wisconsin Medical School
Stephen Dewhurst, University of Rochester Medical Center
Thomas D. Gelehrter, University of Michigan Medical School
Helen Heslop, Baylor College of Medicine
Terry Kwan, TK Associates
Bernard Lo, University of California, San Francisco
Nicholas Muzyczka, University of Florida
Glen R. Nemerow, The Scripps Research Institute
Madison Powers, Georgetown University
Naomi Rosenberg, Tufts University
Robert D. Simari, Mayo Clinic and Foundation
Richard G. Vile, Mayo College of Medicine
Diane W. Wara, University of California, San Francisco
David J. Weber, University of North Carolina, Chapel Hill

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

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

***Ad Hoc* Reviewers/Speakers**

Karl Csaky, National Eye Institute (NEI), NIH
Ellie Ehrenfeld, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Stephen E. Epstein, Washington Hospital Center/MedStar Research Institute
Jacqueline M. Katz, U.S. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services (DHHS)
Raynard S. Kington, OD, NIH

Nonvoting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, DHHS
Stephanie L. Simek, U.S. Food and Drug Administration (FDA), DHHS

[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.

NIH Staff Members

Stephen P. Creekmore, National Cancer Institute (NCI), NIH
Betsy Earp, OD
Donald Everett, NEI
Kelly Fennington, OD
Linda Gargiulo, OD
Kathryn Harris, OD
Robert Jambou, OD
Laurie Lewallen, OD
Jinhua Lu, NCI-Frederick
Maureen Montgomery, OD
Karen Muszynski, NCI
Marina O'Reilly, OD
Gene Rosenthal, OD
Michelle Saylor, OD
Pamela C. Sieving, NIH Library
Paul A. Sieving, NEI
Thomas Shih, OD
Allan Shipp, OD
Santa J. Tumminia, NEI
Anthony R. Welch, NCI
Gisele White, OD
Jason L. Yovandich, NCI

Others

There were 84 attendees at this 2-day RAC meeting. 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.

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

Dr. Wara, RAC Chair, called the meeting to order at 8:30 a.m. on June 15, 2005. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on May 12, 2005 (70 FR 25100). Issues discussed by the RAC at this meeting included public reviews and discussions of five protocols, a data management report, followup from the March 2005 gene transfer safety symposium, and a presentation regarding harmonization of the *NIH Guidelines* with other Federal guidances on biological containment for work with noncontemporary strains of influenza.

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

II. Minutes of the March 16, 2005, RAC Meeting/Drs. Barkley and Muzyczka

Dr. Muzyczka noted that minor problems regarding the March 2005 minutes have been clarified. Dr. Barkley said he was impressed with the quality and clarity of the minutes and noted the educational value of reading them.

A. Committee Motion 1

It was moved by Dr. Muzyczka and seconded by Dr. Barkley that the RAC approve the March 16, 2005, RAC meeting minutes. The vote was 17 in favor, 0 opposed, and 0 abstentions.

III. Certificates of Appreciation

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

Drs. Barkley, Bohn, DeLuca, DeMets, Gelehrter, Johnson (absent for this meeting), Lo, and Simari received certificates of appreciation for their service on the RAC.

IV. Followup from the March 15, 2005, Gene Transfer Safety Symposium: Current Perspectives on Gene Transfer for X-SCID

Presenters: Marina O'Reilly, Ph.D., OBA, and Dr. Wara

Dr. Wara summarized the safety symposium which reviewed the information regarding the third leukemia reported in a participant in the French X-SCID trial, the current trials involving gene transfer for SCID, retroviral integration and insertional mutagenesis, and the use of bone marrow or stem cell transplantation as a treatment for SCID. At the meeting, RAC developed recommendations for gene transfer for X-SCID and other indications using retroviral vectors. The RAC approved the recommendations and the following statement:

Conclusions and Recommendations of the NIH Recombinant DNA Advisory Committee Gene Transfer Safety Symposium: Current Perspectives on Gene Transfer for X-SCID March 15, 2005

On December 5, 2002, February 10, 2003, and March 15, 2005, the NIH Recombinant DNA Advisory Committee (RAC) reviewed the clinical and molecular data concerning three adverse events that occurred in a human gene transfer study being conducted in France to correct X-linked SCID. This study involves engraftment of an autologous bone marrow derived, $CD34^+$ hematopoietic stem cell enriched, cell population transduced with a Moloney murine leukemia retrovirus derived replication incompetent vector encoding the common gamma chain (γc) transmembrane protein subunit shared by receptors for Interleukins 2, 4, 7, 9, 15 and 21. Three children in this study developed T-cell acute lymphoblastic leukemia (T-ALL) almost 3 years after their gene therapy treatment. The leukemias appear to share the common causative mechanism of insertional mutagenesis at or near oncogenes. In the first two participants, the vector inserted at or near the *LMO-2* gene with aberrant production of lmo-2 protein, which contributed to the abnormal growth of the leukemic cells. The integration sites in the cells of the third participant appear to involve *LMO-2,* and three other oncogenes (Science 308: 1735-1736). The unregulated expression of the γc transgene in the vector may also have a cooperative role in the induction of oncogenesis. An analysis of the available data from this and other gene transfer clinical trials for SCID led the NIH RAC to conclude that:

- The majority of children in this X-linked SCID gene transfer study have had major clinical improvement to date.

- Of the nine children in this experimental study who had successful engraftment of their gamma-c (γc) transduced cells, three developed leukemia approximately 3 years after treatment and have required chemotherapy; one participant subsequently died. The overall frequency of this adverse event in this trial cannot be determined at this time.

- The gene transfer was a cause of the leukemias.

- The occurrence of leukemia in this protocol is not a random event and constitutes a serious inherent risk in this study.

- Some subjects in gene transfer studies for non-X-linked SCID experienced mild to moderate clinical improvement.

These findings led the NIH RAC to make the following recommendations, which will be reviewed and potentially revised as new data become available.

- Retroviral gene transfer studies for X-linked SCID should be reviewed, on a case-by-case basis, and limited, pending further data, to patients who have failed identical or haploidentical stem-cell transplantation or for whom no suitable stem cell donor can be identified. Case-by-case review would include appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

- There are not sufficient data or reports of adverse events directly attributable to the use of retroviral vectors at this time to warrant cessation of other retroviral human gene transfer studies, including studies for non-X-linked SCID. Such studies may be justified contingent upon appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

A. Committee Motion 2

The RAC approved the recommendations contained in the March 15, 2005, statement from the safety symposium. The vote was 18 in favor, 0 opposed, and 0 abstentions.

V. Discussion of Human Gene Transfer Protocol #0311-613: A Phase I, Dose-Escalation Study of Intratumoral Herpes Simplex Virus Type 1 (HSV-1) Mutant rRp450 in Patients with Refractory Sarcoma or Neuroblastoma

Principal Investigator:	Timothy P. Cripe, M.D., Ph.D., Cincinnati Children's Hospital Medical Center
RAC Reviewers:	Dr. DeLuca, Ms. Kwan, and Dr. Gelehrter

[Note: Dr. Heslop recused herself because of a conflict of interest.]

A. Protocol Summary

Although the use of modern multimodality therapy has improved survival rates for many cancers, both adult and childhood sarcomas remain a formidable challenge in oncology. The survival of patients with relapsed, refractory, or metastatic disease remains dismal and has not improved significantly with modern therapy. Similarly, metastatic neuroblastoma in childhood remains difficult to cure.

Attenuated HSV mutants engineered with deletions of normally critical genetic functions dispensable in cancer cells are being pursued as therapeutic agents. rRp450 is derived from HSV-1, but deleted in the ribonucleotide reductase gene, causing it to be permissive for replication only in rapidly dividing cells. The vector also expresses the rat CYP2B1 gene, a prodrug converting enzyme. In preclinical studies, rRp450 kills tumor cells by direct lysis and sensitizes them to prodrugs such as cyclophosphamide. A significant anti-tumor effect has been observed in xenograft mouse models.

Research participants will receive vector directly into the tumor by CT-guided injection by interventional radiology and will be able to receive up to four injections at least three weeks apart to determine the safety of single as well as multiple administrations. Adult participants will be enrolled first, and once the dose level has been demonstrated to be safe, children may be enrolled. Studies will include pre-and post

therapy measurements of anti-HSV immunity, serum cytokine expression, and quantitative real time PCR measurements of viremia.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of the oncolytic, replication-competent HSV-1; incorporation of a prodrug-converting enzyme cassette; the enrollment of children as young as 2 years old; limited preclinical data on the efficacy or safety of the HSV mutant to be used in the trial; and the need to include a request for autopsy in the informed consent document.

