RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 7, 8, and 9, 2011

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

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

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

June 7-9, 2011

The Recombinant DNA Advisory Committee (RAC) was convened for its 125th meeting at 11:00 a.m. on June 7, 2011, at the Marriott Suites Hotel in Bethesda, Maryland. Dr. John Zaia (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 11:00 a.m. until 4:30 p.m. on June 7, 8:00 a.m. until 5:35 p.m. on June 8, and 8:00 a.m. until 10:15 a.m. on June 9. The following individuals were present for all or part of the June 2011 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation *(via teleconference on Day 1, in person Days 2 and 3)*
Jeffrey S. Bartlett, The Ohio State University
E. Antonio Chiocca, Ohio State University Medical Center
Hung Y. Fan, University of California, Irvine *(Days 1 and 2 only)*
Yuman Fong, Memorial Sloan-Kettering Cancer Center
Norman Fost, University of Wisconsin–Madison *(Days 1 and 2 only)*
Jeffrey P. Kahn, University of Minnesota
Joseph A. Kanabrocki, University of Chicago *(via teleconference)*
Walter J. Koch, Thomas Jefferson University
Donald B. Kohn, University of California, Los Angeles
Margaret S. Mallino, University Park, Maryland
Anna C. Mastroianni, University of Washington School of Law *(Days 1 and 2 only)*
David A. Ornelles, Wake Forest University School of Medicine
Bernard Roizman, The University of Chicago
Susan R. Ross, University of Pennsylvania
Marcella Sarzotti-Kelsoe, Duke University Medical Center
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute
James R. Yankaskas, University of North Carolina at Chapel Hill
John A. Zaia, City of Hope (RAC Chair)

Office of Biotechnology Activities (OBA)

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

Additional Speakers

Xandra O. Breakefield, Massachusetts General Hospital-East
Heinrich U. Feldmann, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Katherine A. High, Children's Hospital of Philadelphia/University of Pennsylvania School of Medicine
Rudolph L. Leibel, Columbia University Medical Center
Edward H. Oldfield, University of Virginia
Amit Nathwani, University College London Cancer Institute
Arthur W. Nienhuis, St. Jude Children's Research Hospital
Howard I. Scher, Memorial Sloan-Kettering Cancer Center (MSKCC)

[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.

Non-voting Agency Representatives

Kristina Borror, Office for Human Research Protections, U.S. Department of Health and Human Services (DHHS)
Denise Gavin, U.S. Food and Drug Administration (FDA)

NIH/OD/OBA Staff Members

Linda Gargiulo
Chezelle George
Robert Jambou
Erin Luetkemeier
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal
Thomas Y. Shih

Attendees

There were 64 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. Call to Order and Opening Remarks

Dr. Zaia, RAC Chair, called the meeting to order at 11:00 a.m. on June 7, 2011. 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, 2011 (76 FR 28442). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of eight gene transfer protocols, updates on two previously reviewed clinical trials, review of biocontainment for experiments with a defective Lassa virus, and discussion of proposed revisions to the *NIH Guidelines* for work with partial viral genomes in tissue culture (Section III-E-1).

The RAC members introduced themselves by name, affiliation, and research interests.

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

II. Discussion of Human Gene Transfer Protocol #1104-1106 entitled: A Phase I Placebo Controlled Clinical Trial To Evaluate the Safety and Immunogenicity of a Prime-Boost Vaccine Regimen of GEO-D03 DNA and MVA/HIV62B Vaccines in Healthy, HIV-1-Uninfected Vaccinia Naïve Adult Participants

Principal Investigator:	Susan Buchbinder, M.D., San Francisco Department of Public Health
Additional Presenters:	Harriet L. Robinson, Ph.D., GeoVax, Inc.
Sponsor:	GeoVax, Inc.
RAC Reviewers:	Drs. Badley, Kahn, and Ross

Dr. Sarzotti-Kelsoe was recused from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

HIV Vaccine Trials Network (HVTN) 094 is a clinical trial to test the safety and immune responses to a combination of two experimental human immunodeficiency virus (HIV) vaccines developed by GeoVax, Inc.

This trial will assess the safety and tolerability of a heterologous prime-boost regimen consisting of two doses of GEO-D03 DNA vaccine and three doses of GeoVax modified vaccinia Ankara (MVA)/HIV62B (MVA62B) with dose escalation of the GEO-D03 DNA vaccine from 0.3 mg per dose to 3 mg per dose. The GEO-D03 vaccine consists of a 9.9 kb plasmid DNA expressing HIV-1 proteins Gag, PR, RT, Env, Tat, Rev, and Vpu, and human GM-CSF. GEO-D03 was developed from the pGA2/JS7 (JS7) plasmid DNA vaccine that was administered to normal volunteers in two previous HVTN trials. GEO-D03 differs from JS7 by the insertion of a 435 base pair open reading frame for human GM-CSF in the position of a deleted nef sequence. The rationale for co-expressing GM-CSF with the JS7plasmid in the GEO-D03 prime is to modify the response compared to that which is elicited by the JS7vaccine alone to achieve better prevention of mucosal infection believed achievable based on several immune stimulatory functions of GM-CSF. The MVA62B vaccine is a highly attenuated vaccinia virus expressing HIV-1 *gag*, *pol*, and *env* genes from the same HIV-1 sequences present in GEO-D03.

The study will involve 48 healthy adult participants. Some people in this study will get the study products and some people will get a placebo (sterile salt water). Of the research participants receiving the study vaccines, some will receive GEO-D03 at a dose of 0.3 mg (Group 1) and some will receive GEOD03 at a dose of 3 mg (Group 2); all participants who get the study vaccines will receive the same dose of the MVA62B. The investigators will compare the results from participants who received the placebo with results from participants who received the study vaccines. Vaccinations will be given to each participant at months 0, 2, 4, 6, and 8. Participants will receive GEO-D03 vaccine or placebo at months 0 and 2 and MVA62B vaccine or placebo at months 4, 6, and 8.

The main purpose of this study is to ensure that the vaccines are safe and that they cause no serious or bothersome side effects. It is hoped that this clinical trial will contribute to the development of vaccines that can prevent HIV infection or prevent the progression of HIV infection to acquired immune deficiency syndrome (AIDS).

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of the construct that the investigators propose to use in healthy volunteers.

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

Dr. Badley noted that this study is well designed and well constructed, and he expressed little concern regarding the addition of GM-CSF, given the extensive track record of safety of GM-CSF in other vaccine studies, both as a protein and in DNA form. He asked whether anti-GM-CSF antibodies would be monitored pre-vaccination and post-vaccination. Because some research participants in HVTN 065 had measurable CD4 and CD8 T-cell responses to HIV antigens prior to vaccination, Dr. Badley suggested that potential participants for this trial should be screened for preexisting HIV immunity at baseline. Such screening could be used as an exclusion criterion or should be factored in to the final analysis. The proposed virus neutralization assays would test virus neutralization using a variety of laboratory adapted strains of HIV. Dr. Badley asked whether the investigators plan to test neutralization using clinical isolates of both tropisms. Noting that recipients of these vaccines are likely to develop anti-HIV antibodies that could cause false-positive HIV ELISA tests that would influence their future eligibility to receive private health insurance and serve as blood donors, Dr. Badley suggested that these risks be enumerated in the informed consent document.

Dr. Kahn stated that the informed consent document for this study and the consent process described in the protocol are thorough, clear, and carefully constructed. He suggested adding to the informed consent document the specific differences referred to on page 2, section 3, as "This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. The study staff can tell you about the differences." Doing so would better ensure that all potential participants receive this information in a consistent manner across sites. Dr. Kahn requested that the investigators provide information about the range of compensation for participants, which they stated would vary by site, and an explanation for the differences in compensation.

Dr. Ross noted that this protocol is well written and clear, and follows on the previous work of the AIDS Vaccine Network. She suggested that the investigators explain in the informed consent document that most of the studies that concluded that GM-CSF engineered into vaccine regimens is safe were carried out for cancer vaccines in individuals with disease, not in healthy individuals as this protocol proposes to do. Dr. Ross asked whether research participants who are withdrawn or who withdraw voluntarily from this study would continue to receive HIV testing. She noted that the endpoints of the study are to determine the safety and the immunological effects of including GM-CSF in the prime phase of the vaccine by looking at the change in responses during the second and third boosts with the vaccinia construct. Because the (–)GM-CSF arm is not part of this proposed study, Dr. Ross wondered whether the investigators plan to compare the results of this trial with the various adverse reactions or immune responses in the previous vaccine trial, in which the priming construct lacked GM-CSF; she also asked whether the proposed study is powered sufficiently to perform this analysis.

C. RAC Discussion

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

- Regarding reasons that would invoke a safety pause in the trial, Dr. Zaia asked whether the investigators had considered excluding some adverse events that are likely to occur in the first 24 to 48 hours as a reaction to the vaccine. He requested clarification as to whether the unexpected adverse events would trigger the pause.

D. Investigator Response

1. Written Responses to RAC Reviews

Anti-GM-CSF antibodies will be measured at baseline, two weeks after each of the two GEO-D03 DNA vaccine inoculations and 2.5 months after the last DNA vaccine inoculation. The investigators modified the protocol to add a time point at two weeks after the first DNA vaccination.

HVTN 065 was conducted in low-risk participants, so it is unlikely that any immune responses detected in samples from placebo recipients or baseline collections were due to exposure to HIV; therefore, these responses are considered false positives in the assay. The overall false-positive rate for the intracellular cytokine staining assay in HVTN 065 was 4.1 percent, which is consistent with the validation criteria for this assay. The statistical analysis plans and sample size calculations for the current proposed study were conducted with this assay parameter taken into consideration.

The risks of false-positive HIV test results are addressed in the protocol, under Item #20, "There are risks to being in this study," in Appendix A, which is the sample informed consent document. These risks are also discussed in Appendix C of the HVTN Vaccine-Induced Seropositive registry consent.

The investigators plan to do a cross-protocol analysis comparing safety data and select immunological assays from this trial to assays from either HVTN 205 or HVTN 065, or both. The sample sizes from these studies will be sufficient to compare safety and immunological parameters between the GM-CSF and non-GM-CSF adjuvanted DNA priming immunizations.

Participants withdrawn from the study (either by the investigators or their own choice) will continue to receive HIV testing.

In addition to cancer vaccine studies using GM-CSF, there also have been clinical trials of HBV and HIV vaccines with GM-CSF. A malaria vaccine has been tested with a co-inoculated GM-CSF expressing DNA. The investigators believe that the proposed informed consent document addresses the potential risks of GM-CSF, and that adding information about cancer vaccines will not be directly relevant to this trial.

To facilitate consistent communication to sites who will then inform potential participants about the differences between the study vaccine and others, a site educational process called Frequently Asked Questions (FAQs) is utilized. The FAQs are developed for each protocol at the time of the Case Report Form development and provided as part of the training for the sites. The language included in the FAQs concerning the vaccine differences will be developed by the protocol team leadership.

Regarding compensation for research participants at the various study sites, the investigators explained that each clinical research site determines the compensation amount in conjunction with its own institutional review board (IRB). Sites typically compensate at $50 to $100 for each injection visit completed and $50 for each noninjection visit completed. Injection visits are compensated at a higher amount due to additional procedures and the amount of time spent at the clinic. As the sites included in these protocols come from diverse geographic regions of the country with different costs of living, the IRBs ensure that the amounts provided are commensurate with local standards.

2. Responses to RAC Discussion Questions

Dr. Buchbinder explained that only systemic reactions or unexpected adverse events would trigger a safety pause. A Division of AIDS medical monitor plus a clinical monitor provide daily review of adverse events, and a protocol safety review team monitors adverse events on a weekly basis. The standard HVTN pause criteria will be used.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Dr. Zaia stated that the RAC had no existing concerns or comments regarding the design of this protocol because of the investigators' responses to the individual RAC members' written comments and because their presentation at this RAC meeting addressed all of the Committee's concerns. He congratulated Dr. Buchbinder and her team for an outstanding protocol.

G. Committee Motion 1

Although no official motion was made, the RAC members voted to approve that no remaining concerns or comments with regard to this protocol existed. The vote was 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

III. Minutes of the March 8, 2011, RAC Meeting

RAC Reviewers: Dr. Bartlett and Dr. Kanabrocki (*via teleconference*)

Dr. Bartlett stated that the minutes of the March RAC meeting had been reported accurately, with a small number of corrections and comments. Dr. Kanabrocki agreed that the minutes document was accurate.

A. Committee Motion 2

It was moved by Dr. Kanabrocki and seconded by Dr. Bartlett that the minutes of the March 2011 RAC meeting be accepted as written. This motion passed unanimously by voice vote.

IV. Discussion of Human Gene Transfer Protocol #1102-1091 titled: A Phase I Dose Escalation Trial Using *In Vitro* Expanded Allogeneic Epstein-Barr Virus Specific Cytotoxic T-Lymphocytes (EBV-CTLs) Genetically Targeted to the B-Cell specific Antigen CD19 positive Residual or Relapsed Acute Lymphoblastic Leukemia After Allogeneic Hematopoietic Progenitor Cell Transplantation

Principal Investigator:	Nancy Kernan, M.D., MSKCC, and Renier Brentjens, M.D., Ph.D., MSKCC
RAC Reviewers:	Dr. Kohn, Ms. Mastroianni, and Dr. Zaia

Dr. Fong was recused from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy affecting children. Most children with B-ALL are cured with standard chemotherapy regimens. However, a subset of patients will experience relapsed disease and require further therapy with allogeneic bone marrow transplantation (allo-BMT). While this additional therapy may successfully cure a number of these patients, many will ultimately relapse and die from their disease. This latter patient population has a poor prognosis in the setting of current treatment strategies and urgently requires novel therapeutic approaches to treat their refractory disease.

The investigators have developed and extensively evaluated a novel approach to treat these patients with relapsed B-ALL. They have generated a gene (19-28z) encoding for an artificial T-cell receptor that recognizes CD19, a protein expressed on normal and cancerous B cells, including B-ALL tumor cells. By using a noninfectious virus, the investigators can make T cells express this 19-28z chimeric antigen receptor (CAR). The resulting T cells are then capable of killing a patient's tumor cells. A series of experiments have demonstrated that T cells modified to express 19-28z kill malignant B cells *in vitro* and in mice. These results have led the investigators to test this approach in two clinical trials, treating adults with chronic lymphocytic leukemia (CLL) and B-ALL using patient-derived T cells.

This clinical trial proposes to extend CAR-modified T-cell therapy to pediatric participants who have relapsed B-ALL, following allo-BMT. In contrast to the adult protocols that use patient-derived T cells, the investigators propose in this protocol to use donor-derived T cells. Past experience of the investigators has shown that infusion of donor T cells into participants with relapsed leukemia can lead to the development of graft versus host disease (GVHD), wherein the infused donor T cells attack the participant's tissues. In contrast, donor T cells initially selected to target a common virus, Epstein-Barr virus (EBV), have been shown to be safe following infusion. Therefore, dosing relapsed B-ALL participants with EBV donor T cells modified to express the 19-28z CAR is likely to result in minimal toxicity yet allow for optimal CD19 targeted antitumor effect. The primary objective of this clinical trial is to determine the safety and effectiveness of 19-28z+EBV T cells used in pediatric participants with relapsed B-ALL following allo-BMT.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol, Key issues included that it is a pediatric protocol using the novel combination of EBV-specific T cells containing an anti-CD-19 CAR in combination with conditioning chemotherapy. Although such chemotherapy has been used in a number of immunotherapy protocols, which are using CARs, it has not been used in combination with virus-specific CAR T cells. In light of the toxicity seen in the first research participant who received

cyclophosphamide (in a previous trial at the investigators' institution) using anti-CD-19 CAR T cells, and serious adverse events (SAEs) seen in other anti-CD-19 CAR trials, a full discussion of the risks and benefits of proceeding with the current trial design in pediatric participants was deemed warranted.

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

With regard to the clinical protocol, Dr. Kohn suggested that the investigators consider using a dose-limiting toxicity (DLT) rule that considers grade III SAE occurrences or GVHD occurrences (in addition to the grade IV event currently proposed) as dose-limiting. The grade III occurrence could signify a unique complication of this gene-modified product or of the participant population. Additionally, an upper boundary to the extent of relapse that may be present at the time of CTL infusion should be stated. He asked the investigators to clarify whether the eligibility criteria for organ function would be applied at the time of initial enrollment or at the time of admission for cell reinfusion, or at both times. In addition, the investigators should specify which types of allogeneic hematopoietic stem cell transplantation (HSCT) would be eligible. Since the investigators posit that residual EBV antigens in the recipients might support CTL persistence, Dr. Kohn suggested that eligibility be restricted to EBV(+) recipients, as EBV may not be present in some younger children. Since a large number of blood tests are planned (which may be a problem in small pediatric participants), he asked the investigators to delineate the limits on blood volumes for research investigations and the priority of tests to be completed if blood volumes are limited.

Dr. Kohn asked whether post hoc detection of replication-competent retrovirus (RCR) in product or in a research participant would dictate a stopping criterion. He requested justification for the investigators' statement that the lower limit for successful transduction by the CAR is 5 percent, and if cells were transduced at a lower frequency, the participant would be taken off protocol, since a largely nontransduced population is not likely to have prospects for anti-leukemic efficacy benefits but could still experience toxicity from cell infusion and GVHD. With regard to the informed consent document, Dr. Kohn requested that a discrepancy in the number of research participants be clarified, a request for autopsy be included, and a written assent document be added.

Ms. Mastroianni requested an explanation as to why this Phase I study, using donor-derived T cells for the first time in this type of protocol, is proposed to be conducted in a pediatric population first, rather than in an adult population. She enumerated five locations in the donor consent document and three in the participant consent document that needed clarification. In both forms, Ms. Mastroianni advised the investigators to use language that makes clear that this protocol is a Phase I safety study, rather than treatment. She suggested inclusion of a provision for autopsy in the informed consent document and consistency between the reference in the informed consent document to contraceptive use posttransplant and Appendix M-II-B-4-e. Ms. Mastroianni asked about the minimum age for participation, whether there is a maximum age, the rationale for excluding participants older than 25 years, and whether the investigators propose an age range for the donor population. She also requested clarification about the proposed total number of research participants.

Dr. Zaia asked the investigators to explain the planned logistical method for this study, since the protocol proposes a complex implementation scheme. In this study, he noted that donor cell preparation is required for all enrolled recipients even though the chance of donor cells being used is less than 50 percent, due to the 40 percent rate of relapse, which is a significant use of effort and resources; he asked the investigators to explain their rationale for this study design and why an alternative study design would not be more effective. Dr. Zaia requested an explanation for discrepancy between the inclusion criterion of recipients being younger than 19 years of age and the exclusion criterion of recipients being older than 25 years of age. In the informed consent document, Dr. Zaia noted that it appears that the donor is asked to participate prior to the recipient providing consent, and that this may present a potential coercive element for the other independent of who is consented first. Because the risks to the donor are significantly less than those to the recipient, he suggested that the recipient should be consented first, thus lessening the impact of a coercive effect. Noting that the donor could withdraw at any time, Dr. Zaia suggested informing potential donors that, after a certain time point – for example, when the blood collections are complete or when processing is complete – donors will not be allowed to withdraw use of these materials.

