RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 15–17, 2010

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

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

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

September 15–17, 2010

The Recombinant DNA Advisory Committee (RAC) was convened for its 122nd meeting at 1:30 p.m. on September 15, 2010, at the Hilton Hotel and Executive Center, Rockville, Maryland. Dr. John Zaia (Acting RAC Chair) and Dr. Howard Federoff (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 1:30 p.m. until 4:35 p.m. on September 15, from 8:15 a.m. until 5:30 p.m. on September 16, and from 8:30 a.m. until 11:00 a.m. on September 17. The following individuals were present for all or part of the September 2010 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation
Jeffrey S. Bartlett, Nationwide Children's Hospital/The Ohio State University
Michael J. Buchmeier, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center *(Days 2 and 3 only)*
Yuman Fong, Memorial Sloan-Kettering Cancer Center
Norman Fost, University of Wisconsin, Madison *(via teleconference on Day 1, in person on Days 2 and 3)*
Jeffrey P. Kahn, University of Minnesota
Joseph A. Kanabrocki, The University of Chicago
Walter J. Koch, Jefferson Medical College *(Days 2 and 3 only)*
Donald B. Kohn, David Geffen School of Medicine
Margaret Mallino, University Park, Maryland
Anna C. Mastroianni, University of Washington School of Law *(Days 2 and 3 only)*
David A. Ornelles, Wake Forest University School of Medicine
Andre Rogatko, Cedars-Sinai Medical Center
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 Medical Center/New York Head & Neck Institute
James R. Yankaskas, The University of North Carolina at Chapel Hill
John A. Zaia, City of Hope

Office of Biotechnology Activities (OBA)

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

Ad Hoc Reviewers, Presenters, and Speakers

Wilson W. Bryan, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (FDA)
Natasha J. Caplen, National Cancer Institute, NIH
David Claxton, Penn State Milton S. Hershey Medical Center
Ron Crystal, Weill Cornell Medical Center
Hildegund C.J. Ertl, The Wistar Institute
Jeff Fairman, Juvaris BioTherapeutics, Inc.
Audrey Penn, National Institute of Neurological Disease and Stroke, NIH

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Nonvoting Agency Representatives

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

NIH/OD/OBA Staff Members

Chezelle George
Linda Gargiulo
Robert Jambou
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal
Tom Shih

Attendees

There were 35 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, Acting RAC Chair, called the meeting to order at 1:30 p.m. on September 15, 2010. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on August 23, 2010 (75 FR 51827). 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 seven protocols, discussion of pediatric ethics in clinical trials, a proposed amendment to a clinical trial that was discussed publicly at a prior RAC meeting, and an update on another previously discussed clinical trial.

Dr. Zaia introduced the new RAC members by name, affiliation, and research interests: Drs. Badley, Kohn, Rogatko, Strome, and Sarzotti-Kelsoe. The continuing 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. Minutes of the June 16–17, 2010, RAC Meeting

RAC Reviewers: Drs. Buchmeier and Roizman

Dr. Buchmeier noted that the June 2010 RAC meeting minutes were "amazingly accurate" and summarized the document by reference to each agenda item. Dr. Roizman agreed that the minutes document captures a sense of the discussions that took place at the June 2010 RAC meeting.

A. Committee Motion 1

Approval of the June 2010 RAC meeting minutes was moved by Dr. Roizman and seconded by Dr. Buchmeier. The RAC voted unanimously by voice vote to approve the June 16–17, 2010, RAC meeting minutes.

III. Discussion of Human Gene Transfer Protocol #1007-1050 entitled: Phase I Study of an Active Immunotherapy for Asymptomatic Phase Lymphoplasmacytic Lymphoma with DNA Vaccines Encoding Antigen-Chemokine Fusion

Principal Investigator:	Sheeba Thomas, M.D., University of Texas, M.D. Anderson Cancer Center
Additional Presenters:	Larry Kwak, M.D., Ph.D., University of Texas, M.D. Anderson Cancer Center
RAC Reviewers:	Drs. Fong, Sarzotti-Kelsoe, and Yankaskas

A. Protocol Summary

Available therapies for lymphoplasmacytic lymphoma (LPL) provide no survival advantage if started before signs or symptoms of end-organ damage develop; hence, current recommendations are to follow a program of observation while patients are in the asymptomatic phase of disease. A well-tolerated therapy that lengthens this asymptomatic phase, without inducing cross resistance to available agents, would improve the quality—and perhaps length—of patients' lives by extending the interval before chemotherapy is required to maintain disease control.

The investigators hypothesize that by developing a DNA-based vaccine against proteins called idiotypes that are only found on the cancer cells, it might be possible to cause a patient's immune system to recognize these cells as foreign and therefore kill them. They propose to evaluate the safety and feasibility of using such a vaccine in a Phase I (first-in-human) clinical trial of asymptomatic LPL patients.

Studies with an idiotype (Id) protein vaccine have demonstrated specific antibody and T cell responses in animal lymphoma models, and in clinical trials of patients with follicular lymphoma. Investigators now have the ability to clone lymphoma-derived immunoglobulin (Ig) variable region genes and combine them into a single chain antigen format (scFv), allowing more efficient development of novel genetic vaccine delivery systems. DNA vaccines, encoding fusions of idiotype antigen and chemokines, target delivery of tumor antigen to antigen-presenting cells (APCs), leading to activation of selective host immune effectors.

Using idiotypic determinants of a B-cell lymphoma's surface immunoglobulin as a tumor-specific marker, it may be possible to develop patient-specific chemokine-idioptype fusion DNA vaccines that induce an immune response against LPL. By activating the host immune system against the tumor antigen, the investigators postulate that disease control of asymptomatic phase lymphoplasmacytic lymphoma can be maintained.

Chemokine-fused scFv DNA vaccines have been tested in two different murine B-cell lymphomas, A20 and 38C-13, which express surface IgG2a and IgM, respectively. Vaccines were generated by genetically fusing A20 or 38C idiotype single chain with chemokines including murine MIP3α. These tumor models and vaccine constructs are similar to the proposed human treatment. Gene expression was confirmed by the development of idiotype-specific immune responses and vaccine-induced tumor protection. In these experiments, the tumor-free surviving mice were monitored for at least 3 months after vaccination without showing toxic effects.

The investigators now propose to evaluate the safety and feasibility of using such a vaccine, in a phase I clinical trial of patients with asymptomatic phase lymphoplasmacytic lymphoma. Participating patients must be able to provide a lymph node sample of at least 1.5cm in the long axis, or a bone marrow aspiration sample providing at least 5 million CD20 and/or CD38+ cells (approximately 10 ml). The lymphoma immunoglobulin variable regions, VH and VL will be cloned for each patient from the tumor cells by RT-PCR using consensus primers. After DNA sequencing and verification, the mature V region

sequences will be cloned as a single chain antibody gene (scFv - VH and VL are fused in frame with a short linker sequence) into a pUC19 plasmid with kanamycin resistance marker. After verification of the sequence of the scFv, the scFv gene will be fused in frame to the 3' end of the human CCL20 (MIP-3α) gene via a spacer sequence. The sequence of the inserted scFv-chemokine fusion gene will then be verified, and the plasmid DNA will be amplified in *E. Coli*. The plasmid DNA will subsequently be purified from E. Coli according to Good Manufacturing Practices standards, and tested for sterility and endotoxin contamination prior to its use in any patient. The entire vaccine preparation procedure usually takes 4-5 weeks, starting from cloning VH and VL from tumor cells.

Patients will receive 3 doses of DNA vaccine encoding a fusion protein consisting of autologous lymphoma immunoglobulin variable regions (scFv) and a chemokine, human CCL20 (macrophage inflammatory protein-3 alpha - MIP-3α). The proposed study is designed to have 2 dosing cohorts. Cohort 1 patients will receive a dose of 500 µg, and Cohort 2 patients will receive a dose of 2500 µg. Doses will be administered intramuscularly, at 4 week intervals (+/- 3 business days).

The safety profile and maximum tolerated dose (MTD) of the vaccine will be determined in this patient population, as well as the vaccine's ability to generate immune responses in humans. If successful, this vaccine could shift the current paradigm of clinical management for LPL patients in the asymptomatic phase.

B. Written Reviews by RAC Members

Four RAC members voted for in-depth review and public discussion of the protocol due to the novelty of the transgene and the disease.

