RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 19-21, 2007

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

Contents

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

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

June 19-21, 2007

The Recombinant DNA Advisory Committee (RAC) was convened for its 108th meeting at 8:00 a.m. on June 19, 2007, at the National Institutes of Health (NIH), Building 31-C, Conference Room 10, Bethesda, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 5:45 p.m. on June 19, from 8:00 a.m. until 6:00 p.m. on June 20, and from 8:30 a.m. until 3:30 p.m. on June 21. Most of the June 19 meeting date was a symposium on adeno-associated virus (AAV) vectors; the summary of that symposium is provided as a separate document. The following individuals were present for all or part of the June 2007 RAC meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania Medical Center
Stephen Dewhurst, University of Rochester Medical Center
Hildegund C.J. Ertl, The Wistar Institute
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University
Ellen E. Grant, HealthNow New York Inc.
Helen Heslop, Baylor College of Medicine
Jeffrey P. Kahn, University of Minnesota
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Nicholas Muzyczka, University of Florida
Naomi Rosenberg, Tufts University
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities
Scott E. Strome, University of Maryland Medical Center
David J. Weber, The University of North Carolina at Chapel Hill
Lee-Jen Wei, Harvard University

Office of Biotechnology Activities (OBA)

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

***Ad Hoc* Reviewers and Speakers**

Andrew Bakker, Amsterdam Molecular Therapeutics
John Bennett, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Otis W. Brawley, Emory University
Toni Cathomen, Charité Medical School, Berlin, Germany *(via teleconference)*
Nicholas Crispe, University of Rochester
David W. Hackstadt, NIAID, NIH *(via teleconference)*
Katherine A. High, The Children's Hospital of Philadelphia
Anthony Maurelli, Uniformed Services University of the Health Sciences
Janneke Meulenberg, Amsterdam Molecular Therapeutics
Claudia Mickelson, Massachusetts Institute of Technology *(via teleconference)*

[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.

Ellis Neufeld, Children's Hospital Boston *(via teleconference)*
Paul J. Orchard, University of Minnesota
Marina O'Reilly, OBA, NIH
John R. Papp, Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services (DHHS)
R. Jude Samulski, The University of North Carolina at Chapel Hill
Leonard B. Seeff, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH
Susan Wang, CDC *(via teleconference)*
James M. Wilson, University of Pennsylvania
Kimberly Workowski, CDC, DHHS *(via teleconference)*
J. Fraser Wright, The Children's Hospital of Philadelphia

Nonvoting Agency Representatives

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

NIH Staff Members

Valerie Bonham, Office of the General Counsel, OD, NIH
Sandra Bridges, NIAID, NIH
Vijay Camasamudram, National Institute on Deafness and Other Communication Disorders (NIDCD), NIH
Peter Colosi, National Eye Institute (NEI), NIH
Linda Gargiulo, OD, NIH
Mary Groesch, OD, NIH
Kathryn Harris, OD, NIH
Bob Jambou, OD, NIH
Steve Kellstrom, NIDCD, NIH
Laurie Lewallen, OD, NIH
Maureen Montgomery, OD, NIH
Mark Mortin, National Institute of Child Health and Human Development, NIH
Stuart Nightingale, OD, NIH
Michael Pensiero, NIAID, NIH
Sarah Read, NIAID, NIH
Maryann Redford, NEI, NIH
Gene Rosenthal, OD, NIH
Rita Sarkar, National Heart, Lung, and Blood Institute, NIH
Barbara Schuler, National Cancer Institute, NIH
Tom Shih, OD, NIH
Allan Shipp, OD, NIH
Frosso Vougaropoulou, NIAID, NIH
Fei Wang, NEI, NIH
Bruce Whitney, OD, NIH
Yong Zeng, NIDCD, NIH

Others

There were 162 attendees at this 3-day RAC meeting.

Attachments

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

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

Dr. Federoff, RAC Chair, called the meeting to order at 8:30 a.m. on June 19, 2007. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on May 17, 2007 (72 FR 27827). Issues discussed by the RAC at this meeting included public review and discussion of seven protocols, a gene transfer safety assessment board report, presentations and discussions on recent results regarding immune responses to adeno-associated viral (AAV) vectors, and a discussion of proposed experiments involving *Chlamydia trachomatis* that would require a Major Action under Section III-A-1 of the *NIH Guidelines*.

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

II. Minutes of the March 14, 2007, RAC Meeting/Drs. Muzyczka and Wei

Dr. Muzyczka noted that he could find no need for revision of the March 14, 2007, RAC minutes, and Dr. Wei concurred.

A. Committee Motion 1

It was moved by Dr. Muzyczka, without a second, that the RAC approve the March 14, 2007, RAC meeting minutes. The motion was accepted unanimously by voice vote.

III. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Albelda, Federoff, and Heslop

Dr. Heslop noted that of the 26 protocol submissions received by the OBA in the past 3 months, 19 were not selected for public review at this RAC meeting. Of the 19 protocols not selected, 12 are for cancer, 2 are for human immunodeficiency virus (HIV) and related indications, and 1 each are for diabetic peripheral neuropathy, diabetic ulcers, peripheral artery disease, multiple sclerosis, and end-stage renal disease. Dr. Heslop listed the viruses used in those 19 protocols: 6 protocols employ a plasmid vector, 5 employ a retroviral vector, 5 employ an adenovirus (Ad), and 1 each employs a vaccinia virus, a ribonucleic acid (RNA) transfer, and a Venezuelan equine encephalitis virus replicon. During the reporting period, 160 amendments were received by the OBA, including 11 protocol design modifications, 72 principal investigator (PI) and site changes, and 11 responses to *Appendix M-I-C-1* of the *NIH Guidelines*.

Dr. Heslop discussed the adverse events (AEs) during this reporting period. A total of 113 AEs were reported from 26 trials; the majority was unrelated, and 14 newly submitted reports were considered possibly related to the gene transfer products. The Gene Transfer Safety Assessment Board reviewed all of the AEs.

***** NOTE *****

At this point and for the remainder of Day One of this meeting, the RAC sponsored a symposium on immune responses to AAV vectors, in four parts: AAV Virology and Immunology, Immune Responses in Clinical Studies Using AAV Vectors, Animal Models of AAV Immune Responses, and AAV Vector Preparation. A summary of this symposium is provided as a separate document.

IV. Day One Adjournment/Dr. Federoff

Dr. Federoff adjourned Day One of the June 2007 RAC meeting at 5:45 p.m. on June 19, 2007.

V. Day Two Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff opened Day Two of the June 2007 RAC meeting at 8:00 a.m. on June 20, 2007.

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

Presenter: Dr. Patterson

Dr. Patterson awarded a certificate and personal note from NIH Director Dr. Elias Zerhouni to four RAC members who are rotating off the RAC after this meeting: Drs. Heslop, Muzyczka, Nemerow, and Rosenberg. She noted that they had participated in the initial reviews of between 200 and 230 gene transfer protocols. Dr. Patterson made special mention of Dr. Heslop's service on the Gene Transfer Safety Assessment Board, the service of Drs. Muzyczka and Rosenberg on various RAC working groups, and Dr. Nemerow's policy contributions.

VII. Discussion of Human Gene Transfer Protocol #0704-843: A Phase I Study of Autologous T Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 in HIV-Infected Patients

Principal Investigator:	Pablo Tebas, M.D., University of Pennsylvania School of Medicine
Additional Presenters:	Philip Gregory, Ph.D., Sangamo BioSciences, Inc.; Richard Surosky, Sangamo BioSciences, Inc.
Sponsor:	Sangamo BioSciences, Inc.
Regulatory Sponsor:	Carl June, M.D., University of Pennsylvania
RAC Reviewers:	Drs. Kahn, Somia, and Weber
Ad hoc Reviewer:	Toni Cathomen, Ph.D., Charité Medical School, Berlin, Germany (*via teleconference*)

Drs. Albelda, Ertl, and Strome recused themselves from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

HIV requires the expression of two proteins on the surface of CD4 T cells to be able to infect the cell and replicate. In part, the killing of CD4 T cells, once infected by HIV, is believed to be a major contributor to an individual's progression to AIDS. The investigators' hypothesis is that deleting one of these proteins, called CCR5, from CD4 T cells derived from HIV-infected individuals, will protect CD4 T cells from HIV infection and destruction and may postpone or prevent progression to AIDS. Naturally occurring mutations in the CCR5 receptor have been found in certain Caucasian populations; individuals with the mutations have a natural resistance to HIV infection. CD4 T cells from homozygous individuals required a hundredfold higher level of HIV to be infected, and the infection could not spread in culture. In addition, individuals with these mutations showed no evidence that their immune systems were compromised due to the CCR5 defect. In HIV patients who do not progress to AIDS as quickly as the general population, the frequency of CCR5 mutations on a single DNA copy (heterozygotes) is significantly higher, suggesting a protective effect in heterozygotes. Conversely, HIV in infected CCR5 mutation heterozygote individuals has a slower progression to AIDS. The investigators propose that blocking CCR5 expression using genetic editing, rather than by permanent genetic alteration for expression of a CCR5 inhibitor, may be a safer approach.

To accomplish this, the endogenous CCR5 gene in HIV patient derived CD4+ T cells will be disrupted using zinc finger nucleases (ZFNs). ZFNs are hybrid proteins derived from zinc finger proteins (ZFPs) that bind specific DNA sequences and a nuclease domain that cleaves the DNA upon dimerization. ZFN-induced DNA cleavage results in a double strand DNA break (DSB) which is subsequently repaired by the natural DNA repair mechanisms of the cell which is an imperfect process and introduces insertions and/or deletions that can disrupt gene function. SB-728 encodes two ZFNs in a bicistronic transgene expression cassette targeting the CCR5 gene. Each ZFN in SB-728 is comprised of 4 zinc fingers and together SB-728 has a 24-base specificity. The protocol involves the delivery and transient expression of SB- 728 into CD4+ T-cells from HIV patients by Ad5/F35 vectors, resulting in disruption of the CCR5 gene in >20 % of the cells. The modified cell population will be expanded ex vivo.

The proposed clinical study is a dual cohort, open-label pilot study of the safety and antiviral activity of infusions of autologous CD4+ T cells genetically modified at CCR5 gene by zinc finger nucleases SB-728. In cohort 1, HIV-infected patients with detectable plasma HIV RNA who have developed resistance to highly active anti-retroviral therapy (HAART) will be enrolled. If there are no safety concerns following the dosing of the first three patients, cohort 2 will be added evaluating the treatment in six well controlled participants, followed by a structured treatment interruption. To assess the safety, tolerability and potential clinical effects of the treatment, participants will be followed for 9 months after infusion and monitored for CD4 counts, viral load, persistence of ZFN-modified cells, and adverse events.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty and safety of this chromosomal genetic modification process, specificity of the gene editing process, and risks of off-target effects that could occur if the gene editing process goes awry.

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

Dr. Kahn focused his comments on the informed consent document. He noted that, although the document is clear and complete, the consent form needs to more clearly indicate that this is a Phase I trial and therefore will not benefit research participants. Use of the term “treatment” should be modified.

Dr. Somia asked about genotoxicity associated with high-level expression of a zinc finger nuclease in normal cells. He wondered whether the investigators had looked for gross changes at the CCR5 and CCR2 loci and for the percentage of cells that are CCR2 and CCR5 deficient. Preliminary data from the proposed deep sequencing assays and the fibroblast soft agar transformation assay would be informative. Dr. Somia suggested using human CD46 transgenic mice to recapitulate the proposed clinical experiment by isolating murine T cells, transducing with SB-728, and then reinfusing normal mice. He requested information about the sensitivity of the nonbiased assay that looked at the generation of double-stranded DNA breaks, since the goal of the technology is to induce at least two breaks (one at each locus of CCR5). Although the investigators presented data from *in vitro* replication assays that HIV propagated on transduced cells does not evolve to a CXCR4 or dual tropic variant, Dr. Somia wondered whether the investigators had conducted mixing experiments with CCR5 and CXCR4 tropic viruses to examine the dynamics of replication on SB-728 transduced cells. In a scenario in which the newer antiviral drugs (integrase inhibitors and CCR5 antagonists) become available during the enrollment period, he asked the investigators to discuss the predicted detriment of a 2-month delay for the participants in cohort 1. In addition, if structured treatment interruption for participants in cohort 2 results in an antiviral response, Dr. Somia asked the investigators how they will differentiate (for the secondary end points) the effect on infusion of transduced cells from the effect of structured treatment interruption.

Dr. Weber expressed concerns about study design and informed consent for cohorts 1 and 2. Regarding study design, the choice of HIV-infected participants who have developed resistance to HAART may be justified, since such individuals have limited or no treatment options. However, six such participants is an inadequate number to exclude safety concerns. The investigators should justify evaluating treatment in six well-controlled participants after such limited testing. Regarding the informed consent document for

cohort 1, Dr. Weber made several suggestions, including the need for stating explicitly that the participants will receive no benefit from this study, warning participants given diphenhydramine about possible sedation and the hazards of driving if sedated, and advising both male and female participants to use two birth control methods and indicating for how long birth control should be used. The informed consent document should provide more information on the nature of Ad and Ad infections, should more clearly spell out the costs for the participant and what tests are part of the study, and should indicate the amount of radiation likely to be encountered from the required chest radiographs. "Subject," "research subject," or "research participant" should be used in the informed consent document instead of "patient," which implies that the individual would be receiving therapy. The investigators should clarify who will talk with the participants about stopping their anti-retroviral drugs and should use the appropriate terminology to describe that person. The word "low" should not be used to describe the risk of stopping antiretroviral drugs, risks and frequency of those risks (if known) should be provided, and a statement about when participants should restart their antiretroviral drugs should be included.

Dr. Cathomen focused his review on the ZFN technology and gene transfer. A major concern when using ZFNs is specificity—the number of DSBs at the intended CCR5 target locus compared with the number of DSBs at off-target sites. Although DSB-induced apoptosis is not of concern for the proposed study, he noted that other side effects could include translocations and disruption of tumor suppressor genes. On the basis of the clinical studies with integrating retroviral vectors, activation of endogenous protooncogenes by integration of exogenous promoter sequences is of potential concern. Although most of the recombinant Ad (rAd) vector genomes used in this study will not integrate, Dr. Cathomen explained that some residual integration may take place, especially in the presence of DSB-inducing agents such as SB-728. Thus, the detected rAd genomes in the modified T cells after 12 days in culture could represent integration events. In such a case, the strong cytomegalovirus (CMV) enhancer/promoter, used to drive expression of SB-728, would also have the potential to activate endogenous genes. Cytogenic karyotyping to detect translocation events is a low-throughput and laborious process; nonetheless, he recommended an evaluation based on a small number of analyzed cells (about 100) to ensure that translocations are not a common event after the transduction of CD4+ cells with the rAd-SB-728 vector. Because the *Fok1* domain of the ZFNs is of bacterial origin and therefore potentially highly immunogenic, Dr. Cathomen asked the investigators whether they had assessed whether SB-728 expression could be detected in the modified T cells at the end of the 12-day *ex vivo* expansion period (before reinfusion) or whether *Fok1* peptides were presented by the modified T cells.

C. RAC Discussion

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

- Dr. Nemerow asked whether participants would be screened for CXCR4 virus.
- Dr. Wei suggested a possible third cohort made up of treatment-naive participants.
- Dr. Dewhurst requested additional information about the Maraviroc drug data and followup.

D. Investigator Response

With respect to the generation of random DSBs in the genome, the investigators noted that endogenous DSBs are a normal physiologic event resulting from cell exposure to a variety of environmental insults, conversion of single-strand lesions to DSBs, and natural biological processes. To assess potential genotoxicity resulting from the introduction of random DSBs into the genome, the investigators have conducted or will conduct a series of standard drug safety assessment assays. The combination of the standard battery of tests for drug carcinogenicity and toxicity with the ZFN-specific tests of off-target cleavage (unbiased genome-wide approaches as well as sequence-directed evaluation) will permit an accurate assessment of the potential genotoxicity associated with the ZFN approach.