C. RAC Discussion

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

- Dr. Fost elaborated on the ethical point brought up by Ms. Mastroianni. The general notion of invading a child's body where the motivation is primarily research is different than using a child as a donor where the primary motive is for clinical purposes and there exists a reasonable expectation of clinical benefit, even if not directly to that donor. He added that regulations state that it is illegal to do a nonmedically therapeutic procedure on a child that does not directly result in medical benefit to that child. Since there is no conceivable medical benefit to the donor in this protocol, participation of a child in this research is prohibited by regulation unless the participation represents minimal risk to the child.
- Dr. Zaia summarized that the most difficult part of the discussion of this protocol is the inclusion of pediatric research participants. Although the donor is a research participant, the donor is not receiving research and it is solely a therapeutic study for the donor. However, he noted that the RAC resolved this issue by recognizing, in this instance, that donation does not apply more than minimal risk to the donor. The recipient is receiving experimental treatment that is known from other settings to have a potential for being therapeutic, which justifies participation by the recipient.
- Dr. Fost suggested ensuring that the pediatric donors in this trial experience nothing more than minimal risk, as this issue will come up again for the RAC.
- Dr. Zaia asked about the current knowledge of using allogeneic EBV-specific and CD19-specific cells in an adult malignancy.

D. Investigator Response

1. Written Responses to RAC Reviews

At a reviewer's request, the protocol was amended to include the definition of a DLT as Grade III or IV GVHD or a Grade IV toxicity following infusion of the T-cells.

Since this is a Phase I clinical trial, there is no upper limit of tumor burden. However, the potential participant must meet the eligibility criteria for organ function. Definition of those eligibility criteria will be clarified at the time conditioning therapy is initiated. The eligibility criteria for organ function are intended to be applied at the time of infusion; a change has been made in the protocol language to indicate this.

Recipients of all types of allogeneic HSCT are eligible, provided their HSCT donor has agreed to participate in the MSKCC cellular therapy program, except those patients who have received unrelated cord blood transplants. Based on their experience with infusions of EBV-CTL in related and unrelated HLA non-identical pairs, the investigators do not expect to see the development of significant acute or chronic GVHD even in the HLA-disparate donor and research participant setting.

It is not clear whether residual EBV antigens in the recipients will support the CTL persistence or CD19-positive normal B cells in the recipient; it is possible that persistence may not be required. These possibilities of mechanism-of-action will be explored in this protocol. Therefore, at this time the investigators do not wish to require recipients to be EBV seropositive.

The investigators are mindful of the volume of blood that can be drawn safely from pediatric patients and will limit all draws to 5ml/kg, to include both clinically and research needed study draws, with only one draw per day provided the hemoglobin is greater than 8gm/dl.

The investigators' experience with patients who have relapsed post transplantation demonstrates that marrow draws are done monthly to evaluate treatment response in order to change clinical procedures or to determine eligibility for a second transplantation procedure. Therefore, the investigators prefer to evaluate marrows with that same frequency – monthly – if research participants have not had overt progression of disease.

Detection of RCR at any time point will be a stopping criterion for this study; this information has been added to Section 14 of the protocol.

Agreeing with the reviewer regarding the potential for toxicity, the investigators will remove participants from this study (without infusion of T cells) if the transduction frequency is less than 5 percent.

This study is being conducted in a pediatric population first because B-ALL is largely a disease of childhood. From 2004 to 2008, the median age at diagnosis was 13 years. If the study were conducted in adults first, it would take many years to accrue and would exclude the population at greatest risk for developing the disease.

Most donors will be recruited from unrelated adult volunteer donors and parental donors for HLA-disparate transplants. It is possible that a pediatric donor physically large enough to safely donate the volume of blood required would be asked to participate in the study; if so, assent will be obtained as per MSKCC IRB requirement.

After the samples have been tested, they will be retained for the length of the study, which is 15 years.

There is no lower age limit for recipients, as B-cell leukemias have been diagnosed even in infancy. The recipient population must have been diagnosed with CD19+ leukemia at less than 19 years of age. This criterion was selected because the vast majority of patients diagnosed with CD19+ ALL are in this age group. Once the Phase I study is performed in children, the Phase II study would be expanded to include adults who could experience more or less toxicity than children at the same dose levels. With regard to donor age, the investigators agreed to add language stating that unrelated donors should be between the ages of 18 and 60 years, as these are the age restrictions for volunteer unrelated donor registries. There is no upper age limit for a related donor; however, the minimum age for a related donor will be 7 years, as this is the youngest age a person can be considered capable of giving consent to participate in a research study.

Up to 26 research participants (“recipients”) and 26 donors will participate in this study.

Prior production of EBV-CTL was deemed expedient and necessary because the 7- to 9-week time period to generate EBV-CTLs exceeds the 31-day median expected survival of patients developing EBV post-transplant lymphoproliferative disease (PTLPD). Those T-cell lines not used for participants are being evaluated in several protocols for use in “third party” patients with EBV-PTLPD. The “third party” EBV-CTLs are being evaluated in patients lacking a donor-derived cell line or for recipients of cord blood units. These third-party infusions have been the subject of two reports, and demonstrate the efficacy of using “off-the-shelf,” virus-specific, third-party CTLs restricted by human leukocyte antigens expressed by the tumor to treat otherwise lethal EBV-PTLPD.

Donors are frequently asked to participate in studies prior to recipients providing consent; this is true for all cellular therapy protocols presently open at MSKCC. Donors are asked to agree to the use of their cells in generating antigen-specific T cells for one or more of the designated cell therapy trials. Consent from related donors for genetic modification of their EBV-CTLs to express an anti-CD19 CAR will be obtained at the same time consent is obtained from the recipient to receive the genetically modified EBV-CTLs. For unrelated donors, the consent document will be amended to account for the possibility of future genetic modification of the EBV-CTLs. There is no risk to the donor.

The investigators agreed to modify the informed consent document to include the suggested clarifications and language modifications, and to decrease the chances of therapeutic misconception.

2. Responses to RAC Discussion Questions

Dr. Kernan explained that the young blood donors in this protocol would not be at high risk from their donation. They would donate about 40 ml – 8 teaspoons – of blood to start the EBV cell line. Previous

experience with young donors, mostly between the ages of 7 and 12 years, revealed that they are able to donate blood with hemoglobins of 12 or 13, therefore there is minimal risk associated with that donation.

Dr. Kernan agreed that leukophoresis would not be offered to donors who are children, as doing so would pose greater than minimal risk to the child-donors in this Phase I research protocol.

Regarding the current knowledge of using allogeneic EBV-specific and CD19-specific cells in an adult malignancy, Dr. Brentjens stated that the proposed protocol would be the first protocol using these cells in an allogeneic setting. In an autologous setting, nine centers in the United States are performing CD19-targeted CAR-modified therapies. Continued work at MSKCC has treated 10 adult participants with CLL and two participants with ALL, demonstrating marked reductions in lymphadenopathy, although no longterm survival benefit has accrued for those participants (who have bulky disease rather than minimal residual disease). Other groups are collaborating and have promising results, and more than one source indicates that, in an autologous setting, this approach might have clinical benefit.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical/Trial Design Issues

- In other trials, EBV-specific T cells expressing a CAR have been used to prolong the persistence of the CAR-expressing T cells. The rationale is that in a patient who has been exposed to EBV, EBV+ T cells will persist due to continued engagement of the EBV receptor on these gene-modified T cells. One study comparing EBV+ T cells expressing a CAR against a tumor antigen to nonviral-specific T cells expressing the same CAR found the EBV+ T cells did persist for a longer period than nonviral-specific T cells (Pule M.A., et al., *Nature Medicine*, 14(11):1264-1270 (2008)). Only participants with EBV-specific immunoglobulin G (IgG) were eligible for that trial, as this indicated persistent infection and therefore presence of antigen to engage the EBV+ T cells. In this protocol, the investigators will enroll both EBV+ and EBV- patients. In addition to the fact that many pediatric participants may not have had a prior EBV infection, the investigators also note that the exact mechanism for persistence of the EBV+ T cells is not known and that persistence may not be required for efficacy. The protocol should include analyses of the differences in level of activity of these T cells in EBV+ compared to EBV- participants.

Ethical, Social, and Legal Issues

- In this protocol, the research participant will have first undergone a HSCT. The donor for that transplant will be asked to be a donor again for purposes of this protocol. It is not uncommon for pediatric siblings to donate for an HSCT; this is an established medical procedure. However, donation of cells for this protocol would be for research purposes only and of no benefit to the donor. To comply with current regulations regarding pediatric participants in research, the second donation should only entail procedures that are no greater than minimal risk to the donor. According to regulatory criteria, a blood draw to collect the needed cells may be classified as minimal risk but a leukophoresis or any other invasive procedure would likely exceed this standard, as it is not part of common medical care for healthy children. The inclusion of an independent consent monitor would also be advisable to provide further assurance of meaningful assent.

- The participant becomes eligible for the protocol when the participant experiences relapse or persistence of disease following HSCT. The person who donated the cells for the participant's transplant is first asked to consent to donate additional cells for this protocol. The informed consent document for the participant therefore states that that participant is being asked to enroll in this protocol because:

"Your leukemia has:

- Relapsed or leukemia cells are still in your blood or bone marrow after transplant
- Your leukemia cells have a marker called CD19
- Your stem cell donor has already agreed to donate blood for making a white cell line. This cell line may be able to kill your leukemia cells." (underline added)

To avoid any inadvertent pressure on the participant to enroll because the donor has already agreed to provide additional cells, the last bullet should be reworded and perhaps depersonalized; for example, it might simply state that a cell line is available from the donor.

G. Committee Motion 3

Dr. Zaia summarized the RAC recommendations expressing the comments and concerns of the RAC. Although no official motion was made, the RAC voted to approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

V. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Chiocca, Fong, Kohn, Strome, Yankaskas, and Zaia

Dr. Yankaskas reported that the OBA received 19 protocol submissions in the past 3 months, 11 of which were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, seven were oncology protocols, one was for heart disease, one was for coronary artery disease, one was for retinitis pigmentosa, and one was for Fanconi anemia. In these 11 protocols, four used plasmid vectors, two used adenovirus vectors, two used lentivirus vectors, one used a listeria monocytogenes vector, one used a vaccinia virus vector, and one used a retrovirus vector.

Thirteen protocols submitted Appendix M followup information indicating their enrollment. Of trials that had initiated enrollment in the past 3 months, four protocols had been reviewed by the RAC at previous public meetings.

A total of 15 SAEs were reviewed by the GTSAB from 11 protocols, including initial and followup reports. After analysis of these events, the GTSAB concluded that no reports needed additional public discussion at this time.

Dr. Yankaskas noted that a publication resulted from the RAC's June 2010 Symposium on Gene Modified T Cells. In addition, he noted that the RAC Points To Consider was reviewed by the GTSAB and would be posted to the OBA website in the next few weeks.

The RAC previously recommended that the NIH/OBA convene a conference to examine what is known about RNA therapeutics from the clinical experience and the unanswered research questions needed to develop this emerging field with regard to safety, particularly protocols using microRNA (miRNA). A planning committee was formed. The OBA also has received input from industry through the Oligo Safety Working Group. A draft agenda was shown for this December 15-16, 2011, conference, which will follow the December 2011 RAC meeting. Session I will include an overview of current clinical approaches using RNA oligonucleotides; a review of the clinical experience with RNAi; a panel discussion directed at what has been learned about biodistribution, persistence of oligos, and models for off target activity; and the key challenges that need to be addressed to move the field forward. Session II will focus on miRNA basic understanding and potential clinical applications, including an overview of emerging translational research using miRNA, development of preclinical models, supporting technologies and tools, and a panel discussion on assays and models, potential epigenetic effects, and key unanswered questions. The draft agenda will be posted soon to the OBA website.

VI. Discussion of Biocontainment for Scripps Research Institute's Proposed Research Involving Lassa Viruses

Presenter:	Juan de la Torre, Ph.D., Scripps Research Institute (*via web conference)*
RAC Presenter:	Dr. Roizman
Ad hoc Presenter:	Heinrich Feldmann, M.D., Ph.D., NIAID, NIH, Hamilton, MT (*via teleconference*)

A. Presentation by Dr. de la Torre

Dr. de la Torre introduced the arenaviruses, which include a variety of clinically important human pathogens, chiefly Lassa virus (LASV) that causes Lassa Fever Disease. The prototypic arenavirus is called lymphocytic choriomeningitis virus (LCMV), which is a useful laboratory model for studying virus-host interactions. Lassa virus is endemic to West Africa, a vast region that has a large population at risk, with estimates from the Centers for Disease Control and Prevention (CDC) in the range of 500,000 infectious cases per year that result in significant morbidity and mortality associated with the infection. Currently there are no licensed vaccines and the only therapy is an off-label use of ribavirin, an effective nucleoside analog that has side effects and must be administered intravenously. LASV is most prevalent in Nigeria, Sierra Leone, and Guinea; seroprevalence is high in all three countries.

Compelling evidence indicates that one of the best predictors of outcome for LASV infection is viremia. Clinicians report that infected individuals with a very high viremia usually experience a fatal outcome; if the viremia is relatively low, the outcome is usually good. This clinical observation suggests that antiviral drugs do not necessarily need to eliminate the virus but dropping the viral load by one or two logs, may be sufficient to allow an infected individual's own adaptive immunity to control the infection.

Dr. de la Torre and his colleagues want to take advantage of the recent progress in arenavirus genetics as well as new technology involving high throughput screens to identify host cell factors that might contribute to increased resistance against to LASV infection as well as to identify small molecule compounds that could inhibit virus replication. He noted that these types of experiments are difficult to conduct if they must be conducted under biosafety level 4 (BL-4) conditions.

He explained the biology of the arenaviruses, his colleagues' and his research involving reverse genetics of arenaviruses, the research regarding a system that would allow screening for molecules that can inhibit Lassa virus replication or gene expression, and the two-step process they propose for generating the defective recombinant Lassa virus limited to a singleround of infection and incapable of cell-to-cell transmission. This process begins by cloning (using standard molecular biology procedures) and then rescuing a recombinant Lassa that is lacking its surface glycoprotein, having replaced it with green fluorescence protein.

Obvious concerns about general biosafety procedures exist; however, Scripps will have a dedicated space for the Lassa work to prevent any mixing between LASV and LCMV plasmids in the lab, the cells will be checked to ensure they are completely free of LCMV, and only people who are highly trained will be participating in this research.

B. RAC Presentation by Dr. Roizman – Biosafety Working Group Deliberations

Dr. Roizman provided an overview of the Biosafety Working Group (BWG) deliberations. Lassa virus is a Risk Group 4 agent and is a CDC Select Agent. The wildtype virus causes a fatal hemorrhagic fever for which there are no current vaccines or therapeutic options. Dr. de la Torre is requesting review of proposed containment for research with LASV that is modified to prevent cell-to-cell transmission in non-complementing cells. The goals of this research are to develop a virus that can be manipulated at a lower containment level to facilitate research; to use this defective virus to identify compounds that interfere with Lassa virus viral transcription and potentially to identify targets for new therapeutics. Research with LASV must normally be carried out at BL4, but OBA can reduce containment requirements for certain experiments if safety data support that decision. He presented a summary of the proposed research and containment recommendations of the BWG.

Dr. Roizman discussed isolation of genomic RNAs of LASV and generation of LASV complementary DNA (cDNA), noting that purified RNA is not covered under the *NIH Guidelines* as it is not contained in cells; additional biosafety considerations for research with LASV cDNA at BL2; testing for replication-competent rLASVΔGP-GFP; identifying inhibitors of viral replication; and high-throughput screening to identify inhibitors of viral transcription.

The biosafety recommendations from the BWG were summarized as:

- BL-4 containment for all work with potentially infectious LASV, including proof-of-concept work demonstrating that wildtype LASV cannot be rescued from rLASVΔGP-GFP through homologous or non-homologous recombination.
- Production of new batches of rLASVΔGP-GFP will continue at BL4 until sufficient data are obtained regarding the stability of this system to allow production at BL3.
- BL3 containment is appropriate for experiments involving the stable RCV-free rLASVΔGP-GFP in a non-complementing cell line.
- BL2 containment is appropriate for high-throughput screening experiments once the non-complementing cells containing rLASVΔGP-GFP are completely inactivated.

C. Presentation by Dr. Feldmann

Dr. Feldmann commented on the proposed experiments and the BWG recommendations. The Scripps Institute researchers have a great deal of data and much experience with the LCMV system, and there is no reason to expect that LASV would be significantly different from the LCMV system already in use. The BWG recommendations are appropriate. Before the containment level is lowered to BL3, certain safety aspects must be evaluated and verified – similar to what was requested to be done with the Ebola system that came before the RAC previously.

D. RAC Discussion

Dr. Kanabrocki clarified that the BWG is recommending BL2 practices, not using a BL2 laboratory. That means liberal access, not restricted access. BL2 is typically assigned in a clinical center and protection of the workers can be adequate by using appropriate personal protection equipment. BL2 practices could be applied in any wet laboratory space. The BWG is recommending bringing the formaldehyde-fixed samples into a laboratory setting for the high-throughput analysis but using BL2 practices.

Dr. Roizman clarified that, if it can not be rescued from the fixed material, the cDNA should be put in a BL1 laboratory. If it cannot be rescued from glutaraldehyde- or formaldehyde-treated material, then the cDNA is not a biosafety threat.

Dr. de la Torre asked whether samples, in this case cells that have been fixed with 4 percent formaldehyde present a biohazard. If such material is considered infectious or potentially infectious, BL2 would not be appropriate.

Drs. Ornelles and Roizman noted that laser-capture microdissection can isolate RNA from paraffin-embedded, formaldehyde-fixed samples, although the extracted material would not be infectious.

Dr. Kanabrocki elaborated on the BWG discussions. The BWG settled on BL2 for the high-throughput experiments based on a standard of how this work is typically conducted with BL4 agents, similar to the standard that Dr. Feldmann described. His belief is that BL1 would be adequate (instead of BL2) as long as there is control over the material after the reading of the plates and if the material was returned to the laboratory for disposal.

E. Committee Motion 4

It was moved by Dr. Roizman and seconded by Dr. Ornelles that the recommendations of the BSWG be accepted with one change – that BL1 rather than BL2 be required for the fluorescence studies and that

the material would be returned to the laboratory for disposal. This motion passed unanimously by voice vote.

VII. Discussion of Proposed Changes to the *NIH Guidelines for Research Involving Recombinant DNA Molecules* Section III-E-1: Research with Defective Viral Genomes in Tissue Culture

A. Presentation by Dr. Ross

Dr. Ross reviewed Section III-E-1 of the *NIH Guidelines*, entitled "Biocontainment for Research with Partial Genomes of Eukaryotic Viruses in Tissue Culture." She discussed the impetus for review and proposed amendments, the evolution of proposed changes, the revised proposal and outstanding issues for discussion.

Section III-E-1 current requirements allow investigators to initiate research with partial virus genomes in tissue culture at BL1 containment, concurrent with registration of their experiment with the institutional biosafety committee (IBC). The virus must contain less than two-thirds of the genome from any family of viruses and no helper virus can be present. This section is designed to facilitate initiation of low-risk research; the IBC is required to review the research but the research can be initiated before IBC review.

Impetus for the proposed changes stemmed from the RAC review of research with synthetic nucleic acids and associated biosafety concerns. A question arose as to whether synthetic techniques might be able to generate a functional virus containing less than two-thirds of the genome, and there was recognition that rescue of a replication-competent virus could occur in the absence of helper virus through other mechanisms and in particular, via helper function.

Revisions to Section III-E-I were proposed in March 2009. The OBA published a proposal in the *Federal Register* to amend Section III-E-1 by changing the criteria regarding the size of the viral genome deletion from two-thirds to one-half and by requiring the principal investigator to provide evidence that the resulting nucleic acids and tissue culture cells would not be capable of producing a replication-competent virus and to demonstrate the absence of any helper virus. Public comments on this proposal focused on two issues: (1) research has been conducted safely for many years under this section with viruses that contain more than one-half of the genome but less than two-thirds and (2) rather than a quantitative standard based on the deletion size, current understanding of virus biology might allow for a reduction in containment based on functional impairment of a virus.