Dr. DeLuca noted that the main safety issue related to the proposed vector is the extent of attenuation in normal human cells when administered to humans. He expressed concern that the toxicity/safety data with the exact construct to be used in the clinical trial are not extensive enough. Because of the elevated body temperature of the mouse, it is difficult to extrapolate from mice to humans with respect to the pathogenicity of this virus; another nonhuman animal model or nonhuman primate data may be needed. He requested information as to whether the attenuation is due solely to the deletion of the infected cell polypeptide (ICP6) coding sequence. He suggested that given the novelty of the vector for use in humans and the fact that the *p450* gene is not employed in this application, it would be preferable to use a vector that did not express *p450*. This is particularly important because the virus is likely to establish lifelong latency in humans. He asked whether attempts had been made to cycle rRp450 in mice and permissive culture cells to determine whether it is possible to select for second-site mutations that cause reversion to wild-type. Because ICP6 mutants replicate in the brains of young mice, he suggested that lowering the starting dose might be advisable since the participants will be children.

Ms. Kwan asked that the investigators clarify and make consistent the age of exclusion of participants (listed in one section as younger than 1 year old and in another as younger than 2 years old). The terms in the informed consent document that suggest proven effectiveness (e.g., “patient,” “therapy,” and “treatment”) should be changed to more neutral language. The language in the informed consent document is too complex and technical, and although the lay abstract has been modified, it remains quite complicated. She also requested that the informed consent document include a statement about any financial interests held by relevant investigators or institutions and a request for autopsy.

Dr. Gelehrter expressed concern about the lack of safety data in nonhuman primates. He asked about the effect of prior HSV infection or prior heavy chemotherapy on potential toxicity and tumor growth. The appropriately cautious approach of dosing three young adult participants before dosing three children could affect results because different tumor types can occur in adults compared to children. Dr. Gelehrter also asked for more information about the animal model studies.

C. RAC Discussion

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

- Dr. Vile asked whether injections have been attempted in mouse tumors in fully immunocompetent mice or in mice that have a latent HSV infection.

- Echoing concerns expressed by Dr. DeLuca, Drs. Albelda and Muzyczka suggested using a lower dose ($1x10^6$ plaque-forming units [pfu] or lower), especially if no nonhuman primate studies are conducted.

- Dr. Gelehrter asked whether studies had been conducted in which the injections were given 3 or 4 weeks apart, a schedule similar to that in the proposed clinical protocol.

D. Investigator Response

Dr. Cripe responded to RAC questions and concerns in the written reviews and at this meeting with the following information:

- The informed consent document and lay abstract have been modified in the suggested ways.
- Participants younger than two years will not be enrolled and this will be clarified in the protocol.
- The investigators are consulting with the FDA regarding the necessity for non-human primate studies or the use of another appropriate animal model.
- The investigators have not preimmunized or exposed animals to HSV prior to the use of the experimental vector.
- To address the concerns of Drs. DeLuca, Albelda and Muzyczka, a starting dose lower than that originally proposed will be used. In preclinical experiments, efficacy was observed with doses of 6×10^5 pfu/ml.
- Regarding the effect of prior HSV exposure, antibodies are expected to have poor penetration through solid tumors, likely due to high intersitital fluid pressure within the tumors, thus, the presence of anti-HSV antibodies is not expected to impact either toxicity or efficacy. Preexisting humoral immunity would likely limit spread of rRp450 through the bloodstream beyond the tumor site. Individuals previously exposed to HSV will also likely have a more robust antivirus T cell immune response, which may lessen any antitumor effect by limiting virus replication within the tumor. Also It is possible there will be an increased inflammatory effect within the tumor.
- According to the exclusion criteria, no individuals will be enrolled who are known to be significantly immunosuppressed from recent chemotherapy as measured by blood counts. Nevertheless, subjects are expected to be immunocompromised to some degree from prior treatments. Their relative immunodeficiency may limit their ability to mount an anti-viral T cell response. The overall effect may be an increase in efficacy if virus replication proceeds unmitigated within the tumor.
- Because only multiple relapsed or refractory subjects will be enrolled, most adults are likely to have been initially diagnosed during their adolescent years. Therefore, the majority of subjects will have relapsed rhabdomyosacoma, Ewing sarcoma, or osteosarcoma.
- In studies, there has been no indication of a reversion to a wild-type phenotype. Since there is a large deletion in UL39, reversion to a wild-type virus is unlikely. Also, the parental viral strain, KOS, is also fairly attenuated compared to other HSV1 strains.

E. Public Comment

Dr. Borror clarified the regulation regarding research involving children. The four categories of research that are allowed for children are as follows:

- Research not involving greater than minimal risk.
- Research involving greater than minimal risk but presenting the prospect of direct benefit to individual participants.
- Research involving greater than minimal risk and no prospect of direct benefit to individual participants but likely to yield generalizable knowledge about the participant's disorder or condition.

- Research not otherwise approvable that presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children. This research is approvable only the HHS secretary and/or FDA commissioner after consultation with a panel of experts and following public review.

Luis P. Villarreal, Ph.D., University of California, Irvine, noted that rare but potentially significant events may occur with replication competent viruses in which recombination would create a virus that is unlike the initial experimental virus. For example, in trying to eliminate poliomyelitis (polio), attenuated virus strains have picked up sequences from unexpected sources. As a result of these experiences, Dr. Villarreal suggested that the RAC keep in mind that humans harbor many potential sources of viral recombination.

F. Synopsis of RAC Discussion and RAC Recommendations

Dr. Wara summarized the following RAC comments and recommendations:

Scientific/medical/study design issues:

- An HSV-1 mutant similar to rRp450, which also contains a deletion of the gene encoding the virus ribonucleotide reductase, was found to become selectively attenuated in mice (see Jacobson JG et al. *Virology* 173(1):276-83, 1989). Drawing general conclusions about rRp450's safety on the basis of its attenuation in mice might lead to an overestimation of its safety. Additional preclinical studies in a nonhuman primate model should be considered.

- The finding that the HSV-1 construct (rRp450) replicates in the brains of young mice is a safety concern. As such, and given the ongoing concerns about the relevance of the preclinical data from the mouse model, consideration should be given to decreasing the starting dose of the product to less than 10^7 pfu.

- The protocol hypothesizes that deletion of the ICP6 coding sequence is responsible for the attenuation of rRp450. This hypothesis needs to be confirmed, given that similar constructs based on this assumption might be used in future protocols. Consideration should be given to developing a marker-rescued version of rRp450 that would enable studies to be done to confirm the attenuating effect of the ICP6 deletion.

- Another safety question is whether the mutant virus could establish latency in the peripheral nervous system. To assess whether this risk exists, studies should be conducted in the mouse model of cancer to determine the extent to which the mutant virus is able to establish latency in the peripheral nervous system.

Ethical/legal/social issues:

- Since children will be enrolled in the protocol and given that there are concerns that the risks and discomforts to children may be greater than to adults, it is critically important for parents/guardians of prospective participants and the prospective participants themselves to be fully informed about the risks of the study and the procedures involved. The informed consent and assent documents should be rereviewed to be certain that they convey a complete picture of the protocol.

G. Committee Motion 3

It was moved by Ms. Kwan and seconded by Dr. Bohn that the 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. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VI. Discussion of Human Gene Transfer Protocol #0502-699: A Pilot Study of Temozolomide (TEM) and O^6-Benzylguanine (BG) for the Treatment of High-Grade Glioma Using Autologous Peripheral Blood Stem Cells Genetically Modified for Chemoprotection

Principal Investigator:	Lars Martin Wagner, M.D., Cincinnati Children's Hospital Medical Center/University of Cincinnati College of Medicine
Submitter:	Christof von Kalle, M.D., Cincinnati Children's Hospital Medical Center/University of Cincinnati College of Medicine
RAC Reviewers:	Drs. Lo, Nemerow, and Rosenberg

[Note: Dr. Heslop recused herself because of a conflict of interest.]

A. Protocol Summary

Most children diagnosed with high-grade astrocytoma—the most common type of glioma—will not have long-term survival using standard therapies. Although use of the DNA methylating agent temozolomide (TEM) is an encouraging advance in the treatment of this aggressive brain tumor, the benefits are still limited by tumor cell resistance and therapy-related hematological toxicity. The protocol proposes a strategy to overcome both of these limitations.

Methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that removes methyl adducts placed on DNA by TEM, and this pathway has been identified as the mechanism of resistance of tumor cells to TEM. MGMT expression is present in the vast majority of high-grade astrocytomas, thus is a relevant therapeutic target.

O^6-Benzylguanine (BG) is a chemosensitizing agent which effectively reverses MGMT activity, thus increasing sensitivity to TEM. However, concomitant use of TEM+BG in patients causes severe hematologic toxicity, presumably because of inhibition of low levels of endogenous MGMT in hematopoietic cells. This toxicity necessitates the use of lower and likely less effective doses of TEM. If hematopoietic stem cells could be selectively and durably protected against chemotherapy toxicity, then extended courses of intensive treatment could be administered.

A mutant form $MGMT^{P140K}$ has been created which is resistant to TEM+BG. Retroviral transduction of mouse hematopoietic stem cells with $MGMT^{P140K}$ allows for higher doses of TEM+BG to be tolerated without lethal myelosuppression, translating into improved antitumor activity in xenograft models.

The clinical trial proposes to investigate the safety, feasibility, and effectiveness of infusing autologous hematopoietic stem cells transduced with $MGMT^{P140K}$ in participants being treated with TEM+BG. The trial will follow the standard treatments of surgery and irradiation with stem cell reinfusion and six subsequent courses of TEM–BG. Participants who tolerate the therapy well and who demonstrate transgene expression in peripheral blood neutrophils will be allowed to increase the dose of TEM in future courses. The ability to tolerate dose escalation will serve as an important clinical endpoint for the trial. Other important objectives of the study include demonstration of safety and feasibility, assessing the extent and durability of transgene expression in blood and bone marrow, and assessing the antitumor activity.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocol. Key issues included the opportunity to explore additional safety issues related to the X-SCID study conducted in France, further discussion of the approaches to reducing the potential consequences of insertional mutagenesis, the use of recombinant fibronectin (FN) to enhance gene transfer, and further discussion of the potential oncogenicity of the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) that will be contained in the proposed vector.

Dr. Lo asked whether the testing for clonality of insertion sites will be carried out in a time frame that allows the enrollment of subsequent participants to be modified, and whether the survival advantage of transduced cells, due to repeated cycles of chemotherapy, would increase the risk of clonal expansion. He requested further explanation of the theoretical adverse consequences of developing FN antibodies, why the proposed number of participants is 10, and what information the investigators hope to gain from the proposed dose-escalation scheme. Regarding the informed consent process, Dr. Lo asked for a description of the assent process for child participants, a notation of any financial arrangements the investigators may have with the manufacturers of BG or the CD34 cell separator, and modification of language in the informed consent document implying that participants would be expected to live 15 years from the date of gene transfer.

Dr. Nemerow was impressed by the thoroughness of this proposed trial with regard to the multiple stopping points to protect participants. He requested a comparison of the risks for insertional mutagenesis in this protocol compared with that in X-SCID trial. He asked about the amount of contaminating CD34– cells present in the selected population that will be used for retroviral transduction and whether glioma cells may be present. He also asked whether the investigators had considered safety modifications to the vector (e.g., a suicide gene) and whether the MGMT gene has been identified as a potential oncogene in the retrovirus tagged cancer gene database. He requested the results of the study of reconstitution in rhesus monkeys with retrovirally transduced CD34 cells and whether an assessment of germ-line transfer had been done in a large murine study.

Dr. Rosenberg asked several questions regarding the use of the FN component: the mechanism of increased transduction, determination of the number of integrations and the consequences of developing antibodies to FN. She asked how the CD34+ cell population used for infection will be characterized and how those cells might differ from those used in the X-SCID studies. Regarding monitoring of clonally expanded cells, she asked whether the length of time need for testing would affect enrollment of subsequent participants. She also asked for the rationale for the use of the GALV envelope to pseudotype the vector.

C. RAC Discussion

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

- Drs. Lo and DeMets queried whether the number of participants proposed (10) is likely to yield the results needed to meet the trial objective.

- Dr. Patterson asked about the use of the modified woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) in the vector.

- Dr. Albelda asked whether the investigators plan to give one dose of the stem cells, whether they propose to escalate that dose, and how that dose will be chosen.

D. Investigator Response

Drs. Wagner and von Kalle responded to RAC questions and concerns in the written reviews and at this meeting with the following information:

- A single administration of the stem cells is proposed, depending on the number of cells collected. For each participant, a backup of $1X10^6$ CD34 cells per kilo will be kept as an unmanipulated frozen aliquot in case of delayed transfer. Some limitations will be set on the range of stem cell doses to be transduced and administered into the participant, but prescribing a specific dose may be difficult because that number will depend on the number of stem cells available.

- To reduce the likelihood of an insertional mutagenesis adverse event, the transduction efficiency of the primary cell product will be below 50% to avoid multiple-hit integration, cell product dose

will be less than 1 x 10^7 CD34 cells per kg body weight, and serum free-transduction conditions, which is one of the major differences between the French and British X-SCID trials, will be used.

- Based on the available preclinical small and large animal data, the hypothesis behind the trial design is that the MGMT transgene itself does not produce a proliferation or survival advantage other than under the selection conditions with chemotherapy. It may well be possible that with gene modification, more clones survive than normally would after chemotherapy, so that proliferation stress on each single clone may be equal or less compared to following chemotherapy of similar intensity without genetic modification. Insertion site locations can be expected to be selected for sufficient expression levels, but the large animal experience suggests that such expression levels are present in the majority of engrafted clones.

- The GALV envelope is particularly effective in transducing primate and human stem- and progenitor cells. It offers the potential to effectively engraft more immature progenitor and stem cells that contribute to long-term hematopoietic recovery. The French X-SCID trial used an amphotropic envelope, whereas the British trial used a GALV.

- Contamination of the CD34+ by glioma cells is a theoretical concern but occurs only rarely. Multiple studies on the use of autologous stem-cell transplantation for high-grade gliomas have shown the absence of a clinically significant problem. No cases of brain tumor cells contaminating the stem-cell product, resulting in complications, have been reported. The incidence of extracranial metastases at the time of diagnosis (when the stem cells are collected) is exceedingly rare with high-grade glioma—about 1 percent of patients will be found to have extracranial metastases, usually in the lung or lymph nodes, so the bone marrow is not likely to be involved. A review of the literature shows 13 cases of bone marrow involvement with glioblastoma, only one of which was at the time of diagnosis and about 85 percent of which would not have been eligible for the proposed study because of hematologic problems stemming from that bone marrow toxicity.

- Because of the potential for the development of nonhematologic (e.g., liver) toxicity with higher doses of TEM + BG, the investigators prefer to keep participant numbers as low as reasonably possible but high enough to adequately test the proposed approach. Similar gene transfer trials have used only 10 participants.

- The use of the assent process for children ages 11 to 18 years is required by the Cincinnati Children's Hospital Institutional Review Board. Children are informed of their disease and the proposed treatment using age-appropriate descriptions, with family members present during this discussion. Children are given the opportunity to ask questions, and these are answered by the clinician, again using age-appropriate language. After further discussion with the patient and family, the clinician will review the assent document with the child, who will then be given the opportunity to sign the document and enroll on the trial.

- Concerns have been raised about the theoretical risk of oncogenesis associated with the use of the WPRE in gene transfer vectors to increase transgene expression. The WPRE sequence also encodes a truncated X protein which may be involved in oncogenesis. Tumors have been detected in mice injected *in utero* with a lentiviral-WPRE vector expressing Factor IX; however, the mechanism of tumorigenesis is still being investigated. To lower this theoretical risk, the vector includes a modified WPRE altered to disable the WHV X protein promoter and the transcription initiation site for the truncated X protein.

- Regarding the FN, autoimmune disease of the connective tissue could be a theoretical consequence of developing antibodies against fibronectin, but this has never been described in the many clinical trials in which this agent has been used. Patient serum is being archived at specific time points to allow retrospective assessment in the unlikely case of unusual connective tissue symptoms or other evidence of autoimmune disease. This archiving strategy has been

employed in previous clinical gene therapy trials which have used FN. Because of the effectiveness in assisting with gene transfer into hematopoietic stem cells, FN has been frequently used in gene transfer trials.