To address the biological impact of potential cytogenetic abnormalities invoked by SB-728, the investigators propose several *in vitro* and *in vivo* studies—standard *in vitro* assessments of cellular transformation in clonogenicity and soft agar transformation assays, and *in vivo* analyses employing a xenotransplantation model of human lymphocytes in nonobese diabetic/severe combined

immunodeficient (NOD/SCID) mice. The soft agar transformation study of Ad5/F35 SB-728 transduction of the human fibroblast cell line WI-38 is under way; this experiment is designed to test the transformation potential of the replication-defective Ad vector expressing SB-728.

Regarding the concern about newer antiviral drugs becoming available during the enrollment period, Dr. Tebas explained that when the number of treatment options is limited, the clinician must select whether to discontinue a costly and toxic treatment that is not working for the patient or maintain a partially suppressive regimen with the hope that that approach will delay immunologic and clinical failure. Most experienced clinicians would maintain a partially suppressive regimen in a patient with limited therapeutic options, especially if the disease is in an advanced stage. The goal of therapy in this situation shifts from complete suppression of viral replication to partial suppression to prevent immunologic and clinical decline. A patient with a higher CD4 cell count may not be at significant risk for clinical progression, so a change in therapy is optional, and the rate of clinical progression in 2 to 4 months of a partially suppressive regimen should be slow.

Because it is generally accepted that a single treatment interruption in chronically infected individuals does not confer a virologic benefit, this issue should not be a problem for the participants in cohort 2.

Regarding the reasoning for proposing six participants who are well controlled on HAART, the investigators explained that nine previous clinical trials using autologous T cells in HIV have shown a good safety profile and no long-term transformation of these T cells, with followup as long as 10 years. However, in the event of an off-target ZFN-mediated DNA DSB, it is unlikely that such an event would be above what a normal human cell experiences on a regular basis—DSBs are a normal physiologic event. It is important to evaluate this experimental "treatment" in patients who have immune systems capable of mounting an effective antiviral response.

Dr. Tebas stated that participants recruited in the trial for cohort 1 might be screened for the potential presence of CXCR4 virus using the standard assay—the Trofile™ assay (Monogram Biosciences). The sensitivity of that assay is 10 percent to 15 percent, so the CXCR4 virus can be detected only if it is present at a frequency higher than approximately 15 percent. Dr. June added that the investigators will use the Trofile™ assay for this clinical trial and will conduct lookback analysis as more sensitive assays become available.

Dr. Tebas agreed that a third cohort of treatment-naive participants would be a way to test the antiviral activity of this product. However, the investigators decided that, for the first time in humans, they preferred to use cohorts of individuals who are failing currently available treatment.

Dr. Tebas provided more information about the Maraviroc drug trials, for which the success rate has been about 60 percent. Approximately half of the 40 percent who did not respond—20 percent of the individuals who were exposed to the drug—developed CXCR4 virus. Most of those individuals, as it turned out, had CXCR4 virus at levels lower than the Trofile™ assay could detect. The current proposed trial is similar to the Maraviroc trials, but the investigators are not concerned because only one percent of the circulating cells will have the CCR5 knockout.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

<u>Preclinical Issues</u>

- A major safety concern of using ZFNs for genome editing is possible genotoxicity associated with high-level expression of ZFNs in normal cells, which could lead to DNA DSBs at the wrong sites. To further establish the safety of this approach, additional systematic cytogenetic karyotyping analyses on SB-728-treated human CD4+ T cells should be carried out to determine where chromosomal breaks and translocations are likely to occur.

- With respect to the soft agar transformation study of SB-728 transduction of the human fibroblast cell line WI-38, an effort should be made to increase the level of transgene expression that could determine whether overexpression will lead to unpredictable oncogenicity due to breaks in the DNA strands. The preclinical animal studies of the potential oncogenicity of SB-728-modified human CD4+ T cells are also important. Extending this work could result in data that may strengthen findings gathered to date suggesting that the cells are not oncogenic.

Clinical/Trial Design Issues

- Because of its bacterial origin, the *Fok1* domain of the ZFNs may be highly immunogenic. As an added precaution, participants should be monitored for immunogenicity of Fok1.

- CCR5 antagonist drug trials employ an analogous strategy for controlling HIV infection. In those trials, the viral drift caused by the antagonist's selective pressure has resulted in dual tropic HIV infection (i.e., CCXR4 and CCR5 tropic HIV viruses). The selective pressure is expected to be considerably less in this study because only 1 percent of T cells will be CCR5 deficient. Nevertheless, monitoring for viral drift should be an important secondary end point of the study.

Ethical/Social/Legal Issues

- The study proposes a short, structured drug interruption in patients who are doing well using HAART. Although cogent arguments were presented suggesting that a short interruption of HAART may not pose a significant risk, current evidence from the literature indicates that it is not without some risk. The risks associated with interrupting HAART and the lack of potential benefit should be carefully and clearly discussed in the informed consent document and process.

- Even though there is a low probability of the occurrence of viral drift (1 percent), the risk should be discussed in the informed consent document.

G. Committee Motion 2

Dr. Federoff summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 3 recusals.

VIII. Discussion of Human Gene Transfer Protocol #0704-848: A Phase I Study of Intratumoral Administration of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Alloclone-002 Modified for Glucocorticoid Resistance and Interleukin-2

Principal Investigator:	Michael Jensen, M.D., City of Hope National Medical Center and Beckman Research Institute
Additional Presenters:	Philip Gregory, Ph.D., Sangamo BioSciences, Inc.
Sponsor:	Sangamo BioSciences, Inc.
RAC Reviewers:	Drs. Federoff, Kodish, and Vile
Ad hoc Reviewer:	Toni Cathomen, Ph.D., Charité Medical School, Berlin, Germany (*via teleconference*)

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

A. Protocol Summary

Primary brain tumors are the third leading contributor to cancer-related mortality in young adults and the second leading contributor in children and appear to be increasing in incidence in the pediatric and geriatric populations. Gliomas are the most common type of primary brain tumor; annually in the United States, 20,000 cases are diagnosed, and 14,000 glioma-related deaths occur. Gliomas are heterogeneous with respect to their malignant behavior and, in their most common and aggressive forms—anaplastic astrocytoma grade III and glioblastoma multiforme grade IV—are rapidly progressive and nearly uniformly lethal. Currently available therapeutic modalities have minimal curative potential for these high-grade tumors and often exacerbate the already severe morbidities imposed by their location in the central nervous system (CNS).

This Phase I protocol proposes to examine the safety of intratumoral administration of an allogeneic cord blood-derived *ex vivo* expanded CD8+ cytotoxic T lymphocyte (CTL) clone, designated Alloclone-002, that has been genetically modified to 1) express the IL13Rα2-specific IL13-zetakine chimeric immunoreceptor for re-directed glioma targeting; 2) express the HyTK selection suicide fusion protein; and 3) alter both alleles of the glucocorticoid receptor (GR) locus to confer dexamethasone resistance using zinc finger nucleases (ZFNs). This HyTK fusion protein renders T cells susceptible to ganciclovir ablation. Patients with recurrent or recurrent/refractory malignant gliomas who are steroid dependant are to be enrolled.

In the proposed study, 10 research participants with relapsed/refractory malignant glioma will be treated with an allogeneic T cell engineered for glioma killing and dexamethasone resistance. A cytolytic T-cell clone resistant to dexamethasone and expressing the artificial receptor (IL-13-zetakine) and the selection/suicide fusion protein designated *HyTK* will be administered into tumors in a series of four cell doses of 100 million cells along with the cell growth factor IL-2. The safety of this procedure will be monitored closely. Additionally, participants will be evaluated for antitumor responses by serial brain magnetic resonance imaging scans, the immunogenicity of engineered T cells when administered in this fashion, and the ability of ganciclovir to ablate clones should toxicities warrant this maneuver.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of this protocol. Key issues included the following: the novelty and complexity of the method used to modify CTLs and the additional safety concerns raised by the plan to administer IL-2 along with the gene-modified CTL.

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

Regarding participant inclusion criteria, Dr. Federoff asked the investigators to address the parameters to assess inoperability and to include clinical judgment related to multiple site recurrence and/or spread to another previously nonoperated brain region. He requested more detail from the investigators about the data used to determine the number of cells to be infused into participants and what differences they anticipated with Alloclone compared with prior autologous T-cell/zetakine experience. Dr. Federoff was concerned about DNA-modifying events that might occur at sites other than those at the glucocorticoid receptor (GR) locus. He requested further information about the known range of densities of IL-13Rα2 expression among primary malignant gliomas and whether tumors that recur alter their extent of receptor expression. He asked the investigators how they intend to assess the biological effect of the IL-2 infusion and requested specifics about the catheter placement approach that will be used to ensure that Alloclone cells will deliver sufficient levels of IL-2.

Dr. Kodish stated that the informed consent document for this proposed protocol is generally well written and that the section on benefits is appropriately circumspect. On page 109, a paragraph ends with "permission to conduct" but fails to state what is going to be conducted; on page 110, in the list of alternatives, the investigators should consider listing hospice philosophy care, palliative care, or both. He

asked the investigators to include a clinical, scientific, and/or ethical justification for excluding children from eligibility, since these tumors also occur in the pediatric population. The plan to have a research subject advocate (RSA) assess protocol comprehension is excellent.

Dr. Vile stated that one of his major concerns is the degree of inflammatory reactions that might be expected as a result of allogeneic injections along with IL-2. He wondered why the investigators chose the dose of $1x10^8$ cells of Alloclone-002 and what would be the expected toxicity for such a dose. Dr. Vile requested further discussion about the extent of the risk that the Alloclone-002 cells could persist and expand *in vivo* over many generations. He expressed concern related to the findings in the French X-linked severe combined immunodeficiency (X-SCID) trial, in which the transduced cells had a positive selective advantage for expansion in an immune-compromised environment. Although Dr. Vile noted that the clinical situation in the current protocol is significantly different from that for the X-SCID children, he asked for further discussion of the possible dangers associated with transferring the Alloclone-002 cells into an area where they may have some immune privilege and then conferring a positive selection on their growth *in vivo* by giving dexamethasone. Dr. Vile was particularly concerned that the intentional modification of these CTLs to grow under selective pressure would promote their growth and expansion beyond the beneficial effects desired for antitumor therapy. He requested further discussion of the data regarding whether and how frequently the Ad-ZFN treatment modifies other genomic sites, and what toxicities could be expected. Should the cells expand pathologically, the proposed use of herpes simplex virus thymidine kinase (HSV-TK) to ablate the Alloclone-002 cells with ganciclovir is an attractive safety feature, but Dr. Vile asked for more data about the ability to clear 100 percent of transferred cells with HSV-TK *in vitro* or *in vivo* and whether clones emerge that are no longer sensitive to ganciclovir.

Ad hoc reviewer Dr. Cathomen focused on the ZFN technology. A major concern when using ZFNs is specificity, (i.e., the number of double strand breaks (DSBs) at the intended target locus compared with the number of DSBs at off-target sites). He noted that the potential risk of malignant transformation of the modified CD8+ cell clones and other concerns must be balanced against the limited therapeutic options to treat glioma. Dr. Cathomen stated that integration of the strong CMV enhancer/promoter, which drives expression of SB-313 and of *HyTK*, could constitutively activate endogenous protooncogenes in its vicinity; therefore, a soft-agar transformation assay should be conducted to assess the potential for transformation by these vectors. Acknowledging that cytogenetic karyotyping to detect translocation events is a low-throughput and laborious process, he suggested that a small number of cells of the selected CD8+ cell clones should be evaluated to ensure that translocations are not a common event after infection with vector rAd-SB313. Because the *Fok1* domain of the ZFNs is of bacterial origin and hence potentially highly immunogenic, Dr. Cathomen suggested that the investigators assess whether SB-313 expression could be detected in the selected CD8+ T-cell clones or whether *Fok1* peptides are presented by these T cells.

C. RAC Discussion

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

- Dr. Albelda requested more information regarding the investigators' hypothesis that corticosteroids would decrease efficacy of the experimental product—the animal model treated with steroids showed a decreased response, and the animal with a knocked-out corticosteroid receptor showed a better response. He suggested conducting further animal studies along these lines.

- Drs. Albelda and Ertl asked several related questions concerning the side effects of the allogenic T cells that can cross the blood-brain barrier and produce a strong immune response.

- Dr. Ertl asked about the expression of the target antigen—the IL-13 receptor—on nontarget tissue. It is expressed on some lymphoid cells. If there is an immune response in the brain, she wondered whether such a response would cause induction of the target antigen on other cells or whether it would result in destruction outside the brain of cells that would express the target antigen anyway.

- Dr. Muzyczka requested a brief explanation of the convection method the investigators plan to use.

D. Investigator Response

Regarding the number of cells to be infused into research participants, the investigators noted that the differences between the previous autologous T-cell zetakine studies and the Alloclone will be the use of allogeneic Alloclone and the addition of IL-2. This could potentially increase the side effects compared with the autologous T-cell zetakines; however, previous alloreactive T-cell human experience suggests that this is a reasonable starting dose. At least 10 pilot studies involving administration of *ex vivo* activated lymphocytes to MG resection cavities report, in aggregate, an approximate 50 percent response rate in research participants with recurrent/refractory disease, with anecdotal long-term survivors. These studies support the premise that a superior clinical effect of cellular immunotherapy for glioma might be expected with homogeneous, highly potent effector cells. These studies also report on the safety and tolerability of direct administration of *ex vivo* activated lymphocytes and IL-2 into the resection cavity of research participants with MG; even at large individual cell doses as well as high cumulative cell doses, toxicities are modest and typically consist of grade 2 or less transient headache, nausea, vomiting, and fever.

The investigators explained that the longest reported series of patients who have received T cells with a genetically modified TK suicide gene is leukemic patients treated with allogeneic stem-cell transplantation and donor lymphocyte infusions. Even though these patients received retrovirally transduced cells, no evidence of clonal evolution was observed up to 9 years after administration, and a stable gene expression profile, including sensitivity to ganciclovir, was maintained.

To further address the potential for off-target effects, the investigators propose to analyze Alloclone-002 using a standard battery of tests for drug carcinogenicity and toxicity, including karyotyping to assay for any cytogenetic abnormalities, an analysis of IL-2 growth dependence for Alloclone-002, and a soft-agar transformation assay to determine the transformation potential for Ad5/F35 SB-313. Since a single-cell-derived clone is the basis of the proposed Alloclone-002 cell product, the analysis of karyotype represents a valid approach with sufficient sensitivity to assay for any gross cytogenetic impact of ZFN action.

Regarding the exclusion of children from eligibility for this trial, the investigators explained that the IL-13Rα2 expression on glioma subtypes that arise in children has not been fully validated, and the clinical experience in this patient population is limited. The numerical excess of adult candidates versus pediatric candidates for this trial would make adequate pediatric enrollment problematic. In the opinion of the investigators, pediatric development of this therapeutic platform would be best served by a trial limited to pediatric enrollees with cell/IL-2 dosing scaled from what is found to be tolerable in adults.

In response to concerns about the selective pressure on T cells modified for GR disruption in patients on Decadron, the investigators explained that the consequence of Decadron resistance through GR disruption does not confer cell-intrinsic proliferative/survival advantages for the T cell; rather, it presents apoptosis and dysfunction when iatrogenic corticosteroids are present. This is in contrast to the X-SCID experience, in which gamma-C restoration provided T-cell progeny with a growth/survival positive selection by allowing these cells to respond to homeostatic gamma-C cytokines such as IL-7 and IL-15 and antigen-driven proliferation events. In the current proposed study, the transformation of differentiated T cells by a nonviral integrating vector is expected to be limiting, the cell product will be cloned and subjected to release testing that includes failure to survive and proliferate without T-cell receptor stimulation and gamma-C cytokines, and the allogeneic origin of the T cell will eventually lead to rejection.