In April 2010 after consultation with the RAC, OBA proposed additional amendments that retained the prior criterion that work under this section could occur only if one-half of the genome was present but clarified that this requirement applied only to Risk Group (RG) 3 and RG4 viruses, because research with less than one-half of the genome of RG2 viruses is already exempt from the *NIH Guidelines*. Other revisions included functional criteria that would allow reduction of containment to be based on the removal of one or more viral genes that are essential for cell-to-cell transmission and clarification that containment for research with the retroviruses and lentiviruses that have the potential to transduce human cells should not occur at less than BL2.

The BWG discussed additional biosafety concerns, including a suggestion that RG4 viruses be excluded from Section III-E-1, given the potentially serious consequences associated with an automatic lowering of containment for RG4 viruses, that it is prudent to have such high-risk research reviewed prior to its initiation, and that the impact of this proposed change on the research community should be minimal. The question arose about RG2 and RG3 agents, and the BWG discussed potential approaches, one of which was to allow only a step-down approach to containment whereby defective RG2 viral agents could be contained at BL1 and defective RG3 viral agents would be stepped down to BL2 if they met the safety criteria.

The BWG reached some conclusions that research with defective RG4 viruses based on deletion of one or more genes should still be reviewed by the IBC prior to initiation. However, the BWG believes that

further discussion is needed to revise the criteria for RG2 and RG3 virus research that should be subject to Section-III-E-1. The challenge for delineating these criteria is how to articulate guidance for investigators regarding the data needed to lower containment, in light of the differences in risks of recombination among different viruses and the cell lines being used to propagate and maintain them.

Dr. Ross asked for comments and input for the BSWG.

B. RAC Discussion

In response to Dr. Roizman's query about training for workers at the BL-1 level, Dr. Corrigan-Curay explained that training includes strict adherence to good microbiological practices. All persons should have adequate instructions and at a minimum these instructions should include training in aseptic technique and the biology of the organisms used in the experiment so that potential biohazards can be understood and appreciated. For any research group working with agents that are known or potential biohazards, an emergency plan must be in place that describes the procedures to be followed in an accident. The principal investigator is responsible for assuring that all of the potential hazards of the work are understood.

C. Committee Motion 5

It was moved by Dr. Ross and seconded by Dr. Fan that the recommendation of the BWG – that research with defective RG4 viruses based on the deletion of one or more genes should be reviewed by the IBC prior to initiation – be accepted. This motion passed unanimously by voice vote.

VIII. Day 1 Adjournment

Dr. Zaia, RAC Chair, adjourned Day 1 of the June 2011 RAC meeting at 4:30 p.m. on June 7, 2011.

IX. Day 2 Call to Order and Opening Remarks

Dr. Zaia, RAC Chair, called Day 2 of the June 2011 RAC meeting to order at 8:55 a.m. on June 8, 2011. (*Day 2 was scheduled to begin at 8:00 a.m.; however, due to an electrical outage at the Marriott Suites Hotel, the meeting was moved to the NIH campus, Building 1, Wilson Hall. The late start was necessitated by the need to find a suitable meeting location and to move all RAC members and all equipment from the hotel to the NIH.)*

X. Discussion of Human Gene Transfer Protocol #1104-1101 entitled: A Randomized, Double-Blind, Phase III Efficacy Trial of PROSTVAC ± GM-CSF in Men with Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer

Principal Investigator:	James Gulley, M.D., Ph.D., F.A.C.P., National Cancer Institute (NCI)
Additional Presenters:	Wayne R. Godfrey, M.D., BN ImmunoTherapeutics, Inc. (BNIT); Reiner Laus, M.D., BNIT; Heidi Petersen, M.P.H., BNIT
Sponsor:	BN ImmunoTherapeutics, Inc.
RAC Reviewers:	Drs. Kahn, Koch, and Zaia
Ad hoc Reviewer:	Howard Scher, M.D., MSKCC (*via teleconference*)

A. Protocol Summary

BN ImmunoTherapeutics, Inc. (BNIT) is developing PROSTVAC-V/F to treat prostate cancer, which is the second leading cause of cancer deaths in men in the United States. According to the American Cancer Society, approximately 200,000 new diagnoses of, and 28,000 deaths from, prostate cancer occur each year in the United States.

Prostate cancer is an ideal candidate for a treatment such as PROSTVAC-V/F, which stimulates the immune system to target cancer cells. PROSTVAC-V/F consists of two vaccines that use either a vaccinia vector, PROSTVAC-V, or a fowlpox vector, PROTVAC-F. Each vector encodes the gene for the tumor associated antigen prostate specific antigen (PSA) as well as the genes for three different T cell co-stimulatory molecules referred to a TRICOM.

Even in advanced disease, prostate cancer can advance slowly, allowing time for the immune system to be stimulated and, thereby, mount an active immune response. The rationale for the proposed Phase III study stems from more than 15 years of PROSTVAC-V/F research and development programs performed by researchers at the NCI, the former Therion Biologics Corporation, and BNIT.

The foundation for this proposed BNIT-PRV-301 trial investigation is derived from the results of two clinical studies initiated by the former Therion Corporation. The first study was a Phase I trial evaluating the safety and immunogenicity of PROSTVAC-V/F in 10 research participants; the second study was a randomized, placebo-controlled Phase II trial (OBA protocol #0308-600) evaluating the safety and efficacy (as defined by time-to-progression and by overall survival) of PROSTVAC-V/F in approximately 122 men with metastatic castration-resistant prostate cancer (CRPC). Subjects in this study were randomized to receive vaccine with low dose GM-CSF. The control arm was an empty vector plus a placebo for GM-CSF. The primary endpoint for the Phase II trial was progression-free survival but overall survival was an important secondary endpoint active arm received vaccine. The median improvement in overall survival for subjects who received the vaccine with GM-CSF was eight and one half months compared to the control. The results of this study were published in the *Journal of Clinical Oncology* (28(7):1099-1105,2010). This Phase II trial is the basis for the design of this proposed Phase III protocol.

This pivotal Phase III study is designed to confirm the safety and efficacy of PROSTVAC-V/F for treating men with asymptomatic or minimally symptomatic metastatic CRPC in a randomized, placebo-controlled, multicenter, Phase III efficacy trial that will enroll 1200 men ("BNIT-PRV-301"). The primary endpoint for this Phase III study will be overall survival. Other quantitative measurements, including immune responses to tumor antigens, will be performed as exploratory endpoints only. It is hoped that an outcome from these exploratory endpoints will enable assessment of clinical benefit via one of these endpoints in future trials, particularly trials that will evaluate earlier-stage non-metastatic disease.

This proposed Phase III protocol, BNIT-PRV-301, received Special Protocol Assessment concurrence from the FDA on December 3, 2010.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential to be a pivotal trial, both for the treatment of metastatic androgen-resistant prostate cancer and for the field in general. Results from the Phase II trial suggested that, while disease progression did not differ between participants receiving the active agent and those receiving the control vector, the statistically significant survival benefit was clinically meaningful. The RAC recommended a public discussion of this trial with the goal of optimizing both the design and the potential for success of this study.

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

Dr. Kahn focused his comments on the sample informed consent document. He expressed general concern that the consent document frequently uses terms from clinical care in this consent for participation in research. Acknowledging that this consent document is crafted for a Phase III efficacy trial, Dr. Kahn noted that potential participants need to understand that it is still research. He suggested that the investigators avoid language that implies that the vaccine is approved, that it represents treatment, or that the participants will receive care as patients; he provided five such examples. In addition, Dr. Kahn requested that the investigators make clearer whether genomic or other data from samples would be retained and possibly used in future research.

Dr. Koch stated that this well-written Phase III protocol includes extensive documentation supporting the use of this prostate-specific antigen (PSA) based prostate cancer vaccine approach, that the clinical safety of the vaccinia/fowlpox administration with immune booster transgenes appears well tolerated and safe, and that the Phase II trial showed beneficial effects on survival and event-free periods. This three-arm trial proposes to test PROSTVAC-V/F + GM-CSF (arm V+G) against PROSTVAC-V/F + GM-CSF placebo (arm V) and against a double placebo (empty fowlpox vector + GM-CSF placebo [arm P]). Dr. Koch asked the investigators to discuss their expectation that these groups will differ and whether difference will be inferred if only one of the two other groups appears beneficial over the double-placebo group. Because asymptomatic heart failure could be present in this patient population, he wondered whether the investigators had discussed screening for pre-existing left ventricular dysfunction in potential participants. Dr. Koch requested that the investigators discuss their rationale for collecting RNA and DNA from blood for potential gene expression arrays or other genetic or genomic tests.

Dr. Zaia asked the investigators for justification of their plan to use vaccinia vaccine for priming followed by fowlpox vaccine boosters in the two groups receiving the active vaccine, but the double-placebo group is given an “empty” fowlpox vaccine for both the primary and booster immunizations. Since all participants are required to have been previously immunized with vaccinia, any vaccinia-specific (PSA-nonspecific) effects would only occur in the two experimental groups and would not be controlled in the placebo group. He noted two instances within the informed consent document in which safety of the vaccine and potential for benefit to research participants were overstated and should be reworded.

Noting that the product in the Phase II trial was well tolerated with an acceptable safety profile, *ad hoc* reviewer Dr. Scher asked why a fourth arm of this Phase III trial was not proposed, especially in light of the possible disease-modifying effects of GM-CSF. He requested clarification of terms such as complete androgen blockade and radiologic progression, and asked how prior vaccinia experience would be documented. Additional detail was requested about the specific cytokines, chemokines, and nucleic acid analyses that are planned, along with preliminary data to support their inclusion. He noted that understanding the host effects induced by the product is critical to the trial outcome and should be described in more detail. Arms V and P will be compared, but Dr. Scher asked why a similar comparison between arms V+G and V are not included. He suggested that the investigators consider further increasing the proposed sample size. Dr. Scher asked the investigators to address how they will handle the possibility that enlargement of nodal disease might represent successful recruitment of immune effector cells to an area of tumor.

C. RAC Discussion

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

- Dr. Strome asked about the projected cost per patient as this treatment moves forward. In the new U.S. healthcare bill, age is likely to be a consideration relative to cost. Prostate cancer patients tend to be more senior and, with this treatment’s 8.5-month survival and relatively high cost, he wondered if patients would be allowed to use it if they could not provide it for themselves.
- Dr. Kanabrocki commented about biosafety issues and inquired as to plans to protect individuals working on the protocol with regard to preparation and administration of the viral vaccine.
- Dr. Ornelles raised a question about the trial design with respect to the control arm.
- Dr. Zaia asked whether this treatment would affect tumor progression or just overall survival.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators stated that they have revised the language throughout the protocol to better reflect that this vaccine therapy is investigational, to better describe the tests that will be conducted with participant blood samples, and to change “patient” to “subject” to further underscore the investigational nature of this study.

Regarding the trial design, the investigators stated that they expect both PROSTVAC arms to be similar because GM-CSF (which is added to the V+G arm) is a weak adjuvant; they plan to use a low adjuvant dose of GM-CSF, which will minimize the chance of high-dose-related immune suppression. The PROSTVAC/GM-CSF combination in one arm was retained in this Phase III trial because this was the vaccination regimen used in the randomized Phase II trial. The GM-CSF is unlikely to have a major effect on outcome and the investigators have added the PROSTVAC-alone arm to test that hypothesis. The actional difference is whether one or both of the active arms is more effective than control. A finding that low-dose GM-CSF is not required for effectiveness will simplify future treatment in terms of patient administration and cost. This design was vetted with an FDA review team.

The investigators explained that the utility of B-type natriuretic peptide (BNP) measurement for screening for left ventricular dysfunction remains controversial, although mean levels of BNP discriminate well between degrees of heart failure. Use of this measurement in an attempt to screen out asymptomatic left ventricular dysfunction for this study could lead to unnecessary exclusion of otherwise eligible participants. The investigators believe that careful medical history collection and physical examination in combination with thorough screening of potential participants for exclusion criteria would ensure cardiac safety of the study.

PAXgene tubes will be used to collect RNA for evaluation of gene expression, pre- and post-vaccine administration. The investigators plan to monitor for peripheral blood evidence of circulating tumor cells, prostate-specific antigens, or tumor-specific antigen mRNA. The experimental plan is to evaluate expression levels of cellular markers, which have been shown to correlate with prostate cancer specific overall survival.

The investigators explained the rationale for their prime boost strategy (priming with vaccinia and boosting with fowlpox viruses). They have studied extensively the prime-boost strategy in preclinical and clinical settings and have found that this strategy is required for optimal immunologic and antitumor effects. Therefore, this strategy is proposed for the two experimental arms in this trial. With regards to the selection of an empty fowlpox vector as a control, the investigators noted that ideally one would want an inactive placebo. However, if you just give saline there will not be a skin reaction as is seen with the active viral vector and subjects might realize that they did not receive the active agent. This would undermine the blinding of the study and some subjects might leave the trial prematurely. In their assessment the empty fowlpox vector is the best blind since the booster vaccines, which are the majority of vaccine in the active arms, are fowlpox vectors and the skin reactions should be similar in subject receiving the active agents or the control vector. On seven recent clinical trials involving poxviral vectors at the NCI, with more than 1,000 injections given, the investigators had a similar number and severity of injection site reactions (Grade II only) with fowlpox as with vaccinia. In addition, the fowlpox is a non-replicating vector and therefore potentially presents less clinical risk as a placebo. Finally, while the vaccinia vector alone could have nonspecific antitumor effects by cytokine release, studies in multiple mouse models has shown that such effects are minimal, if seen at all. Therefore, they concluded that the optimal trial design for the placebo group (from which individuals have no chance of clinical benefit) in this Phase III study is empty vector fowlpox virus for both the initial and subsequent vaccinations.

GM-CSF is approved for increasing white blood cells (WBCs) in patients treated with chemotherapy. Some small studies have described a modulation of PSA by relatively high-dose GM-CSF when used for 14 days consecutively; however, no study has suggested an improvement in any clinically significant outcome in patients with metastatic CRPC. These cumulative doses are well above the doses needed to induce an increase in WBCs in the average patient. The dose of GM-CSF proposed in this study is designed specifically as a local adjuvant to vaccine and is not one that is associated with significant systemic effect as a single agent. At the dose and schedule proposed in this study, there is no evidence of disease-modifying effects or other systemic effects. When the FDA reviewed and vetted this study design, it was agreed that a GM-CSF-alone arm was not needed.

Regarding documentation of prior vaccinia experience, the investigators explained that, in concurrence with the FDA, it was decided to use either participant-reported smallpox vaccination or medical record

documentation of smallpox vaccination. Each participant will be asked about smallpox vaccination; those who answer “no” or “do not remember” will be excluded from participation in this study. Most people beyond the age of 45 have been vaccinated against smallpox in the worldwide eradication program that ended in the 1970s. Furthermore, ongoing multicenter Phase II studies utilizing PROSTVAC and similar vaccines incorporating vaccinia that are approved by the FDA and the OBA do not require prior vaccinia exposure. The investigators have in place all the CDC guidelines for primary vaccinia vaccination.

The primary endpoint of this trial is overall survival, which is considered the gold standard for assessing the impact of an investigational therapy on disease outcome, and which requires the most time to establish. The investigators have included a secondary endpoint of radiographic event-free survival. In addition to the primary and secondary endpoints, they explained that they are committed to evaluating a number of exploratory endpoints (potential biological/immune markers), which could help to better assess disease activity following dosing with the investigational agents in this trial. The resulting data from these exploratory endpoints, although not critical to the outcome of this study, might support the use of these markers in future trials of this or other investigational treatments and could help to better inform the field of immune therapy.

Because the investigators agree that radiographic event-free survival would be difficult to assess without central review, they plan to have a dedicated Radiology Charter and to store all the images. Upon completion of the study and if the primary endpoint is positive, they will send all the images for central review.

The investigators noted that, since the original RAC submission in April 2011, they have made revisions to the template informed consent document. The revised version, which has been further amended to address a number of RAC reviewer comments, was provided.

2. Responses to RAC Discussion Questions

Regarding the potential cost of this project, Dr. Laus explained that it is an off-the-shelf vaccine, not an individualized vaccine, with a cost similar to a typical pharmaceutical. Pricing discussions have not yet occurred.

Recognizing potential risks for workers administering this vaccine, Dr. Gulley stated that the investigators will follow the CDC guidelines. They are working with all the sites on specific procedures. At Dr. Gulley’s site, preparation of the vaccine will be done in a biosafety cabinet.

With regard to the trial’s control arm, Dr. Godfrey acknowledged that, ideally, an inactive placebo should be used in order to test the effects of the experimental vaccine. However, injecting control-arm participants with saline would result in no skin reaction, and they would know they were receiving the placebo and might leave the study as happened in a previous study. The best blind is the fowlpox because most of the booster vaccines are fowlpox. Because both viruses are being given subcutaneously and the skin reactions are similar between the two, blindedness will be preserved.

Regarding whether this treatment will affect tumor progression or overall survival, Dr. Gulley explained that immunotherapy does not target the tumor directly; it targets the immune system that then targets the tumor, which might take time. In addition, therapeutic vaccines can generate a memory response that could exist for a long time, leading to a prolonged response against a tumor.

E. Public Comment

Dr. Borror commented that the language in the informed consent document was somewhat complicated, and the description of the placebo arm was confusing.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical/Trial Design Issues

- There are two active gene transfer agents being used in the intervention arm; one uses a vaccinia viral vector and the second a fowlpox viral vector. An "empty" fowlpox viral vector has been chosen as a control. A viral vector was chosen as a control, rather than an inert placebo, because it will likely better mask the intervention. The side effects related to vaccination with a viral vector will be similar and may include, for example, local skin reactions. The fowlpox vector was chosen over the vaccinia vector because, unlike the vaccinia vector, the fowlpox vector is unable to replicate and cannot cause disease in humans. These features make it more acceptable from a safety perspective for the placebo participants. Using the fowlpox viral vector as a control instead of the vaccinia will likely facilitate implementation of this large multisite international trial as health authorities in some countries may have concerns with using the vaccinia vector as a placebo control. However, fowlpox does not elicit a strong immune response compared to the immune response generated by vaccinia. The primary endpoint of this study is overall survival, and there are no data indicating that the immune response from a vaccinia vector per se could prolong survival in these patients. Nonetheless, the current design will not control for the potential of the immune response to vaccinia to augment an antitumor response and contribute to survival.

  The RAC recognized the careful analysis that went into this design and that this protocol design is subject to an FDA Special Protocol Assessment (SPA). A SPA provides a mechanism under which the FDA evaluates "certain protocols and issues relating to protocols to assess whether they are adequate to meet scientific and regulatory requirements identified by the sponsor," including efficacy claims that will be part of a biologics license application (Guidance for Industry Special Protocol Assessment, U.S. Department of Health and Human Services, Food and Drug Administration, May 2002 OMB Control Number 0910-0470). Nonetheless, the protocol should more clearly articulate the rationale for choosing the fowlpox vector rather than a vaccination with an empty vaccinia vector followed by repeat vaccinations with an empty fowlpox vector, which would parallel the active arm.

- Although the modified vaccinia virus has been administered safely in a previous trial, implementation of standard operating procedures will enhance the safety of vector administration. In particular, it is important to use procedures that will minimize generation of aerosols when preparing the vector for administration. This can be accomplished by using a biosafety cabinet or, if one is not available, using ethanol-soaked gauze to trap any aerosols generated. All healthcare workers should be properly trained in these procedures. In addition, healthcare workers who will be administering this vector, or interacting with participants who have received the vector, should understand the CDC contraindications to vaccinia vaccination. Any worker with such a contraindication should not participate in these activities.