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

Dr. Fong asked whether clinical markers during the LPL asymptomatic period could predict impending progression to symptomatic disease and how a future trial might be designed to prove clinical efficacy of these vaccines if they are proven safe. He asked about the success rate of vaccine production and whether the bone marrow aspirate and production process would be repeated if needed. Dr. Fong wondered whether the success rate of lymph node harvest would be higher if more than one lymph node were harvested. He queried as to whether the birth control specifications were related to the expectation that research participants will achieve systemically significant levels of MIP3α that would produce deleterious effects on pregnancies. Dr. Fong requested that the investigators explain what will happen to the participants' plasmid DNA after production of the vaccine and whether there is a standard operating procedure for storage of the plasmid DNA. He asked for an explanation as to why Hepatitis B and Hepatitis C carrier states are exclusion criteria and why the investigators propose to test for cytomegalovirus (CMV), West Nile virus, and human T-cell leukemia virus (HTLV).

Dr. Sarzotti-Kelsoe asked why the investigators believe LPL is a good candidate for this experimental treatment. Noting that each vial of participant-specific vaccine would be labeled to include individual identification, she queried as to whether this information is considered protected. She requested an explanation as to why patients' history of Hashimoto's thyroiditis is not listed in the exclusion criteria, the rationale for testing patient sera for anti-MIP-3α antibodies, and the expected retention rate of patients upon enrollment in this clinical trial. She asked the investigators to discuss whether a general guideline exists as to how many LPL cells need to be detected in a bone marrow aspirate or lymph node patient sample to proceed with the purification and cloning of the tumor antigen from such samples.

Dr. Yankaskas asked for an explanation of the basis for selecting the 500 and 2,500 microgram doses, the safety of the lowest dose, and whether any benefits could be expected for doses larger than 2,500 micrograms. He asked for a description of the toxicities observed in the follicular lymphoma idiotypic (Phase III) vaccine trials. Noting that a number of possible mechanisms might explain beneficial or adverse vaccine effects that could affect future development of this and future DNA vaccines, Dr. Yankaskas requested an explanation as to how these mechanisms might be discerned in this study. He

also asked the investigators to explain the basis for limiting the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids in this trial.

C. RAC Discussion

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

- Dr. Rogatko asked why only two doses were selected.
- Dr. Fong requested more information about whether and how the investigators plan to store template DNA for future generation of the experimental product if the research participant relapses during the 15-year followup period, especially if the experimental product is found effective.
- Noting that the proposed study would intervene when a patient is asymptomatic with the hope of adding up to 2 years of increased disease-free survival, Dr. Strome brought up the possibility that this experimental treatment is likely also to increase the risk for infections. Given that scenario, he asked why a patient would opt to participate in receiving the proposed vaccine.
- Dr. Zaia asked whether the investigators would be able to follow a tumor marker to show a biological effect on the tumor.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that LPL is a good candidate for this experimental treatment for two reasons. To produce an idiotype vaccine, the presence of a tumor-specific monoclonal immunoglobulin is a necessary feature. As LPL is a type of B-cell lymphoma characterized by monoclonal immunoglobulin production, this disease is an ideal candidate for study. In addition, LPL is an indolent lymphoma, related to follicular lymphoma, with which the investigators have prior vaccine experience. The indolent nature of LPL allows time for an immune response to be realized, whereas a more aggressive lymphoma might require initiation of chemotherapy before the immune system has time to generate a response.

The two vaccine doses were chosen based on the dosing used in previously reported DNA vaccine clinical studies. These doses were tested in trials of DNA vaccines and were developed for infectious disease and cancer indications. In one idiotype DNA vaccine trial for research participants with follicular lymphoma, vaccination with 1.8 mg of plasmid DNA did not cause acute or longterm toxicities in more than 4 years of followup. Another DNA vaccine, developed against HIV, was studied at doses of 0.5 mg, 2.0 mg, and 4 mg; no additional benefit was noted among those participants who received the 4 mg dose of plasmid DNA compared with those who received the lower doses. However, those who received the 4 mg dose reported increased pain at the injection site compared with those who received lower doses. For these reasons, the investigators have elected not to study doses higher than 2.5 mg and believe the doses selected for this clinical trial are within a safe range.

The Phase III follicular lymphoma vaccine trial results indicated that both the idiotype vaccine and the control were safe and well tolerated. There were no statistically significant differences in the frequency or types of adverse events (AEs) observed between the groups. Grade 1 or Grade 2 AEs were common in both groups but Grade 3 and Grade 4 AEs were rare; there were no vaccine-related deaths. The most common AEs were injection site reactions with erythema and induration lasting for a few days after each vaccination. Fatigue, myalgia, headache, arthralgia, and bone pain were other common AEs observed in both groups.

The investigators explained that their idiotype DNA vaccine differs from those reported previously because the idiotype antigen is being genetically fused with a chemokine. The presence of corresponding chemokine receptors on immature dendritic cells allows *in vivo* specificity in targeting

dendritic cells with the tumor antigen, thereby potentiating vaccine-induced antitumor immunity. Based on their preclinical data, the investigators expect that the vaccine will induce tumor cell death primarily by activating tumor-specific effector T cells. Vaccine-induced production of anti-idiotype antibodies will also help to eradicate tumor cells that express the antigen on their cell surface. In addition, the vaccine might reduce the number of regulatory T cells that have a negative regulatory impact on the effector T cells.

While the 2-week cutoff for NSAID use was chosen somewhat arbitrarily, the investigators' goal was to limit the use of NSAIDs as much as possible and reasonable as these drugs can suppress the immune response induced by the vaccine. In addition, steroids have an anti-lymphoma effect and may impede the ability to determine whether an observed clinical response is due to steroids or the vaccine. In response to Dr. Fong's comments, the investigators agreed to add the chronic use of these agents to the list of exclusion criteria, with the exception that participants would be allowed to receive 81 mg of aspirin for cardiovascular prophylaxis. (This dose of aspirin was allowed in the Phase III follicular lymphoma vaccine trial and did not appear to affect the outcome.)

Since each vaccine is individual-specific, identification is important to safety. To comply with the Joint Commission on Accreditation of Healthcare Organizations' National Patient Safety Goal, MD Anderson requires that each vial be labeled with two identifiers. In addition to identifying each vial with the participant's last name and first initial, the investigators reported that each vial must also include on the label the individual's M.D. Anderson Cancer Center Medical Record Number. The protocol will be amended to reflect this information.

The investigators explained that they do not plan to exclude participants with Hashimoto's thyroiditis because, in the event that worsening of Hashimoto's thyroiditis occurs after vaccination, it may be readily treated with thyroid replacement therapy. In addition, unlike other autoimmune disorders, properly treated Hashimoto's thyroiditis does not cause symptoms, does not affect quality of life, and does not cause cosmetic issues.

The investigators explained that they expect a high retention rate for this clinical trial because patients choosing to participate will be motivated to pursue a potential treatment for asymptomatic phase LPL. Following the experimental treatment phase of the study, participants will be required to follow up at M.D. Anderson Cancer Center every 2 months for 1 year, which is on par with the followup schedule that is recommended to a patient who was not participating on this clinical trial. In the Phase III follicular lymphoma vaccine trial, no participants dropped out of the study by choice, with the only "retention" issue related to those participants for whom a vaccine could not be manufactured (less than 10 percent of participants).

The rationale for testing patient sera for anti-MIP-3α antibodies is to exclude the possibility of autoimmune adverse effects against the self-chemokine protein. In both rodent and nonhuman primate models that received DNA vaccines encoding chemokine-antigen fusions, the investigators did not detect such antibodies in vaccinated animals. This result suggests that induction of autoimmunity against the chemokine motif in the vaccine formulation is unlikely to occur in humans. During the course of the clinical trial the investigators will monitor patient sera for anti-MIP-3α antibodies for information only.

Noting that currently there are no markers that predict impending progression to the symptomatic phase of LPL, the investigators explained that a future trial, designed to prove clinical efficacy, would need to compare the median progression-free interval of research participants receiving the vaccine with that of a control group of asymptomatic-phase LPL participants. To create as uniform a cohort as feasible, participants entering both arms of that trial would need to be newly diagnosed with asymptomatic-phase LPL.

The investigators explained that in the Phase III follicular lymphoma vaccine trial only one lymph node was harvested, which yielded several times the number of lymphoma cells needed for vaccine production. Therefore, they do not believe that more than one lymph node per participant will need to be harvested for vaccine generation in this trial.