As to whether the ZFN treatment modifies other genomic sites, the investigators clarified that the data suggest that off-target action of SB-313 is well tolerated in CD8 T cells. In addition, off-target action of SB-313 is a relatively infrequent event, and the isolation of single-cell-derived clones in which all of the most similar off-target locations genome-wide retain wild-type sequence is straightforward. The investigators reminded the RAC members that they propose to use HSV-TK to ablate the Alloclone-002

cells with ganciclovir should the need arise, which is an attractive safety feature that addresses several concerns about the cells expanding in a pathological, rather than therapeutic, fashion.

In the opinion of the investigators, the role of HSV-TK is to provide for rapid debulking of the T-cell product to ameliorate acute toxicities attributable to T cells should they arise. The allogeneic source of the T-cell product is predicted to result ultimately in the rejection of the cell product, a desired safety feature, provided that tumor eradication has been achieved in the interim.

Regarding the convection method the investigators plan to use, Dr. Jensen explained that there would be a steep falloff of drug concentration in tissue away from where the wafer is deposited. Therefore, to get uniform concentrations of drugs, small molecules, or nanoparticles into larger volumes of brain tissue, convection-enhanced delivery has been developed**.** This technology uses infusion catheters that infuse about 5 microliters per minute and create a bulk flow through the tissue bed in which the drug concentration is not limited by diffusion gradient. This method is currently being used in neurooncology drug trials.

Dr. Jensen stated that sensitive molecular assays have shown a lack of expression of IL-13 receptor either when the brain is quiescent or in models of trauma or inflammation; such is not true outside of the brain. The investigators are infusing a modest number of cells directly into the brain, so the even smaller number of cells leaving the brain and setting up pathology outside the brain is limited. The T cells that could leave the brain would have a very limited lifespan and would be so few in number that it would be difficult to cause pathology; in addition, such pathology would likely be rejected quickly.

Dr. Jensen explained that corticosteroids have an impact on T-cell physiology of adoptively transferred antigen-specific T cells *in vivo* in the clinic.

E. Public Comment

Dr. Borror commented that the informed consent document includes much technical and otherwise complex language. She also expressed concern that the suicide gene was not well described and that the average person would not understand it; yet, this is an important aspect of the trial.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- The expression of the targeted antigen, IL13Rα2, in malignant glioma cells over time, especially with respect to recurrent tumors, is still under investigation in other protocols. As these new data become available on its expression, it should inform and, as appropriate, update the design of this study.

- In using a ZFN to disrupt the glucocorticoid receptor, it is important to determine whether the resulting truncated glucocorticoid receptor expressed in transduced Alloclone-002 cells has any effect on gene expression other than glucocorticoid-receptor-regulated genes.

- A major safety concern of using ZFNs for genome editing is possible genotoxicity associated with high-level expression of ZFNs in normal cells, leading to DNA DSBs at off-target sites. To help address this risk, systematic cytogenetic analysis and karyotyping should be carried out to detect translocation events in the Alloclone-002 cells. Equally as critical is specific testing of Alloclone-002's phenotype with respect to apoptotic pathways other than the glucocorticoid-mediated apoptosis.

- There are no preclinical *in vivo* data to support the plan to disrupt the glucocorticoid receptor as a way of preventing attenuation of the T-cell antitumor response in the presence of systemic high-dose steroids. (Research participants receive high doses of steroids to alleviate intracranial pressure from the tumor.) Conducting animal studies to test this assumption would strengthen the protocol.

Clinical/Trial Design Issues

- Positron emission tomography (PET) can detect cells expressing the reporter gene. PET should be used to further refine the protocol.

- Alloclone-002 consists of gene-modified allogeneic cord blood cells. Monitoring for immunologic reactions to Alloclone-002, particularly since it is to be administered four times, is critical for enhancing the safety of this approach.

Ethical/Social/Legal Issues

- The investigators should make the following changes to the informed consent document:

    - It should be written at an eighth-grade reading level. In particular, it is critically important that the suicide gene technology be described in simple, clear, and understandable terms.

    - Alternatives to participation must be included. Alternatives for these patients are limited to palliation and hospice care.

G. Committee Motion 3

Dr. Federoff summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

IX. Discussion of Human Gene Transfer Protocol #0704-852: A Phase I Open-Label Clinical Trial for the Treatment of β-Thalassemia Major with Autologous CD34+ Hematopoietic Progenitor Cells Transduced with Thalagen™, a Lentiviral Vector Encoding the Normal Human β-Globin Gene

Principal Investigator:	Farid Boulad, M.D., Memorial Sloan-Kettering Cancer Center (MSKCC)
Additional Presenters:	Christopher Ballas, Ph.D., Errant Gene Therapeutics, LLC; Patricia J. Giardina, M.D., Weill Cornell Medical College; Patrick Girondi, Errant Gene Therapeutics, LLC; Richard J. O'Reilly, M.D., MSKCC; Isabelle Riviere, Ph.D., MSKCC; Michel Sadelain, M.D., Ph.D., MSKCC; John Tisdale, M.D., National Institute of Diabetes and Digestive and Kidney Diseases
Sponsor:	Errant Gene Therapeutics, LLC
RAC Reviewers:	Drs. Heslop, Kodish, and Rosenberg
Ad hoc Reviewer:	Ellis Neufeld, M.D., Ph.D., Children's Hospital Boston *(via teleconference)*

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

A. Protocol Summary

The proposed study is a Phase I clinical trial using globin gene transfer technology for the treatment of β-thalassemia major. The β-thalassemias are congenital blood disorders caused by mutations that affect

the β-globin gene and greatly reduce or abolish hemoglobin synthesis. The resulting red blood cells are short lived and are deficient oxygen carriers. The thalassemia syndrome, which includes severe anemia, can be cured by transplantation of blood-forming stem cells from a healthy donor. However, this option is not available to most patients, for whom a matched, related donor is not available. Most patients must settle for palliative therapy based on lifelong transfusions and iron chelation, a pharmacological treatment that aims to delay the inexorable buildup of iron that accompanies chronic transfusion. Despite considerable progress in the management of transfusion therapy, iron accumulation (which causes cardiac, endocrine, and osteoarticular complications) and infectious complications still cause progressive morbidity in a fraction of patients. Death from cardiomyopathy due to iron overload remains the leading lethal complication of this therapy.

The goal of globin gene transfer is to restore the capacity of the subject's own hematopoietic stem cells to generate red blood cells containing sufficient hemoglobin to achieve transfusion independence. CD34+ hematopoietic cells will be transduced with a lentiviral vector derived from HIV-1 encoding human β-globin. The transfer of a regulated β-globin gene has been shown to correct hemoglobin synthesis in several animal models of β-thalassemia. This approach is not restricted by the availability of a donor, since the subject is also the donor. After genetic modification, the blood-forming cells are returned to the patient without the risks of immune rejection and graft-versus-host disease (GVHD) associated with allogeneic bone marrow transplantation. A "reduced intensity conditioning regimen" based on administering an intermediate dose (8 mg/kg) of busulfan will be used to prepare acceptance of the incoming transduced cells.

This protocol will be offered to subjects 15 years of age and older who lack a matched, related bone marrow transplant donor. The postinfusion monitoring will focus on the safety and tolerability of this experimental treatment as well as the molecular monitoring of the persistence and function of the delivered globin gene. The data generated in this clinical study will be reviewed by an independent drug safety monitoring board at each participating clinical site.

B. Written Reviews by RAC Members

Eight RAC members voted for indepth review and public discussion. Key issues included the following: first application of gene transfer for thalassemia and only the second protocol to use lentiviral vectors for a monogenic disease, safety issues that include the risk of insertional mutagenesis because lentiviral vectors integrate into the genome, and the inclusion of children as young as 15 years of age in the study population, thus adding to the protocol's risk profile and risk-benefit calculation.

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

Dr. Heslop suggested that the investigators readdress the rationale for the inclusion criterion regarding lacking a matched sibling donor, given that more recently published studies with unrelated donor transplant show a survival of 80 percent and a disease-free survival (DFS) rate of 66 percent. She expressed concern that many of the research participants for this proposed trial might have splenomegaly and an expanded marrow, and thus the possibility that the risk of side effects might be higher than in normal donors. Although the investigators state that the transduced cells will be frozen while replication-competent lentivirus (RCL) testing and vector number copy determination studies are performed, Dr. Heslop wondered whether the investigators plan to assay viral integration sites before infusion and whether potential integrations would result in the experimental product not being released. Dr. Heslop suggested five enhancements to the informed consent document, including ensuring that the language is at the eighth-grade level, changing wording that implies therapeutic benefit, adding a request for autopsy, and adding information about how participation in this trial might affect the results of subsequent HIV testing.

Dr. Kodish noted that the informed consent document was clear and well written for the intended audience. He requested additional information about the process of using a patient advocate, especially the proposed approach to assent with participants between 15 and 18 years of age, including whether they will be interviewed without their parent(s) present. Dr. Kodish suggested that terminology in the

informed consent document be changed from “gene therapy” to “gene transfer” to avoid the appearance of therapeutic benefit and that all terminology (e.g., “pulmonary fibrosis”) be defined in lay terms.

Dr. Rosenberg asked about the level of β globin production in differentiated human cells compared to normal production. She asked the investigators to provide an update on the linear amplification-mediated polymerase chain reaction (LAM-PCR) analysis from the primate study, which was said to be in progress, to clarify the possibility of clonal expansion of particular subsets of engrafted cells observed in the various mice tested and whether retroviral integration occurred in any of these animals. Although the risk of RCL appears low, Dr. Rosenberg suggested that the investigators comment on the rationale regarding the birth control recommendation, especially at early points in the study. She also asked for further explication of the rationale for the age of the study participants and which aspects might be compromised if the initial study was limited to adults.

Noting that the science underlying this protocol and the murine models that allowed development of this vector are compelling and well presented, *ad hoc* reviewer Dr. Neufeld stated that the protocol and informed consent document overstated issues in regard to the “palliative” alternative therapy of lifelong hypertransfusion and consequent need for iron chelation therapy. He noted the availability of the oral chelator, deferasirox, particularly for patients with heart disease, the decreased risk of Hepatitis C infection, and the improved survival statistics for patients born after 1982. Although the modest accrual goal of three to five research participants per year is justified based on the small North American thalassemia population and the stringent enrollment criteria, he requested that the investigators comment further on the possibility that three per year might be so slow as to threaten the long-term status of the study if the state of the art evolves over a 3-year time horizon. Dr. Neufeld noted that the entry criteria are somewhat ambiguous regarding the number of transfusions, which could allow enrollment of thalassemia intermedia subjects and be a potential problem for the secondary end point of following required transfusion burden after gene transfer. He stated that the survival statistics for transfusion/chelation are relatively misleading and suggested that the investigators revise this wording in the informed consent document to stress the positive survival experience that has been seen in the most recent trials.

C. RAC Discussion

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

- Dr. Weber suggested changing wording in the informed consent document that implies therapeutic benefit.

- Dr. Ertl wondered about the need for additional preclinical studies to better understand whether there is an enrichment or selection for specific clones when passed from mice to mice.

D. Investigator Response

Regarding the rationale for enrolling participants lacking a matched sibling donor in light of recent data on from transplant from HLA-matched unrelated donors, the investigators reviewed that data and asked whether a mortality of 20 percent, a DFS rate of nearly 70 percent, and a GVHD risk of 18 percent are acceptable in patients with thalassemia major.

The investigators stated that viral integration sites would not be assayed before infusions, since there are no available data to assess the reliability and predictive value of such information. However, genomic DNA will be stored for eventual retrospective analyses. Specific integration sites will not be screened even after integration site analysis unless the integration analysis suggests oligoclonal or monoclonal dominance and/or clinical evidence suggests abnormal cell counts or histology—or as otherwise indicated in the appropriate FDA guidance.

Data from preclinical studies conducted by the investigators indicate that the level of expression of β-globin in human cells is similar to that found in mouse cells; additional ongoing studies aim to confirm

whether similar levels of expression are to be expected in research participants. Taken together, data gathered and from ongoing studies suggest that transduced hematopoietic progenitor cells harboring one or two vector copies should, on average, express β-globin in a therapeutic range.

With regard to their study of TNS9.3 in rhesus macaques, the investigators averred that low-level marking in the animals had so far precluded reliable amplification of integration sites due to competition by internal vector sequences. Analysis of murine integration sites indicated that integration of the globin vector was similar to the expected pattern for lentiviral vectors (i.e., frequent integration within genes, including some oncogenes).

Besides the aspects of the gene transfer, research participants in this proposed study will receive a chemotherapy agent (busulfan) as part of the preparative regimen. For that reason, the investigators included in this protocol a recommendation for the use of birth control.

In response to questions about the age range of potential participants, the investigators explained that their experience with transplanting thalassemia major patients indicates that patients who are 10 to 16 years of age already show extensive iron deposition in the liver as well as evidence of fibrosis. Recent studies have demonstrated that the median age for the onset of severe complications of thalassemia and its treatment is 16 years. Patients between the ages of 15 and 18 years with evidence of early complications most probably will have higher risks of complications with allogeneic transplantation based on organ toxicity, especially if they receive grafts from unrelated donors. In addition, the age of 15 years also was chosen because, by that age, the probability of subsequently having a human leukocyte antigen (HLA)-matched sibling born to the family is radically decreased.

The investigators explained the proposed use of a patient advocate, a process that was discussed with and approved by the chair of their institutional review board (IRB). An experienced senior IRB member will be present at the time of the consent discussion with potential research participants and their families to ensure that the information provided is not biased and that everyone adequately understands the trial, the experimental treatment to be given, the risks associated with this experiment, and the assessments and tests required prior to and following the collection, modification, and administration of stem cells.

Dr. Sadelain explained about additional preclinical studies that are ongoing. In one cohort of approximately 300 mice, the investigators are looking at integration sites and for the presence of enrichment or selection for specific clones. In principle, because the β-globin vector is tissue specific and not expressed in hematopoietic stem cells, there is no expectation that it will promote preferential clonal expansion.

The investigators agree to clarify the protocol and informed consent document regarding Dr. Neufeld's comments about the oral chelator, risks of infection and younger cohort survival.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

<u>Preclinical Issues</u>

- Insertional mutagenesis is a safety concern for any protocol using a retroviral vector in hematopoietic cells. Although the planned studies of vector integration in mice should yield useful safety data, it is not clear what steps will be taken if clonal expansions due to insertional oncogenesis are seen. Further consideration should be given to this possibility, and a contingency plan should be developed for the protocol.

- Contaminants in the vector, such as adenoviral E1a or SV40 T-antigen transcripts from the packaging cells, could increase the risk of oncogenesis. Screening the vector with a sensitive assay such as reverse transcriptase polymerase chain reaction (RT-PCR) should be considered.

Clinical/Trial Design Issues

- It is not clear what steps will be taken if clonal expansions due to insertional oncogenesis occur at the clinical stage. A contingency plan addressing this possibility is needed.

- Studies are currently under way that will provide data on the safety of administering GCSF in thalassemia patients undergoing transplantation. Since data from the studies will be relevant to this protocol, it would be important to obtain the data as soon as the study is completed.

- Since only patients with thalassemia major are eligible for the protocol, the inclusion criterion related to the blood transfusion requirements needs to be clarified.

- In clinical practice, one of the goals of hypertransfusion in thalassemia is suppression of endogenous erythropoiesis, for example, by keeping the hemoglobin level at 10 g/dL or higher. Transfusion dependency is one of the study end points, but if transfusions are continued in the postgene transfer period using the same hemoglobin threshold, it will be difficult to determine the effect of the gene transfer. The transfusion criterion for the 3- to 5-month period following vector administration should be specified in the protocol and discussed in the informed consent document.