Ethical, Social, and Legal Issues

- The investigators were asked to clarify in the informed consent document whether any genetic or genomic information would be collected through blood samples. In addition, the informed consent document should articulate who will have access to research samples and for what purposes.

G. Committee Motion 6

Dr. Zaia orally summarized the RAC recommendations to include a variety of clinical and ethical, social, and legal concerns that will be included in the letter to the investigators, expressing the comments and concerns of the RAC. Although no official motion was made, the RAC members voted to approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XI. Discussion of Human Gene Transfer Protocol #1104-1104 entitled: AAV-BDNF Gene Therapy for Obesity

Principal Investigators:	Joan Han, M.D., National Institute of Child Health and Human Development (NICHD) (*not present*); Jack Yanovski, M.D., Ph.D., NICHD; and Matthew During, M.D., D.Sc., Ohio State University Medical Center
RAC Reviewers:	Drs. Fong, Fost, and Sarzotti-Kelsoe
Ad hoc Reviewers:	Rudolph Leibel, M.D., Columbia University, and Edward Oldfield, M.D., F.A.C.S., University of Virginia (*not present*)

Drs. Bartlett and Chiocca were recused from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

The investigators propose a clinical trial for individuals with severe obesity due to specific genetic predispositions. They plan to enroll four research participants who have Prader Willi Syndrome (PWS) and eight participants with severe obesity as a result of a genetic mutation of a protein called melanocortin 4 receptor (MC4R).

Studies of severely obese people in the United States suggest that more than 500,000 individuals have a mutation in the MC4R gene that is responsible for their excessive body weight. PWS is a rare disorder, estimated at 20,000 within the United States, that is associated with several features including poor muscle tone, reduced mental capacity, and an insatiable appetite starting at 1 year of age, leading to excessive weight gain and obesity. Both conditions are associated with a relative deficiency of a protein called brain-derived neurotrophic factor (BDNF), which leads to the aggressive food drive and slow metabolism.

The protocol proposes to administer an adeno-associated virus (AAV) vector expressing brain-derived neurotrophic factor (BDNF) directly into the ventral medial hypothalamus (VMH) of research participants with morbid obesity either as a result of mutations in the melanocortin 4 receptor (MC4R) or those with Prader Willi Syndrome. The MC4R appears to act as an intermediary, functioning downstream of leptin and upstream of BDNF signaling, and is believed to play an important role in appetite regulation. Mutations in the MC4R gene, found in ~2.5% of severely obese individuals, leads to both an aggressive desire for food (including binge eating), and reduced metabolic rate. Prader-Willi syndrome is caused by the lack of expression of paternally-derived genes on chromosome 15q11-13, and, of relevance to this protocol, is characterized by rapid onset of weight gain and severe hyperphagia between ages one to five. Both conditions are associated with an impairment of hypothalamic control of appetite and metabolism, with a relative deficiency of hypothalamic (and serum) levels of BDNF.

The over-expression of BDNF is hypothesized to restore MC4R signaling to increase metabolism, and facilitate the loss of excess body weight. To help decrease BDNF protein expression as weight loss progresses and prevent cachexia, the vector contains a molecular autoregulatory system using a specific microRNA targeting BDNF. Production of this microRNA element will be controlled by a promoter that is responsive to Agouti-related peptide. In preclinical models, production of this protein increases in response to BDNF-induced weight loss.

The trial will study 12 subjects in total, six with a low dose and six with a high vector dose. In each dose cohort, two subjects will be enrolled with PWS, and 4 with MC4R mutations. The study endpoints include assessments of safety and tolerability as well as body weight and fat mass.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion the protocol. Key issues included the use of a novel transgene for two new disease indications together with a novel regulatory system using miRNA.

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

Dr. Fong requested that the investigators present the preclinical toxicity studies using this construct, and discuss whether mortalities and unexpected toxicities occurred in the animal studies. One criterion for exclusion is listed as "anorexiant or body weight altering medication in the preceding six months" and he asked about the six month timeframe and whether such medication would alter efficacy or interfere with measures of metabolism in this study. Noting that the investigators list as a safety measure the possibility of using a vector with Cre recombinase gene to inactivate the gene of interest, Dr. Fong requested additional specifics about how far along the investigators are in producing and testing such a clinical-grade vector, and suggested that this vector should have standard operating procedures associated with it. He asked for a more detailed description of the research plan in terms of enrollment of subsequent participants and escalation to next dose levels. Noting that one of the problems with ongoing studies will be tying eventual observations in changes in metabolism to the gene delivery, Dr. Fong suggested that having a marker to indicate gene expression and persistence would aid in moving forward this line of research. He asked the investigators to discuss whether there exists a better construct with a marker gene that will more quickly prove/disprove utility of the proposed approach. He asked whether the investigators have formulated a plan for dealing with technically poor magnetic resonance followup scans, and whether anesthesia or sedation would be used in obtaining such scans.

In view of the novel product and rescue strategy, Dr. Fost opined that it would be desirable to conduct the first human studies in patients with a clear clinical need and to include high standards for consent. Therefore, he suggested that the inclusion criteria might better be restricted to participants with clinical complications of their morbid obesity, which would improve the benefit/risk analysis (participants would have a greater likelihood of benefit) and provide a secondary outcome measure (improvement in the comorbidity). The importance of high standards for consent would suggest excluding participants with PWS for this study, given the association of PWS with low cognitive functioning, even with the investigators' planned reliance on surrogate consent. In general, Dr. Fost noted that participants unable to consent are not enrolled in Phase I studies because of low prospects for direct medical benefit, unless those participants have an immediate life-threatening condition. If early studies show promising results, he stated that there would be time enough to expand subsequent trials to include participants with PWS. To enhance the likelihood that participants have a high degree of understanding of the benefits, risks, and alternatives, Dr. Fost suggested a consent monitoring system that would assess potential participants' understanding of the basic elements of consent by someone independent of the research team. It would also be desirable for potential participants to have the opportunity to obtain an independent second opinion – to talk with someone knowledgeable with these conditions who is not part of the research team; Dr. Fost suggested formalizing this assistance. He requested clarification of two sections of the informed consent document, who will be conducting the surgery, and how participants will be recruited to this study.

Dr. Sarzotti-Kelsoe asked whether biological measures of safety should be measured, especially in case of an adverse event, such as a bleeding incident during gene delivery, that poses a risk of systemic exposure. She asked the investigators to provide more details about the intriguing ability to reverse the potential overexpression effect of the gene transfer by treating the participant a second time using a Cre recombinase-expressing vector. She also asked why the adeno-associated virus (AAV)-BDNF was only tested in mice and not in nonhuman primates before proposing a clinical trial. Dr. Sarzotti-Kelsoe suggested that possible risks associated with virus or gene spreading to distant sites be discussed in the clinical protocol, not merely in Appendix M-II. She suggested clarification of two terms in the informed consent document, and asked that the investigators describe how the "hole in the skull" would be sealed at the end of the procedure and what would happen to the blood samples drawn during the trial if the participant opted to withdraw from the study.

Ad hoc reviewer Dr. Liebel asked about the rationale for not first testing this procedure in nonhuman primates, for additional details from the preclinical studies, why cerebrospinal fluid would not be monitored in the research participants, and why the investigators have not chosen to conduct more extensive, prolonged inpatient studies on these participants before and after receiving the AAV vector.

He asked the investigators to discuss the counseling that would be given to participants regarding intentional weight loss after the procedure. He noted that decreased serum BDNF in obese, healthy children could simply be a response to high leptin or insulin suppressing BDNF. Dr. Liebel indicated a described peripheral effect that might be a cautionary point for this study – a study in which investigators found that the use of the BDNF-related peptide Neurotropin-4, when given intravenously to nonhuman primates, resulted in the opposite effect (increase in body weight) compared to central (directly into the brain) administration that led to marked suppression of food intake.

Ad hoc reviewer Dr. Oldfield noted the general need for more information about the procedure to determine its potential risks and accuracy. He requested additional information about the targeting, delivery, and distribution of AAV-BDNF; the proposed catheter and whether it has been shown not to bind AAV-BDNF, which would potentially affect delivery; and whether the proposed catheter has been demonstrated to be safely placed in the hypothalamus without producing injury. Dr. Oldfield asked the investigators to discuss the accuracy of targeting the VMH with the proposed approach, how it will be determined that the targeting is accurate, and what studies in animals with large brains have been conducted to show that the VMH can be targeted accurately. Noting that the VMH is a small nucleus and is in an area surrounded by other important nuclei of the hypothalamus, Dr. Oldfield asked what had been done to show the distribution of delivery of AAV-BDNF and expression of BDNF in this region, whether delivery and expression can be accomplished precisely within the VMH using this approach, whether the absence of distant gene expression has been investigated, and what has been done to show the accurate delivery of the desired volume to the target.

C. RAC Discussion

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

- Dr. Leibel stated that, despite the investigators' arguments, he would still strongly favor use of a recombinant protein before going to a trial using an AAV vector to deliver the protein. In addition, he suggested not going forward with the viral study without good data in a nonhuman primate, including an attempt to reverse the effect of the vector by whatever method is proposed.
- Dr. Kohn requested to see more information about the reliability of the autoregulatory sequence, since it is essential as a safety component.
- Dr. Kahn expressed concern about the PWS population participating in this study. He stated that participants in this protocol should be able to make a voluntary decision to participate and should be individuals who do not have good or any options.
- Noting that obesity is a serious, life-threatening problem, Dr. Fost opined that PWS patients should be included in this intervention as soon as it seems reasonably sensible to do so, based on preliminary studies that show it is safe and effective and that the system works as intended.
- Dr. Fost reiterated his recommendation that, given this first proof-of-concept study, all participants ideally should be at the very high end of capacity to consent. This recommendation is particularly salient for the PWS patients who might want to participate.
- Dr. Zaia summarized discussion about the need to conduct nonhuman primate studies as (1) protein studies in nonhuman primates are not needed, vs. (2) a study should be conducted in nonhuman primates using the vector to mimic the approach that will be followed in humans.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding preclinical toxicity studies, the investigators explained that the majority of the preclinical animal studies were published in *Nature Medicine*. They have carried out additional studies including a longterm study following mice to 18 months and a repeated injection study, and have looked at toxicity with high-dose vector, general behavioral analyses, and effects of ectopic expression. To date no significant toxic effects from the BDNF gene transfer have been observed.

Requiring 6 months of no anorexiant or body-weight altering mediation is standard in trials evaluating novel anti-obesity treatments. Any acute effect from a previous experimental drug would be washed out much earlier; however, if a drug had efficacy on body weight, then the 6 months allows re-establishment of a new baseline.

Cre recombinase has not been used clinically in a human gene transfer protocol. Although the investigators have no formal plan to use a Cre vector, they have included the loxP sites to flank the transgene because doing so provides the possibility of a rescue procedure. Such a rescue would be carried out only in the setting of a severe and ongoing adverse event, otherwise untreatable and related directly to the overexpression of BDNF. The likelihood of such an event is extremely low, based on the investigators' record in related protocols and their animal studies, and any such intervention would be implemented on a humanitarian case-by-case basis, without formal approval of a Cre recombinase product. Animal data suggests that it would be possible to delete approximately 80 percent of the transgene expression with a single AAV-Cre injection, and a similar rescue efficiency could be expected with a cell-permeable recombinase protein.

With the unregulated vector as well as the regulated BDNF vector, weight loss is already significant at one week, although the peak effect took approximately three weeks before plateau. Therefore, the investigators agreed to include in the protocol a delay in enrollment of six weeks between doses, and within each dose cohort enrollment will be limited to two participants per month.

The vast majority of human gene transfer trials of neurological disease have not used tagged genes or additional marker genes. While the investigators agree that it would be useful to be able to monitor noninvasively the expression of the transgene, doing so would add to the complexity of the construct and introduce additional regulatory concerns. Unique safety issues might be associated with such a marker gene and, in the brain, overexpression of marker genes (e.g., GFP) has been associated with toxicity and potential immunogenicity. Therefore, the investigators must be dependent on indirect measures or surrogate markers of gene expression. Animal studies suggest that plasma leptin is highly sensitive to BDNF gene transfer, even with relatively mild weight loss. Therefore, in addition to body weight, fat mass, and other comprehensive metabolic studies, the investigators will look at plasma adipokines including leptin and adiponectin.

While the investigators do not plan to include children in this study, they do expect some degree of cognitive impairment among the PWS individuals, who may be as young as 18. Radiological departments and magnetic resonance imaging (MRI) units are highly experienced with individuals who are overly anxious, have cognitive impairment, or are simply unable to comply with requests to lie still. Mild sedation is typically administered in such situations, and would be considered for participants in this protocol if needed. Typically, images are reviewed during the MRI procedure and, if technically poor, repeat scanning is carried out.

In view of the novel product and the rescue strategy, it would be desirable to conduct the first human studies in research participants with a clear clinical need, and to include high standards for consent. The investigators agree that this intervention should be performed only on those with a real need, and the participants they propose all have obesity that is associated with significant morbidity.

The investigators agreed to modify the inclusion criteria to require a significant clinical complication of obesity (e.g., impaired glucose homeostasis, steatohepatitis, moderate to severe sleep apnea, severe hypertension) in addition to BMI greater than 40. The risks of this experimental intervention, based on experience to date with AAV gene transfer in the brain, should be no greater than those of bariatric surgery, for which a BMI of 35 or greater with comorbidity or a BMI greater than 40 regardless of comorbidity are the approved indications. Such criteria are also appropriate in this protocol; since most individuals with BMI greater than 40 will have some associated comorbidity, adding such an additional inclusion requirement is unlikely to affect recruitment.

The investigators received significant encouragement from the PWS community and foundations to include PWS participants in this trial. Orphan diseases tend to be neglected, and there might be unique

safety and tolerability issues in PWS that would not be apparent if MC4R-related obesity were studied alone. However, the investigators agreed to ensure that they include only individuals who can give fully informed consent in this Phase I study, a requirement that is likely to exclude the vast majority of the PWS population.

Regarding consent monitoring, the investigators agree that a structured interview is of value and they agreed to modify the consent process to include such an audit/monitoring. They also agreed to build into the consent process an independent participant's advocate. This is not the same as an obesity expert or endocrinologist, but this advocate will have the requisite training to understand the condition, the therapeutic options, and the risks and potential benefits of the gene transfer. Individuals are referred to the NIH obesity research program because they have attempted, and failed, management of their obesity. Thus, all participants will have consulted with experts on obesity prior to agreeing to be evaluated for participation in this trial at the NIH.

After the experimental infusion, surgical complications necessitating a prolonged hospital stay are not expected. With a previous study using the identical infusion system and with this proposed procedure carried out under local anesthesia, the investigators found they could safely discharge participants on the day of surgery. However, all participants in this protocol will be kept in the hospital for a minimum of one night and will be discharged from 24 to 48 hours following surgery, based on their clinical state.

The investigators explained how participants would be recruited. Drs. Han and Yanovski have already established systems for recruitment of participants with significant obesity, including PWS and MC4R mutations. These systems include flyers sent to any physician who has ordered sequencing of the MC4R through Athena Diagnostics, informing them of the availability of studies at the NIH for those with function-altering mutations of MC4R. Drs. Yanovski and Han have an existing single-visit evaluation study of both disorders funded by the Prader-Willi Syndrome Association, and all potential research participants are likely to come from this previously identified sample.

The investigators intend to carry out a toxicological nonhuman primate study under Good Laboratory Practices (GLP) conditions as part of their toxicology submission to the FDA to obtain an active investigational new drug (IND) designation. They have carried out similar studies in nonhuman primates in support of their epilepsy and Parkinson's AAV gene transfer protocols, resulting in no significant toxicological concerns. The primate data was not critical for these previous protocols in terms of issues that were raised by the NIH RAC; therefore, the investigators believed it was appropriate to receive expert feedback and public comments prior to instigation of the primate experiments, since the RAC input might guide these final preclinical experiments.

Although the investigators do not believe that spread of the vector is likely, considering the method of delivery, the total volume, and the titer of the vector, they agreed to insert a sentence in the protocol to describe vector spread or mistargeting as a potential risk.

Regarding the possibility of using less-alarming terminology to describe the proposed procedures, the investigators explained that it is difficult to euphemize the language for a neurosurgical procedure involving a burr hole. In previous protocols, their experience indicates that participants are not overly alarmed when the procedure is fully explained and they understand that a burr hole cover is placed on the skull to ensure that no hole remains in the skull.

The investigators agreed to include a statement to let each participant know that, if they withdraw from the study, stored samples will be used for research unless they request to have them destroyed.

In experimental animal studies to date, including normal lean mice, DIO mice, and db/db mice, the investigators have seen a consistent effect on body weight. Both MC4R-mutation individuals and PWS individuals are more likely to be modeled by the db/db mice, since the leptin receptor is upstream of MC4R, and therefore a similar sensitivity to BDNF would be expected.

The protocol is quite comprehensive in terms of metabolic monitoring and attempts to minimize participant burden. Total Daily Energy expenditure estimated over one week by the doubly labeled water method allows participants to participate in their normal daily activities at home, rather than be restricted to a metabolic chamber. Indirect calorimetry at rest is also already being obtained in this study. Intake is studied with laboratory meal studies that allow examination of ingestive behavior. More prolonged inpatient studies, including chamber calorimetry and functional MRI, might be proposed in the future should initial results suggest efficacy for this approach.

It is likely that BDNF will result in an improved ability to comply with a diet, largely because BDNF will help with satiety and will reduce appetite. Hence, for this Phase I study, the investigators do not want to intervene with food intake but prefer to monitor appetite and food ingestion, with definitive testing of appetite and weight effects obtained in a controlled Phase II study.

With chronic obesity, it is likely that some resistance to the high levels of circulating leptin develops, particularly marked in some individuals. This resistance to leptin might result in a fall in circulating BDNF, as has been observed in some individuals without known MC4R mutations. The investigators expect that such individuals would also be highly sensitive to hypothalamic BDNF gene transfer. Low plasma levels of BDNF in obesity could also potentially be a marker of leptin resistance.

The investigators provided details of the catheter design. The device is a proprietary device developed by Medtronic and is known internally as the MANTIS (Medtronic Acute Neurosurgical Therapeutic Infusion System). It has undergone extensive evaluation, including studies involving binding to AAV, and uses materials approved by the FDA for human investigational use. There is no issue with biocompatibility, leachables, or vector binding. The device is flexible with extremely low dead space and is constructed of PEEK with a 1 cm, 200 micron stainless steel tip. To reach a deep target, guide tubes similar to those used for deep-brain stimulation surgery are used; these guide tubes are engineered especially by Medtronic for use with the MANTIS. It is designed for acute implantation (up to 24 hours) within the human brain, and uses a novel burr hole cover that is able to lock down on the catheter and then subsequently be unlocked based on a release cord accessible without need to return to the operating room. The device has been used without complications in 22 humans targeting another deep nucleus with similar dimensions. Targeting the hypothalamus versus the subthalamic nucleus does not represent a significantly greater challenge, and is well within the expertise of a well-trained stereotactic and functional neurosurgeon.

The catheter tip that penetrates the hypothalamus is only 200 microns in diameter, and is in place for only about two hours. In studies on Parkinson's disease, targeting an even smaller brain region, the investigators observed no injury based on a combination of imaging (MRI) and PET studies.

The target region for this proposed study is much larger than the subthalamic nucleus (STN). The investigators propose to have the tip of the cannula within the midregion of the VMH and, as a general rule, stereotactic surgery by the average functional surgeon is accurate within 23 mm 70 percent to 80 percent of the time. That degree of precision is acceptable for the current protocol. Even though the investigators reported imprecision in experimental animal studies of approximately 0.5mm, which would translate in the human brain to a centimeter or more, no problems were associated with imperfect targeting.