The birth control specifications in this trial are in place solely as a precaution, since it is the first time that this vaccine will be studied in humans. The investigators do not expect systemically significant levels of MIP-3α and have no prior knowledge (and have not found any literature) that would lead them to expect a deleterious effect of the vaccine on pregnancies. The birth control specifications are in place for the duration of the experimental phase and up to 4 weeks after the final vaccination, for a total time frame of approximately 4 months.

Regarding the exclusion of potential participants who are hepatitis B or hepatitis C carriers, the investigators explained that available data suggests that levels of CD8-positive T cells can vary during the course of hepatitis B infection. Similar findings might be seen in individuals with hepatitis C. Since T-cell response is integral to immune response to the DNA vaccine, individuals with hepatitis B and hepatitis C, in any stage, have been excluded from study participation.

While the investigators will not exclude potential participants who have been exposed to CMV, West Nile virus, and HTLV, they propose to collect exposure information to evaluate whether prior exposure to these viruses affects participants' immune response to the DNA vaccine.

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

With regard to why LPL was chosen, Dr. Thomas explained that the investigators can easily identify a monoclonal immunoglobulin and Dr. Kwak has prior experience with other low-grade lymphomas and follicular lymphoma. If the treatment concept works in LPL, it could be translated to other diseases.

Dr. Thomas explained that the dose levels were chosen based on experience from two other studies. In one of those studies an HIV vaccine was given at 0.5 mg, 2 mg, and 4 mg; the two lower doses were found to be as efficacious as the highest dose, which had increased side effects. Therefore, the two lower doses were chosen for this proposed trial because the investigators believe they are as likely to produce response but without incurring additional side effects. The dose decision was also based on financial reasons, as this vaccine is expensive to manufacture.

Regarding product release criteria, Dr. Kwak explained that the final product would be developed with the guidance of the FDA as well as the good manufacturing practices (GMP) laboratory that will make the product, whether a commercial vendor or the MD Anderson GMP facility. The current plan is to have the MD Anderson GMP facility make these products. The FDA has reviewed the manufacturing process for products for two clinical trials made by the MD Anderson GMP facility and no corrective actions were required as a result of either of those reviews.

Dr. Kwak noted that anti-chemokine antibodies remain only a theoretical concern, as none were encountered in any of the investigators' rodent studies. In a limited nonhuman primate study conducted with a similar but not identical DNA vaccine, the investigators also looked for and did not find anti-chemokine antibodies. However, even though they do not believe these antibodies will be present, the investigators intend to assay for them for information only.

Dr. Thomas stated that the investigators have no reason to expect or be concerned that the proposed vaccine would increase the rate of infections.

Dr. Kwak explained that the mechanism of the vaccine effect would be measured using state-of-the-art quantitative assays for T-cell immunity against the antigen, using either ELISpot or intracellular cytokine staining, with the hope of correlating the results with clinical outcome. Determining that correlation might be challenging in this trial because there will be only 12 research participants. However, immune assay results from the investigators' Phase III vaccine trial will be extrapolated to this trial.

In response a query about storing more template DNA for future generation of the experimental product, Dr. Kwak stated that the investigators would like guidance from the RAC on this question. He noted that for the Phase III protein vaccine study the sponsor has stored samples of the starting tumor cells. However, in the majority of cases, even though starting material will be stored, the investigators would prefer to re-biopsy at the time of relapse in case the antigen has changed.

Dr. Thomas responded to a question about dosing participants while they are asymptomatic by pointing out that symptomatic LPL patients often receive nucleoside analogs and alkylating agents, sometimes in combination. That treatment could affect T-cell function and then an immune response could not be able mounted. Such a scenario would be avoided by evaluating the experimental regimen in asymptomatic research participants, with the hope of delaying time to progression.

Dr. Thomas stated that tumor markers will be followed in serum and urine as well as via computed tomography (CT) scan imaging to look for a biological effect on the tumor.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical/Trial Design Issues

- A traditional dose escalation design is being used; however, only two doses are planned: 0.5 mg and 2.5 mg. If a dose-limiting toxicity is observed at the higher dose, the dose escalation rules would dictate de-escalation to the lower dose. While it is not anticipated that the higher dose would lead to a dose-limiting toxicity based on experience with DNA vaccines, the investigators should consider incorporating the option to test an intermediate dose and determine what that dose might be in advance.

- This study involves the administration of participant-specific plasmid vaccines, which will incorporate lymphoma immunoglobulin variable regions (V_H and V_L) from each participant. It is conceivable that, given the long latency of the asymptomatic phase of this disease, future administration(s) of this vaccine may be desirable. A plan should be developed for the preservation (for example, storage of plasmid-containing bacterial stocks) of these participant-specific constructs. The informed consent document should then discuss the option of long-term preservation of the participant's specific study agent.

- A previous vaccine study for individuals with follicular lymphoma involving administration of a participant-specific protein idiotype as an antigen showed promising clinical results. Of note, a statistically significant difference was observed in the number of infections in the group receiving the protein idiotype compared to the control group. Even though the infections observed were Grade 1 or Grade 2 by the Common Terminology Criteria for Adverse Events, it is important to implement appropriate monitoring in this study for these types of events.

Ethical/Social/Legal Issues

- While some participants will require only a single bone marrow biopsy to stage and obtain cells to produce the vaccine, other participants may require more than one biopsy. The informed consent document should discuss this possibility.

G. Committee Motion 2

Dr. Zaia summarized the RAC recommendations that will be included in the letter to the investigators. It was moved by Dr. Yankaskas and seconded by Dr. Fong that the RAC approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Discussion of Human Gene Transfer Protocol #1007-1052 entitled: Pilot and Feasibility Study of Hematopoietic Stem Cell Gene Transfer for Wiskott-Aldrich Syndrome

Principal Investigator:	Sung-Yun Pai, M.D., Children's Hospital Boston, Harvard Medical School
Additional Presenters:	Anne Galy, Pharm.D., Ph.D., Genethon (France); Luigi D. Notarangelo, M.D., Children's Hospital Boston, Harvard Medical School; and Adrian J. Thrasher, Ph.D., M.D., FRCP, MRCPCH, FMedSci, Great Ormond Street Hospital for Children (United Kingdom)
Sponsor:	David A. Williams, M.D., Children's Hospital Boston, Harvard Medical School
RAC Reviewers:	Drs. Kahn, Kohn, and Ross

A. Protocol Summary

Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency caused by mutations in a single gene, WAS, mapping to chromosome Xp11.22-Xp11.3 and coding for the Wiskott- Aldrich Syndrome Protein (WASP). WASP is a critical regulator of actin signaling with expression limited to hematopoietic cells, and thus is required for multiple functions including T cell activation, dendritic cell migration and podosome formation, and B cell terminal development and function. WAS is characterized by micro-thrombocytopenia, recurrent infections, eczema and associated with a high incidence of auto-immunity and of lymphoid malignancies. Classic or severe WAS, is generally observed in patients with nonsense mutations or insertions/deletions resulting in frameshift or splice-site mutations or missense mutations and resulting in unstable protein.. With few exceptions, WASP-negative patients have classical disease. Affected patients have a severely reduced life expectancy.

Currently, the only curative option for WAS patients is hematopoietic stem cell transplantation (HSCT). This treatment is most successful when an HLA-identical sibling or matched unrelated donor is available and results in correction of microthrombocytopenia and immune dysfunction, even when stable mixed chimerism occurs. However, even patients undergoing matched HSCT can suffer from considerable morbidity and mortality due to graft versus host disease (GVHD) and many patients lack an HLA-identical donor. The outcome of mismatched related HSCT is consistently poor with survival of approximately 50%. Gene transfer is an attractive alternative treatment for WAS. Successful gene transfer using autologous gene-corrected HSC would overcome clinical complications linked to GVHD and its treatment. Furthermore, in contrast to allogeneic HSCT, gene transfer would not be limited by the availability of compatible donors. Several lines of evidence indicate that partial reconstitution with gene corrected cells may be sufficient to ameliorate the disease.