- The protocol overstates the inadequacies of current therapy in two ways. First, the document fails to acknowledge that the disease is well controlled in some patients. Second, the document overstates the risk of infection from blood transfusions. Improvements in screening practices have reduced the risk of transmission of infectious diseases such as hepatitis C, and it is no longer considered a "significant" risk even for patients undergoing multiple transfusions.

Ethical/Social/Legal Issues

- As a safety study, the potential benefits of the protocol are theoretical at best, a point made adequately in the informed consent document. However, the discussion of gene transfer studies that have been successful is misleading and should be deleted.

G. Committee Motion 4

Dr. Federoff summarized preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

X. Consideration of Proposed Major Action: Under Section III-A-1 of the *NIH Guidelines*: Tetracycline Resistance and *Chlamydia trachomatis*

Principal Investigator:	Daniel Rockey, Ph.D., Oregon State University
Additional Investigator:	Walter E. Stamm, M.D., University of Washington (UW) School of Medicine
RAC Moderator:	Dr. Kirchhoff

A. Presentations

Dr. Corrigan-Curay introduced this proposed Major Action, starting with thanks to the expert consultants: John Bennett, M.D., NIAID, NIH; David W. Hackstadt, Ph.D., NIAID, NIH; Anthony Maurelli, Ph.D., Uniformed Services University of the Health Sciences; Claudia Mickelson, Ph.D., Massachusetts Institute of Technology; John R. Papp, Ph.D., CDC; Susan Wang, M.D., M.P.H., CDC; and Kimberly Workowski, M.D., CDC.

She explained that a major action under section III-A of the NIH Guidelines applied to research involving "the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such an acquisition could compromise the use of the drug to control disease agents in human, veterinary medicine, or agriculture." Dr. Corrigan-Curay reviewed the progress of this proposed Major Action to date, including the notice published in the May 9, 2007, *Federal Register;* a review by the RAC Biosafety Working Group; a review by outside expert consultants; and questions asked of and responses received from PIs regarding risks and benefits and possible risk mitigation strategies. She also reviewed the agents, practices, safety equipment, and facilities required for biosafety levels (BSLs) 2, 2+, and 3.

Dr. Bennett, Head, Clinical Mycology Section, NIAID presented the public health implications of chlamydial infection and the current treatment options. Dr. Rockey proposes to use several strains of *C. trachomatis* that cause distinct clinical presentations, L-serovars, which cause lymphogranuloma venereum (LGV) and E and G serovars, which cause primarily genital infection (i.e. cervicitis, pelvic inflammatory disease, urethritis and prostatitis). In addition, vertical transmission from infected women can lead to conjunctivitis or pneumonia in infants. All strains of *C. trachomatis* used are transmitted by infected genital secretions through sexual contact, or for infants, in the birth canal. Ocular transmission by contact with genital secretions is also possible.

LGV is a disease that is seen predominately in developing countries. The disease starts as a painless ulcer on the male genitalia or in the female genital tract. As the bacteria spread, lymph nodes in the area become swollen and tender (buboes). The lymph nodes may break open and drain through the skin. Years later, edema from scarring can lead to lymphatic obstruction. In the United States, proctitis caused by the L-serovar of *C. trachomatis* has been seen in certain populations, mainly in men who have sex with men. It is characterized by severe rectal pain, and often rectal discharge and/or bleeding and can be mistaken for inflammatory bowel disease. First line treatment for both diseases is doxycycline for 21 days. An alternative is erythromycin. While there is some evidence that other antibiotics may be effective treatment alternatives, the Centers for Disease Control and Prevention has concluded that, in the absence of data from controlled treatment trials, alternative antibiotics cannot be recommended at this time.

Serovars E and G of *C. trachomatis* cause genital chlamydia infections, which is the most common sexually transmitted disease in the United States. The majority of these infections are asymptomatic. In symptomatic cases there may be vaginal discharge in women and in men urethral symptoms or scrotal pain in men. Despite the usually benign nature of the infection, it can result in long-term medical complications, such as infertility and, according to the CDC, accounts for over one billion dollars annually in medical costs. CDC treatment guidelines for genital chlamydial infections include doxycycline or azithromycin as first line antibiotics and erythromycin or fluoroquinolones as second line treatment.

Dr. Rockey discussed transfer of a tetracycline resistance gene into *C. trachomatis*. He provided background on chlamydial disease in humans and other species Dr. Rockey noted that the most significant challenge to progress in chlamydial research is the lack of a genetic system; progress in vaccine design, the study of pathogenesis, and studies of basic biology are all seriously limited by the lack of a genetic system. Tetracycline resistance was chosen because a gene encoding this resistance has been identified on a stably integrated genetic element in the chromosome of certain strains of *Chlamydia suis,* a non-human pathogen that infects swine. Dr. Rockey believes he will be able to transfer this resistance to *Chlamydia trachomatis* in these proposed experiments because *C. trachomatis* and *C. suis* are able to occupy the same intracellular vacuole simultaneously, thereby favoring the genetic exchange of DNA between them. *Chlamydia muridarum,* a mouse pathogen can also occupy the same vacuole as *C. suis* and transfer of tet^R from *C. suis* to *C. muridarum* could be attempted as a proof of principle. However, because of differences in generation time between these latter two species (*C. suis*

and *C. trachomatis* have similar generation times of approximately 20 hours, whereas for *C*. *muridarum* it is approximately 50 hours), this approach is likely to be technically more challenging since it would require that the stages of intracellular growth cycles between both species be appropriately synchronized for a favorable outcome. Importantly, moreover, Dr. Rockey ultimately would like to manipulate the human pathogen.

He described the various precautions that will be taken in the OSU laboratories, stating that training on the clinical signs of laboratory infection will be routine in the laboratory and that trained personnel will conduct all work in a BL-2 environment. The natural route of infection in this system is by direct contact with infected secretions, so that any secondary transfer of the pathogen is highly unlikely if a laboratory worker becomes infected. All sonication is conducted in a biosafety cabinet, centrifugation is in a BL-2 environment, and no tubes or culture vessels containing viable *C. trachomatis* will be opened outside of a biosafety cabinet. Chlamydial culture expertise is available at UW and basic biology expertise at OSU; any recombinant strains grown at OSU will be transferred between laboratories via automobile in doubly contained frozen vessels. All strains will remain sensitive to azithromycin. The investigators plan to work with *C. trachomatis* LGV-434 (serotype L2) and a small number of clinical isolates.

On behalf of the RAC Biosafety Working Group, Dr. Kirchhoff presented a summary of risk assessment of *C. trachomatis* (wild-type versus the proposed *C. suis*) in terms of strains, pathology, antibiotic treatment, laboratory acquired infection (LAI) route, risk group, and minimal BL level. He also reviewed the agents and practices related to BL-2 and BL-3.

Dr. Kirchhoff also noted that the RAC was cognizant of the work of another NIH federal advisory committee, the National Science Advisory Board on Biosecurity and the emerging dialogue on the potential for scientific research to be misused to threaten public health. In looking at the project proposed by Drs. Rockey and Stamm through this lens, the RAC noted that while the creation of tetracycline resistant *C. trachomatis* raises important public health issues, two important characteristics limit the ability to use tetracycline resistant *C. trachomatis* as a potential bioweapon. First, the clinical manifestations are neither immediate nor life-threatening, in contrast to agents such as anthrax or smallpox. Second, the normal mode of transmission of this obligate intracellular pathogen is sexual or by other intimate contact (e.g. the birth process) making the pathogen an unlikely candidate for a bioweapon.

Dr. Silvio P. Mariotti, Coordinator, WHO Alliance for the Elimination of Trachoma commenteds focused on the use of tetracycline to treat ocular infections involving *C. trachomatis*. Dr. Marriotti stated that in 50 countries, blindness due to *C. trachomatis* infection is a major public health problem, especially in developing countries. Tetracycline,is often the only available drug in the pharmacies of poor, rural areas where *C. trachomatis* infection typically occurs. Dr. Mariotti did not express concern regarding the introduction of tetracycline resistance into strains that cause only sexually transmitted forms of disease. Dr. Rockey indicated that he does not work with the ocular strains in his laboratory. and the RAC recommended this as a stipulation of the research being approved..

B. RAC Discussion and Investigator Responses

The discussion focused on two main potential biosafety risks: transmission of tetracycline resistant *C. trachomatis* to laboratory workers and transmission of tetracycline-resistant *C. trachomatis* to the public. The RAC discussed in detail both of these risks and potential mitigation strategies.

It was agreed that the most likely mode of transmission in the laboratory would be through aerosols. It was noted that there is a dearth of case reports, but that this may reflect considerable underreporting of such events. Therefore, there are limited data on the clinical presentations of persons with laboratory-acquired chlamydial infections, but given the entry route it would likely present with signs and symptoms of pulmonary disease. In one report, a lab worker developed pleuritis after apparent exposure to aerosolized *C. trachomatis*. In addition to respiratory infection, ocular infection is possible, even with the non-ocular strains, through direct inoculation. However, with standard containment practices, the probability of this occurring is extremely low.

It is not known whether some laboratory infections would remain subclinical, as is the case with genital infections. The incubation period for the genital chlamydia infections commonly seen in the U.S. is not clearly known due to the large numbers of asymptomatic cases. For symptomatic infections, one to two weeks appears to be a reasonable estimate, but in infants, the incubation period can be as long as two months. Therefore, advising laboratory workers about the incubation period for infection will be difficult. This is more likely to be the case with respiratory infections, since ocular infections are more likely to be clinical with an injected conjunctiva and crusting around the eye.

Regarding treatment of laboratory-acquired infections, despite the potential removal of doxycycline due to transfer of tetracycline resistance to *C. trachomatis* it was generally agreed that alternative antibiotics would be effective in treating the disease, but the discussants emphasized that a mechanism would have to be developed to ensure that treating medical personnel be made aware of the laboratory worker's exposure to tetracycline resistant *C. trachomatis*.

The risk to the public would either come from transmission from infected laboratory workers or through inadvertent escape of *C. trachomatis* from the laboratory. *C. trachomatis* is an obligate intracellular pathogen that dies after being on a surface for several hours. Therefore, its ability to escape the laboratory and infect the general public is remote if not impossible. The possibility of transmission of tetracycline resistant organisms from infected workers to the public, either during the incubation period or during clinical or asymptomatic infection, remains an open question. Because of the lack of reports of laboratory-acquired infections, there are essentially no data on the possibility of transmission of such infections to close contacts. It is conceivable that a laboratory acquired ocular infection could be transmitted to a close contact, in a manner similar to what occurs with the ocular disease trachoma. Nonetheless, making such comparisons may not be valid since trachoma, which as noted is a major problem in many developing countries, is quite different clinically from the ocular disease caused by genital strains of *C. trachomatis*. Laboratory-acquired infections contracted through aerosols may produce a respiratory infection. Despite the lack of data, however, the fact that genital forms require transfer of fluids and that the organism's natural infection route is not respiratory, the possibility of laboratory infected workers spreading the organisms to close contacts was felt to be quite remote.

C. Public Comment

Public attendees offered no comments.

D. Committee Motion 5

The RAC voted 15 in favor, 1 opposed, and no abstentions to recommend to the NIH Director that Drs. Rockey and Stamm be allowed to proceed with this line of research using the containment level described below with additional stipulations. This recommendation applies only to the research proposed by and to be conducted by Drs. Rockey and Stamm. Other investigators who wish to conduct similar experiments would need to submit their proposed experiments to the Office of Biotechnology Activities for RAC review and NIH Director approval.

Recommendations/stipulations following the RAC's discussion

Containment for Dr. Rockey's and Dr. Stamm's Experiments:

- All research involving the introduction of tetracycline resistance into *C. trachomatis* must be performed at biosafety level (BL) 2 using BL3 practices (referred to as BL2+). The *NIH Guidelines* articulates requirements for BL2 laboratory facilities and equipment in Appendices G-II-B-3 and G-II-B-4 while BL3 practices are described in Appendices G-II-C-1 and C-2 of the *NIH Guidelines*. The RAC specifically emphasized the following BL3 practices:
    - Access must be restricted to well-trained personnel whose presence is required for the conduct of this work, and
    - The investigators must use sealed centrifuge rotors and tubes.

Additional Recommendations:

- The investigators must use cup sonication rather than probe sonication to separate the infectious form [elementary bodies (EB)] from the metabolically active [reticulate bodies (RB)] form of the bacterium.
    - If possible, the investigators should consider the use of other techniques that do not involve the potential for the generation of aerosols, such as freeze-thaw, to separate EBs from RBs.

- Any work with the *Chlamydia* serovars A, B, or C, which cause the ocular disease trachoma must not be conducted in the same laboratory in which tetracycline resistance is being introduced into *Chlamydia trachomatis* serovars that cause genital disease (L, E and G).

- When tetracycline resistant *C. trachomatis* are transferred to other laboratories, the investigators must ensure that the practices and procedures being employed are identical to those set by the NIH Director. As noted, however, since the NIH Director's approval will only apply to experiments conducted by Drs. Rockey and Stamm. Any work involving the introduction of tetracycline resistance and *Chlamydia* proposed by investigators other than Drs. Rockey and Drs. Stamm would need to be reviewed by the RAC and specifically approved by the NIH Director.
- The investigators should develop an assay to detect the tetracycline resistant genetic element so that in the event of a laboratory acquired infection, a determination could be made as to whether the source was the genetically modified strain of *Chlamydia.*

- The RAC made a number of recommendations concerning the health surveillance program for individuals working with tetracycline resistant *C. trachomatis.* These were:

    - In addition to being trained on proper biosafety practices, laboratory workers must be provided education on the possible clinical manifestations of a chlamydial laboratory acquired infection.

    - Both laboratories should have a detailed written action plan outlining the specific steps to be taken in the case of a laboratory exposure or infection. This plan should include at a minimum:
        - Identification of key personnel who would provide diagnostic testing and treatment;
        - Instructions on managing exposures or infections discovered during off hours (after close of business, holidays, weekends, etc.);
        - Specific recommendations for azithromycin allergic or sensitive lab workers, including excluding all lab workers with known macrolide antibiotic allergies to work on these experiments;
        - Specific recommendations for treatment of infected laboratory personnel who develop side effects while being treated with azithromycin, and
        - Specific precautions to be taken by infected laboratory workers with respect to close contacts (e.g. family members).

    - It is likely that not all laboratory workers would be treated by physicians and other healthcare providers who have direct knowledge about the investigators' research. Therefore, the investigators should develop an outreach program to educate frontline healthcare workers in the diagnosis and treatment of laboratory workers who might be infected with tetracycline-resistant *Chlamydia.*

    - As part of these efforts, a medical card should be developed that would be carried by all laboratory workers. Minimally, this card should include at least the following:
        - Identification of key personnel responsible for providing diagnosis and treatment;
        - A CDC telephone number for reporting the infection and obtaining treatment recommendations; and
        - A twenty-four hour contact number for the principal investigators.

Dissenting Opinion

Dr. Scott Strome, Professor and Chairman, Department of Otorhinolaryngology-Head and Neck Surgery, University of Maryland voted against allowing these proposed experiments to proceed. He concluded that the current risks of proceeding outweighed the benefits because the investigators have an alternative model using murine and swine *Chlamydia* strains that would pose substantially less risk for human disease and would possibly provide insight into both subsequent experiments using human strains and to the biology of *Chlamydia* in general

XI. Discussion of Human Gene Transfer Protocol #0704-849: A Phase I Study Evaluating the Use of Allodepleted T Cells Transduced with Inducible Caspase 9 Suicide Gene after Haploidentical Stem Cell Transplantation

Principal Investigators:	Malcolm K. Brenner, Ph.D., M.B., Baylor College of Medicine (BCM) and Helen Heslop, M.D., BCM *(via teleconference)*
Additional Presenters:	Gianpietro Dotti, M.D., BCM; Robert Krance, M.D., BCM; Heidi L. Weiss, Ph.D., BCM *(via teleconference)*
RAC Reviewers:	Dr. Ertl, Ms. Shapiro, and Dr. Strome
Ad hoc Reviewer:	Paul J. Orchard, M.D., University of Minnesota

Drs. Heslop and Rosenberg recused themselves from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Hematopoietic stem-cell transplantation is an established form of treatment for a number of cancers and serious blood disorders. Transplant patients receive two types of cells from their donors: stem cells, which replace the damaged bone marrow, and immune cells, which protect against subsequent infections and may also help eliminate the cancer. One type of immune cell, the T lymphocyte, also can attack the recipient's tissues to produce GVHD. Some patients develop a severe form of GVHD that can be fatal.