With respect to the Parkinson's disease Phase II study, the cases of implantation failure were not true mistargeting. One instance was the result of medical misadventure when the surgeon did not change the frame coordinates when he switched from one side of the brain to the other, and so the second catheter went directly across the midline to the same STN. In the other participants, the problem was predominantly on one hemisphere and was due to the device not being fixed properly by the burr hole cover. Therefore, the targeting errors were due to the device either falling out (when the drapes were removed) or it partially withdrew along its insertion trajectory. A problem with the locking mechanism was corrected.

Original data showed that changes occur in BDNF expression within the arcuate nucleus, and the investigators then extended their analysis to the VMH. Animal studies showed expression that extends throughout the ventrodorsal coordinates of the medial hypothalamus, including the arcuate, the VMH, and the dorsomedial hypothalamus. BDNF is expressed throughout this region (and in almost all other hypothalamic nuclei) and is also found in the efferent regions. AAV is not able to jump synapses or spread from neuron to neuron; therefore, once AAV is taken up by a cell, it is unable to infect a second cell. The BDNF transgene can be released by the soma, dendrites, and axons of any given transduced neuron, and with the rich interconnections of the hypothalamus there is no concern about local spread.

2. Responses to RAC Discussion Questions

In response to RAC concerns that the preclinical animal studies still raise questions about safety, Dr. During acknowledged that the investigators have not conducted a GLP primate study. Such a study would be a major undertaking so it will be important to ensure that it is well planned and will meet appropriate scientific criteria for the RAC and the FDA. The investigators were waiting to begin a nonhuman primate study until after raising various issues at this RAC meeting, but they reminded RAC members that they have an enormous amount of data on mice and rats. A lot of data exists with regard to high-dose BDNF in humans and in primates, but the investigators acknowledge the need to conduct their own nonhuman primate study. While they did not agree that there is a need to do a recombinant protein primate study, the investigators agree about the need to do a viral vector gene transfer study in nonhuman primates.

Dr. During stated that the investigators want to include the PWS patients in this protocol from the start, as they exhibit the greatest need – they do not have any options, they cannot have bariatric surgery, and they die of obesity. The most severe genetic cause of obesity is PWS. PWS patients represent a unique population that has an identified, clear BDNF deficiency syndrome based on serum levels, which is the scientific rationale for why they should be allowed to participate in this study.

Dr. Yanovski explained that PWS patients have differing degrees of mental capacity. The NIH has an active consent department, and the investigators understand the desire to protect those potential participants who might not be able to make a fully informed consent decision. He agreed that it might be possible to start this protocol with MC4R patients first and then, if safety is determined, to proceed to enrolling PWS patients.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Preclinical Issues</u>

The RAC appreciates that the investigators submitted the protocol for review early in the process of development so that they could take the Committee's recommendations into account for future preclinical studies and trial design. However, it is difficult to assess the safety of the proposed vector until additional animal studies, including nonhuman primate studies, are conducted. The RAC noted that the investigators are welcome to present the results of these studies at a future RAC meeting to receive additional input.

- Prior to clinical studies, a preclinical study mimicking the clinical study should be done in nonhuman primates and this study should attempt to test the ability of the miRNA-based autoregulatory system to control BDNF expression in the context of weight loss induced by the initial overexpression of BDNF. Such a study will also allow evaluation of the proposed technique for administration to the VMH in a larger animal model.

- The longterm reliability of the miRNA autoregulatory system is critical to the safety of this study. Preclinical studies longer than 6 months in duration should be conducted to determine the stability (e.g., lack of silencing of the AgRP promoter) of the miRNA autoregulatory mechanism.

Clinical/Trial Design Issues

- While severe obesity contributes to the development of a number of chronic conditions, including diabetes, cardiovascular disease, and sleep apnea, and is associated with earlier mortality, the approach proposed in this protocol is nonetheless an aggressive approach for this chronic health condition. Therefore, optimizing the evidence to support the safety of this approach is critical. Preclinical studies in nonhuman primates will provide additional safety data. However, presently there are no data in humans demonstrating that increasing BDNF levels in the VMH will lead to weight loss. One way to test this hypothesis would be by administering recombinant BDNF protein using the identical surgical technique as a proof of concept for the vectored delivery of the protein. However, the RAC acknowledged the challenges to conducting such a study, as already noted by the investigators, including the availability of the proprietary recombinant BDNF protein and the limitations of such a study in predicting the effect of the protein delivered by a vector that should provide continued expression of BDNF. Nonetheless, the protocol and informed consent document should include information regarding the rationale for proceeding directly to the gene transfer trial rather than testing administration of the protein alone, including a discussion of how data from other studies, for example in Parkinson's disease, have informed this decision.

- To provide another level of safety, the vector is designed with the BDNF transgene flanked by loxP sites that might allow deletion of the transgene by cre recombinase should the miRNA autoregulatory mechanism not to be effective in halting BDNF expression. This Cre-lox approach has never been used clinically. The Cre recombinase mediated rescue is not part of this research protocol and is intended to be used only on a "compassionate use" basis in the event of a severe and ongoing adverse event directly attributable to overexpression of BDNF. If this occurred, the investigators would consult with the FDA regarding administering a TAT-Cre recombinase protein. The potential use of this alternative safety mechanism in case of a severe adverse event should be discussed more explicitly in the protocol and informed consent and preclinical models should validate the approach as it may be used.

- If cerebral spinal fluid is collected, BDNF levels along with those of AgRP and neuropeptide Y, which were significantly upregulated in the preclinical models, should be measured to increase understanding of the effect of these proteins on clinical outcomes. Some data may be obtainable from the nonhuman primate studies.

- While systemic release of BDNF is not expected following delivery to the brain, peripheral antibodies to BDNF should be monitored.

Ethical, Social, and Legal Issues

- This protocol is very complex with respect to its science and may be difficult to describe simply to potential research participants. PWS is associated with low cognitive function and enrollment in this protocol should be limited to those participants, including PWS participants, with higher cognitive function and the capacity to understand these complexities. Initiating enrollment with MCR4 research participants, who do not have any genetic predisposition to intellectual impairment, and gathering additional safety data in this population should be considered prior to enrollment of PWS research participants.

- To enhance the likelihood that the research participants have a clear understanding of the risks, benefits, and alternatives to enrollment in the protocol, a consent monitoring plan should be considered. This might include the use of an independent consent monitor and/or written assessments of the participants' understanding before they are enrolled.

G. Committee Motion 7

Dr. Zaia orally summarized the recommendations expressing the comments and concerns of the RAC. Although no official motion was made, the RAC voted to approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 2 recusals.

XII. Presentation of Certificates of Appreciation

Presenter: Amy Patterson, M.D., OD, NIH

Dr. Patterson expressed the deep appreciation of the NIH for the work of this committee in general. She listed the many accomplishments of the RAC over the years. First convened in 1975, the RAC continues to be a body held as a model of scientific review and transparency.

Dr. Patterson presented four retiring RAC members with certificates of appreciation from the NIH, and listed the individual RAC-related accomplishments of each during their tenure on this committee. Drs. Bartlett, Fan, and Kahn were recognized for their four years of RAC membership, and Dr. Zaia was recognized for his four years of membership plus his chairing of the RAC for the past year.

The new RAC chair will be Dr. Fong.

XIII. Presentation of Results from Phase II Protocol #0710-877 entitled: Phase II Safety and Efficacy Study Evaluating Glutamic Acid Decarboxylase Gene Transfer to the Subthalamic Nuclei in Subjects with Advanced Parkinson's Disease

Presenter: Matthew During, M.D., D.Sc., Ohio State University Medical Center

A. Presentation

Dr. During provided a brief background and then focused his presentation on the recently completed Phase II data.

The investigators are developing AAV vectors expressing glutamic acid decarboxylase (GAD) for potential treatment of people with advanced Parkinson's disease (PD). An enormous amount of preclinical and clinical experience shows that AAV in the brain is both safe and stable. The completed Phase I study involving 12 research participants was started in 2003 with results published in *Lancet* in 2007. This Phase I study featured unilateral surgery and was the first gene transfer study for adult neurodegenerative disease. The randomized, blinded, Phase II study showed no side effects; this study was followed by a longterm followup study that is now in the crossover phase in which those participants who received the sham treatment are now eligible to receive the experimental gene transfer. The original Phase II study met its primary endpoint, which is why the crossover arm is about to commence.

PD is mostly considered a disease in which the substantia nigra in the midbrain degenerates, dopamine is lost, and the dopamine neurons send out axons into the caudate putamen. Most therapeutic strategies relating to PD – and the main treatment – focus on drugs that facilitate dopamine neurotransmission in the brain. Downstream of the striatum and the caudate, where dopamine works, is the subthalamic nucleus, which is a key relay nucleus that signals further downstream to areas that output to the thalamus and cortex. It has been known for many years that manipulating the subthalamic nucleus by inserting deep brain stimulation electrodes or lesioning the subthalamic nucleus can result in remarkable efficacy because of the altered imbalance in the circuitry that controls movement.

The major differences between the Phase I and Phase II studies are that the Phase II study was a randomized double-blinded trial, dosed bilaterally to both hemispheres (versus unilateral dosing for Phase I), uses the Medtronic brain infusion system, uses a single fixed dose of 3.5×10^{10} vector genomes,

employs multiple sites, and uses product produced under much tighter GMP conditions. One of the key problems is that every biological therapy for PD that looked promising in successful Phase I studies – including transplants, adrenal transplants, pig cell transplants, and recombinant protein infusions – failed in Phase II. It has been posited that lack of homogeneity in the study population is partly responsible for these failures, so Dr. During and his colleagues instituted strict inclusion criteria; they also included well-experienced study sites.

The Phase II blinded phase was conducted in the first 6 months, and then an open phase was conducted from month 6 out to 12 months; the trial is currently just beyond the 12-month time point. While there is a slight gender bias toward males in PD, enrollment in this Phase II trial is heavily skewed toward men. Dr. During explained that almost every experimental trial on the brain experiences great difficulty in recruiting females, possibly because families do not like to volunteer their mothers or their sisters and are more protective of the females in the family. The protocol included a completely new infusion system that included a pump, a catheter, and a locking system. Because this device had not been tested in a bridging study and therefore the investigators expected some problems with the device, they defined as part of the protocol that if they did not hit the correct target, those participants would be excluded prior to breaking the blind. Dr. During reviewed the specifics of how this surgery was performed.

Phase II results at 6 months showed that 50 percent of the research participants met the stated criterion of a Unified Parkinson's Disease Rating Scale (UPDRS) Part 3 value of 9 or greater, which translates to a moderate to large change and is considered a clinically meaningful effect. Only 14 percent of the sham-treated participants met that standard. The 12-month data shows the same consistent improvement overall in the UPDRS scores persisting out to 12 months with 62.5 percent of the participants meeting the UPDRS Part 3 value of 9 or greater. (Sham-treated participants' scores also increased somewhat at 12 months compared with their 6-month scores.) Secondary endpoints that showed improved results for GAD-treated versus sham-treated participants included the amount of time during the day that the participants respond well to medications (effect started at one month, was robust at three months, and fell off a little at six months), and the UPDRS Part 4, a composite score of dyskinesias related to medications, which did not improve in the shams but showed progressive improvement over time in the GAD group. These outcomes are comparable to deep brain stimulation, which is the gold standard and currently the only FDA-approved surgical treatment for PD.

Regarding adverse events that occurred with 20 percent or greater frequency, during 12 months, 35 percent of the sham group got significantly worse, an expected result because PD is a progressive degenerative disorder. However, not a single GAD research participant experienced that complication.

Dr. During enumerated the investigators' plans going forward. The Phase II open-label study is commencing in June 2011 – the research participants who got the sham treatment are now getting the GAD treatment. The long-term follow-up study is continuing. The investigators have met with the FDA, and the FDA has accepted the Phase II study results as being sufficient to move forward on a Phase III study. The Phase III study will move ahead under special protocol assessment in 2011.

B. RAC Discussion

Dr. Zaia asked whether the investigators had experienced difficulty recruiting research participants. Dr. During explained that participants were recruited within eleven months, with no apparent difficulty. The risk of this surgery is very low, and PD patients are highly motivated to undergo experimental treatments. After removing six of the GAD-treated participants due to mistargeting, the investigators ended up with 16 GAD-treated versus 22 sham-treated participants.

Dr. During stated that the investigators have not yet decided whether the Phase III trial will feature randomization of best standard of care versus the GAD treatment or whether sham treatment will be included. The FDA has stated that they would prefer the Phase III trial to include sham treatment, but the investigators prefer to test the GAD treatment against standard of care.

XIV. Discussion of Human Gene Transfer Protocol #1101-1088 entitled: Phase I Study of Intra-pleural Administration of GL-ONC1, a Genetically Modified Vaccinia Virus, in Patients with Malignant Pleural Effusion: Primary, Metastases, and Mesothelioma

AND

Discussion of Human Gene Transfer Protocol #1101-1089 entitled: Phase I Trial of Attenuated Vaccinia Virus (GL-ONC1) Delivered Intravenously with Concurrent Cisplatin and Radiotherapy in Patients with Locoregionally Advanced Head and Neck Carcinoma

Principal Investigators:	Valerie Rusch, M.D., MSKCC (for 1101-1088) Loren Mell, M.D., University of California, San Diego School of Medicine (for 1101-1089)
Additional Presenters:	Kevin J. Harrington, M.D., Ph.D., Royal Marsden Hospital, London, U.K.; Aladar A. Szalay, Ph.D., Genelux Corporation; Tony Yu, Ph.D., Genelux Corporation
Sponsor:	Genelux Corporation
RAC Reviewers:	Drs. Ornelles, Roizman, Strome, and Yankaskas

Drs. Fong and Kohn were recused from discussion of these protocols due to conflicts of interest.

A. Protocol Summary for #1101-1088 and #1101-1089

GL-ONC1 is a genetically stable attenuated Lister (LIVP) strain of vaccinia virus designed to enter, colonize, locate, and destroy cancer cells without harming healthy tissues or organs. Vaccinia virus strains have been used safely in millions of people as a vaccine against smallpox. Researchers at Genelux Corporation have modified the LIVP strain of vaccinia virus to increase its safety by reducing its toxicity and to increase its tumor selectivity and antitumor activity without limiting its ability to replicate in cancer cells.

GL-ONC1 carries a unique fluorescent/luminescent fusion protein designed to provide noninvasive, realtime imaging capabilities, including tumor diagnosis and localization, microscopic analysis of tumor biopsies, cancer staging, and followup monitoring. Scientists developed this protein by combining two proteins, one from glowing sea pansies and one from jellyfish. After the virus finds and enters a tumor, it begins to replicate, proportionally emitting light throughout the viral amplification process. The combinatory effects of direct viral killing of tumor cells and immune activation by the host result in an antitumor response induced by the virus while sparing normal tissue cells from harm. GL-ONC1 has demonstrated efficacy and safety in animal models when administered both as a single-agent and when administered in combination with chemotherapy and other licensed products in eradicating or stabilizing tumors in more than 25 different major types of human tumors.

Currently, an investigational trial is being conducted at the Royal Marsden Hospital in Surrey, United Kingdom, as a first-in-humans Phase I open-label, dose-escalating study of the safety, tolerability, and tumor-specificity of intravenous administration of GL-ONC1 in patients with solid organ tumors. An additional endpoint of this trial is the recommendation of dose/schedule for future trials. Research participants are treated for six planned cycles beginning with a dose of $1x10^5$ plaque-forming units (pfu) and escalating to a final dose concentration of $5x10^9$ pfu per cycle of treatment. Standard clinical procedures are conducted to follow participants through the course of treatment and posttreatment follow up. Optional tumor biopsies are obtained to assess presence of virus in tumor tissue. GFP imaging is performed on suitable participants with superficial tumor lesions to test for presence of virus. To date, 21 participants have been dosed in cohorts one through seven with no DLTs observed. Two participants in cohort 5 ($1x10^9$ pfu/cycle) experienced vaccinia-related skin rash that resolved without treatment at the end of cycle one. Typical adverse reactions were flu-like symptoms. Only one serious adverse event (arterial emboli) was determined to be possibly related to dosing and no deaths have been related to GL-ONC1 administration. Minimal viral shedding occurred in one participant following cycle one only (cohort

five). Best response was RECIST stable disease for longer than six months (n=1) and for three months to six months (n=5).

Based on the tolerability of GL-ONC1, the sponsor is initiating other early-stage trials in Europe and in the United States in several oncology indications, with different routes of GL-ONC1 administration.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocols. Key issues included the novelty of the poxvirus vector, which contains multiple reporter genes, and the potential for unexpected interactions among the gene products of the reporter genes.

Four RAC members provided written reviews of these proposed Phase I trials.

Although the mechanism by which this virus shows selectivity toward tumor tissue over normal tissue remains uncertain, Dr. Ornelles noted that the severity and life-threatening nature of the malignancy targeted by this protocol is commensurate with the uncertainties associated with the mechanism of action. He voiced three major concerns. The limited information on the human biology of this modified virus and the apparent absence of preclinical studies in nonhuman primates warrant that special attention is made to monitor for effects that are unique to humans that could arise to create a permissive niche for virus replication and persistence. A high degree of plasticity to the vector genome is indicated by attenuation of GFP expression after three to six passages in a non-primate cell line compared with GFP expression that was sustained for more than six passages in CV-1 cells, and the nature of possible variants developed under selective pressure in a human are impossible to predict. Dr. Ornelles also was concerned that the viral progeny produced by multiple rounds of replication would have had a greater opportunity to silence reporter gene expression, and therefore detection by imaging methods alone could lead to a false negative conclusion. He raised several minor concerns including the increased liver pathology observed in rats that received multiple administrations of GL-ONC1, the need for clarification of the two studies on adverse events associated with smallpox immunization carried out in 1968, and an explanation as to why there is no statistical basis for determining the maximum tolerated dose.

Dr. Roizman discussed four fundamental problems arising from the proposed studies. Modifications made in the GL-ONC1 do not specifically target the virus to tumor cells, and the data provided suggest that the virus can infect normal cells but that its ability to replicate in normal cells is drastically reduced. While the virus is purported to induce an immune response to the tumor, Dr. Roizman wondered if the virus also would induce an immune response to normal cells. Vaccinia induces a strong, effective immune response; he asked whether GL-ONC1 should be used only once and, given that GL-ONC1 did not kill 100 percent of the tumor cells in cell culture, he asked about the rationale for using an agent that potentially can be given only once without serious adverse reactions. Dr. Roizman requested that the investigators explain their rationale for testing research participants after days two and three but not at a later time to determine how long the infectious virus persists in the thoracic cavity. He noted that participants will undergo video-assisted thoracic surgery for biopsy collection, and that this surgery extends the proposed study from safety to elements of efficacy at potential risks to the participants. Thus, he asked how the investigators would determine which individuals could undergo this thoracic surgery.

Dr. Strome asked why three computed tomography (CT) scans would be necessary. In addition, he noted that, at the highest dose level, 4 of 30 animals died, which is a significant percentage. Also of concern is the liver histopathology, particularly the increased mitosis. The numbers of grade I and II adverse events are significant but tolerable and the thromboembolic event is significant; however, given this pathology, which is uniformly fatal, awareness and early intervention make both acceptable. Dr. Strome believed that stating that an intravenous dose of $1x10^8$ is safe is an overstatement because 4 of 30 animals died, persistent liver cellular change and mitosis were identified, and, in a clinical trial, a potentially life-threatening embolic phenomena was observed. In addition, he suggested that the informed consent document be revised to clarify several terms and processes, and expressed his concern about the statement that “the study will not pay for medical treatment.”