The protocol proposes a pilot and feasibility study of *ex vivo* gene transfer using a lentiviral vector (LV) to transduce autologous bone marrow derived CD34+ HSC. Cells will be infused into patients conditioned with cytoreductive chemotherapy. Collaborating investigators in Europe have developed a LV encoding the human WAS cDNA under control of the WAS promoter and pseudotyped with the Vesicular Stomatitis Virus glycoprotein (VSVg) envelope. This w1.6_hWASP_WPRE (VSVg) LV (abbreviated as w1.6W) has been shown to be efficacious in both *in vitro* and *in vivo* pre-clinical models. Safety, including cellular toxicity, insertional mutagenesis and tumor formation, has been studied by a number of methods including: 1) a sensitive *in vitro* transformation assay, 2) toxicity studies in transduced human CD34+ cells, 3) examination of the insertional pattern in transduced murine cells, and 4) long-term observation and secondary transplant studies in mice. In the United States, the investigators plan to enroll 5 boys with classic WAS who lack a matched related or unrelated donor. Parallel studies using the same LV produced in the same facility, Genethon, will be conducted in London, UK (5 subjects) and Paris, France (5 subjects). The primary objective will be to demonstrate feasibility and safety. The secondary objective will be to assess therapeutic efficacy.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of the protocol. Key issues included a new pediatric disease using an integrating vector in CD34+ cells, raising the possibility of insertional mutagenesis. While preclinical data indicates lentiviral vectors are likely to be less mutagenic than the retroviral vectors that led to leukemias in the X-SCID trials, the lack of clinical data to support this theory means that the risk/benefit assessment for such protocols deserves careful consideration. While the investigators have attempted to define a population that is least likely to benefit from the proven alternative therapy (BMT), the RAC believed that the risk/benefit aspects of this experimental approach in pediatric research participants should be discussed.

Three RAC members provided written reviews of this proposed pilot and feasibility study.

Noting that the informed consent document is well written, in language that is clear and appropriate for the research participant population and with sufficient detail and explanation, Dr. Kahn suggested that the consent should be targeted to the parents of potential participants and the language made consistent for that audience. He requested that the investigators include wording to indicate that this is a Phase I trial and that it represents first-in-human research. Dr. Kahn also suggested enlarging the brief statement regarding storage of blood for future research tests, including the envisioned future uses, whether it would be used for general research related to WAS, and whether samples would be stored in a biobank. If samples will be banked for future unrelated research, he stated that an opt-out provision should be included within the informed consent document.

Dr. Kohn asked the investigators to justify the criteria wherein availability of a 9/10 adult donor excludes participation in this trial but a 5/6 cord blood unit of sufficient cell dosage does not. Noting that the pretransplant conditioning regimen proposed represents about 75 percent of a standard fully ablative conditioning regimen, he requested information about precedent for this regimen and whether it poses an increased risk for incomplete cytoablation and possible persistence of autoreactive cells. Regarding the parallel study sites in Europe, Dr. Kohn asked whether the production of the vector in France using fetal calf serum (presumably not of U.S. origin) would pose any acceptance issues; he also suggested adding how the parallel trials would cross-inform about serious adverse events (SAEs), including reporting of sentinel events in any trial to the appropriate sponsors and oversight entities. He cautioned that participants who have received the gene-modified CD34+ cells and have less than 0.1 percent gene marking at 6 weeks should not be taken off study—and should be followed for the full 15 years—as they still may be at risk of insertional oncogenesis events. With regard to the informed consent document, Dr. Kohn noted that it is well written and strikes a good balance between completeness and readability. He suggested adding the risk of partial immune reconstitution with resultant autoimmunity because autoimmune complications could result in significant morbidity. In addition, he suggested that the informed consent document include information for potential participants about the results from the German trial of retroviral-mediated gene transfer for WAS; preliminary outcomes show positive clinical effects but concerning integration site patterns with the first-generation gamma retroviral vector.

Dr. Ross expressed concern about some of the background data and whether it is sufficient to initiate a human trial, particularly one that would enroll pediatric participants. She asked how likely it would be that a patient with expression of endogenous WAS protein would be included in this study, whether the investigators are concerned that the transgene expression would downregulate endogenous protein expression in these individuals, and whether the investigators intend to perform genotyping and WAS protein expression analysis on potential participants prior to their inclusion in the trial. Noting the strong need for correction in more than just T cells, Dr. Ross requested an explanation for why engraftment in other compartments is considered a secondary endpoint for this trial. Given that the investigators emphasize that patients with WAS older than 5 years do not do well with allogeneic stem cell transplants, she asked the investigators to explain why participants older than 5 years are proposed to be included in this study and whether they believe that engraftment of transduced CD34+ cells would be more successful than allogenic transplants in this population. Noting that the investigators stress that the disease in the knockout mice differs from the clinical disease in humans, Dr. Ross requested that the investigators provide a more comprehensive justification of the use of this vector in humans given the

data in mice. She asked whether it might be possible that the weak WAS promoter, while preventing toxicity, is not sufficiently strong to rescue the phenotype or whether mice are simply not a good model for testing the efficacy of this experimental therapy.

C. RAC Discussion

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

- Dr. Fong asked why three trials were being proposed in three different centers, and whether there would be an overlap in the Data and Safety Monitoring Boards (DSMB).
- Dr. Zaia asked whether the vectors to be used in all three trials are exactly the same and whether the same producer will make them.
- Dr. Kahn suggested that the RAC recommend full harmonization among the three sites for this trial, and queried the investigators as to whether such a recommendation would be helpful.
- Noting that it is possible to optimize trials globally, Dr. Rogatko expressed concern that the investigators would be tripling the cost of the knowledge gained from this trial by conducting three separate trials.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding storage of blood samples, the investigators explained that the blood discussed in the section entitled “What do I have to do if I am in this research study?” refers to study-related blood samples, primarily samples for replication-competent lentivirus and retrospective analysis of insertional mutagenesis, should myelodysplasia or leukemia occur. The section of the protocol entitled “Are there other things I should know about?” refers to banking of remaining samples for possible future use, which could be used for unrelated research or for general research related to WAS. This section includes an opt-out provision.

The investigators agreed that few data allow ranking of the success rates for unrelated donors of various degrees of mismatch. It is possible to extrapolate from leukemia research data that a 9/10 adult bone marrow match and a 5/6 cord blood donor match are equivalent. Justification for excluding potential participants who have a 5/6 cord blood donor is based on the limited published experience with cord blood in WAS and the far greater experience with mismatched unrelated donor BMT for WAS and other diseases; the high rate of reactivation of viral infections in cord blood recipients is especially problematic in patients with WAS. Because there exists few published outcome data for these patients following cord blood transplant and cord blood transplant results in a high rate of infection, the investigators believe it is suitable to offer those patients with a 5/6 cord blood donor the option of enrollment on this trial.

The pretransplant conditioning regimen was chosen to accommodate the restrictions of culture time and to allow administration of cells without the need for cryopreservation. Two lines of data based on prior studies of chronic myelogenous leukemia and WAS patients lead the investigators to believe that cytoablation will be achieved with this regimen—120 mg/m^2 of fludarabine and 12 mg/kg of busulfan—in the majority of cases. However, because of the risk of incomplete ablation and persistence of autoreactive cells, the investigators agreed to modify the informed consent document accordingly.

With regard to bone marrow harvest, the investigators explained that the maximum size of harvests and minimal timing between the proposed harvests were based on the National Marrow Donor Program guidelines, which recommends up to 20 ml/kg with an absolute maximum of 2 liters and a minimum of 4 weeks before re-collection if a second donation is requested. In this trial the investigators believe it is important to have the maximum number of cells for both genetic modification and re-infusion as well as

for backup in case of poor CD34 cell recovery after transduction, given the anticipated ablative nature of the conditioning regimen.

Production of the vector by Genethon in France involves the use of fetal bovine serum (FBS). The FBS is from a geographic region compatible with U.S. Department of Agriculture requirements for importation to the United States. This was not an issue at the meeting with the FDA prior to filing the investigational new drug (IND) application, and all relevant documentation pertaining to the animal-derived components as well as certificates of analysis of all materials will be provided in the IND submission. The investigators do not anticipate any issues with acceptance of the FBS used for this vector.

The investigators agreed that the 6-week time period is premature to remove a participant who shows, off study, less than 0.1 percent gene marking in the peripheral blood. Therefore, the protocol will be modified to indicate that participants who have received transduced cells (with or without backup harvest co-infusion) will be taken off study if they have less than 0.1 percent gene marking on two consecutive time points occurring after the 6-month assessment.

With regard to how the three international trials will interact, information regarding participant safety in the European trials will be transmitted by the European study sponsor (Genethon) to Dr. Williams in a fashion similar to communication with European national agencies. Dr. Williams is expected to report all safety issues to Genethon in the same manner. In addition, the investigators agree that available information regarding the Hannover, Germany, trial conducted by Dr. Christoph Klein should be included in the informed consent document and, at the time of IND submission, they will revise the informed consent document to include any available published data regarding clinical effects and preliminary integration site analysis.