- Results from insertion site testing will be known within 7-14 days from the initiation of testing. The expected enrollment is one participant per month. If testing shows evidence of clonality of insertion sites, then repeat testing will be done immediately, and further evaluation will be performed to include assessment of the bone marrow for evidence of leukemia by morphology or flow cytometry. The DSMB will be notified immediately of this evaluation, and study accrual will be halted until they make a final assessment regarding risks to subsequent patients.

E. Public Comment

There was no public comment.

F. Synopsis of RAC Discussion and RAC Recommendations

Dr. Wara summarized the following RAC comments and recommendations:

- The purity of the transduced cell product being administered is an important safety issue. Additional efforts should be made to characterize non-CD34+ cells in the product. The presence of tumor cells, which should be screened for using an appropriate cell-surface marker for glioma cells, would be of particular concern.

- The rationale for studying 10 research participants is not clear. More thought should be given to what useful data will be gleaned from studying 10 research participants, and the basis for selecting the sample size should be outlined in the protocol.

G. Committee Motion 4

It was moved by Dr. Rosenberg and seconded by Dr. Lo that the 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. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VII. Data Management Report/Drs. Albelda, Heslop, Simari, and Wara

Dr. Simari reported that there had been 16 protocol submissions since March 2005, three of which were selected for in-depth review and public discussion. Of the 13 trials not selected, 11 were for cancer, one for rheumatoid arthritis and one for peripheral artery disease. Regarding vector usage, six protocols used plasmids, two used AAV, one used adenovirus, one used fowlpox and vaccinia, and one used RNA transfer. No AEs reported were considered necessary for public discussion.

Dr. Heslop reported that 136 protocol amendments had been filed in the past three months, of which 16 were principal investigator (PI) or site changes, 9 were protocol design modifications, 17 were status changes, 65 were annual updates, 3 were responses to the *NIH Guidelines, Appendix M(1)C(1)*, and 26 were other modifications or amendments. None warranted public discussion.

VIII. Discussion of Human Gene Transfer Protocol #0504-703: A Phase I, Safety, Dose-Escalating Study of MultiGeneAngio in Patients with Peripheral Arterial Disease (PAD)

Principal Investigator:	P. Michael Grossman, M.D., University of Michigan Hospitals and Health Centers
Sponsor:	Moshe Y. Flugelman, M.D., Lady Davis Carmel Medical Center/MultiGene Vascular Systems, Ltd.
RAC Reviewers:	Drs. Dewhurst, Powers, and Simari
Ad Hoc Reviewer:	Stephen E. Epstein, M.D., Washington Hospital Center/MedStar Research Institute

[Note: Dr. Gelehrter recused himself because of a conflict of interest.]

A. Protocol Summary

An estimated 8 million individuals in the United States and 16 million worldwide suffer from PAD. PAD is a clinical syndrome in which patients with blocked arteries in the legs suffer from consequences of reduced blood supply to the legs secondary to the arterial blockage. Reduced blood supply to the legs can cause discomfort or pain while walking (claudication) or can result in gangrene, requiring amputation. The severity of signs and symptoms depends on the degree of arterial blockage and on the development of collateral circulation, a natural process that bypasses the blocked artery. In most individuals with blocked arteries, natural bypasses develop to some extent. Unfortunately, those collateral arteries are very small, cannot replace the arteries that are blocked, and therefore cannot provide a sufficient amount of arterial blood to the legs. A significant number of these patients can be treated with exercise programs and drugs. Nearly 500,000 PAD patients in the United States are treated by surgery or balloons and stents. Every year, 200,000 U.S. patients who are not amenable to current treatments undergo leg amputation or are crippled by pain and are unable to walk even short distances. Many of these individuals also suffer from diabetes mellitus.

MultiGene Vascular Systems, Ltd., has developed a therapeutic approach to enhance arteriogenesis, and increase blood flow around the occluded artery, thereby, improving the blood supply to the ischemic organ. Angiogenesis and arteriogenesis require ongoing input from gene-activated endothelial and smooth muscle cells (SMC). $VEGF_{165}$ and angiopoietin-1 (Ang-1) genes play a central role in the process. Autologous endothelial and SMC cells are isolated from a short venous segment that is stripped from the arm under local anesthesia. The endothelial cells will be transduced with a retroviral vector expressing Ang-1 and the SMC will be transduced with a $VEGF_{165}$ vector. The cell-based product will be injected at the site of arterial blockage using a routine catheterization procedure. Product safety will be tested in 12 participants with PAD, with an escalating dose of the participant's own cells injected at the site of arterial blockage in the leg. The primary focus of this study is product safety, and the secondary goal is to document any therapeutic effect.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Thirteen RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novel *ex vivo* gene transfer approach for PAD, use of a new transgene, use of systemic expression of two growth factors, use of retrovirally transduced cells expressing molecules that may dysregulate cell growth, and the anti-apoptotic potential of the novel transgene. Several RAC members noted additional scientific issues and concerns related to the informed consent process and document.

Dr. Dewhurst asked the investigators to comment on any concerns about the possibility that stem or progenitor cells may be present in the endothelial or SMC populations and could also be transduced with the Ang-1 or $VEGF^{165}$ vectors, given the potential growth-promoting or anti-apoptotic activities of the transgenes. He also asked about the potential effects of Ang-1 on neural cells. Regarding the preclinical data, he requested additional information on the rabbit in which injected cells were found in the lung and insight as to whether a similar outcome could occur in humans. Regarding the clinical protocol, Dr. Dewhurst requested additional details about the technical aspects of the cell isolation process and

suggested that a 1-year cancer screening followup might be replaced with a more proactive approach that would include an annual followup for a lengthier period. His comments on the informed consent document included the inappropriate use of "therapy," additional language regarding the possibility of participants needing a repeated vein-stripping procedure, and a more specific description of the risks of cell embolization.

Dr. Powers stated that the informed consent document misleadingly suggests a therapeutic benefit to participants, referring to "therapy" and similar language. He inquired about the relationship between the sponsor and the PI, whether the PI has any financial interest in the investigative agent, and whether the sponsor has any control over the monitoring and reporting of AEs or the termination of the study for safety reasons.

Noting the complexity of the protocol, using two different cell types and gene products, Dr. Simari requested further information about several critical assessments not included in the preclinical studies: the levels and duration of *in vivo* transgene expression, biodistribution, the purity of the transduced cell populations, survival of delivered cells, dose response, and the oncogenic potential of Ang-1. He also asked for clarification of the role of each type of transduced cells and the unmodified cells. The participant population also should be clarified since it was described both as patients with no options and chronic, stable claudicants, who would have treatment options. He also expressed concern that the cell populations may contain proliferative progenitor cells, which as in the X-SCID trial, may become transformed by insertional mutagenesis. Dr. Simari also noted informed consent issues, including the presence of therapeutic misperception, the lack of information about the relationship of the PI to the sponsor, possible underestimation of the risk of harvesting, and concerns about the potential presence of progenitor cells and retroviral insertion.

Dr. Epstein expressed concern about the insertion of a retroviral vector into cells that persist for long periods of time, given that prolonged unregulated expression of VEGF and Ang-1 can result in hemangioma. He asked the method being used to detect hemangiomas since routine histological analysis of tissues may not be adequate. He suggested performing either angiography or CT of the hind limb at 3 months and 6 months after cell administration. Dr. Epstein asked for additional information about the statistical analysis in the rabbit studies that showed efficacy and more information about studies on the miniature pigs (minipigs). He also noted that intra-arterial injection makes it difficult to demonstrate cell persistence and the length of time the cells express their transgene products; if cells were injected intramuscularly and then sampled serially, the result would provide data about transgene persistence and expression.

C. RAC Discussion

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

- Dr. Albelda expressed concern that this trial is not designed to analyze gene transfer or VEGF levels, thus, if a physiological response is not observed, it will difficult to identify problems and determine next steps.

- Dr. Dewhurst asked whether there is any inherent difference in the mouse model versus the rabbit model and which one is more reliably predictive of human reaction.

- Ms. Kwan asked whether this protocol, as a multicomponent study, is designed so that if results are positive but serious AEs occur, it will be possible to parse out the culpable component.

- Dr. DeMets noted that, for a Phase I, dose-limiting toxicity (DLT) study, the investigators plan on making many measurements. He expressed his opinion that, if the goal is to find the DLT, all of those measurements may not be necessary at this stage.