Dr. Yankaskas asked the investigators to explain the putative mechanisms for selective targeting of GL-ONC1 to tumor cells as compared to normal cells and for decreased replication of GL-ONC1 in normal cells as compared to tumor cells. He suggested that minimum Absolute Lymphocyte Count, lymphocyte subset counts, and/or immune function assay criteria be specified for inclusion and exclusion criteria to reduce the risks to study participants. Noting that liver, lung, lymph node, and spleen toxicities were observed in the rodent experiments and that serum chemistries and blood counts will be measured during this protocol, Dr. Yankaskas requested an explanation of the strategies to distinguish whether observed abnormalities are due to vaccinia virus or to cisplatin or other drugs.

C. RAC Discussion

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

- Dr. Zaia asked why vaccinia acts as an oncolytic virus.
- Dr. Strome suggested changing the inclusion criteria so as not to include individuals with untreated disease. He expressed preference for adding administration of this virus as a possibility if a patient fails either surgery and radiation or traditional chemoradiation therapy.
- Dr. Kanabrocki expressed concern for healthcare workers about exposure to vaccinia when preparing or administering the study drug. Healthcare workers should be made aware of counterindications for exposure to the study drug and, if they have conditions that might predispose them to infections, they should be allowed to decline participation in this protocol. If possible, the study drug should be prepared in a biological safety cabinet and, if that is not possible, methods for aerosol control should be used in preparation of the study drug.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that they have tested the hypothesis as to whether sites of inflammation could create a permissive niche for virus replication and persistence, and found that the safety profile of GL-ONC1 is not altered when combined with multiple does of irradiation. Focal irradiation treatment in nontumor-bearing mice does not cause viral colonization of healthy organs after systemic injection of GL-ONC1. Multiple doses of irradiation given to healthy tissue have no effect on viral colonization of that particular tissue or of other organs. Combining GL-ONC1 treatment with irradiation does not appear to alter the safety profile of the virus. In addition, systemically injected GL-ONC1 virus does not colonize an induced inflammatory site.

Vaccinia viruses have a long history of use in humans and the safe use of vaccinia vaccine has been well documented. The LIVP vaccinia virus, from which GL-ONC1 was derived, has been used as a live vaccine against smallpox and administered to millions of people in the late 1960s and 1970s. No virulent revertants from vaccinia vaccine strains have ever been reported; therefore, the investigators do not expect GL-ONC1 to revert to a more pathogenic strain. In the current ongoing Phase I trial being conducted at Royal Marsden Hospital, where GL-ONC1 is administered intravenously to research participants, no outbreak of progressive vaccinia infection has been reported. So far, doses up to $3x10^9$ pfu have been administered to participants and this dosing has been well tolerated. More potential participants are being evaluated in this United Kingdom trial for safety of and tolerability to the virus.

The investigators agreed that detection by imaging methods alone could lead to a false negative conclusion. Therefore, they agreed to add viral plaque assays using samples from tumor biopsies and pleural drainage in addition to GFP imaging of tumor and surrounding tissue.

Liver toxicity was observed only in rats with intravenous administration of repeated dosing of GL-ONC1 (twice weekly for 4 weeks). Diffused perilobular single cell necrosis and fibrosis was seen in the high-dose group ($1x0^8$ pfu) and, in some animals, an increased hepatocellular mitosis was evident. However, these changes were partially reversible after a 2-week recovery period, and the number of mitoses was

no longer increased at the end of 2-week recovery compared to untreated controls – the increased mitosis in the liver was temporary and the change was reversible. In contrast to the repeated-dose toxicity study, the single-dose application of GL-ONC1 did not cause any detectable liver toxicity in the investigators' preclinical studies at 1-week or 2-month endpoints. Clinical protocol #1101-1088 proposes a single dose for all cohorts, and only individuals with adequate hepatic functions will be eligible to be included in this study. The investigators believe there is no significant concern about potential liver toxicity based on their preclinical data, and vaccinia virus induced liver pathology is not a known common adverse event in people receiving smallpox vaccine. Since GL-ONC1 is a derivative of a vaccinia vaccine strain, increased liver pathology is not expected to be of concern in humans.

As an assessment of eligibility and for patient safety, the amount of free pleural space to permit infusion of the experimental drug needs to be ascertained by CT scan during the screening phase to determine if drainage is needed. Three CT scans are necessary to accomplish this assessment.

In a preclinical study, animals in the high dose group ($1x10^8$ pfu per animal) virus was administered intravenously as two large volume boluses , which may explain the cause of death of the animals in this group. The animals in the low and medium dose groups received a much lower volume. No other compound-related mortalities and no other signs of toxicity were recorded within 14 days post-dose for any dose or control groups in this study. Other reasons for the animal deaths are possible. The investigators repeated this experiment with high doses of virus delivered intravenously into mice; no animals died during that study and all animals gained weight at expected rates during the 59-day follow-up period after virus injection.

Because the route of intrapleural administration of GL-ONC1 has not been investigated in a Phase I trial, protocol #1101-1088 was designed as a single-agent dose to determine the safety of this administration route. Chemotherapy, large-field radiation therapy, or any other type of anti-cancer treatment is not allowed so as not to confound the results. Because the first-in-human Phase I trial in the United Kingdom was conducted in participants who received a single-agent dose of GL-ONC1 administrated intravenously, protocol #1101-1089 was designed as a combination experimental treatment schema to investigate if GL-ONC1 could be safely administered intravenously in combination with chemoradiation.

The investigators agreed to add to the informed consent document the following information about a serious adverse event that occurred in the Royal Marsden study in the United Kingdom: "In an ongoing GL-ONC1 Phase I trial conducted at the Royal Marsden Hospital in Surrey, United Kingdom, a patient developed a blood clot in an artery in the leg. Although cancer patients in general have a propensity for forming clots, we cannot rule out that this serious adverse reaction was possibly related to study medication, GL-ONC1. The clot was surgically removed and the patient recovered."

In response to concern about the statement that "the study will not pay for medical treatment," the investigators explained that the informed consent document included in the current version of the clinical protocol is a suggested template, and this section might be required to be revised to conform to the IRB requirements for product liability reimbursement for any injury that is determined to be definitely related to the study drug. If the IRB does not require that the sponsor pay for product liability costs, the investigators agreed to highlight the statement about not paying for medical treatment, to ensure that potential research participants would be aware of this policy.

To help explain the experimental drug's predicted effect on tumors and not on normal cells, the investigators explained that the immune system continually surveys the body for the presence of abnormal cells, which are destroyed when recognized. However, tumors have multiple mechanisms for evading immune responses. The major argument to support oncolytic viral therapy is that even a small initial dose of a replication-competent oncolytic virus will amplify through successive rounds of viral replication, resulting in eventual eradication of the entire tumor. Ultimately the combined effects of viral oncolysis and activation of the immune system lead to tumor cell destruction. Normal cells infected with virus will also elicit an immune response; however, the immune response is largely directed toward viruses rather than normal cells. Consequently, infected normal cells will be cleared and uninfected

normal cells will be spared. On the other hand, the oncolysis of tumor cells will expose specific tumor antigens that are otherwise undetected by the immune system because of the evading mechanisms. As a result, an adaptive immune response will develop against these specific tumor antigens, triggered by viral infection.

The first cycle of dosing is anticipated to be important for virus delivery to the tumor bed; however, the subsequent cycles of viral dosing could reinforce a sustained antitumor effect by immune activation. In this case, activated immune response against the virus is a friend, not a foe, to the oncolytic viral therapy of cancers. In a previous human trial for hepatocellular carcinoma using a different strain of vaccinia virus, in humans, a second antitumor response occurred after the second administration of virus, providing human data that vaccinia virus can be used a second time with effectiveness even in the setting of established immunity.

The research participants will also be tested for virus on the day of pleurodesis, which is sometime between Day 3 and Day 7. After that, easy access to the pleural cavity will no longer be available since the chest tube will be removed. Therefore, the investigators plan to stop assaying for virus not because they expect the virus will no longer persist but because the pleural cavity will no longer be accessible.

Video-assisted thoracic surgery (VATS) is a standard medical practice to reduce the potential for a recurrence of pleural fluid in this patient population. As such, VATS is not solely a procedure being conducted as part of this research and therefore would not expose participants to additional risks in order to collect biopsies to test for the presence of virus. Fever or flu-like symptoms would not be a determining factor in delaying thoracic surgery except in cases of sepsis.

Vaccinia virus has shown a natural tropism to tumors, yet the mechanism of this tropism remains to be established. Two levels of tropism that might have contributed to the selective “targeting” of GL-ONC1 to tumor cells as compared to normal cells are: (1) The intracellular events that differentiate tumor from normal cells – including the cell-cycle status of the infected cell, the lineage and differentiation state of the infected cell, the availability of trans-acting transcription factors from the host cell that are required as components for intermediate and late viral transcription, and the intrinsic antiviral state of the infected cell; and (2) GL-ONC1 is a large virus particle and would require leaky vasculature for transfer out of the circulation. The aberrant angiogenic signaling in tumors results in a vasculature that is leaky and tortuous. Therefore, a preferential delivery or “targeting” of GL-ONC1 to tumor after a systemic delivery of the virus is not surprising. In addition, the compromised immunosurveillance in tumor tissues or the immunosuppressive nature of certain tumor types also favors the delivery and spread of GL-ONC1 in the tumor tissues.

Absolute neutrophil count is a standard eligibility criterion in trials using biologics. In the principal investigator’s opinion, the existing exclusion criterion based on absolute neutrophil count should be an indication of adequate immune system function and therefore subject study participants to lower risk.

2. Responses to RAC Discussion Questions

Dr. Szalay explained that the investigators were surprised that vaccinia acts as an oncolytic virus. They do not know why tumor cells support virus replication and why the virus either does not replicate in healthy cells or replicates vastly differently. They have yet to understand what genes are necessary to trigger virus replication in tumor cells.

Dr. Szalay justified the inclusion of patients with untreated disease by stating, on behalf of Dr. Harrington, that without starting this trial with the combined experimental treatment, it never will be known which experimental treatment is most effective for treating head and neck cancer.

Regarding biosafety issues involving the administration and preparation of the study drug, Dr. Rusch stated that MSKCC has guidelines for protection of staff involved in contact with research participants. An infectious disease expert will address those issues to ensure staff safety.

E. Public Comment

With regard to the informed consent document for protocol #1088, Dr. Borror noted that the investigators should make it clear that none of the experimental processes and drugs are "part of regular cancer care." In protocol #1089, some terminology is too technical and references to treatment should be rephrased.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations – Protocol #1088

Clinical/Trial Design Issues

- Preclinical models indicate that this modified vaccinia virus selectively replicates in tumor cells, but the biology underlying this selective replication is not well understood. Any studies that can be performed in this clinical trial that might further elucidate the mechanism of action of this virus should be undertaken.

- Although the modified vaccinia virus has been administered safely in a previous trial, implementation of standard operating procedures will enhance the safety of vector administration. In particular, it is important to use procedures that will minimize generation of aerosols when preparing the vector for administration. This can be accomplished by using a biosafety cabinet, or if one is not available, using ethanol soaked gauze to trap any aerosols generated. All healthcare workers should be properly trained in these procedures. In addition, healthcare workers who will be administering this vector, or interacting with participants who have received the vector, should understand the CDC contraindications to vaccinia vaccination. Any worker with such a contraindication should not participate in these activities.

Ethical, Social, and Legal Issues

- The informed consent document states that administration of the modified vaccinia virus is part of the standard of care. As an experimental agent is not part of standard of care this should be clarified.

G. Synopsis of RAC Discussion and RAC Observations and Recommendations – Protocol #1089

Clinical/Trial Design Issues

- The decision to select this patient population for a Phase I trial should be reconsidered. Under the proposed protocol, individuals with newly diagnosed, locoregionally advanced, non-metastatic and unresectable stage III or IV head and neck cancer will receive the modified vaccinia virus in addition to standard chemotherapy and radiation. This raises two issues. First, the protocol does not outline the criteria to be used for determining whether a patient has unresectable disease. Resecting the cancer will likely improve the prognosis and therefore it is important that the criteria used be uniformly applied without consideration of enrollment in this protocol. Second, and more importantly, even those participants with unresectable disease may have a fairly good prognosis with chemotherapy and radiation. A recent publication from the Cleveland Clinic on a series of patients who would likely meet the criteria for enrollment into this protocol found that 5-year survival with chemotherapy and radiation was as high as 60 percent (Adelstein D.J., Saxton J.P., Rybicki L.A. *et al*. J. Clin Oncol. 2006, 24: 1064-1070). Even when looking at older data, the survival rate with chemotherapy and radiation alone in this population is at least 30%. These data suggest that a number of patients who may enroll in this trial would otherwise have good responses to standard therapy. The rationale for exposing these patients to an experimental therapy when the toxicities of the vector, and its potential interactions with standard therapy, are not known needs to be articulated clearly or the investigators should consider enrolling only those individuals who have failed initial surgery/radiation or chemotherapy/radiation. This population clearly has a poor prognosis and administration of this modified vaccinia virus will be in the context of a salvage therapy.

- A similar concern is raised by the enrollment of patients with newly diagnosed salivary cancer. The optimum treatment for salivary cancer is currently being studied in an ongoing Phase II trial. Consider excluding this population until more data is available regarding their prognosis with chemotherapy/radiation alone.

- Core biopsies of neck lymph nodes done for research purposes only should avoided, as core biopsies increase the risk of seeding the tumor. Ultrasound guided needle aspirations will provide a safer alternative.

- Patients undergoing chemotherapy and radiation for head and neck cancer experience a number of treatment related toxicities. This may make it difficult to distinguish toxicities caused by the modified vaccinia virus from the significant toxicities of chemotherapy/radiation. Obtaining historical data on similar patients, if available, will aid in interpretating safety data.

- Preclinical models indicate that this modified vaccinia virus selectively replicates in tumor cells, but the biology underlying this selective replication is not well understood. Any studies that can be performed in this clinical trial that might further elucidate the mechanism of action of this virus should be undertaken.

- Although the modified vaccinia virus has been administered safely in a previous trial, implementation of standard operating procedures will enhance the safety of vector administration. In particular, it is important to use procedures that will minimize generation of aerosols when preparing the vector for administration. This can be accomplished by using a biosafety cabinet, or if one is not available, using ethanol soaked gauze to trap any aerosols generated. All healthcare workers should be properly trained in these procedures. In addition, healthcare workers who will be administering this vector, or interacting with participants who have received the vector, should understand the CDC contraindications to vaccinia vaccination. Any worker with such a contraindication should not participate in these activities.

Ethical, Social, and Legal Issues

- The informed consent document contains several complex terms, such as “dose cohort,” that should be simplified. Also, any references to administration of the modified vaccinia virus as a “treatment” should be removed.

H. Committee Motion 8

Dr. Zaia summarized the recommendations expressing the comments and concerns of the RAC. Although no official motion was made, the RAC approved these summarized recommendations. The single vote for both protocols was 15 in favor, 0 opposed, 0 abstentions, and 2 recusals.

XV. Discussion of Human Gene Transfer Protocol #1103-1095 entitled: A Phase I/II Study of the Safety and Feasibility of Administering T Cells Expressing Anti-EGFRvIII Chimeric Antigen Receptor to Patients with Malignant Gliomas Expressing EGFRvIII

Principal Investigator: Steven A. Rosenberg, M.D., Ph.D., NCI
Additional Presenters: Steven A. Feldman, Ph.D., NCI; Howard A. Fine, M.D., NCI; Carolyn M. Laurencot, Ph.D., NCI; Richard A. Morgan, Ph.D., NCI
RAC Reviewers: Drs. Badley, Chiocca, and Fan *(via teleconference)*

A. Protocol Summary

This study will be performed in research participants who have glioblastoma multiforme (GBM) with no other effective treatment options available. Epidermal growth factor receptor (EGFR) overexpression leads to unregulated cell growth and malignant transformation. Increased EGFR signaling via overexpression or mutation is the most frequent genetic alteration associated with GBMs, occurring in 40 to 50 percent of these tumors. The most common EGFR mutant is the EGF receptor variant III (EGFRvIII), which harbors an inframe deletion of exons two through seven. This deletion results in a truncated extracellular ligand binding domain, but renders the protein constitutively active in a ligand-independent fashion. EGFRvIII expression has been shown to enhance tumorigenicity, promote cellular motility, and confer resistance to radiation and chemotherapy. EGFRvIII is present in 24 to 67 percent of GBMs but not in normal tissue, making it an attractive target for therapy. The investigators have developed an experimental procedure for treating patients with cancer that uses their own blood cells genetically modifying their T cells to express a chimeric antigen (CAR) receptor that targets EGFRvIII A CAR consists a single chain from the variable reqions of the heavy and light chains of a monoclonal antibody that is attached to the signaling domains in T cells. These modified T cells have the ability to recognize antigen on the basis of an antibody that signals the T cell to mediate antitumor activity. These anti- EGFRvIII T cells are grown in the laboratory and would infused into research participants.

The investigators have engineered peripheral blood lymphocytes (PBLs) from two donors with the EGFRvIII CAR and have cocultured these transduced cells with glioma tumor stem cells. The EGFRvIII CAR-engineered cells from both donors produced interferon gamma following coculture with the tumor stem cell lines. These results further support the use of EGFRvIII CAR-engineered T cells as a potential immunotherapy for glioma patients.

The primary objectives of this study are to determine the side effects of this experimental treatment and to determine the six month progression-free survival of research participants. The secondary objectives of this study are to determine the survival of the infused cells in the participant's body and to evaluate radiographic changes after dosing.

This study will be conducted in two phases. The first phase will determine a dose that can be given safely to participants. The second phase will determine if the experimental treatment is able to result in a six month progression-free survival of 30 percent rather than the historical value for recurrent glioblastoma of 10 percent (based on a recent study). Individuals will be accrued to both the Phase I and Phase II portions of the trial in two groups: research participants with recurrent malignant glioma requiring steroid use at the start of this trial and research participants with recurrent malignant glioma not requiring steroids at the start of this trial. Up to 160 individuals may participate in this experimental study.

Initially, lymphocytes will be collected through leukapheresis. The lymphocytes will be grown in the laboratory and transduced with the anti-EGFRvIII CAR genes. Once these cells are grown in the laboratory, research participants will be given chemotherapy (cyclophosphamide and fludarabine) for 7 days to suppress the immune system. One to 4 days after the chemotherapy, all participants will be given the experimental cells intravenously in a 20- to 30-minute timeframe, followed within 24 hours with intravenous interleukin 2 (IL-2) every 8 hours for up to 15 doses, depending on tolerance. Participants will be given appropriate medications to treat the side effects of this dosing regimen and to prevent infection secondary to the immune suppression caused by the chemotherapy.

Participants will return to the NIH after four to five weeks to have their tumor(s) evaluated. If the tumor shrinks, participants will continue to return to the NIH to have their tumors evaluated.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. Key issues included the utilization of a novel target for this third-generation design CAR. While EGFRvIII is a mutated EGFR and the investigators' preclinical data demonstrated that T cells expressing this anti- EGFRvIII CAR specifically targets that mutant, the RAC believed that the potential for activation of the anti-EGFRvIII CAR T cells by EGFR expression on normal tissues deserved further discussion.