In this protocol, the investigators propose to improve the safety of donor T cells by removing the cells that are capable of causing GVHD while leaving behind the T cells that fight infection and cancer. They propose giving larger doses of T cells that can be destroyed after they are infused into the research participant, if those T cells start to cause GVHD.

The protocol proposes to increase the safety of the approach by incorporating a suicide gene, inducible caspase 9 (iCaspS), in the allodepleted T cells, permitting their destruction should administration have adverse effects. iCasp9 consists of a pro-apoptotic molecule, human caspase 9, joined to a drug-binding domain derived from human FK506-binding protein; addition of a small molecule synthetic drug leads to homodimerization of caspase 9, activation of the caspase pathway and apoptosis of the transduced cells within 24 hours. The dimerizer, API 903, has successfully completed safety-testing in human volunteers. A retroviral vector will express iCasp9 and a selectable marker (truncated CD19) to enable enrichment of transduced cells to >90% purity. The protocol is intended to provide a safe method for boosting transplant recipients' immunity to reduce relapse and infections while providing effective treatment for the minority of recipients who develop severe GVHD. The investigators will insert this suicide gene into donor T cells using a retroviral vector as the carrier.

More than 70 individuals have been treated with this process over a 10-year period, using a retroviral vector, without harm. Research participants in this proposed protocol will be followed for up to 15 years to make sure that AEs do not occur as a result of the clinical trial.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol because it involves a novel suicide gene system for the ablation of donor T cells.

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

Dr. Ertl requested that the investigators explain two points regarding this complex regimen to improve the outcome of T-cell-depleted stem-cell transplantation. First, the protocol will deplete antihost allo-reactive T cells by isolating donor cells and then transforming them with Epstein-Barr virus (EBV). Upon irradiation, the EBV-transformed host lymphocytes will be used to activate the donor T cells, and activated cells then will be depleted based on CD25 expression. This process will deplete donor T cells directed against HLA determinants of the host, but it should also deplete T cells against EBV. Because of the risk that some of the EBV-infected host cells may be transfused back into the host, which will receive T cells depleted of those that could protect against EBV, Dr. Ertl asked the investigators how the risk of uncontrolled EBV infection would be addressed. Second, the dose-escalation study proposes that two research participants will be enrolled for escalating doses of T cells, and then six participants will be enrolled for the highest dose. The highest dose has a 30-percent probability of causing GVHD; thus, only one or two participants would be expected to require drug treatment. Dr. Ertl asked the investigators to discuss how an N of 1 or 2 would allow for firm conclusions about the safety of the procedure.

Ms. Shapiro requested that the investigators expand on their risk-benefit evaluations. She noted that the selection of classes of research participants places adults before children as "a matter of social justice," based on the judgment that children ought not to bear the burdens of research unless absolutely necessary. Ms. Shapiro also stated that the informed consent document does not—but should—include an assent section, which should include whether the permission of both parents will be required, whether obtaining valid assent from all prospective child participants (regardless of age) will be possible, and whether a prospective child participant's dissent will be respected if his or her parent favors the participation of the child.

Dr. Strome asked the investigators to clarify the statement in the proposal that lymphoblastoid cell lines (LCLs) will not present any tumor-specific antigens, since these are B cells that have been exposed to tumor *in vivo*. He was concerned that this LCL activation/depletion strategy could potentially deplete tumor-reactive T cells. Dr. Strome also asked the investigators to clarify how this strategy would provide an additional layer of participant protection; specifically, how it would provide protection against an uncontrolled autoimmune T-cell response, whether proliferating T cells would carry the suicide gene, and how this strategy would help with chronic GVHD. In the paper published in *Blood* (Straathof et al. 2005[2]), Dr. Strome noted that those investigators performed *in vivo* studies to test their strategy in NOD/SCID mice, administering the drug 4 days after T-cell transfer. He asked whether the investigators of this proposed protocol have additional safety data to support the efficacy of this strategy at delayed time points and whether any other animal studies might be available for the RAC to review.

Dr. Orchard noted that, based on current eligibility criteria, groups are eligible for this protocol that would not necessarily be considered "high-risk" patients, such as those with acute leukemia or those with chronic myeloid leukemia (CML). Although patients may be at higher risk based on the inability to identify a well-matched related or unrelated donor, data suggest that transplant using a cord blood graft could be performed relatively expediently and could be expected to provide an outcome comparable to a matched unrelated donor transplant in patients with acute leukemia. In addition, CML patients may be successfully treated with imatinib and other molecular agents for an extended period. As a result, Dr. Orchard asked the investigators to discuss the decisionmaking process in enrolling research participants in contrast to providing those individuals with more conventional transplants using cord blood grafts or other therapies. Noting that the transduced cells maintained *in vitro* for 3 or 4 weeks appeared to lose transgene expression associated with a decrease in the ability to eliminate the cells, Dr. Orchard asked the

[2] Straathof KC, Pulè MA, Yotnda P, et al. An inducible caspase 9 safety switch for T-cell therapy. *Blood* 105(11):4247-54, 2005.

investigators to discuss available evidence that this loss of expression would not occur *in vivo*. He also asked about the possibility that a second or third course of AP1903 might be administered if GVHD persists, and he wondered whether sufficient numbers of cells could be generated to propose repeated doses of genetically modified T cells at doses of $1x10^7$ cells/kg for up to five infusions. (A total number of $5x10^9$ cells will be needed if research participants up to 100 kg in weight are eligible to participate in this trial.)

C. RAC Discussion

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

- Dr. Ertl recommended that, if the investigators encounter GVHD, that they analyze the cells that are causing GVHD to make sure that those cells express the dimerization.

- Dr. Wei asked why the investigators believe the highest dose should be used with the depleted T cells.

- Dr. Kodish queried the investigators as to whether the haploidentical transplants would be conducted under an IRB-approved study.

D. Investigator Response

The investigators explained that, in principle, primary B cells may act as antigen-presenting cells, although their ability to process and present "external" tumor antigens *in vivo* is limited. However, the investigators do not use primary B cells for allodepletion. The lymphoblastoid cell lines (EBV-LCLs) used for this process have been cultured and expanded *ex vivo* in the absence of any tumor antigens. Therefore, even if the primary B cells had been exposed to tumor antigens *in vivo* and had processed and presented any of them, the time and dilution effects of culture would reduce the level of antigen below the threshold for measurable presentation.

Because the investigators are using a cotransferred selectable marker (CD19), the suicide gene will be present in the transferred T cells. If any of those transferred cells proliferate to cause autoimmune disease, they will be susceptible to killing by the dimerized small molecule. If autoimmunity arises from residual T cells in the primary graft, the suicide gene activation will not be of benefit. This study will help sort out these possibilities.

The investigators explained that it is unusual to develop chronic GVHD in the absence of acute GVHD, which will be treated in this protocol by suicide gene activation. If such chronic GVHD does arise *de novo* and if gene-modified cells remain detectible in the circulation, the investigators will be able to activate the suicide gene. It is unknown whether activation of the suicide gene will benefit chronic GVHD, since the pathogenesis of this disorder is imperfectly understood and the direct and continuing contribution of T cells may be limited. In general, anti-T-cell therapies are less effective for treating chronic compared with acute GVHD; therefore, most efforts focus on preventing the acute episodes since they usually forecast the appearance of chronic GVHD.

The investigators recently developed a SCID mouse model in which the behavior of adoptively transferred T cells can be followed *in vivo* for several weeks using an *in vivo* imaging system. Using this system, it has been shown that the transgene is functionally expressed for at least 3 weeks, a period of time during which acute GVHD from the transferred cells will most likely occur.

Regarding eligibility for this gene transfer protocol, the investigators explained that this protocol is an optional add-on study for individuals who lack a major histocompatibility complex identical donor and who have already consented to receive a haploidentical transplant. This protocol, which aims to boost immune reconstitution following such transplants, is discussed with potential research participants only after the decision has been made that their best available donor is a haploidentical family member and that such a transplant is indicated for their underlying diagnosis. Therefore, potential participants will

have a haploidentical transplant regardless of whether they elect to participate in this proposed study. The options for each patient are considered individually at biweekly transplant team meetings, and decisions are made after extensive consultation with the referring physician, the patient, and the prospective donor.

Gene expression dwindles as T cells enter their resting phase and increases again upon activation. Therefore, the investigators hope that cells that become activated as they cause GVHD will express higher levels of the transgene and will become vulnerable to killing. Although the investigators do not know whether activation during delayed GVHD will increase gene expression in humans, the proposed study is intended to address this question. The investigators acknowledged that it is possible that this approach will be of benefit only for GVHD occurring within the first 2 to 4 weeks after T-cell administration.

Regarding the proposed use of frozen/thawed cells, the investigators explained that their functional studies have been performed on frozen/thawed cells, and there has been no evidence of functional impairment. Although they have never infused frozen/thawed CD19 selected cells into humans clinically, the investigators' *in vivo* data using frozen/thawed T cells expressing other transgenes show excellent functionality.

In response to concerns about the ability to generate sufficient numbers of cells, the investigators stated that they have scaled up to $5x10^8$ using appropriate tissue culture bags. The additional scale-up needed to obtain the maximal dose in this proposed protocol will use the same technology but with a greater number of bags and technologists. The investigators' production facility routinely handles numbers of cells an order or magnitude higher than what is needed for this protocol, so generating sufficient numbers of cells will be readily accomplished.

Regarding the justification for enrolling children as research participants in this proposed protocol, the investigators explained their belief that the benefits outweigh the perceived risks. Infection due to slow immune reconstitution is a major cause of morbidity and mortality after haploidentical transplant. Several studies have shown that immune recovery can be enhanced and antiviral responses restored by adoptive transfer of T cells, but this approach carries a risk of GVHD. The suicide gene system proposed in this study will provide a "safety switch" should this AE occur; studies using a different suicide gene system have shown that transduced cells can be ablated if they cause GVHD. The suicide gene system proposed in this protocol has a potential advantage: The system is humanized and does not require administration of a therapeutically efficacious antiviral drug for activation. Relapse is the other major cause of mortality after haploidentical transplant; donor T cells can mediate antitumor activity at least in some malignancies, thus raising the possibility of potential benefit. Development of a more effective safety switch will have benefits for other gene transfer and cell therapy applications. Although the major risk is insertional mutagenesis, this complication has not been observed in any studies with gene transfer to mature T cells. Different results may be obtained in pediatric versus adult recipients, since there are differences in the types of viral infections seen posttransplant; for example, pediatric patients have a much higher incidence of adenovirus infection. For all of these stated reasons, the investigators believe that this proposed protocol meets the requirements of the Common Rule since the potential benefits outweigh the risks and since the benefits for children may differ from those for adults.

Dr. Krance explained that a child life specialist who has no connection with this protocol will be present during the consent discussion to act as an advocate for the child and to ensure that the parents (and child, to an age-appropriate level) understand the study and have all their questions answered. If there is a disagreement between parents on whether to participate or if the child life specialist believes the child does not want to participate, the investigators will not proceed to enroll that child.

Responding to concerns about the risk of uncontrolled EBV infections, the investigators noted that the donor and recipient are only haploidentical, thus sparing T cells reacting to EBV antigens presented by the shared haplotype. In subsequent clinical studies, the investigators observed excellent reconstitution of T-cell immunity to EBV after infusion of allodepleted T cells and saw no evidence of lymphoproliferative disease in any individual.

Regarding the risk of infusing recipient LCL, the investigators explained that recipient LCLs are cultured in acyclovir for 2 weeks prior to use as stimulator cells to prevent their production of infectious EBV. The cells also are irradiated prior to use, and the responding T cells are manipulated by allodepletion, transduction, and CD19 selection, with several washes in each step prior to cryopreservation. With those safeguards, it is unlikely that residual B cells will be found in the final product. In addition, one of the release criteria for the final product is that it lacks residual B cells by phenotyping, and the investigators plan to use real-time PCR to monitor recipients for EBV reactivation.

In response to concerns about the proposed number of research participants not being large enough to allow for firm conclusions regarding the safety of the experimental procedure, the investigators reiterated the primary purpose of this protocol—to establish the safe maximal tolerable dose (MTD) of gene-modified allodepleted T cells that can be given without inducing GVHD. In collaboration with their statistician, the investigators are implementing the Continual Reassessment Methodology, a model-based strategy for estimating dose-toxicity response. Extensive simulations have shown the investigators that they can expect better operating characteristics with this design, in terms of higher probabilities of selecting the correct MTD and comparable toxicity rates with the standard 3+3 design.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Clinical/Trial Design Issues

- Although the proposed strategy for depleting alloreactive donor T cells prior to infusion to the research participant is well designed, it will not lead to deletion of all of the alloreactive cells. Cells then will be transduced and selected for cells that express the inducible suicide gene. Again, this selection will not result in a 100-percent pure population, and the end population will contain cells that do not carry the suicide gene. Some of these cells may be alloreactive and may initiate GVHD. In participants who develop GVHD, the investigators are encouraged to identify the reactive cells to establish if they are transduced and can thus be eliminated with the drug treatment or, instead, if they are derived from non-transduced cells that are resistant to elimination with the drug.

- The iCasp9 consists of the human caspase 9 gene fused to a slightly mutated human FK506-binding domain, which will produce a drug-binding protein. Although all of the components of the molecule used in this suicide gene are derived from human proteins, the chimeric nature of the molecule (i.e., the use of a drug-binding site on the caspase 9 gene) could prove to be immunogenic. Efforts should be made to detect such reactions by developing assays that are sensitive enough to allow analysis of the immunogenicity of potential neoepitopes derived from the transgene product.

- A continual reassessment statistical method will be used in the dose-escalation plan. The detailed statistical plan for this part of the protocol was not available for review prior to the meeting. The plan will be reviewed by the RAC upon submission.

- Under the DHHS regulations governing research involving children (45 CFR 46 Subpart D), research involving more than minimal risk must, among other things, hold out the prospect of direct benefit for the individual research participant. No issues were raised about the determination that the protocol meets the criteria outlined under those regulations.

Ethical/Social/Legal Issues

- The modified informed consent document is more clear and understandable.

G. Committee Motion 6

Dr. Federoff summarized the RAC recommendations to include a variety of clinical and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XII. Day Two Adjournment/Dr. Federoff

Dr. Federoff adjourned Day Two of the June 2007 RAC meeting at 6:00 p.m. on June 20, 2007.

XIII. Day Three Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff opened Day Three of the June 2007 RAC meeting at 8:30 a.m. on June 21, 2007.

XIV. Discussion of Human Gene Transfer Protocol #0704-842: A Randomized, Controlled Phase III Trial of Replication-Competent Adenovirus-Mediated Suicide Gene Therapy in Combination with Intensity-Modulated Radiation Therapy (IMRT) Versus IMRT Alone for the Treatment of Newly Diagnosed Intermediate-Risk Prostate Cancer

Principal Investigators:	Svend O. Freytag, Ph.D., Henry Ford Health System (HFHS); Benjamin Movsas, M.D., HFHS
Additional Presenters:	Mei Lu, Ph.D., HFHS; Anthony Luznik, gene therapy patient
RAC Reviewers:	Drs. Flint, Grant, and Wei
Ad hoc Reviewer:	Otis W. Brawley, M.D., Emory University

A. Protocol Summary

Prostate cancer is the most commonly diagnosed malignancy in men. Although conventional therapies (surgery and radiation therapy) produce high cure rates of early-stage prostate cancer, many tumors recur and metastasize. There is a need to develop new therapies that may improve the effectiveness of conventional cancer therapies.