- Dr. DeMets suggested collaboration with a biostatistician to ensure that the planned protocol will be able to reach its stated goals.

D. Investigator Response

Drs. Flugelman and Grossman responded to RAC questions and concerns in the written reviews and at this meeting with the following information:

- Biodistribution studies demonstrated that vector containing cells were present in the ischemic, treated muscle tissues 6 month following dosing. In several animals injected with the high dose, cells containing vector sequence were found in the lungs. No transduced cells were found in other organs or tissues.

- Two different methods to test effectiveness were used in the preclinical studies, one to assess perfusion and one to assess flow. To assess flow, the investigators used a Doppler flow meter that was positioned on the femoral artery of the affected leg and compared those results with the other leg that was not operated on and not treated. To assess perfusion, the investigators used a laser Doppler system that sends a laser beam to the muscle and traces the velocity of the red blood cells in the tissue. Measuring flow in just one artery may miss important information, but combining perfusion and flow should provide a fuller picture.

- All participants enrolled in this study will be individuals who have been on optimal medical therapy, have been offered and have undergone exercise therapy, have been counseled to stop smoking and have stopped smoking, and have been evaluated for revascularization and, for a variety of reasons, are not optimal candidates for that procedure.

- The vein harvest is done on an outpatient basis under local anesthesia. A literature review regarding the vein harvest procedure indicates the relative ease with which it is done and the lack of complications associated with vein harvesting from the forearm.

- In the pre-clinical studies of MultiGeneAngio, the issue of efficacy and the need for each component of MultiGeneAngio was addressed by injecting unmodified cells and each cell type after transduction separately. The efficacy study showed that the combination of the two cell types transduced with the angiogenic genes was superior in regard to flow increase, perfusion increase, and improved vascularization as evident by angiography to the use of the unmodified cells, and the use of single type of transduced cells.

- The investigators have followed rabbits for up to 6 months to exclude tumorigenicity of the injected cells, with no sign of any tumor or outgrowth of any cell types.

- Regarding concerns about hemangioma, the full pathological report showed no signs of hemangioma from hundreds of slides (15 slides from each leg), 72 angiographies and at least 25 angiographies at 6 months.

- Both the minipig and the rabbit were used as models so that the investigators would feel more confident about carrying this trial to humans; neither is more predictive of human reactions than the other.

E. Public Comment

Although agreeing that the informed consent document had been improved as a result of a rewrite, Dr. Borror noted that complex language remains that would not be understandable by all participants.

F. Synopsis of RAC Discussion and RAC Recommendations

Dr. Wara summarized the following RAC comments and recommendations:

Scientific/medical/study design issues:

- Preclinical studies should be conducted, using the same product to be used in the clinical trials, for the following reasons:
    - To help clarify the effects due to expression of the two proposed transgenes by investigating the contributions of the nonmodified smooth muscle and endothelial cells, including survival of those cells in ischemic tissues and any effects they may have on improving perfusion of the ischemic tissues. In addition to the nonmodified cells, other controls should include each cell type transduced with a retroviral vector expressing a marker gene.
    - To clarify the purity and identify the types of nontargeted cells present in the cell populations.
    - To conduct biodistribution studies to assess the distribution, viability, and ultimate fate of delivered cells. The persistence of the cells and the gene products should be determined utilizing real-time, reverse transcriptase polymerase chain reaction. It is important to follow messenger RNA levels as well as virus DNA levels. Data on the tracking of delivered cells in at least one nonhuman animal, which were referenced during the presentation, should be provided to the OBA.
    - To clarify the dose response for both the cells and the gene products.
- The investigators should conduct an additional statistical analysis of the preclinical data to clarify the efficacy of the proposed approach. In particular, variance analysis would assist in the evaluation of the supplemental preclinical data provided prior to the RAC meeting.
- The investigators should clarify the eligibility criteria for including patients with chronic claudication who have received usual clinical care such as exercise. Who will make enrollment decisions? A clinician not involved in the protocol should be engaged to discuss the available options and to provide advice about enrollment decisions.
- The investigators should clarify the risk of the injected cells lodging in the pulmonary vasculature and reference this information in the informed consent document.
- The investigators should clarify the plans and responsibility for long-term followup, including the appropriate followup for malignancies.
- It is not clear whether the determination of DLT is a study objective and, if so, how DLT will be defined. The investigators should clarify this and define the criteria for proceeding with dose escalation.
- The investigators should consider using an imaging technique to monitor the potential risk of hemangiomas.

Ethical/legal/social issues:

- The reading level of the informed consent document is too high, and the investigators should simplify the language to increase comprehensibility.

G. Committee Motion 5

It was moved by Dr. DeLuca and seconded by Dr. Simari that the 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. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

IX. Day One Adjournment

Dr. Wara adjourned Day One of the June 2005 RAC meeting at 4:45 p.m. on June 15, 2005.

X. Day Two Opening

Dr. Wara opened Day Two of the June 2005 RAC meeting at 8:00 a.m. on June 16, 2005.

XI. Discussion of Human Gene Transfer Protocol #0410-677: A Phase I Trial of Ocular Subretinal Injection of a Recombinant Adeno-Associated Virus (rAAV-*RPE65*) Gene Vector in Patients with Retinal Disease Due to *RPE65* Mutations

Principal Investigator:	Samuel G. Jacobson, M.D., Ph.D., Scheie Eye Institute/University of Pennsylvania Health System
Additional Presenters:	Gregory M. Acland, Ph.D., Cornell University; Jean Bennett, M.D., Ph.D., University of Pennsylvania; Terence R. Flotte, M.D., University of Florida; William W. Hauswirth, Ph.D., University of Florida
Submitter:	Philip J. Cross & Associates, Inc.
RAC Reviewers:	Ms. Kwan, Dr. Heslop, and Dr. Vile
Ad Hoc Reviewer:	Karl Csaky, M.D., Ph.D., NEI

[Note: Drs. Albelda, Muzcyzka, and Johnson (absent for this meeting) recused themselves because of conflicts of interest.]

A. Protocol Summary

Leber congenital amaurosis (LCA) is an incurable and untreatable group of early-onset, molecularly heterogeneous, retinal degenerative diseases that cause severe vision loss. One of the LCA subtypes is caused by mutations in the RPE65 gene. RPE65 encodes a protein in retinal pigment epithelium (RPE) cells critical to the retinoid (vitamin A or visual) cycle: specifically RPE65 is necessary for 11-cis retinal to be synthesized and available to form light-absorbing pigment in photoreceptors. In studies in RPE65 knockout mice and dogs with a naturally occurring mutation, administration of rAAV-RPE65 by subretinal injection resulted in restoration of visual function that persisted for at least three years in the dog model.

The protocol proposes to extend this work to humans with blindness due to *RPE65* mutations. Participants with known *RPE65* mutations will receive a surgical injection in the retina (one eye only) of rAAV-*RPE65.* The initial trial is one of safety, and the participants will be monitored by clinical eye examinations for any side effects.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a novel transgene, administration of the construct by subretinal injection, lack of available preclinical biodistribution data using this transgene, risk of autoimmune response to the transgene, inclusion of research participants with non-life-threatening disease with varying lifespans following the clinical trial, the need for explicit tests to detect germ-line biodistribution of the AAV vector, and the potential for unexpected complexity when expressing wild-type *RPE65* in persons with a mutation in this gene.

Ms. Kwan noted that, unlike the majority of gene transfer protocols submitted for RAC review, this protocol addresses a devastating but nonfatal disease. She requested a thorough discussion of the actual and theoretical long-term safety issues that could be associated with the proposed gene transfer, at least some of which should be presented in simple lay language that might be used for discussion with

a potential study participant (who could be as young as 18 years old). Ms. Kwan also requested that the discussion include the potential restrictions and followup responsibilities that would be incurred by participants.

Noting that this study has the potential to improve vision in a group of individuals with a genetic disease resulting in severe vision loss, Dr. Heslop stated that a number of safety concerns are inherent in the proposed delivery method of this transgene to a participant population with a nonfatal (although debilitating) disease. She requested additional data on the biodistribution of this construct, particularly in the central nervous system (CNS) and germ-line tissues, and the duration of transgene expression in the target retinal cells. She also called for discussion of the risks of an autoimmune response or inflammation (as seen in some of the canine and nonhuman primate models) and the possibility that vision might worsen as a result of this study. Dr. Heslop asked the investigators to provide more details about the degree of visual impairment of the participants enrolled in this study. She also asked the investigators to provide more detail about their plan to follow study participants over the long term.