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

Noting that this study will yield important information, Dr. Badley asked the investigators whether individuals enrolled in this study would be allowed to receive radiotherapy followed by temozolomide – the standard of care for gliomas – during the study. In the work of other investigators using EGFRvIII-specific CAR in a mouse model, where inhibition of intercranial tumor growth was observed, he asked whether off target effects of the CAR-expressing T cells were noted and whether off target effects of the CAR to be employed in this study had been noted in preclinical animal models. In the Phase I dose escalation study, Dr. Badley noted that participants would not receive preconditioning with nonmyeloablative cyclophosphamide and fludarabine and would not receive high-dose IL-2 following CAR-transduced T-cell administration, but in the Phase II study such preconditioning and IL-2 will be included. He asked the investigators to explain why this difference is proposed, especially given that the toxicities of the CAR-transduced T cells might be related or possibly altered by the presence of preconditioning and/or IL-2. Dr. Badley suggested that the Phase II arm include only individuals who would receive steroids; doing so would reduce this second arm of the Phase II study, thus reducing the time required to complete the study, reducing the cost, and potentially exposing fewer research participants to an as-yet-unproven experimental treatment.

Dr. Chiocca asked the investigators to provide justification for inclusion of patients with a Karnofsky Performance Status (KPS) of 60 or more, noting that most protocols require a KPS of at least 70 because individuals with a lower score perform poorly. Dr. Chiocca inquired as to whether individuals with previous implantation of Gliadel or use of the Novocure electrical fields system would be excluded from participation in this study, and why history of coronary and/or cerebral ischemia is an exclusion criterion. He asked the investigators to provide evidence that initially diagnosed EGFRvIII tumors remain EGFRvIII positive at recurrence. Dr. Chiocca requested clarification as to whether participants on steroids during the trial could be removed from steroid use in the midst of the protocol if they are clinically performing well, and whether participants initially not on steroids would be able to be placed on steroids if clinically required – and how both situations would affect subsequent data analysis. In addition, Dr. Chiocca requested that the investigations include a statement that the RAC/OBA would be able to review the results of the Phase I trial before accruing to Phase II commences.

Dr. Fan inquired about whether the EGFRvIII CAR vector has been tested for efficacy or toxicity in any animal tumor models. He asked the investigators to provide information on how the backbone provides advantages to the CAR in terms of enhanced effectiveness or survival of the CAR T cells, and whether there are theoretical concerns that this design could enhance risk. Dr. Fan wondered about the rationale for the starting dose of 1x107 CAR T cells and whether any preclinical data informed that choice.

C. RAC Discussion

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

- Dr. Roizman asked to what extent the tumor cells are dependent on EGFRvIII for survival, and whether it is possible that, as a consequence of this experimental therapy, the investigators would simply select cells that are no longer dependent on expression of EGFRvIII.

- Dr. Zaia asked the investigators about their rules for de-escalation in case a dose-limiting toxicity is encountered, especially in the first cohort, which is planned to include only one individual.

D. Investigator Response

1. Written Responses to RAC Reviews

To clarify inclusion/exclusion criteria, the investigators stated that research participants must have failed previous therapies such as radiotherapy and temozolomide, must be at least four weeks past radiation therapy, and cannot be treated with other agents while on this protocol. They must be at least six weeks from nitrosoureas, four weeks from temozolomide, three weeks from procarbazine, two weeks from

vincristine, and four weeks from last bevacizumab administration. In addition, they must be at least four weeks from other cytotoxic therapies and two weeks for non-cytotoxic agents (such as interferon and tamoxifen), including investigative agents.

Two publications reported using anti-EGFRvIII CAR-transduced T cells in mice that had received intracranial glioma xenografts. Neither report described any off target toxicity associated with the treatment. EGFRvIII is a tumor-specific mutation that has never been reported in normal tissues, so it is unlikely that ontarget/offtumor toxicity will be observed.

Both the Phase I and Phase II studies propose to use the identical preconditioning and IL-2 administration. Specifically, all research participants will receive a non-myeloablative but lymphocyte-depleting preparative regimen consisting of cyclophosphamide and fludarabine, to be followed by intravenous infusion of *ex vivo* tumor reactive, EGFRvIII CAR gene-transduced peripheral blood mononuclear cells (PBMCs), plus intravenous aldesleukin.

The protocol was designed to evaluate the potential response to EGFRvlll CAR dosing in research participant populations with and without co-administered steroids. Because the investigators do not believe it is possible to predict which of the two populations would respond best to the proposed dosing, they would like to retain the current design that includes both groups in Phase II.

Regarding using a KPS cutoff of 60, the investigators explained that more than 30 intramural NCI IRB-approved glioma Phase I and Phase II clinical trials and the vast majority of NCI brain tumor consortia trials use that KPS cutoff. They want to maintain the same criterion, since they intend to compare their clinical outcome in this protocol to historical data based on these prior trials.

The investigators agreed that excluding potential patients with a prior history of Gliadel placement is a good idea since, as a foreign body, it could invoke and/or potentiate some unknown immunological reaction. However, they do not believe there is any medical or scientific rationale to exclude potential participants with a prior history of treatment using Novocure's electrical field system. History of coronary and/or cerebral ischemia is listed as exclusion criteria due to the toxicities associated with high-dose IL-2 therapy.

The investigators stated that they are not aware of any data suggesting that EGFRvlll-positive glioblastomas recur as EGFRvlll-negative tumors, except possibly in the face of anti-EGFRvIII immunotherapeutic interventions (i.e., vaccine) in the interim. Given that EGFRvIII is thought to instill a more aggressive and invasive phenotype, EGFRvlll-positive cells are expected to predominate at the time of recurrence.

If a participant's cells do not expand to sufficient quantities to be placed in the currently accruing cohort, that person will be treated with the lower cell number and will be enrolled in the appropriate cohort for the number of cells. Participants have completed the lymphodepleting chemotherapy regimen by the time the final cell count is known, and the investigators believe it would be inappropriate to withhold treatment with the cells at this point since the participant has already experienced the risks from lymphodepletion. The FDA has requested a starting dose of $1x10^7$ cells in other recent cell therapy protocols and the investigators have seen stable disease in some individuals treated with this dose of cells, and therefore they believe that this dose is justified.

Volumetric criteria have been evaluated for assessing response to therapy and patient outcome in trials of malignant gliomas and have never been shown to be superior to bidirectional or even unidirectional measurements, so volumetric criteria are not routinely included in their assessments. By definition, true pseudoprogression is a self-limited event within the first four weeks to eight weeks following the completion of radiation and progressive radiographic; clinical worsening defines true progression. Radiographic worsening following the induction of a potent immunologic response against the glioma is expected to be self-limited, whereas true tumor progression will not be. That distinction is the reason they have chosen a six-month progression-free endpoint as the primary measure of efficacy, rather than radiographic response.

Regarding whether the vector has been tested for efficacy or toxicity in animal tumor models, the investigators stated that they do not believe that immune-deficient mice are an informative animal model to support or negate human clinical trials. Toxicity studies would be invalid due to the lack of EGFRvIII expression in normal tissue. Efficacy in these immunodeficient xenograft models would not predict success in humans due to the lack of endogenous lymphocytes – both effector and negative cells are absent in the mice.

Multiple independent publications have reported that incorporation of 4IBB signaling domains enhances the *in vivo* cell persistence and effectiveness of CAR-transduced cells in murine tumor treatment models. There have been no reports documenting that incorporation of these signaling domains in CAR vectors is a safety concern.

The starting dose is one log lower than what has been shown to be effective in patients treated with CD19-specific CARs. The investigators believe this dose to be the most appropriate starting cell number for this first-in-humans trial.

2. Responses to RAC Discussion Questions

Dr. Fine explained why the primary endpoint is not radiographic response but rather is progression-free survival. The investigators' assumption is that, by six months, the inflammatory effect will have begun to die down. By six months, tumor progression without further dosing will be obvious compared to the appearance of an acute inflammatory response. In the unique situation of an ongoing inflammatory response for six months, other clinical manifestations would be evident to indicate an inflammatory response for that length of time.

Dr. Rosenberg explained that antigen variant loss is possible, but that it appears that this particular mutation constitutively turns on the protein kinase activity of these cells and therefore it is likely that the mutation and the constitutive activation are essential for the malignant phenotype. In that sense, it might represent an ideal tumor antigen if the tumor cannot survive unless it has the constitutive activation of the protein kinase. Dr. Fine added that, if this protocol eradicates the EGFRvIII expressed in cells and selects for a subpopulation that only expresses the wildtype EGFR, the investigators likely will have conferred a significant benefit to the research participants. Significant clinical efficacy will likely be seen, because the vast majority of tumor cells will have been eradicated and there will be selection for a less proliferative invasive population of cells.

Regarding dose de-escalation for the first cohort if toxicity is encountered, Dr. Rosenberg stated that the investigators have not written a de-escalation plan into the protocol. The initial dose of $1x10^7$ is such a small dose of cells and they have not seen toxicity at that low dose. However, he agreed to include in the protocol that if toxicity is attributable to the cells at the $1x10^7$ dose the dose will be reduced to $3x10^6$. However, he stated that such a cell number is nearly invisible in a test tube, so if toxicity occurs at the $1x10^7$ dose the investigators might not de-escalate the dose to the lower numbers.

E. Public Comment

No comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Dr. Zaia stated that the RAC had no existing concerns or comments regarding the design of this protocol because of the investigators' responses to the individual RAC members' written comments and because their presentation at this RAC meeting addressed all of the Committee's concerns. He congratulated Dr. Rosenberg and his team for an outstanding protocol.

G. Committee Motion 9

Although no formal motion was made, the RAC voted to recommend that there were no remaining concerns with regard to this protocol. The vote was 16 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XVI. Day 2 Adjournment

Dr. Zaia, RAC Chair, adjourned Day 2 of the June 2011 RAC meeting at 5:35 p.m. on June 8, 2011.

XVII. Day 3 Call to Order and Opening Remarks

Dr. Zaia, RAC Chair, called Day 3 of the June 2011 RAC meeting to order at 8:00 a.m. on June 9, 2011.

XVIII. Discussion of Human Gene Transfer Protocol #1104-1100 entitled: A Phase I Study of the Treatment of Recurrent Malignant Glioma with rQNestin34.5v2, a Genetically Engineered HSV-1 Virus, and Immunomodulation with Cyclophosphamide

Principal Investigator:	Antonio Chiocca, M.D., Ph.D., Ohio State University Medical Center
Sponsor:	Antonio Chiocca, M.D., Ph.D., Ohio State University Medical Center
RAC Reviewers:	Dr. Fong, Ms. Mallino, and Dr. Yankaskas
Ad hoc Reviewer:	Xandra Breakefield, Ph.D., Massachusetts General Hospital

Because this protocol was presented by Dr. Chiocca, a RAC member, Dr. Corrigan-Curay explained the OBA policy for this situation. When a RAC member presents a protocol, it is OBA policy that that person excuses himself/herself from the protocol presentation; this is not required by NIH ethics rules however. After discussions, the OBA agreed that Dr. Chiocca would be the best person to present this protocol. Dr. Corrigan-Curay described the unique process for this situation. Dr. Chiocca would present his protocol and then the RAC would go through the reviews. Reviewers and the RAC members were requested to focus their questions on the science and any technical questions. After those questions were answered, Dr. Chiocca would leave the room and reviewers and RAC members would discuss and formulate recommendations.

Drs. Bartlett, Chiocca, and Roizman were recused from discussion of this protocol due to conflicts of interest.

A. Protocol Summary

Treatments for the most malignant type of brain tumors (malignant glioma/glioblastoma multiforme [GBM]) fails to provide long-lasting control, with 50 percent of afflicted patients dying within 15 months of diagnosis. A variety of experimental treatments have been tested. One area of experimental therapy involved the use of oncolytic (tumor-killing) viruses, whose infection and replication into tumor cells has been engineered for tumor-selectivity. The oncolytic virus modified for this protocol is based on herpes simplex virus type 1 (HSV-1), a pathogen that is widespread in humans. The genetically modified HSV-1 was rendered glioma-selective through the following manipulations:

1. One of the genes that encodes for a viral protein (ICP6) has been removed. Without this gene, HSV-1 has to utilize factors within the infected cells to grow and replicate efficiently. The investigators have shown that such factors exist in cells that are mitotically active or that have a defect in a cellular gene (p16) that is missing in most gliomas.
2. The two copies of the viral gene that encode for a protein (ICP34.5) needed for robust viral growth in an infected cell have also been removed, and one copy was reinserted under control of a nestin promoter that is also selectivein adult human gliomas.

These two manipulations allow the new HSV-1 (designated as rQNestin34.5v.2) to be relatively selective in destroying gliomas specifically, and not normal brain cells; which has been confirmed in cultured cells and in animal models.

One additional manipulation allows rQNestin34.5v.2 to be highly effective in injected gliomas. Animals (and likely humans) are able to rapidly counteract HSV-1 in the brain through their initial immune responses. Brain cells, such as microglia, and systemic immune cells, such as natural killer (NK) cells and macrophages, can blunt and effectively ensure that viral replication and its undesirable consequences on normal brain does not occur. Such initial innate host defenses also exist in the context of gliomas injected with rQNestin34.5v.2 that curtail the biodistribution of the virus within the tumor and limit its therapeutic effectiveness. In animal models, the investigators have discovered that a single dose of cyclophosphamide (CPA), a commonly utilized agent that modulates the immune response, administered two days before rQNestin34.5 significantly enhances viral biodistribution, replication, and efficacy, therefore effectively reducing the dose of virus required to produce a survival effect by two orders of magnitude.

Given this background, the investigators propose to utilize rQNestin34.5v.2 with CPA in clinical trials for human malignant gliomas. The first proposed trial will be in individuals who have failed all other standard-of-care treatments and whose gliomas are recurring/progressing. If the tumor is thought to be resectable, research participants will undergo injection of rQNestin34.5v.2 into the resected cavity of the tumor; if the tumor is not resectable, the agent will be injected following a biopsy confirming recurrence of the tumor. All participants will receive one dose of intravenous cyclophosphamide, 2 days before injection of rQNestin34.5v.2 to provide immunomodulation and improve the intratumoral biodistribution of the agent.

Since the trial is designed to find the maximum tolerated dose that is not toxic, cohorts of three participants will be enrolled for injections with increasing doses of the agent. When the maximum dose is determined, three participants who have been diagnosed with a resectable tumor will receive an intratumoral injection of the agent, followed four days to eight days later by tumor resection to obtain the tumor tissue and show that the agent is distributing well. The tumor cavity will then be reinjected with the agent. Except for injection of the agent and administration of CPA, all treatments in the trial are considered to be standard of care.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential for off target effects on nestin-positive neuronal stem cells. This oncolytic herpes virus is constructed for robust replication in tumor cells; in a CPA immunologically modified state, the potential for off target replication in neural stem cells containing low levels of nestin could increase.

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

Dr. Fong asked the investigators to respond to the following questions:

1. What is the preclinical toxicity data for combined use of rQNestin34.5 and CPA?
2. Are there nontumor tissues in human adults that might trigger Nestin-34.5?
3. Should parallel cohorts be performed for HSV Ab+ and HSV Ab- participants because of the possibility of a difference in toxicity and efficacy between these two groups?
4. How will the investigators distinguish between the physical effects of injection from viral infection in the MRI studies?
5. For how long will participants be required to use birth control and what type?
6. Should HSV shedding be assessed in the first days after injection instead of the planned assessment on day 28? Should assessment of HSV by vaginal or rectal swab be performed sooner than the planned day 28?
7. CPA is given 2 days before injection of virus; should new baseline blood counts be obtained immediately prior to injection of virus?

The investigators stated that if the DLT is not reached, the maximum tolerated dose (MTD) would be the highest dose reached in this study; Dr. Fong suggested a term other than MTD be used to describe this dose. He asked for clarification of the stopping rules since this protocol proposes to halt the trial if one or more participants experience a DLT but, in classic Phase I design, DLT in one of three participants would lead to enrollment of three more participants at the same dose to be sure that the DLT was not serendipitous. Dr. Fong also suggested a standard operating procedure for use of antivirals in case of toxicity, to ensure that antivirals are used to protect participants and to prevent inadvertent non-indicated use that might mask potential efficacy.

Ms. Mallino stated that the abstracts as presented are clearly stated and easy to understand, and the proposed clinical protocol is thorough and well organized. She noted a few typographical errors in the proposed informed consent document and she requested clarification of the number of blood draws suggested in month nine and month 12, but otherwise she found the text of the document acceptable.

Dr. Yankaskas noted that the rQNestin34.5v.2 virus was generated by removing the green fluorescence protein gene and that it is important to know whether this virus infects tumor and/or normal cells in humans *in vivo*. He asked the investigators what methods were utilized to analyze the biodistribution of virus and to distinguish infections of tumor and normal cells in tissues excised post virus injection in Arm A of this protocol. CPA will be given 2 days prior to tumor biopsy or resectioning, and rQNestin34.5v.2 will be injected if the biopsy reveals the presence of high grade or malignant tumor cells; he asked whether cyclophosphamide administration is the standard of care for patients who may not receive the virus if the biopsy does not identify tumor cells and whether the potential toxicity is justified in these individuals. Dr. Yankaskas requested that the investigators explain the distribution of virus injected into the brain or tumor with the proposed technique, which is 0.1 ml per approximately 1 cm^3 volume and leaving the needle in place for one minute after injection. He suggested that descriptions of the protocol be simplified to improve communication with potential participants and their families, and that information about collection of samples from saliva and semen or vaginal secretions (to assess rQNestin34.5v.2) be included in the informed consent document.

Ad hoc reviewer Dr. Breakefield asked the investigators to indicate what information in the literature supports lack of nestin expression in neural stem cells in normal brain. She suggested saving frozen brain tumor tissue from participants for genotyping of glioma-associated mutations, in case the genotype is found to affect the response to this experimental intervention. Dr. Breakefield asked about the extent of nestin-positive tumor cells within a tumor in GBM, the current data on effect of CPA treatment prior to virus injection in tumors in animal models, and the perceived involvement of steroids on the proposed treatment. She suggested that the possibility of death, even though unlikely, should be listed in the informed consent document as a possible serious adverse event.

C. RAC Discussion

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

- Dr. Zaia suggested redefining the stopping rules to be initiated if Grade IV – rather than Grade III – toxicities are encountered. It is likely that many Grade III toxicities, such as somnolence and ataxia, will occur with these neurological symptoms, therefore using Grade III might make it difficult to complete the study. He also suggested defining encephalitis within the protocol, so it is clear at what point the stopping rules would be initiated if encephalitis occurred. Dr. Kohn agreed with Dr. Zaia that relatively common postoperative complications should not be a cause for stopping this trial.

- Dr. Kohn asked whether the virus could be toxic if it is amplified in the nestin-positive cells follow by migration into nestin-negative cells.

- Dr. Kanabrocki asked about the investigators' plans for monitoring participants with regard to biohazard concerns.

D. Investigator Response

1. Written Responses to RAC Reviews

Preclinical toxicity data for combined use of rQNestin34.5 and CPA are not yet available, as these experiments are currently in progress.

With the planned intraglioma or periglioma administration of rQNestin34.5v2, the most significant concern for nontumor expression of ICP34.5 triggered by nontumor activation of the nestin promoter would be in surrounding brain tissue. In addition to their own data with postmortem immunohistochemistry showing relative lack of nestin immunopositivity in the subventricular zone, the investigators noted very little evidence, if any, in the literature of nestin expression in the neural stem cell niche of older human adults.

As to whether parallel cohorts should be performed for HSV antibody-positive and HSV antibody-negative participants, the investigators stated that published data from previous clinical trials do not appear to show that seronegative patients experience toxicity differently than seropositive patients.

Birth control, using barrier-type methods, should be practiced by research participants until there is no evidence of shedding in blood, saliva, or semen/vaginal secretions.