Regarding the likelihood of enrolling WAS protein-expressing participants, the investigators stated that they anticipate that most enrollees will be WAS-protein null. At most 1 of 5 participants are expected to have detectable endogenous WAS protein expression. While possible, there is little data regarding down-regulation or dominant negative forms of WAS.

In this pilot and feasibility study the investigators have restricted the primary endpoints as much as possible to safety endpoints. While multi-lineage engraftment is the goal, the population targeted by this study is composed of individuals who have or are at risk of having severe immune complications of WAS (including autoimmunity and malignancy). Mixed and split chimerism occurs frequently after BMT for WAS, and preservation of donor T cells with loss of myeloid chimerism is a common pattern. Such individuals generally are thought to be protected from T-cell-related complications, including severe infections, autoimmunity, and Epstein-Barr virus lymphoma. Even with low-level myeloid donor engraftment resulting in thrombocytopenia, additional therapies such as splenectomy could be utilized to increase the platelet count to a safe range. For these reasons, the investigators prefer to keep T-cell engraftment as a primary endpoint, with myeloid and other cell engraftment as secondary endpoints.

Patients with WAS older than 5 years have poorer survival after allogeneic transplant, particularly if an unrelated donor is used. As GVHD and complications of its treatment are major factors in this poor survival, engraftment of transduced autologous cells would be expected to be more successful as this strategy obviates GVHD.

The prominent features of WAS in leukocytes in both the knockout mice and in humans are primarily functional deficits (T-cell activation, antibody responses especially to carbohydrate antigens, dendritic cell migration) rather than numerical deficits. Therefore, the investigators have relied on assays of functional reconstitution in mice models to demonstrate the adequacy of transgene expression rather than improvements in overall B-cell, T-cell, or granulocyte numbers. These defects are present in the mouse model and in humans. Thus the investigators believe that the mouse is a good model to test efficacy of this vector, and that the endogenous promoter is sufficient to drive WAS protein expression after gene transfer.

With regard to rescue of the phenotype, the investigators believe the murine model is a good model for testing efficacy via functional *in vivo* reconstitution. In addition to the efficacy evidence of the vector, the data also show correction of myeloid cell function and resolution of autoimmune colitis with the WAS gene transfer approach. These results, obtained with more than 100 treated mice, demonstrate that the 1.6 kb endogenous promoter is sufficiently strong to rescue the WAS cellular phenotype.

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

Regarding the three trials being conducted at three different sites, Dr. Williams explained that it is difficult to conduct a cross-Atlantic trial under one IND, so the investigators have formed a consortium to work on this three-site trial. While the two European sites are under no obligation to report under the U.S. IND, they have agreed to provide information about their SAEs and to report their AEs as if they were obliged to do so under FDA rules. Because the NIH is insistent that their grant money not be used to support European trials, the best arrangement is to have parallel trials with the same protocol and an agreement to collate trial data for reporting purposes and to collate safety data on an ongoing basis during the trial.

Dr. Williams responded that the vectors will come from different lots but will be made in the same facility with the same safety data. The trials are essentially identical, although he added that the U.S. trial site has not gone through full FDA or NIH review yet, which could result in changes to the protocol that will not necessarily be changed in the two European sites. While acknowledging that the various regulatory agencies are aware of the importance of harmonization, Dr. Williams averred that it would be helpful if the RAC reinforced that idea. Full harmonization among the three trial sites is the aim of the consortium formed to conduct this three-site trial. The end result is intended to be a combining of the same trial in multiple participants, with data collected at the trials' end that would be published jointly.

Dr. Williams explained that Genethon is a not-for-profit patient disease organization that has laboratories and GMP facilities.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

<u>Clinical/Trial Design Issue</u>

- The total enrollment planned for the trial in the United States is five participants; however, an additional 10 participants are planned to be enrolled in two parallel European trials that will use the same vector and, as currently planned, the same enrollment criteria and protocol procedures. Given the small population eligible for these trials, having a single oversight body, i.e., a single DSMB, review the data across the three trials should be considered to optimize the conduct and safety of these trials.

G. Committee Motion 3

Dr. Zaia orally summarized the comments and concerns of the RAC. It was moved by Dr. Strome and seconded by Dr. Kohn that the RAC approve these summarized recommendations. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Biosafety Update

Presenter: Dr. Kanabrocki

Dr. Kanabrocki updated the RAC members on the status of some of the changes to the *NIH Guidelines* that have been discussed at past RAC meetings.

With regard to synthetic nucleic acids, a draft *Federal Register* notice will be provided for review to individual members of the Biosafety Working Group as well as to RAC members to ensure that it accurately reflects the final recommendations that were discussed at the June 2010 RAC meeting.

With regard to transgenic rodents, a *Federal Register* notice detailed the proposed changes to Appendix C-VII, which will read:

> Generation of BL-I transgenic rodents via breeding:
>
> The breeding of two different transgenic rodents or the breeding of a transgenic rodent with a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL-1 containment and will be exempt from the *NIH Guidelines* if:
>
> Both parental rodents can be housed under BL-1 containment and neither parental transgenic rodent contains the following genetic modifications:
>
> (a) More than 50 percent of the genome of an exogenous virus from a single Family of viruses; or
>
> (b) A transgene that is under control of a gammaretroviral long terminal repeat (LTR), and
>
> It is anticipated that the transgenic rodent that would result from this mating will not:
>
> (a) Contain more than 50 percent of an exogenous viral genome from a single Family of viruses.

A suggestion was made to change the language slightly for (a) "Incorporation of more than 50 percent of the genome…" and for (b) "Incorporation of a transgene…" to increase accuracy of the descriptions.

Nine comments were received, all of which were supportive of this proposal. One commenter asked for clarification of how much gammaretroviral LTR sequence must be present to not be exempt. In the final *Federal Register* notice, additional explanation will be added to clarify the sufficient gammaretroviral LTR sequence to control expression of the transgene (not smaller fragments of homologous sequence not acting as a promoter).

VI. Update on December 2010 Symposium: "Retroviral and Lentiviral Vectors for Long-Term Gene Correction: Clinical Challenges in Vector and Trial Design"

Dr. Corrigan-Curay discussed a symposium being planned for December 9–10, 2010, that would immediately follow the December 2010 RAC meeting. The draft agenda was provided and a number of speakers are already confirmed. She asked that the RAC members attend this symposium and that comments about the agenda be addressed to the OBA. The agenda will be posted to the OBA web site soon after this RAC meeting.

VII. Day 1 Adjournment

Dr. Zaia, Acting RAC Chair, adjourned Day 1 of the September 2010 RAC meeting at 4:30 p.m. on September 15, 2010.

VIII. Day 2 Call to Order and Opening Remarks

Dr. Zaia, Acting RAC Chair, called Day 2 of the September 2010 RAC meeting to order at 8:15 a.m. on September 16, 2010.

IX. Pediatric Ethics: A Regulatory Framework

Presenter: Dr. Kahn

A. Presentation

Dr. Kahn provided brief context to the history of pediatric ethics as driven primarily by exploitation of individual research participants, noting that events spawned ethical debate that led to regulatory response. Two trials are of particular note: the Tuskegee Syphilis Study (1932 to 1974) in which African-American men were recruited into a trial of the natural history of syphilis and were told they would benefit therapeutically by their participation, which was not the case; and the Willowbrook State School study (mid-1950s to early 1970s) in which institutionalized mentally retarded children were recruited into "the research wing" of the school where they were intentionally dosed with hepatitis A in an effort to better understand the immunology of hepatitis. Both studies came to light in the media and caused public scandal.

Two ethical issues were prominent in these studies. One is that the parents of the Willowbrook children were told that there was no room at the institution unless they were willing to consent to their child's participation in research by living in the research wing. The second issue, which is more common in historical stories related to research, was that participants were harmed without the potential for offsetting medical benefit to them as individuals. As a result of these two infamous studies, in 1974 Congress formed the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (National Commission), which was active for 4 years and focused on protection of human research participants.

Two international codes that do not have the force of regulation or law are influential with regard to these issues. The Nuremberg Code (1948), taken literally, says that research on children is prohibited because research participation requires the voluntary consent of the participant "as absolutely essential." The Declaration of Helsinki (World Medical Association, 1964 and later revisions) states that research involving legally incompetent participants must procure "the consent of the legal guardian."