Dr. Vile noted the importance of addressing fully the risk-benefit issues since the disease being studied is not fatal. One of his major concerns related to the direct toxicology of the vector/injection following subretinal injection, and he suggested that the outcome of the nonhuman primate histopathology study be examined to determine the local effects of both the injection itself and any inflammatory reactivity associated with the vector. In addition, Dr. Vile requested ongoing updates on the long-term effects of expression of the human *RPE65* protein from the ocular histopathology of the remaining 11 monkeys involved in the 3-month study. He also asked whether expression of a foreign gene in the retinal epithelium could lead to the priming of effective immune responses against that protein. He requested discussion of this in context with the different types of RPE65 mutations (i.e., possible different responses for null vs. missense mutations).

Dr. Csaky requested a more complete discussion of the biodistribution of an AAV vector injected subretinally in research participants, including the possibility of inflammation and cerebritis and whether MRI scanning and neurologic exams should be performed on participants. He noted that a more complete discussion of the implications of uveitis following injection should be addressed in the protocol and in the informed consent document. Regarding direct ocular toxicity of the vector, Dr. Csaky asked whether the large amount of total virus particles delivered to more diseased retinas might cause damage, and what percentage of the preparation were empty capsid particles. He also noted that genotype and phenotype variations in LCA warrant addressing questions related possible different effects such as autoimmune reactions more likely to occur in participants with nonsense mutations while participants with missense mutations possibly not responding to the proposed gene replacement.

C. RAC Discussion

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

- Ms. Kwan suggested that the investigators engage the assistance of a psychologist or social worker who can help evaluate the likelihood of a participant's ability to fulfill the enrollment obligations over the long term as well as over the short term.

- Dr. Weber asked whether 3 weeks is adequate to fully assess safety in terms of inflammation, retinal detachment, and other potential AEs.

- Dr. Lo asked whether the participants, having received the proposed subretinal injection and retinal surgery, are then at greater risk for long-term AEs such as posterior subcapsular cataract, retinal detachment, or retinal hemorrhage.

- Ms. Kwan noted that the informed consent document was unusually well written, including language that was crafted carefully so as not to suggest benefit that was not likely to occur and detailing plans to speak with the participants to assess their comprehension.

- Dr. Csaky asked about the possibility of toxicity in the optic nerve or optic chiasm for these participants once they receive a substantial dose of AAV vector and expressed concern that the participants might not be aware of the appearance of such toxicity because their visual function is already severely impaired.

- Dr. Lo stated that the risk section of the informed consent document appeared to emphasize the short-term risks due to short-term complications of the procedure. He suggested adding a sentence about the possibility of long-term risks, including unanticipated long-term risks, since some of the participants will be quite young when they undergo this procedure.

D. Investigator Response

Dr. Jacobson and his colleagues responded to RAC questions and concerns in the written reviews and at this meeting with the following information:

- Regarding possible toxicity in the optic nerve or optic chiasm, the investigators had planned to look carefully at the 3-month nonhuman animals for such evidence. For the research participants, the investigators had planned to conduct neurological exams but agreed that perhaps a neurologist should examine the participants in anticipation of that toxicity.

- Some of the experimental dogs are showing an untreated mild inflammatory response. In response, the investigators will discuss whether to pretreat research participants before surgery to ward off any potential inflammatory response.

- Regarding the possibility of an immune reaction to the RPE65 protein, the investigators noted that no antibodies to RPE65 were detected in 28 dogs injected with the human RPE65 cDNA.

- While it is known that LCA-causing mutations are found across the entire coding sequence, little is known about the effects of the different mutations on the RPE65 protein. It is not know which mutations result in true null effects vs. truncated non-functional proteins. The trial stopping criteria could be modified to address "lead indicators" of adaptive immune responses, such as rising titers of antibodies against RPE65 and/or rising values on the lymphocyte proliferation assays on exposure to the specific RPE65 antigen, which is the marker for cell-mediated immune response.

- In the monkey biodistribution study, the vector copy number detected in the retina was much higher than that detected in other tissues. Lower copy numbers were detected in the optic nerve. Other areas of the brain related to vision could also show low copy numbers, but the data were not uniform for dose or location. No vector was detected in the gonads.

- Patients with these retinal degenerative diseases are prone to subcapsular cataracts as a result of the basic disease process. Whether that process will be accelerated by the proposed intervention is unknown. There was no such evidence in the monkeys at 2 months.

- Regarding the duration of gene expression, RNA is detected in RPE of injected dogs (after RT-PCR of laser capture microdissected cells) at least through 8 months. The long-term ERG/visual success to date is indirect *in vivo* evidence of expression.

- Longterm follow-up will occur for 15 years after study agent administration. Annually, subjects will be contacted by mail or by telephone and information concerning any cancer, neurological, autoimmune or hematological disorder that developed or worsened since the previous contact will be obtained. The subjects will also be asked for information concerning hospitalizations or use of medications. At the time of death, no matter what the cause, permission for an autopsy will be requested of subjects' families. Subjects will be asked to advise their families of this request and of its scientific and medical importance.

- Local inflammation can occur after delivery of AAV – partly due to the surgical procedure and maybe partly due to delivery of the AAV itself. A single incident of severe ocular inflammation occurred 4 years ago in an RPE65-mutant dog injected with incompletely purified vector. There have been no further incidents of uncontrolled ocular inflammation. In the non-human primate experiments under GLP conditions using surgical procedures that approximate those in human operating rooms, ocular inflammation to date has been detectable but mild at early times post-op. Further explanation of this potential problem will be added to the protocol and consent.

E. Public Comment

Dr. Borror recommended that participants be given an audiocassette tape of the protocol description and the informed consent document, and if necessary, the equipment to listen to the tape so that they can review the information when they go home and after the study starts. This information could be provided in Braille to participants who can read Braille. It is important that participants not be dependent on other people when they need to refer to the protocol and the informed consent document.

F. Synopsis of RAC Discussion and RAC Recommendations

Dr. Wara summarized the following RAC comments and recommendations:

Scientific/medical/study design issues:

- A toxicology study of six cynomolgus monkeys has been carried out to determine the short-term sequelae of vector administration. Eleven other monkeys are involved in a longer term (3 months) evaluation. When the 11 monkeys are evaluated, ocular histopathology should be carefully reviewed for the local effects of both the injection procedure and any inflammatory reactivity associated with the virus. The histopathology data should inform the design of the clinical trial.

- In preclinical canine and nonhuman primate studies, the vector migrated to the optic nerve and chiasm and, even more concerning, to the visual cortex, suggesting a possible nonspecific flow of vector to other brain regions. Therefore, a careful clinical assessment of participants is essential. It is critical to determine whether biodistribution also will occur in humans and, if so, whether it causes toxicities. Because toxicities in clinical studies cannot be assessed through visual field testing in these visually impaired participants, the use of high-resolution MRI scans of the optic nerve should be considered to assess whether migration occurred to the optic nerve and chiasm.

- Injection of the vector construct, rAAV-*RPE65*, caused ocular inflammation in the nonhuman animal model. As an immunogen, the *RPE65* protein could trigger an immune response in the human eye as well. As such, levels of ocular inflammation should be measured, and toxicity thresholds should be determined. In addition, consideration should be given to administering periocular steroids prophylactically prior to surgery.

- The protocol calls for a 3-week interval between dose cohorts. Since this interval may not be long enough given the safety issues, it would be better to determine the pacing of the study after the data from the preclinical toxicity studies have been reviewed and to allow for further adjustments as the clinical trial proceeds if toxicities occur in trial participants.

Ethical/legal/social issues:

- A psychologist or social worker should be involved in the consent process to help evaluate the capacity of the prospective participants, particularly younger ones, to adhere to the protocol requirements, including long-term followup tests and assessments.

- The informed consent document does not currently state that one of the risks of the protocol is increased or complete loss of vision. This omission is serious and should be corrected. Long-term risks such as retinal detachment also should be discussed, and the informed consent document should include a statement about the possibility of other unforeseen risks.

- Given the prospective participants' visual limitations, a Braille or audiocassette version of the informed consent document should be developed and offered to participants.

G. Committee Motion 6

It was moved by Dr. Heslop and seconded by Dr. Lo that the 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. The vote was 15 in favor, 0 opposed, 0 abstentions, and 3 recusals.