In the protocol, a replication-competent adenoviral vector (rcAd) will be used to deliver a cytosine deaminase (CD)/herpes simplex virus thymidine kinase (HSV-1 TK) fusion gene to prostate tumors. Preclinical studies have demonstrated that the replication competent adenovirus itself has potent anti-tumor activity by replicating in and preferentially destroying human cancer cells. The therapeutic effect of the replication competent adenovirus can be enhanced significantly by invoking two suicide gene systems (CD/5-FC and HSV-1 TK/GCV), which render malignant cells sensitive to specific pharmacological agents and, importantly, sensitizes them to radiation.

The safety and potential efficacy of the approach has been evaluated in four Phase I/II clinical trials without and with conformal radiation therapy (CRT).The proposed Phase III study will involve men with intermediate-risk prostate cancer to determine whether gene transfer will improve the effectiveness of radiation therapy providing another therapeutic option for select patients with newly diagnosed prostate cancer. The primary end point of this proposed trial is local tumor control as determined by prostate biopsy at 2 years; secondary end points include time to clinical/biochemical progression, time to development of distant metastases, survival, toxicity, and quality of life. At least two centers will conduct this study—the Henry Ford Health System and the Fox Chase Cancer Center.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. Key issues included the following: consideration of the adequacy of the current safety and efficacy data that underpin the decision to proceed directly to a Phase III study, concerns about the safety of the vector construct, questions about the study design, particularly the primary end point, and the limited experience of the gene transfer field with Phase III studies.

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

Dr. Flint noted that this proposed study differs from the considerable experience accumulated using Ad vectors because of its use of a replication-competent vector and because it would be a Phase III trial. Noting that, of the nine research participants dosed in the Phase I trial of Ad5-yCD/*mut*TK_{SR39}*rep*ADP plus IMRT, only three participants (cohort 3) received two intraprostatic injections of the highest dose ($1x10^{12}$ viral particles, which is the dose to be used in the proposed Phase III study), Dr. Flint asked the investigators to describe in detail the data that led to the conclusion that no additional toxicity was associated with a second Ad injection and to describe any new toxicity data that have been collected. She also expressed concern about proceeding to a protocol in which 80 participants will receive two injections with only limited evaluation of the safety of the Phase I vector and asked the investigators to explain their rationale. Dr. Flint asked the investigators to discuss the data from preclinical or clinical experiments that demonstrate that Ad5-yCD/*mut*TK_{SR39}*rep*ADP kills tumor cells more efficiently than a replication-incompetent Ad vector that carries the same fusion of suicide genes. Noting that the replication of Ads that cannot direct synthesis of the E1B 55kDa protein is cell-type dependent and has been reported to vary even among normal human cells, Dr. Flint asked how the investigators propose to assess such possible outcomes. She asked the investigators to provide a current summary of assessment of the efficacy of Ad5-yCD/*mut*TK_{SR39}*rep*ADP in the Phase I trial, because most of the originally submitted information about efficacy pertains to participants who received the parental yCD/Tkrep Ad vector. Dr. Flint also requested that the investigators discuss the rationale for selecting tumor biopsy at 2 years as the primary outcome measure.

Dr. Grant's review was read into the record by Dr. Federoff. [She was not present on this day of the June 2007 RAC meeting.] Dr. Grant had no additional comments on scientific or clinical issues and focused her review on the informed consent document. She offered two suggestions to clarify comprehension of the stages of the study. Under section 2, "What Will Happen If I Take Part in this Research Study?," the investigators should consider separating Group 1 requirements and Group 2 requirements, at least in the first mention of this information in the first few pages. In this same section, the investigators should consider moving the subsection titled "Pre-Treatment Evaluations" toward the beginning of the section.

Dr. Wei noted that the primary end point is local tumor control at year two based on biopsy results and suggested that the investigators analyze the data with respect to "treatment failure," since potentially not every participant would be followed for the full two years due to death, a serious adverse event (SAE), or other reasons. Therefore, "success at year two" would be defined as a research participant still taking part in the followup phase of the trial and having a negative biopsy and no distant failure. With an N of 90 (assuming 5-percent loss to followup), the final 95-percent confidence interval for the difference of two "failure" rates is expected to be 0.07 and 0.37. The lower bound of this interval is only 7 percent, which may not be clinically interesting if SAE issues arise. He noted that the planned study size may be small, even for an optimistic assumed treatment difference of 20 percent. For the interim analysis, Dr. Wei stated the possibility of reestimating the sample size for the study. The investigators offered no detail about how sample size reestimation would be accomplished, whether the study would be blinded or unblinded with respect to the two arms, and what the violation-of-error rates would be. He also suggested that a futility analysis be added to the interim analysis in case it is not feasible to obtain a significant result at the end of this study.

Ad hoc reviewer Dr. Brawley noted that the investigators have chosen a population with a known quantifiable likelihood to relapse after traditional IMRT or IMRT and hormones, and they argue successfully that better treatment is needed. He stated that he understood and accepted the rationale of

using the result of a biopsy at two years after completion of therapy as a reasonable end point. Although the proposed virus is a second-generation, rcAd that has been used in a smaller number of research participants and appears to be safe, Dr. Brawley asked the investigators to discuss further the fact that only a limited number of research participants have received two injections of this vaccine. He also asked the investigators to discuss why they propose to take participants with a Karnofsky Performance Status of 70, since such individuals are quite ill. In standard practice, most men of intermediate risk with high-volume disease are treated with hormonal therapy for 2 to 3 months, followed by radiation for 6 to 8 weeks and then 6 months of hormonal therapy using a leuteinizing hormone-releasing hormone agonist; Dr. Brawley wondered whether the investigators would choose to include men who would not be candidates for hormonal therapy.

C. RAC Discussion

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

- Dr. Brawley suggested that the investigators structure this proposed trial so that the first 50 enrollees (25 of whom would receive the experimental drug) would be viewed as a randomized Phase II safety study. Then, when the Data Safety Monitoring Board (DSMB) approves the safety issues, the investigators could continue to the Phase III trial using those initial 50 enrollees in the Phase III analysis. Such a trial would be deemed "Phase II/III" and would need to have an experienced DSMB.

- Noting that toxicity will be based mainly on what leaks out of the injection site and will be influenced by the presence of virus-neutralizing antibodies in blood, Dr. Ertl suggested that the investigators measure preexisting immunity in the research participants before dosing.

- Dr. Ertl suggested that it will be important for the investigators to test their hypothesis that the success they have seen is likely to be caused by an immune response induced by the vector and the tumor cells. She stated that this important point should be demonstrated before proceeding to a Phase III trial, in part because such testing would be difficult to accomplish with the large numbers of Phase III participants.

- Dr. Ertl expressed concern that only three participants (in other trials) have received the two-dose regimen, although a total of 49 individuals have been dosed under this gene transfer regimen. Her concern was based primarily on the possible presence of preexisting immunity and antibodies, which could make the side effects more severe.

- Dr. Brawley explained that the expected toxicities would occur very early on, so a Phase II/III trial would adequately take care of the safety concerns. Concern about the PSA doubling time issue is mooted in this trial because the endpoints will be biopsy at two years and time to progression—the investigators will be generating data that may validate the prostate specific antigen (PSA) doubling time as a secondary end point.

- Dr. Strome objected strongly to the use of Phase I data to make statements about efficacy.

- Dr. Strome reminded the investigators that, since there are more than two treatment options, they should make sure that all of the people involved have consented the participant and were part of the consent process. This includes a radiation therapist, a medical oncologist, and an urologist, all of whom should sign off for each individual that there is generalized agreement that this form of therapy is appropriate.

- Dr. Somia requested additional information about the efficacy of the second injection of virus, especially as this relates to the investigators' experience in animal studies and how those might relate to the human experience.

- Dr. Albelda suggested that the investigators perform imaging after the first dose and again after the second, so that each research participant is her or his own "control."

- Noting that one of the hallmarks of Ad vector escape in viremia is the production of proinflammatory cytokines, Dr. Nemerow suggested that the investigators look at the presence of IL-6 and/or IL-10 in the first 20 or 25 research participants.

- Dr. Wei reiterated that the investigators should ensure that the principal outcome measures are well understood by the DSMB. The investigators and their statistical team should address the number of research participants required to obtain those measures.

D. Investigator Response

Regarding the use of rcAds as gene transfer vectors, the investigators explained that, because of their replicative properties, rcAds result in much greater therapeutic gene expression than do replication-defective adenoviruses and have the potential to infect a greater number of tumor cells. rcAds also may result in greater tumor antigen presentation (via their cytolytic effects) and provide a greater "danger signal" to the immune system (via *de novo* viral gene expression) than replication-defective Ads.

In response to concerns about additional toxicity associated with a second Ad injection, the investigators shared data showing that there were 4.3 treatment-related AEs per participant per gene transfer cycle with one cycle of gene transfer but only 3.0 AEs per participant per gene transfer cycle with two cycles; two cycles of gene transfer did not increase the incidence or the severity of treatment-related toxicities per research participant. All participants who received two Ad injections developed grade 1 flu-like symptoms, including low-grade fever, chills, and muscle aches; these events lasted less than 2 days, the symptoms were treated with Tylenol, and none were considered clinically significant. The most common side effects attributable to the radiation therapy were gastrointestinal and genitourinary events plus fatigue, none of which were exacerbated by two cycles of gene transfer.

Explaining their decision to enroll only intermediate-risk patients in this proposed Phase III study, the investigators stated that, in both Phase I trials, all intermediate-risk participants were negative for adenocarcinoma at their last biopsy yet a fraction of the high-risk participants were still biopsy positive following dosing.

The investigators explained that, owing to the long natural history of prostate cancer, it is not feasible to use "survival" as the primary end point in clinical trials of newly diagnosed prostate cancer. It would take 15 years to reach that primary end point, a major reason why pharmaceutical companies do not undertake the development of new treatments for newly diagnosed prostate cancer. However, the FDA has recently eased its position on this issue and will now accept "time to progression" as the primary end point in Phase III registration trials. Based on recent discussions with the FDA, the investigators changed their primary end point to "time to progression," and prostate biopsy status at two years will be a secondary end point. In addition, the investigators are committed to collecting data on 190 participants to obtain more information and to study secondary and exploratory end points, even if the trial does not meet its primary end point.

Short-term (6 to 8 months) hormone therapy is not included in this Phase III trial because its efficacy is not considered to be well tested, since it is based on one small Phase III study; however, the investigators will make potential participants aware of this study and will provide hormone therapy if requested. In addition, hormone therapy obfuscates posttreatment biopsy results and would lengthen the time to PSA failure (a secondary end point). Excluding the use of hormone therapy in this proposed study will result in a "cleaner" trial that will allow the investigators to study the effect of the gene transfer without the confounding effects of hormone therapy.

The investigators explained that low-grade, regional peritonitis was observed in their preclinical mouse toxicology studies but was not seen in the Phase I clinical trials. The peritonitis was due to leakage of the injected Ad into the abdominal cavity due to the small size of the mouse prostate gland.

Explaining that they are not interested in the absolute difference in positive biopsy rate, the investigators explained that the proposed Phase III trial has the goal of a 50-percent reduction in positive biopsy rate at two years in the investigated therapy arm compared with the control arm. With 50-percent relative reduction and 40-percent positive biopsy rates in the control group, the investigators anticipate a 95-percent confidence interval for the relative reduction 0.31 to 0.81, which they believe is significant for clinical practice.

Dr. Movsas explained that the investigators are working with a multidisciplinary prostate options clinic at the HFHS and the Fox Chase Cancer Center. Patients are seen within that setting, are given all their options, meet a variety of experts, and then are allowed to go home and think about it. Patients who are considering participating in a gene transfer trial meet with this group several times before they are enrolled in the trial. They also meet with the research nurse separate from one of the trial's investigators, so they have an opportunity to decline participation.

Dr. Freytag discussed the animal data related to the proposed second dose of virus. In general, the animal data, which have been accumulated for 13 years, indicate that results are improved when multiple injections are delivered to an animal tumor. Although there is no direct evidence in humans that the second injection helps, it provides a second opportunity to target the tumor. The injection algorithm is a first injection that treats the entire prostate, skewing the Ad dose to the affected regions that have tumor, and the second injection is targeted only where the tumor is located, thus giving a boost to the areas with high tumor burden.

E. Public Comment

Dr. Takefman noted that a document on oncolytic viruses is being written for the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. He commented on oncolytic viruses and shedding and noted that the investigators' data are somewhat atypical, leading to a question about the timing of PCR imaging analysis.

Anthony Luznik, a gene transfer patient, was diagnosed with prostate cancer in October 2005; his cancer has now been rendered benign. He reported that his two injections of Ad gene therapy were astonishing free of the side effects normally associated with cancer treatment, and he described what happened to him physically and psychologically at each of the two injection time points. Mr. Luznik reiterated the importance of new therapies and commended the RAC for its work.

Dr. Borror suggested that the investigators avoid using terms such as "therapy" and "treatment" throughout the informed consent document. Other language suggesting therapeutic benefit also should be changed, such as "these genes have the ability to convert nontoxic drugs…that will help destroy cancer cells" should be changed to "that we hope will help destroy…". She also noted that some of the terminology is confusing, especially that related to preenrollment testing and investigator review of the followup tests.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

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

- Questions were raised about the appropriateness of moving from a Phase I study to a Phase III study that will enroll 250 research participants. Although the decision to proceed to Phase III is based on what was characterized as strong efficacy data, the adequacy of the data is in question. The data consist of the results from three participants in the Phase I study and data from several other Phase I trials, some of which used a different vector construct. Not only is caution warranted in interpreting efficacy data from safety studies involving so few participants, but

also the safety profile of the vector construct is based on the limited experience of a small Phase I study. SAEs that do not occur in sufficient frequency cannot be detected during small Phase I trials.

- To address these concerns, the investigators should consider modifying the study design as follows: Revise the protocol as a Phase II/III study; begin by enrolling a limited number (e.g., 40 to 50) of participants and focus on safety; present the Phase II unblinded results to an independent DSMB prior to proceeding to Phase III; and make the safety profile the primary criterion for proceeding. Since the decision to proceed to a Phase III trial will nonetheless involve a risk-benefit analysis, the development of a statistically meaningful safety and efficacy end point for Phase II is also strongly encouraged.

- There is very limited clinical experience in using two doses, and because all participants will receive two doses, it will be difficult to assess the efficacy of the second dose. Consideration should be given to employing single photon emission computed tomography (SPECT) imaging modalities that may help determine the relative efficiency of the second dose.

- Phase I data demonstrated that certain participants experienced a prolonged delay in progression as measured by PSA doubling time, an unvalidated surrogate marker. Although the delay was thought to be indirect evidence of "anti-tumor immunity" from the gene transfer, no data to support the hypothesis were presented. A more thorough analysis of the immunological and inflammatory responses in participants will help elucidate the potential immunological contribution to efficacy as well as better characterize the systemic responses to the conditional replicating Ad administration delivered locally to the prostate. This could be done in a limited number of initial participants and is an additional reason to consider redesigning the study as a Phase II/III trial.

- Although viremia was not detected in the Phase I study, the investigators should consider employing more sensitive and validated methods for detecting replicating virus that might enable a more definitive assessment of the degree to which the Ad vector escapes from the local administration site.