The core ethical issues with regard to including children in research are:

- Splitting apart of risk and benefit: When individual participants agree to be exposed to risk, sometimes there is potential for offsetting therapeutic benefit to them but sometimes all benefit goes to others, either members of the group from which they come or society in general. In any event, there is a strong duty to protect children from harm.

- Problem of consent: Children lack decision-making capacity, and proxy consent by parents or guardians should be motivated by the best interests of the child.

- When research involves greater than minimal risk and either limited prospect for, or no intended medical benefit to participants, only two choices exist. Either children should be protected from any greater than minimal risk, at the cost of acquiring possible information—there is a strong duty to protect from harm but a much less clear obligation to produce benefit—or some current children can be exposed to risk to promote the well-being of future children—the prospect of great future good outweighs the risks imposed on a few research participants in the present. Regulatory efforts have attempted to allow both choices to exist.

In 1978, the National Commission issued the following recommendations:

- Research is valuable and necessary for the health and well being of children, and can be performed ethically.
- Research must be scientifically sound and significant.
- Studies must first be performed on animals and adults, and older children before infants, if possible.
- Risks must be minimized.
- Privacy and confidentiality must be protected.
- Selection of participants must be equitable.
- Permission of parents and assent of children, where they are capable, must be obtained.
- Increased risk requires potential for offsetting therapeutic benefit to individual participants.

Since 1978, policies have focused on protection, including institutional review boards (IRBs) and prospective review, risk-benefit balancing, and an emphasis on informed consent. Additional protections for research on "vulnerable" participants includes children due to their inherent lack of capacity to consent and the greater potential for exploitation, which effectively means that adults should participate in research before children.

For research involving children, the Code of Federal Regulations (45 CFR 46 subpart D) states that research that is not greater than minimal risk can be conducted on children when there are adequate provisions for assent of the child and permission of the parents or guardians. For research that holds greater than minimal risk, with potential for direct medical benefit to the participants, the risk must be justified by the anticipated benefit to the participants and the anticipated risk-benefit must be at least as favorable to the participants as in available alternative approaches. Research that holds greater than minimal risk without anticipated benefit to participants is permitted only if the risk is a minor increase over minimal risk, the intervention is reasonably commensurate to what participants would experience, and the research is likely to yield generalizable knowledge about or information of vital importance to understanding the participant's disorder or condition.

Research otherwise unapprovable can be appealed through the Section 407 process. In this process, the DHHS Secretary may determine, after consultation with a panel of pertinent experts and opportunity for public review and comment, that the research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of children; the research will be conducted in accordance with sound ethical principles; and adequate provisions exist for assent of participants and permission of parents or guardians.

The key issue is how to balance the protection of children with the advancement of research on children's health. Current regulations are an attempt to manage the competing interests of protection versus access to benefits. What is most needed to determine at the Federal policy level, in IRBs, and in the RAC is how to create equitable access to the benefits of research while providing adequate protection for those who are involved in it.

X. Challenges in the Regulation of Pediatric Clinical Trials

Presenter: Wilson W. Bryan, M.D., Office of Cellular Tissue and Gene Therapies (OCTGT), Center for Biologics Evaluation & Research, FDA

A. Presentation

Dr. Bryan discussed the therapeutic development process, the drug development process, the regulatory process, and the ethical issues and special protections of pediatric studies.

The objective of drug development is to find drugs and biologics that are safe and effective for treating a given indication. Preclinical research and clinical research in Phases I, II, and III represent a rational, orderly process in which each phase builds on the information provided in the step(s) before. It is

important to get the proper information at the preclinical stage to guide the development in Phase I, and for the information from Phase I to guide subsequent studies.

The regulatory process involves submitting an IND that goes to a review team in the OCTGT. The FDA's primary objective in reviewing an IND, no matter what the phase of the investigation, is to assure the safety and rights of the participants. One of the instruments used to assure the safety and rights of the participants is a "clinical hold," which is an order issued by the FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. A Phase I investigation may be placed on clinical hold if the FDA finds that human participants are or would be exposed to an unreasonable and significant risk of illness or injury; that the clinical investigators named in the IND are not qualified; that the investigator brochure is misleading, erroneous, or materially incomplete; or that the IND does not contain sufficient information to assess the risk to participants of proposed studies. Most clinical holds are based on participants being exposed to an unreasonable significant risk or that the sponsor does not provide enough information for the FDA to assess the risk to the participants.

Children are a vulnerable population because they are not able to give true informed consent and, therefore, the FDA asks sponsors to study adults unless it is scientifically necessary to study children. "Scientifically necessary" is determined by assessing whether studies in adults would be relevant, ethical, and feasible, and whether they would substantially accomplish the study goals. If a study in children is scientifically necessary, sponsors are asked to consider studying older children first, because older children generally are better able to give consent than younger children, especially compared to infants.

The OCTGT believes that gene transfer trials generally have more than a minor increase over minimal risk. So the OCTGT regulates gene transfer trials in children with consideration of the principles of 21 CFR 50.52: there must be a prospect of direct benefit to the individual participants and that children may be involved as participants only if the IRB finds and documents that the risk is justified by the anticipated benefit to the participants. To provide evidence of the prospect of direct benefit, the OCTGT often asks IND sponsors to provide proof-of-concept data from preclinical and/or previous human studies.

Regulatory challenges in pediatric clinical trials for the sponsor include:

- Minimizing risks while maintaining prospect of direct benefit and an acceptable risk-benefit ratio—determining what constitutes sufficient evidence of a prospect of direct benefit, what is the appropriate study population, and how the starting dose can be determined.
- Considering available nonclinical data and previous human experience with the product or related products and determining what study procedures are acceptable.
- Considering the risk of the procedure, the benefit (if any) of the procedure to the participant, and the value of the resulting data.

For pediatric studies, the OCTGT asks the sponsor to describe how the study meets the requirements of Subpart D and why the study of children is scientifically necessary.

Regulatory challenges in pediatric trials for the FDA include a determination of when it is appropriate for a pediatric study to be a first-in-human study for a new experimental gene transfer and if adults must be studied to provide initial evidence of safety before proceeding with a study in children and the number of adults that must be studied.

Regulatory challenges in pediatric trials for the IRB include:

- Determining to what degree IRBs defer assessment of the science and ethical/human participant protection issues to other entities (e.g., the FDA, the RAC).
- Assessing how IRBs determine whether early-stage gene or cell transfer studies have a prospect of direct benefit.
- Considering how IRBs help investigators and participants and their families avoid a therapeutic misconception when communicating risks and anticipated benefits.

Because of these questions and to increase the awareness of the scientific, ethical, and regulatory issues, the OCTGT (along with several partners including OBA) is sponsoring a workshop on November 2, 2010, in Bethesda, Maryland. The goal of this workshop is to gather information from stakeholders regarding best practices related to cell and gene transfer clinical trials in pediatrics including evaluating these novel therapeutic products prior to initiating pediatric clinical studies, identifying and minimizing risks associated with the administration of cell and gene transfer products in pediatric studies, obtaining informed consent and assent, and conducting continuing review and oversight of cell and gene transfer products in pediatric studies.

B. RAC Discussion

Dr. Bryan clarified that whether an acceptable risk/benefit ratio exists for children is a different question than whether or not it is scientifically necessary to study children at this time; the FDA currently has an ethical principle that adults must be studied first even if the risk/benefit ratio in children is acceptable. Therefore, the FDA asks sponsors to explain why is it scientifically necessary to study children, not merely whether the risk/benefit ratio is acceptable. Dr. Kahn added that the risk/benefit ratio is not relevant if the benefit will not accrue to the children enrolled in that trial.

Dr. Rogatko mentioned the challenge of therapeutic misconception, especially for people with cancer who have exhausted all known treatment possibilities. These potential research participants see the possibility that this new experimental drug might help them, and they think the probability of success is much higher than what is stated in any informed consent form. Dr. Kahn concurred, stating that the data on therapeutic effect is dismal in Phase I cancer trials, but cancer patients believing they might get therapeutic benefit in a Phase I safety and toxicity study is major reason why people participate in these trials—and is a significant problem.

Dr. Fost explained that the “adults first rule” is an FDA policy that is based on a reasonable set of arguments. However, some people think the policy is too restrictive and that the policy makes it very difficult to develop new technologies that might benefit children.