XII. Biological Containment for Work with Noncontemporary Strains of Influenza: Harmonization of the *NIH Guidelines* with Other Federal Guidances

Introduction: Kathryn Harris, Ph.D., Contractor, OBA
Presenter: Jacqueline M. Katz, Ph.D., U.S. Centers for Disease Control and Prevention (CDC), DHHS

Dr. Katz described the process to revise *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*), a joint NIH and CDC publication which provides guidelines for the safe handling of infectious agents in laboratories. The BMBL is updated approximately every 5 years and the 5th edition is expected to be published in fall 2005. As part of that process, a committee has updated the agent summary statement for influenza. In response to the inadvertent inclusion of H2N2 influenza virus in a proficiency testing kit, the CDC and NIH decided to post interim guidelines in advance of publication of the BMBL.

The 4th edition of the BMBL recommended BSL2 facilities and practices for influenza viruses, including non-contemporary human strains. It did not address work with high- and low-pathogenicity avian influenza viruses (HPAI) or genes of the 1918 influenza virus. The 5th edition will recommend BSL2 for contemporary human strains. BSL3 or ABSL3 with respiratory protection will be recommended for non-contemporary human virus strains (e.g., H2N2). BSL3 or ABSL3 with respiratory protection and shower out will be recommended for work with reassortant or recombinant viruses including genes from the 1918 virus or HPAI viruses. Risk assessment for work with recombinant or reassortant viruses should be protocol driven and consider the gene constellation, the number of years since the viruses circulated among humans, biocontainment level recommendations for the parental strains, evidence of clonal purity and phenotypic stability, and any evidence for attenuation in appropriate animal models.

Dr. Harris summarized modifications to the *NIH Guidelines* to harmonize with the interim guidelines and revised BMBL. Currently Appendix B lists all influenza viruses as Risk Group 2 agents. While the *NIH Guidelines* do state that containment levels higher than the risk group classification may be appropriate, to avoid confusion, Appendix B will be changed to address contemporary and non-contemporary strains. While contemporary strains of influenza virus will retain their classification as Risk Group 2 agents, the classification for noncontemporary human strains of influenza virus (e.g, H2N2, 1918) and HPAI (e.g., H5N1) in *Appendix B* of the *NIH Guidelines* would be changed to Risk Group 3. In addition, specific biosafety recommendations for research with noncontemporary strains of influenza virus will be added to *Section II-A-3* of the *NIH Guidelines*.

A. RAC Discussion

Questions and comments from RAC members and others included the following:

- Dr. Dewhurst asked for a definition of "noncontemporary." Dr. Katz responded that the H2N2 influenza viruses that circulated between 1957 and 1968 are classic examples, because

individuals born after 1968 have no immunity to them. However, the H3N2 and H1N1 influenza viruses currently circulating are undergoing antigenic variation, so individuals with recent exposure to H3 or H1 influenza viruses (the majority of the population) will have some unknown level of immunity to the earlier strains. "Noncontemporary" refers to subtypes that have not circulating in the human population for a period of time and this should be considered in the risk assessment.

- Dr. Dewhurst requested that the detailed and clear slides from both presenters be posted on the Web site since they contain useful information for IBCs. Links to the presentations have been included in two sections of the OBA web pages: RAC meeting section and with the resources included in the Safety Symposium for recombinant DNA research with pathogenic viruses section.

- For the benefit of IBCs, Dr. DeLuca recommended that both the BMBL and the *NIH Guidelines* clarify that risk assessment of recombinant viruses start with the containment level of the parent virus in the higher risk group, and then lower containment can be considered after a protocol-driven, comprehensive risk assessment.

XIII. Discussion of Human Gene Transfer Protocol #0504-707: A Dose-Finding and Safety Study of an Oncolytic Poliovirus/Rhinovirus against Malignant Glioma

Principal Investigator:	Darrell D. Bigner, M.D., Ph.D., Duke University Medical Center
Submitter:	Matthias Gresh Gromeier, M.D., Duke University
RAC Reviewers:	Drs. DeLuca, Johnson *(written review)*, Lo, and Wara
Ad Hoc Reviewer:	Ellie Ehrenfeld, Ph.D., NIAID

A. Protocol Summary

The protocol proposes the use of polio virus (PV) for the treatment of malignant brain tumors. PV exhibits a natural tropism for neuroectodermal tumors which overexpress the PV receptor, CD155. The presence of the PV receptor renders the tumor cells susceptible to infection and lysis by PV. To target PV to tumor cells and prevent infection of spinal cord neurons, the PV vector was modified by exchanging the PV internal ribosome entry site (IRES) with its counterpart from human rhinovirus 2 (HRV2) The chimeric vector, PVS-RIPO, lost the ability to replicate and cause damage to cells of neuronal lineage, probably due to incompatible cell type-specific RNA:protein interactions, but rapidly propagated in and caused lysis of malignant glioma cells. PVS-RIPO demonstrated strong cytolytic properties in established glioma cell lines as well as in cell cultures established from patients' tumors in *in vitro* or xenograft animal models.

PVS-RIPO will be studied in clinical investigations against glioblastoma multiforme (GBM), the most severe form of malignant glioma. The virus will be injected directly into the tumor mass, and participants will be thoroughly followed for up to 1 year thereafter to determine the safety of the approach and detect potential antitumor effects of virus administration.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Sixteen RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential of the study agent to mutate to a more virulent form upon replication in permissive cells and concerns about the safety of administering a modified PV into participants with GBM since such individuals are prone to immunodeficiency and since published reports have noted the occurrence of *Pneumocystis carinii* pneumonia in this population. Additional scientific questions and concerns included safety, efficacy, and outcome data in a cynomolgus monkey model; dosing in the preclinical studies; data on the genetic stability of the PVS-RIPO construct; and additional issues related to the informed consent document.

Dr. DeLuca asked about the status of the nonhuman primate studies and the mouse model. He noted that the Sabin vaccine strains of PV can mutate to more pathogenic forms and asked whether studies have

assessed the frequency of vector mutation and how the proposed clinical doses compare with those found in vaccine doses. Dr. DeLuca suggested that the use of human embryonic kidney 293 cells as a model neuronal cell line may not be appropriate and that an alternative neuronal model should be explored. He also suggested a slight modification in the dose escalation to a threefold increase at each increment, rather than the planned fivefold followed by twofold.

Dr. Johnson's review was read into the record by Dr. DeLuca. Dr. Johnson expressed concern that the nonhuman primate studies cited in the protocol were not performed with the agent currently proposed for use and that the virus used in the nonhuman primate studies was delivered intraspinally rather than intracerebrally, as proposed for this study. He suggested that the a nonhuman primate study with the proposed agent should be completed before further evaluation of the protocol is undertaken. With regard to the proposed nonhuman primate study, he asked how the number of nonhuman animals to be studied had been chosen, why the maximum dose would not be higher by one log than the proposed human dose in an attempt to find toxicity, and whether different regions of the nonhuman primate brain should be injected and studied since GBM is not likely to be confined. Dr. Johnson also requested an explanation of how the rhinovirus internal ribosomal entry site functions only in dividing cells.

Dr. Lo primarily discussed precautions that should be taken regarding several categories of persons in addition to the research participant who may be at risk in this study. Operating room staff members (who might be at risk for a needlestick containing PVS-RIPO) and nursing and custodial staff members (who might be exposed to PVS-RIPO excreted in feces) perhaps should be tested for antibodies to PV, and perhaps those who are immunocompromised should be excluded from caring for participants in this study. The informed consent document and discussions related to the consent process should include a section on risks to family members and other household contacts. Dr. Lo suggested some changes to the protocol to minimize transmission risks, such as adding "having a family contact who has not been vaccinated against polio" as an exclusion criterion and not allowing hospital staff members who have not been vaccinated against polio to care for these participants. He also asked what steps would be taken with research participants who excrete virus beyond the expected 30-day shedding period.

Dr. Wara's also asked for clarification of the results of the nonhuman primate study. She asked whether potential participants for this protocol should undergo a screening evaluation for secondary immunodeficiency, given the rare occurrence of *Pneumocystis carinii* pneumonia in this group, and whether antibody to PV is an adequate screen. Dr. Wara noted that a secondary group possibly at risk from this protocol is family members and care providers and suggested that they be checked for antibody to PV. She also suggested that the informed consent document include a statement regarding death as a possible consequence of participation in this trial.