- SPECT imaging data demonstrated radiographic evidence of Ad in the glands and tissues of the head and neck, suggesting that rcAd vector could appear in saliva. Since this could present a safety risk to close contacts, the protocol and consent should be modified to address the possibility of transmission to close contacts.

- Because the primary study end point for this protocol is "time to disease progression," the statistical calculations, which were used to determine sample size and were based on the prior end point of prostate biopsy results at 2 years, are no longer valid. A new statistical plan will be developed. When using an end point such as progression of disease, confidence intervals rather than *p* values may be more helpful in determining the correct sample size.

- Given the questions about safety and efficacy of the current design, the DSMB will have a critical role in protecting the participants in this study. A copy of the DSMB charter is to be sent to the OBA.

Ethical/Social/Legal Issues

- To avoid therapeutic misconception, the term "gene transfer" should be used instead of "gene therapy."

- Simplify the description of the series of tests and treatments that are part of the protocol.

- Clarify or delete the reference to "routine tests that would be performed whether or not you decide to participate in this research study."

G. Committee Motion 7

Dr. Federoff summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XV. Discussion of Human Gene Transfer Protocol #0704-853: A Phase I, Open-Label, Dose-Escalation, Multiple-Dose Study of the Safety, Tolerability, and Immune Response of CRS-207 in Adult Subjects with Selected Advanced Solid Tumors Who Have Failed or Who Are Not Candidates for Standard Treatment

Principal Investigator:	Elizabeth M. Jaffee, M.D., The Sidney Kimmel Cancer Center at Johns Hopkins University
Additional Presenters:	Dirk G. Brockstedt, Ph.D., Cerus Corporation; Thomas W. Dubensky, Jr., Ph.D., Cerus Corporation; Joseph J. Eiden, M.D., Ph.D., Cerus Corporation; Rodney A. Prell, Ph.D., DABT, Cerus Corporation
Sponsor:	Cerus Corporation
RAC Reviewers:	Drs. Dewhurst, Kahn, and Kirchhoff

Drs. Albelda, and Federoff recused themselves from discussion of this protocol due to conflicts of interest; as a result, Dr. Dewhurst became temporary Chair of the RAC during the discussion of this protocol.

A. Protocol Summary

Malignant mesothelioma, non-small cell lung cancer (NSCLC), and cancers of the pancreas and ovary are among the most aggressive and lethal malignancies. Collectively, new cases of these cancers are diagnosed in approximately 200,000 Americans each year and cause nearly as many deaths. In addition to their severity, these tumors share the common feature of high-level expression of a protein called mesothelin on the cell surface. Cerus Corporation has developed a vaccine-based approach that is designed to stimulate the immune response to mesothelin in participants given this candidate therapy to target and kill malignant cells that overexpress mesothelin. Mesothelin meets three important criteria that favor its potential use as an immune target in the development of therapeutic vaccines for individuals with malignant mesothelioma, NSCLC, and carcinomas of the pancreas or ovary: (1) It is widely shared by most ovarian and pancreatic cancers; (2) it has limited expression in normal tissues, and cytotoxic T-lymphocyte responses can be induced following vaccination; and (3) these responses correlate with clinical benefit in individuals at high risk for disease recurrence. The vaccine, CRS-207, is based on the demonstrated antitumor activity in nonhuman animal models of cancer. The Phase I study will evaluate the safety, tolerability, and immune response to CRS-207 in adult research participants who have malignant mesothelioma, advanced NSCLC, or advanced carcinoma of the ovary or pancreas that is refractory to standard treatment.

The CRS-207 vaccine is based on a live-attenuated form of the bacterium *Listeria monocytogenes (Lm)*, which is often found as a food-borne contaminant. In addition to causing a generally mild and self-limited form of gastrointestinal disease, the form of *Lm* usually found in nature also can occasionally cause a more severe illness known as listeriosis. Cancer patients, particularly those with hematologic malignancies or severe immunosuppression of their immune system, may be at increased risk for listeriosis. To address this potential safety issue in the proposed trial, the Cerus Corporation has developed a live attenuated strain of *Lm* that is more than a thousand-fold less virulent when administered in mice. The safety and tolerability of this attenuated strain, CRS-100, are currently being evaluated in a Phase I clinical trial. *Lm* is recognized as a powerful activator of nonspecific immune responses (innate immunity), which in turn facilitates development of specific or adaptive immunity.

The investigators propose to conduct a Phase I, open-label, dose-escalation, multiple-dose study of the safety, tolerability, and immune response of CRS-207 in adults with malignant mesothelioma, NSCLC, or advanced carcinoma of the ovary or pancreas who have failed or are not candidates for standard therapy. The primary study objective is to determine the MTD of CRS-207 and gather data regarding the safety profile following a multiple-dose regimen of administration of CRS-207. The secondary objective is to explore the immunological response to and the biodistribution and clearance of CRS-207 and evaluate tumor status prior to and after administration of the investigational agent. CRS-207 will be administered by intravenous (IV) infusion every 21 days up to a total of four dosings. Each participant will receive by IV, a total of four doses (an injection once every three weeks) at one of three dose levels of CRS-207: $1x10^8$ colony-forming units (cfu), $1x10^9$ cfu, or $1x10^{10}$ cfu. Each IV administration will take approximately two hours to complete, and participants will be observed closely during and after each injection. Beginning on Day seven after the last study dose, a 10-day course of oral antibiotics will be provided to all study participants.

B. Written Reviews by RAC Members

Nine RAC members voted to for in-depth review and public discussion of the protocol. Key issues included the following: the novelty of the construct, the death of one macaque monkey in the preclinical repeat-dose safety study of CRS-207, the relative lack of safety data on the use of this *Listeria* strain in humans, and the safety of this attenuated strain of *Listeria* in potentially immunocompromised research participants with cancer.

Three RAC members provided written reviews of this proposed Phase I trial. Two reviews (by Drs. Dewhurst and Kahn) were submitted in time to allow for a response from the investigator.

Noting that some of the high-dose macaques shed the agent in urine—not a major concern because of the fecal-oral route of transmission and the high infectious dose—Dr. Dewhurst requested that the investigators discuss potential implications for spread of the agent in the environment or possible recombination with the wild-type organism. He also requested more in-depth discussion of the implications for the human trial of the fatal reaction in one high-dose macaque in the repeat-dose safety study of CRS-207; this animal had a severe reaction on the first dose of the agent, which manifested in liver, renal, and hematologic changes. This fatal reaction brings to the fore questions about the dose levels in the human trial and the reliability of the titering of this organism, since a postdose reevaluation found that the real dose administered to the macaque that died was more than threefold higher than what was originally thought. In addition, the monkey that ultimately died experienced only a modest increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which raises a concern about reliance on AST and ALT as measures of safety; Dr. Dewhurst suggested the possibility of adding cytokine analysis to the clinical study. Information about the death of this macaque should be provided to potential research participants in the risks section of the informed consent document. He requested that the investigators discuss more fully the proinflammatory response in the cynomolgus monkey study, which is strikingly nonlinear at the high dose, for IL-7 and tumor necrosis factor-alpha (TNF-α) in particular. Combined with the fatality data, this is reminiscent of the steep toxicity curve with high-dose Ad vectors. Dr. Dewhurst suggested that the investigators consider adding an assessment of hematologic, clinical laboratory, and liver enzyme function at day 14 after each vector dose.

Dr. Kahn focused his comments on the informed consent document. He suggested that it be made clear that this is a Phase I trial and that the primary goal of this trial is to assess safety; this information should be included in sections 1 and 6 of the document. Information about the death of the macaque should be included in section 4 of the document. The information in section 9 of the document about the amount of compensation has been left blank; this information should be filled in, and an indication should be included as to whether the IRB has approved a specific amount. Section 12 of the informed consent document limits the sponsor's responsibility to payment for "only the costs of acute medical care arising from injuries that are the result of your participation in the study *to the extent that such injuries are due to the negligence of the Sponsor*…". Dr. Kahn wondered whether this wording is acceptable to the IRB and

expressed hope that the sponsor would pay for any injuries incurred as a result of participating in a Phase I trial.

Dr. Kirchhoff expressed particular concern about the death of one of the macaques in the CRS-207 trial. The stimulation of serious side effects can be nonlinear, so the proposed Phase I dose-escalation clinical trial may expose participants to substantial risk for serious complications. He noted that it appears likely that CRS-207 caused the monkey's death, and an additional issue is the striking persistence of the recombinant *Lm* in the inoculated—and immunocompetent—monkeys; this finding may diminish the crispness of the dose-escalation approach, since injections after the first one will be given to participants already heavily infected with the bacteria. Dr. Kirchhoff noted the complex ethical issue of how information about the death of the macaque should be integrated into the process of informed consent, given that the potential research participants are cancer patients who are likely vulnerable to disregarding the death of this animal. He asked whether the full information on the CRS-100 trial would be completed, analyzed, and presented to the RAC before the CRS-207 trial begins, since data are currently available only for three participants in that trial. Revision of the wording should be made in the submitted responses to the issues raised in Appendices M-II through M-V (of the *NIH Guidelines*). The investigator incorrectly states that this study does not involve the administration of recombinant DNA, when clearly it does.

C. RAC Discussion

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

- Dr. Kirchhoff asked how the investigators know that the antimesothelin responses are not simply a marker for something else, so that in targeting mesothelin the investigators would be going after the wrong target.

- Dr. Ertl requested more information about the toxicity curve of *Listeria* in animals, particularly in monkeys. Toxicity is known to be quite different in mice compared to monkeys and compared to humans; although it is impossible to kill a mouse with Ad, monkeys are more sensitive but still about 10 times less sensitive than humans. The other problem with Ad is that the toxicity is not linear, with no warning signs prior to serious problems. Dr. Ertl asked whether toxicity studies have been conducted with *Listeria* to understand the toxicity curve and the relative sensitivity of different species.

- Dr. Ertl suggested that individuals with immune-compromised family members should not enroll in this trial.

- Several RAC members wondered whether at least some participants should complete all four doses before escalating the dose for the next cohort.

- Because some of the macaques had possible neurologic signs, Dr. Dewhurst expressed concern about possible *Listeria* in the CNS in individuals who have a compromised blood-brain barrier. He suggested excluding individuals with brain metastases.

D. Investigator Response

In response to requests from RAC members for updated information on the Phase I study of CRS-100, the investigators described the study and some of the latest data. To determine the MTD and to explore the safety profile of single-dose administration of CRS-100, this first study in humans provides for IV administration of increasing amounts of CRS-100 to successive cohorts of study participants. Increase in dose level between cohorts is based on the absence of dose-limiting toxicity (DLT) possibly or probably related to CRS-100 among the first three participants in each cohort or observation of no more than one DLT among the first six participants in a cohort. Participants receive a single IV dose of the study investigational agent, injected over a 2-hour period. No SAEs have been reported during the study, and no investigational new drug safety reports have been generated. One participant died due to progressive

disease after completion of the study. The second cohort is currently open for enrollment. *Lm* was not detected following administration of CRS-100 in cultures of sputum, urine, feces, or blood.

Regarding possible spread of the experimental agent in the environment or by recombination, the investigators explained that infectivity of wild-type *Lm* has not been studied in humans under controlled clinical conditions. The best estimates of an infectious dose of the wild-type organism for humans are provided by investigations of food-borne outbreaks. These studies suggest that the oral dose required to cause disease in 90 percent of the normal population is about $1x10^9$ cfu; the infectious dose in individuals with compromised cellular immune responses is estimated to be $1x10^7$ cfu. Based on the estimated infectious dose of wild-type *Lm* and the shedding observed in nonhuman primates, human-to-human transmission of an infectious dose of CRS-207 appears unlikely. Epidemiological studies have shown that the principal mode of transmission of *Lm* for both epidemic outbreaks and sporadic infections is contaminated food; although person-to-person spread via the fecal-oral route is also postulated as a route for transmission of *Lm*, such occurrences appear to be very uncommon. Regarding potential implications for spread in the environment, CRS-207 does not have a selective advantage for growth in the environment compared with the wild-type organism. The possible recombination of CRS-207 with wild-type *Lm* is also highly unlikely, as is reconstitution of a wild-type phenotype by incorporation of foreign DNA into CRS-207.

In response to concerns about one macaque's severe reaction to the first dose of the experimental agent, the investigators explained that the studies with macaques define a syndrome of DLTs, including thrombocytopenia, anemia, and leukopenia, that are likely the result of an inflammatory response to high doses of attenuated *Listeria*. The results of the CRS-207 study suggest that, although one high-dose animal was more sensitive to systemically administered *Listeria* and exhibited a more pronounced response when compared with animals that received an equivalent dose, the adverse clinical responses occurred at a dose above the previously defined MTD for the parent strain CRS-100. No such sequelae have been observed in monkeys given doses equal to or lower than $1x10^{10}$ cfu, which would be more consistent with a hypersensitive response. An additional measure of safety is provided by a 2-hour infusion time during the clinical trial compared with the 30-minute infusion employed in the toxicology studies. Longer infusion times have been shown in preclinical studies to lessen the acute response to IV administration of live bacteria. In addition, the proposed CRS-207 clinical trial has been revised to limit the dose escalation to ten-fold increases between cohorts, unlike the toxicology studies that were performed with thirty-fold increases, which provides an additional safety measure.

The investigators responded to concerns about potential failure to detect DLT with the current phlebotomy schedule by agreeing that the protocol would specify that additional clinical laboratory assessments, other than at the times specified in the protocol, are expected to be performed if the laboratory results indicate the likelihood of continued progression and potential for DLT with the current phlebotomy schedule. Cytokine and chemokine analyses have been incorporated into the protocol, and although are not themselves the basis for defining DLT, these assays will be evaluated for potential correlation with other clinical signs and symptoms during the course of the study.

Noting that many anesthesias are potentially hepatotoxic, Dr. Jaffee agreed that the investigators should consider adding to the inclusion criteria that participants should have no elective surgery within a certain time related to their enrollment in this trial, including dental surgery—except in an emergency.

Dr. Brockstedt explained that the investigators intend to measure, as a batch analysis, TNF-α and IL-6 cytokines at 4 hours postdosing, 24 hours postdosing, and 4 and 7 days postdosing. The peak responses are usually seen within 4 hours postdosing.

Dr. Jaffee explained that the CRS-100 study would be conducted essentially in parallel to this proposed CRS-207 study; the CRS-100 study is targeting liver lesions. She offered to keep the RAC apprised of the progress of the CRS-100 study.

Regarding whether the tumor rejection antigen is the appropriate target, Dr. Jaffee stated that the investigators have shown that there is a correlate; the T cells can lyse tumor-expressing mesothelin *in*

vitro, but it is unknown whether these cells circulate and cause regression in patients. She stated that this is the beginning of a platform that, if found safe, would target multiple antigens in any disease. There is no current way to prove that mesothelin is the rejection target other than through the *in vitro* data and mouse models that show that immunization against mesothelin causes tumor regression.

Dr. Prell explained that the investigators analyzed the histopathology for the macaque that died, and nothing was above and beyond what other animals in that dose group showed. Unfortunately, neither tissues nor serum was collected for bacterial culture.

Dr. Dubensky explained that the rationale behind the gene deletions was not to change the biodistribution profile but to remove the ability of the *Listeria* to grow and spread efficiently within the context of the liver while not removing its ability to be phagocytized. Dr. Prell added that *Listeria* does not accumulate following multiple injections; the profiles look comparable after multiple injections in terms of acute clearance and biodistribution in the tissues that were tested in mouse studies.

E. Public Comment

Public attendees offered no comments.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Clinical/Trial Design Issues

- The following recommendations relate to the monkey that was found in a moribund condition following a repeat dose study of CRS-207. The animal was euthanized (after a second dose of the study agent was administered), and postmortem evaluation showed systemic evidence of a severe reaction either to the anesthesia, to CRS-207, or to both.