Dr. Fost opined that characterizing all Phase I trials as having absolutely no prospect of medical benefit is not accurate. The prospect of direct benefit to participants is extremely low but not zero. For a child who is desperately ill with a life-threatening disorder, waiting years for the adult studies may not be in the child’s interest. This issue surfaced in the early days of the AIDS epidemic in which people with HIV did not want to wait for results from the placebo-controlled randomized trials.

XI. Proposed Amendment on Protocol #0904-977 entitled: Direct CNS Administration of a Replication Deficient Adeno-Associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human CLN2cDNA to Children with Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL)

Presenter: Ron Crystal, M.D., New York Presbyterian Hospital/Weill Cornell Medical Center

A. Presentation

Dr. Crystal explained that LINCL is caused by mutations in the CLN2 gene and is a classic lysosomal storage disease. It is autosomal recessive and rare, with only about 200 cases in the world at any one time. Children with LINCL are normal until ages 2–4 years and then develop cognitive impairment, visual failure, seizures, and deteriorating motor development, leading to a vegetative state and death by ages 8–12 years. These children lose neurons and have loss of brain volume.

LINCL is a fatal childhood disease for which there is no therapy and no prenatal or newborn screening. If these parents do not bring their children to participate in this trial, they may go to China and pay up to $75,000 for unproven stem cell therapy. Despite the fact that this experimental therapy requires an invasive neurosurgical procedure, significant therapeutic misconception occurs (which is true in any

childhood disease). These parents have no choice—they know their child is going to die and they feel guilty because their genes have caused this disease. Dr. Crystal explained that even if death is in bold letters on the front of the informed consent document as a possible consequence of the experimental procedure, therapeutic misconception remains a significant problem requiring a variety of strategies to be used during the consent process, such as patient advocates.

The investigators' first trial used an adeno-associated virus type 2 expressing CLN2. The vector was administered through six 2 mm burr holes. The primary problem was with regard to controls, as sham neurosurgery is universally disallowed in a pediatric population. The lack of placebo or blinded controls confounded the interpretation of the results on a clinical scale.

After screening AAV serotypes, a new vector was developed derived from nonhuman primate rh10AAV. Preclinical data using knockout mice showed that the saline-treated mice die in 120 to 130 days. (There is no knockout nonhuman primate model for this disease.) Survival in the vector-treated mice increases the earlier they are treated, so it is likely that the earlier these children are dosed, the higher the possibility that benefit could accrue. In the investigators' original trial, they first enrolled children with severe stage of disease and then some children with moderate stage of disease; in the current trial they have enrolled children with mild and moderate stage of disease.

The investigators treated the first child about 5 weeks ago. That child is 7 years old and is a homozygote for one of the common genotypes. The child was in the intensive care unit for 2 days and was discharged at day 4. The family stayed at a hotel on campus for 2 weeks, as requested, to facilitate evaluation of the child, who was discharged and has now been home for approximately 3 weeks with no serious adverse effects. The trial has been designed to have 16 children who can be evaluated with efficacy parameters but who will not be dosed (to serve as "controls") and 16 research participants who will be dosed, divided into two dose cohorts. A total of nine children have been screened to date. Controls are expected to be provided by children who complete the separate screening protocol but whose parents decide not to proceed to the gene transfer protocol.

Participants are evaluated using quantitative magnetic resonance imaging (MRI) (ventricular volume, gray matter volume, and the cortical apparent diffusion coefficient), the Mullen Score, and the Quality of Life Questionnaire. New assessments being developed are magnetic resonance spectroscopy and diffusion intenser imaging. There exists a critical need to develop quantitative phenotypes to assess efficacy because the quantitative clinical scales currently available are crude, at best.

The investigators now propose to obtain cerebrospinal fluid (CSF) from these children. While there is no direct benefit to these children in doing so, this trial represents a unique opportunity. No therapies are being developed for LINCL anywhere else in the world, so all these children are coming to these investigators for screening. The plan is to archive the CSF samples while developing the biomarker methodology. The investigators will evaluate the screening samples as a function of age, genotype, and disease severity, and they will have the pre- and post-transfer efficacy data. The plan is to archive the samples until the methods are established, which currently are being developed in the knockout mouse. Once the best parameters are chosen in the mouse, the investigators will examine the screening samples as a function of age; once the best parameters are chosen from that examination, the investigators will examine the archived samples of the children.

B. RAC Discussion

Dr. Zaia clarified that the purpose of this presentation was to educate the RAC about future protocols. The RAC only requires that investigators inform the RAC of new amendments to their protocols; amendments are not brought to the RAC for specific recommendations.

Dr. Crystal reiterated that the reason for collecting the CSF now is to store it for future use if and when the investigators develop a biomarker, which is in the early stages of development. He also explained that harvesting the CSF would be accomplished via a spinal tap, which would occur while the children are

anesthetized for the MRI studies as part of the research plan for this protocol. As a result, discomfort from this additional procedure would not occur and the risk from the procedure would be minimal.

Dr. Mastroianni opined that whether this CSF collection procedure should be considered a protocol amendment or a separate protocol depends on whether it is a minor increase over minimal risk. She suggested that the RAC ask the IRB to take into consideration that the RAC considers this added procedure a minor increase over minimal risk.

Dr. Crystal averred that presenting this amendment to the RAC at a public meeting is not a significant burden. While, he expressed no doubt that this amendment will be approved by his institution's IRB, he believed it was important that the proposed amendment become part of the public record and that relevant public discussion occurs and is useful.

Noting that each spinal tap is different and the procedure is not generic, Dr. Fost stated that an 8-year-old being asked to contribute to normative data normally would not meet the regulations. However, the procedure being proposed with this amendment would meet those regulations—the psychological risk of the spinal tap is zero since the children are anesthetized anyway and the medical risk is trivial. Therefore, in the context of this study, these spinal taps, while having no prospect of benefit for the children enrolled in the study, meets a minimal risk standard, both medically and psychologically.

Drs. Kahn and Mastroianni stated that while it is not the RAC's responsibility to approve or deny protocols or amendments, it is the RAC's role to pay attention to issues that might otherwise be missed. This responsibility exists for every level of review of clinical protocols.

XII. Discussion of Human Gene Transfer Protocol #1007-1049 entitled: Phase 1/2 Open-Label, Single-Center, Multiple-Dose, Dose-Escalation Study to Evaluate the Safety and Tolerability of SNS01-T Administered by Intravenous Infusion in Patients with Relapsed or Refractory Multiple Myeloma

Principal Investigator:	John A. Lust, M.D., Ph.D., Mayo Clinic
Additional Presenters:	John Thompson, Senesco Technologies, Inc.
Sponsor:	Senesco Technologies, Inc.
RAC Reviewers:	Dr. Bartlett, Ms. Mallino, and Dr. Strome
Ad hoc Reviewer:	Natasha Caplen, Ph.D., National Cancer Institute, NIH

A. Protocol Summary

Multiple myeloma (MM) is an incurable blood cancer that causes an accumulation of cancerous plasma cells (a type of B cell that is responsible for antibody production) in the bone marrow. Senesco has developed a gene transfer platform for treating advanced MM based on the eukaryotic translation initiation factor 5A protein (eIF5A).

The anti-tumoral activity of SNSO1 -T is based upon modulation of eIF5A, a translation factor and mEWA shuttle protein that is regulated by post-translational hypusine modification. Hypusine-modified eIF5A is an important regulator of cell survival and has been identified as a marker of neoplastic growth. Paradoxically, numerous studies indicate that the non-hypusine-modified eIF5A protein is a potent inducer of apoptotic cell death in malignant cells, including myeloma cells.

SNSO 1 -T consists of two active components: a B-cell-specific expression plasmid expressing a non-hypusinable mutant of eTF5A (eTFSAK5OR): and a small interfering RNA (siRNA) which targets an untranslated region of native human eIF5A mRNA. These two components are combined with a linear 22-kDa polyethyleneimine (PEI) cationic polymer to form nanoparticles approximately 70 nm in diameter. The mode of action of the combined product, SNSO1-T; is to use the specific siRNA to deplete the abundant hypusine-modified eIFSA, while using the plasmid to express the pro-apoptotic eIFSAKSOR protein.

Depletion of hypusinated eIFSA has been demonstrated to impair myeloma cell growth and to sensitize the cells to apoptosis.