Dr. Ehrenfeld noted that the proposed virus should be safer than the vaccines used in the human population as the IRES modification is being added to the already-attenuated phenotype of the Sabin type 1 vaccine strain. The requirement that participants must have humoral immunity to be included in this study is an important precaution because the experience with the Salk vaccine suggests that humoral immunity is sufficient to protect against polio. She requested discussion at this RAC meeting as to whether family members and care providers should receive a vaccine boost as a precaution. Dr. Ehrenfeld also noted that there was no disclosed basis for the assertion that excretion of virus from participants is anticipated not to exceed 30 days.

C. RAC Discussion

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

- Dr. Vile requested data on curing gliomas in mice.

- Dr. Bohn asked whether the investigators had conducted studies of primary neurons *in vitro* using tissue from either rats or humans.

- Dr. Weber noted that the investigators propose that health care workers or clinicians who are immunocompromised would be excluded from taking care of the participants in this trial. Although the investigators can inform them of the risk and reassign them if they want to be voluntarily reassigned, they cannot exclude those individuals against their will, since to do so would violate Federal antidiscrimination laws.

D. Investigator Response

Dr. Gromeier and his colleagues responded to RAC questions and concerns in the written reviews and at this meeting with the following information:

- The non-human primate toxicology studies are a stringent requirement before progressing to the clinical trial and are being designed in collaboration with the NCI-Rapid Access to Intervention Development program. The toxicology plan includes four monkeys (an equal number of males and females) for each of the three dose groups. Using direct intracerebral injection, there will be a vehicle control, a dose of $1X10^6$ pfu per monkey, followed by a dose of $1X10^9$ pfu. Two sacrifice times will be used—day 29 and day 58. The clinical dose will depend on the design and outcome of the toxicology trials.

- The CD155 transgenic mice develop classic polio when infected by different routes of administration; therefore, the mice provide an alternative model to non-human primate neurovirulence testing.

- While the possibility of staff exposure to the vector is remote, the suggested exclusions and preventative measures will be included.

- The informed consent document will be modified to include a section describing risks to household contacts.

- The Sabin vaccine is routinely tested in non-human primates by intraspinal injection as a requirement for batch release. This model has generated considerable data regarding the attenuated neuropathogenecity of PVS. Any RNA virus is inherently genetically unstable. However, the IRES modification in PVS-RIPO is much less likely to revert to neurovirulence than the point mutations in the parental Sabin strain.

- Regarding whether family members and care providers should be boosted with Salk vaccine as a precaution, the investigators responded that they would consider changing the instructions on the informed consent document to recommend that people be informed that the booster is available. Dr. Gromeier stated that Duke University Medical Center does not believe mandatory boostering is necessary.

- HRV2 IRES-mediated neuro-attenuation appears to result from incompatible RNA:protein interactions at the IRES/viral 3'UTR resulting in translation repression at the HRV2 IRES in neuronal cells.

- Based on the known propensity of GBM to arise multifocally, any form of administration that would spread the agent evenly over a wider distribution area could aid targeting and enhance oncolytic potential. However, when convection-enhanced delivery studies in an athymic rat GBM model were performed, the investigators did not observe significant differences in clinical response compared to single stereotaxic injection.

- The investigators have conducted and published xenograft studies in rats and in mice (including CD155 transgenic mice), using both subcutaneous and intracerebral delivery. The results show that every tumor in every CD155 mouse has been cured.

- The investigators agreed that the dose escalation would be changed to successive three fold increases.

- Regarding the use of 293 cells as a neuronal model, 293 cells were established from embryonic kidney transformed with adenovirus. However, the cells are of neuronal origin and may have been preferentially transformed with the adenovirus. The 293 cells express a range of neuronal markers and may be a preferred model compared to neuroblastoma cells, which often convert to fibroblast-like cells during passage.

E. Public Comment

Karen Muszynski, Ph.D., Rapid Access to Intervention Development Program, Biological Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI-Frederick Cancer Research and Development Center, briefly discussed two preclinical safety studies conducted to address the issue of reversion to neurovirulence phenotype. Conducted by Eckard Wimmer, Dr.Rer.Nat., Ph.D., State University of New York, Stony Brook, one of these safety studies injected PVS-RIPO into the brains of CD155 transgenic mice and then harvested the virus from the brain and spinal cord at different timepoints. The intention was to isolate any neurovirulent variants that occurred, sequence, locate the mutations, and determine the rate of reversion. Dr. Wimmer was unable to recover any neurovirulent variants over a 6-week period. His conclusion was that the virus grows very slowly in the CNS of mice, and he showed that the virus is cleared from the CNS after approximately 4 weeks. A second neurovirulence study in mice was conducted with the FDA. That study was conducted using the World Health Organization protocol for releasing vaccine lots. None of the mice that received PVS-RIPO showed any signs of neurovirulence in contrast to the Sabin vaccine control mice, which experienced some paralysis and/or death at a low rate. The conclusion based on these two studies was that PVS-RIPO is more stable than the Sabin strain.

Dr. Borror noted that the informed consent document contains some complex language that would not be understandable to all participants. The April 2005 version describes the procedure for virus administration by stating that it "works its way into the cancer cells to kill them." This wording implies therapeutic benefit, and she suggested that the investigators change the wording to be more tentative.

F. Synopsis of RAC Discussion and RAC Recommendations

Dr. Wara summarized the following RAC comments and recommendations:

Scientific/medical/study design issues:

- The gene transfer product to be used in the clinical protocol, PVS-RIPO, a poliovirus/rhinovirus recombinant, will be tested in nonhuman primate studies at doses of up to 10^9 pfu. The biodistribution and toxicity results from these studies should be used to guide the design of the clinical study. The preclinical study results should be submitted to the OBA.

- Since GBM typically is not confined to one area of the brain, it would be useful to gather data from the nonhuman primate studies about the effects of injections in multiple areas of the brain.

- The investigators should consider assessing the capacity of PVS-RIPO to replicate in glial tumor cells in the presence of specific antibodies to the virus.

- Although the vector is a highly attenuated form of PV, it is, nonetheless, an infectious agent, and as such, the investigators should consider offering a booster of the Salk polio vaccine to family members and care providers.

Ethical/legal/social issues:

- The investigators should revise the informed consent document as follows:

    - Given that this is the first time a PV vector will be injected into the human brain, the informed consent document should indicate that death, although not anticipated, is a possibility.

    - The investigators should rewrite the informed consent document. The reading level is too high. In addition, care should be taken to avoid overstating the potential benefits of participating in the study. (For further information, please refer to the *NIH Guidance on Informed Consent for Gene Transfer Research* at <http://www4.od.nih.gov/oba/rac/ic/>.)

G. Committee Motion 7

It was moved by Dr. Bohn and seconded by Dr. Lo that the 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. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XIV. Closing Remarks and Adjournment/Dr. Wara

Dr. Wara thanked the participants and adjourned the meeting at 12:30 p.m. on June 16, 2005.

[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.
Acting 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 in the minutes after that meeting.

Date: _____

_____
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Attachment I
Recombinant DNA Advisory Committee Roster

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Attachment II
Public Attendees

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Ruth S. Turner, Genzyme Corporation
Luis P. Villarreal, University of California, Irvine
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Lars Martin Wagner, Cincinnati Children's Hospital Medical Center/University of Cincinnati College of Medicine
Carolyn Wilson, FDA

Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
AE	adverse events
Ang-1	angiopoietin-1
BG	benzylguanine
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BSL	biosafety level
CDC	U.S. Centers for Disease Control and Prevention
CNS	central nervous system
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
FDA	U.S. Food and Drug Administration
FN	fibronectin
GaLV	gibbon ape leukemia virus
GBM	glioblastoma multiforme
HRV2	human rhinovirus type 2
HSV-1	herpes simplex virus type 1
IBC	institutional biosafety committee
ICP6	infected cell polypeptide 6
LCA	Leber congenital amaurosis
MRI	magnetic resonance imaging
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	NIH Office of Biotechnology Activities
PAD	peripheral arterial disease
pfu	plaque-forming unit
PI	principal investigator
polio	poliomyelitis
PV	poliovirus
rAAV	recombinant adeno-associated virus
RAC	Recombinant DNA Advisory Committee
RNA	ribonucleic acid
RPE	retinal pigment epithelium
TEM	temozolomide
VEGF	vascular endothelial growth factor
X-SCID	X-linked severe combined immunodeficiency disease
WPRE	woodchuck hepatitis virus posttranscriptional regulatory element