    - Under the proposed protocol, at least two participants are to receive two doses (out of a total of four) prior to proceeding to the next cohort. In view of the questions surrounding this preclinical event, it may be prudent to administer all four of the planned doses of the study agent to at least two participants in each cohort prior to proceeding to the next cohort.

    - Given that the anesthesia may have played a role in the monkey's illness, the protocol should explicitly exclude anyone who has recently had anesthesia for any reason as well as persons intending to undergo any procedure requiring anesthesia (including elective minor dental surgery).

    - Although pathological changes in the brain were not found on autopsy, the monkey exhibited some neurological symptoms. Although the protocol plans to exclude participants with known brain metastases, it may be prudent to screen potential participants who have a high risk of brain metastases (e.g., individuals with adenocarcinoma) using imaging tests rather than a clinical history or physical exam only.

- A Phase I trial of CRS-100 using an attenuated strain of *L. monocytogenes* that is nearly genetically identical (it does not contain the gene that encodes mesothelin) to CRS-207 is ongoing and may provide important safety information on the use of the attenuated *Listeria* platform. To facilitate the monitoring of these two trials, annual reports for Protocol #0704-853 should include the safety data from the CRS-100 trial.

- It is known that *L. monocytogenes* is more likely to cause disease in immunocompromised individuals. Despite CRS-207 being an attenuated strain of *Listeria*, consideration should be given to excluding anyone who would be in close contact with immunocompromised persons

during the study period.

Ethical/Social/Legal Issues

- The following three changes to the informed consent document should be made:
    - The introductory section should clearly state that the protocol is a Phase I study and that its primary objective is to establish safety and tolerability.
    - Section 4 ("Risks") describes the moribund state and euthanization of the monkey in the preclinical study as follows: "In one study, a monkey had to be put to sleep (euthanized) when it became very sick after receiving CRS-207 (at a dose higher than the doses planned in this study) followed by a sedative to have blood drawn, and it is unknown if this was due to the CRS-207." The phrase "...it is unknown if this was due to the CRS-207" should be changed to read "...a contribution by CRS-207 cannot be ruled out."
    - It is misleading to state that this trial does not involve the administration of recombinant DNA, since it clearly does. A statement along the following lines should be added, where appropriate: "The protocol involves the injection of genetically modified bacteria that contain recombinant DNA."

G. Committee Motion 8

Dr. Dewhurst summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 9 in favor, 0 opposed, 0 abstentions, and 3 recusals.

XVI. Discussion of Human Gene Transfer Protocol #0704-846: A Phase I, Dose-Ranging Study to Assess Safety and Distribution of GT-111 in Patients with Advanced Metastatic Cancer

Principal Investigator:	Pierre L. Triozzi, M.D., Cleveland Clinic Foundation
Additional Presenters:	David T. Curiel, M.D., Ph.D., The University of Alabama at Birmingham; Dror Harats, M.D., Vascular Biogenics Ltd.; David A. Snyder, M.D., Vascular Biogenics Ltd.
Sponsor:	Vascular Biogenics Ltd.
RAC Reviewers:	Drs. Albelda and Nemerow and Ms. Shapiro

Drs. Strome,, Kodish, and Kahn recused themselves from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

Anticancer therapy—including chemotherapy, radiotherapy, immunotherapy, and other modalities—usually fails when the disease is advanced and the tumor burden is large. Therapies targeting every cancerous cell tend to fail at this stage, both because it is not feasible to successfully target every cancer cell and because the tumor cell has the ability to become modified genetically and therefore to acquire drug resistance. Within the past decade, there has been an effort to develop new therapeutic modalities that target either the tumor as an organ or its unique biology.

Formation of new blood vessels (angiogenesis) is a major biological process that enables the tumor to grow beyond its initial microscopic size and spread via metastases, resulting in the morbidity and mortality associated with cancer. A successful antiangiogenic therapy not only would inhibit new vessel formation and pruning of immature tumor vessels but also would target newly formed vessels, allowing normalization and stability of existing blood vessels.

GT-111 is a non-replicating adenoviral vector, which contains a modified murine pre-proendothelin promoter and a Fas-chimera transgene (Fas and human Tumor necrotizing factor (TNF) receptor). The modified murine promoter is able to target expression of the transgene to angiogenic blood vessels leading to targeted apoptosis of those vessels. The chimeric TNFR can trigger the Fas pathway specifically to the tumor by binding TNFα, abundant in the microenvironment of tumors.

In vitro and in vivo studies have shown GT-111 caused cell apoptosis specific to angiogenic endothelial cells. Studies indicated transgene expression was restricted to tumor-bearing organ., Safety toxicology studies in tumor- and nontumor-bearing mice showed no significant toxicity issues.

This Phase I study in individuals with advanced metastatic cancer will assess the safety of the gene transfer drug GT-111 for the first time in humans and will evaluate changes in markers for cancer as a result of this drug.

B. Written Reviews by RAC Members

Thirteen RAC members voted for indepth review and public discussion. Key issues included the following: the novelty of the approach, the tissue specificity of the transgene given the implications if apoptosis in nontumor blood vessels was triggered, and the potential reduction of efficacy of this vector due to the presence of neutralizing antibodies to the Ad.

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

Dr. Albelda requested additional information regarding the specificity of the promoter, and details of the biodistribution (RT-PCR) study. He also asked for data to support the statement that tumors have high levels of TNF-α.. He expressed concerns about the potential efficacy of the approach, including IV administration of vector to subjects with pre-existing Ad neutralizing antibodies, sufficient trafficking of vector to tumor and studies indicating vascular disruption may only temporarily slow tumor growth. Dr. Albelda asked the investigators whether studies were conducted in animals that had inflammation or other conditions in which angiogenesis might be present (such as wound healing and regenerating endometrium). He also asked them to explain their reasoning for proposing to observe participants for only 14 days before dosing the next group of participants and for following participants for only two months on study. He suggested excluding research participants with any type of imflammatory disease and screening for Ad neutralizing antibodies. Dr. Albelda expressed concern that the informed consent document was not specific to gene transfer and included neither an explanation of how the GT-111 agent is supposed to work nor a description of risks, the primary one being expression of the transgene in nontumor endothelium.

Dr. Nemerow asked, given that mice developed anti-TNF receptor antibody following vector administration, whether an anti-TNFR antibody reaction would lead to signaling events that damage normal vasculature or other normal tissues. He asked about the level of TNF-α following vector injection in mice and how the level of this cytokine in blood compares with that in the tumor microenvironment. He asked about the mechanism by which non-tumor endothelial cells were killed by high concentrations of vector in the absence of TNFα in preclinical studies. Dr. Nemerow also asked the investigators to discuss their reasoning for the highest dose anticipated for participants being only a 2.8-fold safety margin for IV administration, given the safety issues related to this transgene and the ability of Ad vectors to stimulate TNF-α production. He suggeted that the informed consent document should include a description of the potentially serious AEs previously encountered with systemic administration of Ad vectors.

Ms. Shapiro stated that the risk-benefit evaluation would be enhanced by additional discussion of safety concerns raised by questions surrounding tissue specificity of the transgene and the possibility of triggering apoptosis in nontumor cells as well as concerns that efficacy may be marginal using IV administration. She queried whether risks would be minimized further by refining exclusion criteria to exclude individuals with atherosclerosis. With respect to the informed consent document, Ms. Shapiro asked the investigators to discuss risks more fully, specifically the possibility of triggering apoptosis in

nontumor cells, and she suggested that additional discussion be included regarding the use to which the research blood and urine samples would be put and how and for how long those samples would be maintained. Ms. Shapiro noted that the informed consent document states that there will be no payments to cover treatment of research-related injury; however, she stated that compelling ethical arguments could be made to compensate participants for such injuries.

C. RAC Discussion

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

- Dr. Ertl asked for more information about the sensitivity of the assay to test expression in nontarget tissue and whether levels of TNF would be detected, and she suggested that the investigators use the most sensitive assay available, which is nested RT-PCR.

- Dr. Albelda, Dr. Kirchhoff, and Ms. Shapiro expressed the opinion that the investigators should have a dialog with their institution regarding research-related injury reparations to research participants.

D. Investigator Response

Regarding the specificity of the promoter, in vivo studies using various reporter genes did not detect expression in non-angiogenic endothelial cells. The biodistribution study using Q-PCR detected vector DNA in all tissues which largely cleared by day 91 except for hepatic lymph node. Transgene expression was only detected by RT-PCR in the lungs of tumor bearing mice.

Dr. Harats clarified the exclusion criteria regarding co-morbidities. Potential subjects with an acute cardiac event within the last year, vascular disease, prolererative and/or vascular retinopathy, active chronic diseases other than cancer, or recent surgery would be excluded. GT-111 was studied in a murine model of rheumatoid arthritis, disease severity appeared reduced by the reduction of angiogenci activity.

There is variability in TNF levels within tumor microenvironment and it is difficult to quantify differences between blood and tumor because TNFα is a soluble factor.

Dr. Harats addressed the efficacy concerns. Regarding neutralizing Ad antibodies, GT-111 is envisioned as a one-time administration, perhaps as a neo-adjuvant prior to surgery, and it is expected to act rapidly before the generation of new neutralizing antibodies. Comparisons of local and systemic delivery of GT-111 in preclinical studies indicated systemic delivery was more efficacious. In prior studies of anti-angiogenic factors, key limitations to effectiveness included rapid drug clearance and lack of localization. The targeted expression of the transgene may help address these issues. Lower doses may achieve efficacy in humans as compared to mice because murine cells may be refractory to adenovirus and human TNF has a higher affinity for the transgene.

The 14 day observation period and two month follow-up were selected because minimal toxic effects were observed in the preclinical studies by day 5 with a trend toward recovery by day 31. Subjects will be followed until disease progression.

The anti-TNFR antibody reaction is not expected because a human transgene is being used in humans rather than human in mice. Having a significant titer of antibodies specific to the binding site is unlikely.

The killing of normal EC may be due to potential auto dimerization or trimerization of receptor, irrespective of TNF presence.

To obtain a better answer on efficacy and toxicity, Dr. Harats agreed to ensure that each cohort includes at least one participant with high levels of preexisting Ad neutralizing antibodies and at least one participant with low levels.

The investigators indicated that the informed consent document would be revised to comply with institutional requirements and the suggestions of the RAC reviewers.

E. Public Comment

Dr. Borror noted that the language in the informed consent document was overly complex, especially on the first page. References to "treatment" should be avoided unless modified by "experimental," and references to "gene therapy" should be modified so as not to imply therapeutic benefit.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- One of the limitations of systemic administration of Ad vectors is preexisting neutralizing antibodies. To better elucidate the effect of preexisting Ad neutralizing antibodies on the response to the investigational agent GT-111, further studies using animals preimmunized with the vector should be considered.

- In a preclinical study, nonangiogenic endothelial cells were destroyed following the administration of the GT-111 vector at high particle concentration (multiplicity of infection of 1,000) in the absence of TNF-α. Because this may be a significant safety issue in the clinical study, additional preclinical studies should be conducted to investigate this finding.

- Although the PPE-1-3x promoter is highly specific and designed to target only tumor angiogenic endothelial cells, additional animal studies would provide further data on its specificity. Using a sensitive assay, such as nested RT-PCR, could determine whether the transgene is expressed in transduced nonmalignant endothelial cells or other cell types.

- To better determine the safety of systemic administration of the higher doses of the Ad vector, it would help to conduct nonhuman primate studies using the highest doses to be used in the clinical study.

Clinical/Trial Design Issues

- Although antiangiogenic agents prevent formation of angiogenic vessels, the investigational agent is expected to disrupt existing angiogenic vessels. As such, the protocol and consent document should consistently refer to GT111 as a "vascular disruption agent" rather than an antiangiogenic agent.

- Even though an immune response to the transgene product is not expected because the transgene product is not a foreign protein, participants should nonetheless be monitored for such reactions.

- The safety of a dose may differ from one participant to another depending on their weight, especially in a late-stage oncology trial like this one where some participants are likely to be cachectic. As an additional safety measure, it may be prudent to consider the participant's weight when determining his or her dose.

- Although it may be difficult in a Phase I trial to stratify participants according to tumor type, this should be considered in the analysis of results.

- Preexisting Ad neutralizing antibodies may interfere with vector administration. The titers of anti-Ad antibodies that interfere with transduction should be determined in preclinical studies, and the data should be used to screen prospective participants for antibody titers lower than that expected to cause significant interference.

Ethical/Social/Legal Issues

- The following changes should be made in the informed consent document:
    - The investigators should specify the purposes for which samples obtained for research will be used and how and for how long the samples will be retained.
    - The reading level is too high and should be simplified.
    - All references to "gene therapy" and "treatment" should be replaced with "gene transfer" and "investigational agent" to prevent misunderstanding about the potential benefits of the study.
    - Systemic Ad vector administration caused the death of a research participant in a gene transfer trial in 1999. This event and others should be described.
    - The assertion (on page 1) that "This modified virus is able to specifically insert a gene into angiogenic blood vessels (growing vessels due to metastases) which will lead to the destruction of these vessels" is misleading. It should be made clear that this is the study hypothesis.

G. Committee Motion 9

Dr. Federoff summarized the RAC recommendations to include a variety of preclinical, clinical, and ethical/social/legal issues, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No official motion was made or seconded regarding these summarized recommendations. The vote was 9 in favor, 0 opposed, 0 abstentions, and 3 recusals.

XVII. Closing Remarks and Adjournment/Dr. Federoff

Dr. Federoff noted that the RAC Clinical Trials Working Group has met twice and will begin developing its working agenda for the next 9 to 12 months. More information will be forthcoming to RAC members via e-mail.

Dr. Federoff thanked the participants and adjourned the meeting at 3:30 p.m. on June 21, 2007.

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

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

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: _____

_____
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Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
Ad	adenoviral, adenovirus
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCM	Baylor College of Medicine
BSL	biosafety level
CDC	U.S. Centers for Disease Control and Prevention
cfu	colony-forming units
CML	chronic myeloid leukemia
CMV	cytomegalovirus
CNS	central nervous system
CTL	cytotoxic T lymphocyte
C. trachomatis	*Chlamydia trachomatis*
DFS	disease-free survival
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
DSB	double-strand break
EBV	Epstein-Barr virus
FDA	Food and Drug Administration, DHHS
GCSF	granulocyte colony-stimulating factor
GR	glucocorticoid receptor
GVHD	graft-versus-host disease
HAART	highly active antiretroviral therapy
HFHS	Henry Ford Health System
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSV-TK	herpes simplex virus thymidine kinase
HyTK	hygromycin phosphotransferase-thymidine kinase
iCasp9	inducible caspase 9
IL-13Rα2	a glioma-specific cytokine receptor
IL-2	interleukin 2
IMRT	Intensity-Modulated Radiation Therapy
IRB	institutional review board
IV	intravenous
LAI	laboratory acquired infection
LCL	lymphoblastoid cell line
Lm	*Listeria monocytogenes*
MG	malignant glioma
MSKCC	Memorial Sloan-Kettering Cancer Center
MTD	maximal tolerable dose
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIDCD	National Institute on Deafness and Other Communication Disorders, NIH
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
NEI	National Eye Institute, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NOD/SCID	nonobese diabetic/severe combined immunodeficiency
NSCLC	non-small cell lung cancer

OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
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PCR	polymerase chain reaction
PET	positron emission tomography
PI	principal investigator
PSA	prostate specific antigen
RAC	Recombinant DNA Advisory Committee
rAd	recombinant adenoviral, adenovirus
rcAd	replication-competent adenoviral, adenovirus
RCL	replication-competent lentivirus
RNA	ribonucleic acid
RSA	research subject advocate
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SPECT	single photon emission computed tomography
TNF-α	tumor necrosis factor-alpha
USUHS	Uniformed Services University of the Health Sciences
UW	University of Washington
X-SCID	X-linked severe combined immunodeficiency
ZFN	zinc finger nuclease