Regarding the stopping rules based on only one participant experiencing a DLT, the investigators explained their rationale for inclusion of Grade III instead of Grade IV. Grade III toxicities in nervous system, psychiatric, or infectious disease categories (that would be listed as an SAE) are severe events necessitating medical intervention, limiting self-care daily living activities , and/or causing significant impairment of cognitive performance from baseline. The investigators were not comfortable continuing the trial if a single participant suffered such an event that was attributed to the trial agent.

The investigators agreed to add a standard operating procedure for use of antivirals in case of toxicity.

Because all participants will have been previously diagnosed with malignant glioma, the investigators stated that it is highly unlikely that new growth observed on MRI (i.e., recurrence) is another type of tumor. The only differential could be radionecrosis. Although CPA is not a frontline chemotherapy agent in patients with malignant glioma, it has been used for tumor recurrence as salvage chemotherapy.

The proposed injection method has been employed extensively in other studies. Because these injections were performed into the tumor bed after tumor resections, there was no tissue available to examine; when tumors recurred several months later, the injection sites would not exist since the recurrent tumor would have distorted and destroyed the previous injected tissue architecture.

The investigators offered to have their Comprehensive Cancer Center IRB, which includes lay representation, provide recommendations related to sections of the protocol where language should be simplified.

Consistent with their own postmortem immunohistochemistry data revealing relative lack of nestin immunopositivity in the subventricular zone, there is very little evidence, if any, in the literature of nestin expression in the neural stem cell niche of older human adults. The areas that appear to contain nestin positive neurons are not areas from which gliomas commonly arise.

An active biorepository program exists at the investigators' Comprehensive Cancer Center and, generally, most resected gliomas are stored frozen and are cultured through this program.

Reports indicate that nestin immunopositivity correlates with tumor grade. Grade IV gliomas possess highly positive immunostaining throughout the tumor. One investigator estimated in 69 high-grade gliomas that 58 percent of tumors contained between 30 and 60 percent staining positive cells, and 33 percent of tumors had more than 60 percent staining positive cells.

In animal trials, researchers have shown that CPA leads to increased survival when administered to mice with brain tumors treated with oncolytic HSV (oHSV) and allows for reduction of effective doses of the oHSV; this result is likely due to CPA's effect on rapid reduction of microglial/macrophage activation. The investigators have also shown that CPA's effect limits NK cell activation/infiltration in glioma models.

Similar to other trials of oHSV injection in recurrent malignant gliomas and because this recurrent glioma population is likely steroid dependent, the investigators do not plan to exclude potential participants based on their steroid intake.

2. Responses to RAC Discussion Questions

Regarding whether the virus could be toxic if it got into nestin-negative cells, Dr. Chiocca responded that mere entry into the cell might cause a cell death; however, the virus would not replicate so the infection would not spread in normal brain. Some collateral damage to normal tissue is a possibility – this result is seen in some of the animal data – but surgery or radiation causes the same kind of damage to immediately surrounding tissues.

Regarding biohazard concerns, Dr. Chiocca explained that the investigators are following typical BL-2 parameters and personnel will use traditional bodily fluid precautions. At present, the protocol does not include specific information about isolating research participants; viruses in glioblastoma patients have not shown evidence of person-person transmission. Infection control procedures will be utilized.

The investigators do not know if injecting the virus into a large tumor will result in a large level of biodistribution. That possibility will be tested in this trial.

Radiation is not administered using the gamma knife. GBM patients usually are administered external field radiation, in which the tumor bed is radiated at a distance of 2 cm to 3 cm. The gamma knife is used as salvage radiation at recurrence in some cases; it has not been shown to affect overall progression-free survival.

E. Public Comment

Dr. Borror noted that the informed consent document uses overly optimistic wording in the section that describes the virus, how it was formed, and what the investigators believe the virus will do, referring to the virus as treatment and as being able to kill cells. Stating her concern that these statements could foster therapeutic misconception, she suggested they be revised.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- The primary goal of this trial is to assess the safety and determine the MTD of the vector when injected into recurrent malignant gliomas. The MTD will be determined by a single DLT, and the study will be stopped when one participant experiences a DLT. A DLT is defined as any Common Terminology Criteria for Adverse Events v4.0 Grade IV or Grade V toxicity, except for Grade IV lymphocyte, neutrophil, or white blood cell decrease. In addition, the study will be stopped and the MTD defined by a single Grade III toxicity for encephalitis, encephalomyelitis, meningitis infections/infestations, as well as any Grade III toxicity for nervous system disorder, which includes somnolence, ataxia, and depressed level of consciousness. The rationale for using a single Grade III event from these categories is that they are severe events necessitating medical intervention, limiting self care for activities of daily living, and/or causing significant impairment of cognitive performance from baseline. However, in this population, which is undergoing surgery for a recurrent brain mass, the investigators should consider whether a DLT in two out of six patients should be required before the trial is stopped and the MTD determined. Additionally, given the difficulty of separating the etiology of a single neurosurgical toxicity from

the administration of the virus, the investigators should reconsider whether it is appropriate to use Grade III neurological events to define the DLT, or whether certain Grade IV neurological events should be considered a DLT. Any major neurological events should, however, be reviewed with the data and safety monitoring board.

- This is a replication competent virus that has been modified to target malignant glioma cells. While replication of the virus is critical to its oncolytic function, replication may lead to adverse events that will require administration of appropriate antivirals to kill the virus. It is important to have a specific plan outlining the clinical indications for antivirals, which antivirals will be administered, and the doses. This will help assure that antivirals are employed in a standard manner within the trial.

Ethical, Social, and Legal Issues

- The informed consent document contains several overly optimistic descriptions of the virus. For example, the virus is referred to as treatment and there is a reference that the virus is able to kill tumor cells. These statements might foster therapeutic misconception and should be revised to emphasize the experimental nature of this approach.

G. Committee Motion 10

Dr. Zaia orally summarized the recommendations expressing the comments and concerns of the RAC. Although no formal motion was made, the RAC voted to approve these summarized recommendations. The vote was 12 in favor, 0 opposed, 0 abstentions, and 3 recusals.

XIX. Update on Protocol #0707-864 entitled: An Open Label Dose-Escalation Study of a Self Complementary Adeno-Associated Viral Vector (scAAV2/8-LPl-hFIXco) for Gene Therapy of Hemophilia B

Presenters: Arthur Nienhuis, M.D., St. Jude Children's Research Hospital, and Katherine A. High, M.D., Children's Hospital of Philadelphia *(via teleconference)*
Additional Presenter: Amit Nathwani, M.B., Ch.B., University College London Cancer Institute, United Kingdom *(via teleconference)*

Dr. Zaia provided a history of this protocol. He noted that the RAC publicly reviewed this protocol in December 2007 with the discussion focusing on the novelty of the vector and the proposal to use immunosuppressive drugs in the event a research participant experienced an immune-related transaminitis. In a previous trial for Hemophilia B, T-cell responses to AAV capsid had resulted in transient transaminitis and elimination of the transduced cells. Immune responses to AAV was the topic of a safety symposium convened by the RAC in June 2007.

A. Presentation by Dr. Nienhuis

Dr. Nienhuis provided an updated clinical summary for this trial that has been enrolling participants since January 2010.

Hemophilia B is a deficiency of clotting Factor IX that affects about 1 in 30,000 males. Its frequent spontaneous hemorrhage can be lethal; during the course of this trial, one potential participant experienced a lethal central nervous system bleed. The rationale for gene transfer includes that longterm expression of only one protein is required, moderate increases greater than 1 percent will impact symptoms, and tight regulation of expression is not necessary. AAV does not cause human disease and requires a helper virus for replication, which adds another dimension of safety. It can transduce nondividing cells and was thought less likely to stimulate an immune response. Animal studies indicate that long-term transgene expression is possible. After a single intravenous injection, Dr. Nienhuis stated that expression in monkeys has persisted for 7 or 8 years.

To date the investigators have treated six participants – two at the low dose, two at the intermediate dose, and two at the high dose. Participants range in age from 27 to 64 years. They all have severe hemophilia, several have missense mutations, all had severe disease as reflected by the need for prophylaxis, and each had spontaneous hemorrhages throughout the year, with their distribution of hemorrhages as expected in hemophiliac patients with severe disease. Vector shedding was detected in a number of fluids but clears quickly within two to three weeks without reoccurrence. There is a primary immune response – IgM spikes and then IgG increases over time, which is expected in individuals who had not previously been sensitized to the vector.

Dr. Nienhuis showed data from the six participants. Participants 1, 2, 3, and 4 all have received intermittent prophylaxis, have gone without prophylaxis for several months, and have a Factor IX level of 2 percent to 3 percent, which reflects vector-derived Factor IX production. A Factor IX level of 1 percent to 2 percent is adequate to prevent most bleeding episodes. The fifth participant, who was dosed five months ago, developed asymptomatic transaminitis with a 10-fold increase in transaminases and his Factor IX level declined from six percent to a range of two-three percent. In accordance with the protocol, the investigators started participant 5 on prednisolone when the transaminases increased; it was tapered over 8 weeks and currently he is off steroids and his Factor IX level is at 3 percent. The sixth and final participant was dosed in December 2010 and he also developed a small increase in his transaminases at around days 60 and 70. As a result of the investigators' experience with participant 6, they are amending the protocol such that if the transaminase doubles and then increases again the next day, prednisolone will be started even though the transaminase may remain within the normal range. Participant 6 has now been tapered off prednisolone completely for four weeks. His Factor IX level is in the nine percent range, where he is at minimal risk for bleeding and would be considered a very mild hemophiliac.

In summary, six participants received scAAV2/8-LPl-hFIXco**,** two each at three dose levels. Peripheral vein administration of scAAV2/8-LP1-hFIXco was well tolerated without any acute side effects; persistent, stable Factor IX expression occurred in all six participants; four of the six participants were able to stop prophylaxis and two were able to extend the interval between prophylaxis; and vector was present in excretions/secretions but cleared by two-three weeks. Preliminary results are encouraging and a protocol amendment is in progress to expand the high-dose cohort.

B. Presentation by Dr. High

Dr. High presented the results of the immune studies involving the six research participants in this trial. The aim of the analysis of PBMCs was to monitor T-cell responses in the context of the study. The hypothesis from the immunologic standpoint was that the use of a more efficient vector might achieve therapeutic levels of transgene expression at lower vector doses and might evade the immune responses seen in the previous AAV-2 trial.

The immune studies used interferon gamma ELISpot on PBMCs from the participants before and at a series of time points after vector infusion to assess T-cell responses to three groups of antigens. One group of antigens was derived from the AAV-8 capsid sequence, one group was derived from the human Factor IX transgene product, and a third group was derived from alternate open reading frames within the cDNA of their vector. All six participants showed no documented response to any peptides from Factor IX or any peptides derived from the alternate open reading frame within the cDNA cassette; all of the responses were to the AAV-8 capsid.

The results from the two participants in the low-dose cohort were negative. The results from the two participants in the mid-dose cohort showed T-cell responses to peptides derived from the AAV capsid but neither of these participants had elevated transaminase levels. For the high-dose participants, the results for participant 5 for the first six weeks showed no or minimally detectable T-cell responses; however, at eight weeks a response was seen and then a gradual tapering off occurred as he started on high-dose prednisolone. For participant 6, there were initially no detectable responses; unfortunately, the investigators encountered poor cell viability when the sample arrived from London, so they do not have a complete dataset on participant 6.

In summary, the lowest doses of vector that were tested were not associated with detectable T-cell responses to the capsid in the PBMCs. Fractionation of the cells showed CD4 and CD8 T-cell responses to capsid following infusion of an AAV vector at doses greater than or equal to $6x10^{11}$ vector genomes per kilogram. Dose escalation to $2x10^{12}$ was associated with elevation of liver enzymes concomitant with the detection of capsid-specific T cells in PBMCs in one research participant. No response was elicited in any of the participants to Factor IX transgene product or to alternate open reading frame translation products. These findings are in agreement with the findings from the previous AAV-2 Factor IX study for hemophilia B for vector delivered to the liver, and the findings suggest that, for liver-directed gene transfer, there may be a threshold vector dose at which detection of capsid T-cell responses are clinically relevant to the outcome of gene transfer. One of the most important conclusions from the study of the high-dose participants was that the clinical data suggests that the response, if it occurs, can be blunted by a course of prednisolone that is initiated when the transaminases begin to rise.

C. RAC Discussion

Dr. Zaia asked why T-cell activation occurs two months after exposure to the antigen with the high dose and occurs almost immediately with the lower dose. Dr. High responded that timing of responses has been an issue of continuing interest for everyone involved in AAV-mediated, liver-directed gene transfer. Dr. High and her colleagues have no explanation for the response variability they witnessed in their trial, although part of the difference between humans and animal models might be attributable to the fact that humans are natural hosts for the wildtype virus from which this vector is derived and might harbor memory T cells that are reactivated when the vector is infused. However, she acknowledged that the answer to this question is speculation and is not known.

In light of the importance of the high-dose results, Dr. Sarzotti-Kelsoe inquired as to whether it might be possible to rescue any more cells from Participant six. Dr. High explained that the investigators are receiving more cells from that participant, but at later time points; the batch of cells that was of poor quality was from weeks four through eight.

Dr. Bartlett asked the investigators to comment on the fact that the Factor IX levels do not seem to be related either to the rise in transaminases or to the T-cell responses in Participant six. Dr. Nienhuis explained that there may have been a prednisolone effect as well, because when the prednisolone was tapered the Factor IX level slid back to a baseline of three percent. About half of the Factor IX expression was lost in this individual as a consequence of the transaminitis.

Dr. Zaia requested that the investigators comment on the decrease over time in the use of supplementary Factor IX. Both Dr. Nienhuis and Dr. High explained that hemophiliacs typically infuse themselves at home, often in preparation for activity or trauma. As they gain confidence that they are producing Factor IX, they extend their infusion intervals. There is more extensive use of prophylaxis in the adult hemophilia population in the United Kingdom than in the United States, where most adults are managed “on demand” – they treat themselves when they bleed.

Dr. Kohn asked whether the investigators are considering giving prophylactic prednisolone courses; if that were done for one or two weeks soon after infusion, it might be possible to blunt this response and not suffer the kind of loss to a participant in which their Factor IX level drops from the early T-cell response. Dr. Nienhuis responded that the investigators have rewritten that portion of the protocol and would prefer to find out how frequently such a reaction might occur, rather than administering prednisolone on a routine basis. Dr. Nathwani concurred, noting the investigators believe it is important to dose a few more participants to determine if this finding is consistent in the high-dose cohort and whether or not those participants require steroids.

Dr. Yankaskas inquired as to whether the investigators were considering focusing their enrollment at the intermediate dose, since they did not exhibit the adverse event reporting for the high-dose group but did have substantial benefits in their Factor IX levels. Dr. Nienhuis responded that the investigators have chosen to propose to expand the high-dose cohort. They want to have as their goal a Factor IX level of

five percent, rather than the two percent or three percent experienced at the intermediate-dose level. Dr. Nathwani agreed, stating that a recent publication in *Blood* showed that life expectancy in hemophiliac patients can be improved significantly if levels of Factor IX expression are above five percent, compared to lower levels.

Noting that participants might cease to produce therapeutic levels of Factor IX, Dr. Zaia wondered if the investigators are prepared to amend the protocol to redose them. Dr. Nienhuis stated that the investigators have said in the informed consent document that they would hope to treat with an alternative serotype; a serotype 5 is being developed as a potential possibility. Dr. Nathwani elaborated that solid data in animals indicate that it will be possible to use a different serotype; however, switching serotype and redosing these individuals to maintain Factor IX expression should be done in the context of a different study.

XX. Closing Remarks and Adjournment

Noting that the presentation by Drs. High and Nienhuis represented the second time at this meeting that the RAC had been presented with protocol updates, Dr. Zaia encouraged these updates to continue at future RAC meetings.

Dr. Zaia thanked the RAC members and the OBA staff, and adjourned the June 2011 RAC meeting at 10:15 a.m. on June 9, 2011.

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

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

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

This Minutes document will be considered formally 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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Recombinant DNA Advisory Committee

Attachment I: RAC Roster
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Attachment II
Public Attendees

[This list includes only those individuals who are not elsewhere identified.]

Audrey Alivio, Neurologix, Inc.
Ira Berkaven, CBER/FDA
Gwen Binder, Adaptimmune, Ltd.
Roman Brantsom, MSKCC
Kevin Curran, MSKCC
Heather Embree, Lentigen Corp.
Jane Fountain, NINDS/NIH
Kevin Harrington, Institute of Cancer Research (United Kingdom)
Nancy Kernan, MSKCC
Arifa Khan, CBER/FDA
Carrie Lamenat, NCI/NIH
Joshua Lu, FDA
Francesco Marincola, Magnuson Clinical Center, NIH
Maritza McIntyre, ReGenX Biosciences
Loren Mell, University of California, San Diego
Rick Morgan, NCI/NIH
Laissa Ouedraogo, Henry M. Jackson Foundation for the Advancement of Military Medicine, Division of Acquired Immunodeficiency Syndrome
Harriet Robinson, GeoVax, Inc.
Valerie Rusch, MSKCC
Christine Sapan, Neurologix, Inc.
Reinhard von Roemeling, Genelux Corp.
Chris Yankaskas
Jack Yanovski, NICHD/NIH

Attachment III
Abbreviations and Acronyms

AIDS	acquired immune deficiency syndrome
allo-BMT	allogeneic bone marrow transplantation
B-ALL	B-cell acute lymphoblastic leukemia
BDNF	brain-derived neurotrophic factor
BL	biosafety level
BNIT	BN ImmunoTHerapeutics, Inc.
BNP	B-type natriuretic peptide
BWG	Biosafety Working Group
CAR	chimeric antigen receptor
CLL	chronic lymphocytic leukemia
CPA	cyclophosphamide
CRPC	castration-resistant prostate cancer
CT	computed tomography
DLT	dose-limiting toxicity
DHHS	U.S. Department of Health and Human Services
EBV	Epstein-Barr virus
EBV-CTLs	Epstein-Barr virus specific cytotoxic T-lymphocytes
EGFR	epidermal growth factor receptor
EGFRvIII	EGF receptor variant III
FAQs	frequently asked questions
FDA	Food and Drug Administration, DHHS
GAD	glutamic acid decarboxylase
GBM	glioblastoma multiforme
GLP	Good Laboratory Practices
GM-CSF	granulocyte macrophage colony-stimulating factor
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft versus host disease
HIV	human immunodeficiency virus
HSCT	hematopoietic stem cell transplantation
HSV-1	herpes simplex virus type 1
HVTN	HIV Vaccine Trials Network
IBC	institutional biosafety committee
IgG	immunoglobulin G
IL-2	interleukin 2
IND	investigational new drug
IRB	institutional review board
KPS	Karnofsky Performance Status
LASV	Lassa virus
LCMV	lymphocytic choriomeningitis virus
MANTIS	Medtronic Acute Neurosurgical Therapeutic Infusion System
MC4R	melanocortin 4 receptor
miRNA	microRNA
MRI	magnetic resonance imaging
MSKCC	Memorial Sloan-Kettering Cancer Center
MTD	maximum tolerated dose
MVA virus	modified vaccinia Ankara virus
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NICHD	National Institute of Child Health and Human Development, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*

NK	natural killer
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
oHSV	oncolytic HSV
PBLs	peripheral blood lymphocytes
PBMCs	peripheral blood mononuclear cells
PD	Parkinson's disease
pfu	plaque-forming units
PSA	prostate-specific antigen
PTLPD	post-transplant lymphoproliferative disease
PWS	Prader Willi Syndrome
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
SAE	serious adverse event
SPA	Special Protocol Assessment (FDA)
STN	subthalamic nucleus
UPDRS	Unified Parkinson's Disease Rating Scale
VATS	video-assisted thoracic surgery
VMH	ventromedial hypothalamus
WBCs	white blood cells