Preclinical studies showed that when Senesco's nanoparticles are administered systemically to immune-compromised mice bearing subcutaneous human myeloma tumors, they routinely inhibited tumor growth by greater than 90 percent. Currently, Senesco is conducting extensive toxicology studies with SNS01-T.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Key issues included the strategy of expressing a modified initiation factor together with an siRNA designed to inhibit the expression of the endogenous molecule via a novel nanoparticle. It was also determined that the potential for off-target toxicities by the siRNA or expression in nonmyeloma cells deserved further discussion.

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

Regarding biodistribution, Dr. Bartlett noted that a clear demonstration of effective and sufficient delivery to the bone marrow is lacking in this proposed protocol, and he requested that the investigators present data about their completed biodistribution and toxicity studies. As the majority of SNS01-T is expected to be taken up in the lung, liver, and spleen, biodistribution should be assessed with precision to guide interpretation of the observed biological responses. Because the investigators base tumor selectivity primarily on the assumption that the enzyme deoxyhypusine synthase (DHS) is overexpressed in MM compared to normal tissue, Dr. Bartlett asked the investigators to share their evidence for that over-expression. He requested that the investigators comment as to the potential consequences of off-target expression of eIF5A as well as potential biomarkers that might assist in evaluating these consequences. Dr. Bartlett asked whether the investigators have data regarding the inflammatory profile of their plasmid, whether they have compared inflammatory profiles of eIF5A expression plasmids with and without B-cell specific promoter/enhancer elements, and why they have decided not to assess proinflammatory cytokines at early time points. He asked for the investigators how the starting dose was chosen.

Ms. Mallino stated that the nontechnical abstract provided by the investigators was too technical in its presentation, and noted particular terms that needed explanation for the lay reader. Within the informed consent document for both the original study and the followup study, she requested that the investigators drop the reference for signing the document on behalf of someone else as the inclusion criteria state that a potential research participant must be at least 18 years old and able to sign informed consent documents. Ms. Mallino also pointed out a time-related inconsistency with regard to the procedures at followup visits as detailed in the informed consent document.

Dr. Strome focused his review on patient safety issues. He asked the investigators to share the preliminary results in the dog model, including efficacy and toxicity. Within the informed consent document, Dr. Strome noted that the information on participant entry suggests randomization by dose. To alleviate the potential misunderstanding that this protocol is a primary efficacy study, he suggested presenting the information in the informed consent document in the same format as in the clinical protocol, with the first subjects getting the lowest dose and dose progression only after AEs are evaluated. Because variability in detailing currently available therapeutic regimens could occur, he suggested including a more detailed discussion and mentioning other experimental trials for comparison. Dr. Strome suggested wording changes related to the benefit from participating in this trial, differences between human bone marrow and the bone marrow of mice, and the description of duration of side effects. He asked for clarification about the length of time costs of medical treatment for a direct, severe complication of product would be covered by the sponsor. Dr. Strome also suggested that recruitment of participants be limited based on a participant's travel time to the Mayo Clinic as participants will be required to return six times during a 6-month period.

Dr. Caplen asked the investigators whether it would be feasible to assess the expression of DHS and/or the levels of hypusinated eIF5A in relevant samples from patients with MM, and whether either could be

developed as a biomarker for treatment selection. She requested more quantitative data regarding cell lines representing non-B-cell types found with the bone marrow niche, for example osteoclasts or osteoblasts or bone marrow endothelial cells, and for nontransformed cells. Dr. Caplen asked about the likely effect of silencing eIF5A expression in cells within lung, liver, and spleen. She stated that the use of an additional preclinical model—such as an orthotopic model in which cells that can be imaged, expressing the luciferase gene, are administered systemically—would help assess the potential outcome of administering SNS01-T, especially with the dosing schedule proposed, which might be ineffective in an orthotopic model that will feature additional delivery challenges. Since it is estimated that injection of SNS01-T will be taken up by 2.5 percent of cells in the bone marrow (as measured by siRNA uptake), Dr. Caplen noted that use of an orthotopic model would help determine if a transfection efficiency of 2.5 percent affects tumor growth within the bone marrow.

C. RAC Discussion

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

- Dr. Rogatko asked the investigators to defend their choice of dose-escalation design, and suggested that the investigators discuss this design with their statistician.

- Dr. Ornelles expressed concern that knocking out—and failing to replace—eIF5A in cells such as T cells could result in those cells losing the ability to undergo death and, therefore, in the face of an apoptotic challenge, those cells might not die.

- Dr. Caplen suggested that the investigators look at the growth patterns of the T cells under stress.

D. Investigator Response

1. Written Responses to RAC Reviews

Data from the biodistribution arm of the mouse toxicology study has confirmed that the plasmid is present in the highest quantities in the site of injection (tail), followed by the liver, spleen, and lung. However, the plasmid has been detected in all analyzed tissues, including brain, heart, kidney, liver, lung, spleen, tail, testes/ovaries, blood, and bone marrow in both males and females, indicating a widespread distribution of SNS01-T in mice following tail-vein injection. Biodistribution of the siRNA is also being analyzed in the mouse toxicology study; the data are not yet available but will be included in the IND submission.

Since plasmid DNA has been detected in all tested organs and tissues, Senesco anticipates that the siRNA will have a similar biodistribution throughout the body. Consequently, Senesco included additional groups in the mouse toxicology study to address the safety of the siRNA. Since the siRNA component of SNS01-T is specific to human eIF5A and will not affect expression of the mouse eIF5A protein, a well-described siRNA specific to mouse eIF5A was included. Two groups of mice were treated with PEI nanoparticles containing the mouse-specific siRNA. In one group the mouse-specific eIF5A siRNA was combined with a nonexpressing plasmid to assess effects related to suppression of mouse eIF5A. In the second group, the mouse-specific siRNA was combined with the plasmid in order to assess effects of suppressing mouse eIF5A in the presence of eIF5A* (human eIF5A containing a lysine to arginine substitution at amino acid position 50 which prevents hypusination). Results of these studies will be included in the IND submission.

Senesco considered orthotopic MM models and concluded that they may not be any more predictive of human efficacy than are xenograft models. Preclinical data demonstrates that myeloma cells take up SNS01-T, that SNS01-T treatment inhibits growth of subcutaneous MM tumors, and that SNS01-T reaches the bone marrow of healthy mice. MM xenograft models have been used to assess the potential efficacy of a wide spectrum of MM therapeutics, some of which have been approved by the FDA.

Section M-II-A-1a of the sponsor's submission was unclear and appears to have prompted the inference that tumor selectivity is attributable to over-expression of DHS in myeloma cells. To clarify, the investigators explained that over-expression of DHS has been reported in multiple metastatic tumor types but has not been reported in MM. Senesco has demonstrated that depletion of hypusinated eIF5A in multiple myeloma cells leads to an inhibition of nuclear factor kappa B (NF-κB) activity. Constitutive activation of NF-κB is a common feature of multiple myeloma and inhibition of NF-κB activity is associated with increased apoptosis in myeloma cells. Treatment with SNS01-T is expected to reduce hypusinated eIF5A and thus reduce NF-κB activity, thereby rendering the tumor cells more susceptible to apoptosis induced by expression of eIF5A.

Although ectopic expression eIF5A* is not expected to injure nonmalignant tissues, Senesco will be using traditional clinical chemistry markers to monitor participants for signs of organ damage. Extensive toxicological testing of SNS01-T is currently ongoing in mice and dogs. In addition to the histopathology, liver, kidney, and other organ systems will be evaluated by a variety of clinical pathology parameters.

Two dose levels of SNS01-T, 0.375 mg/kg/day and 0.75 mg/kg/day, were tested in the dog pivotal toxicology study. Analysis of the 2-week recovery period is ongoing and will determine if any observed changes are reversible. Biomarkers of toxicity due to SNS01-T will be forthcoming as more data from the toxicology studies become available.

Senesco recognizes that seriously ill patients may respond differently to the treatment than healthy mice and dogs. As such, patients receiving SNS01-T will be carefully monitored during the study for changes in vital signs, physical examination, and laboratory values. Any clinical chemistry markers found to be relevant to monitoring side effects due to SNS01-T will be included in patient monitoring.

Since no deleterious effects attributable to an inflammatory response resulting from systemic delivery of the pExp5A plasmid were observed in mice, the investigators did not examine the inflammatory profile of dosed animals beyond the cytokine analysis being performed as part of the pivotal toxicology studies. Senesco has not compared the inflammatory profiles of the different plasmids used in the preclinical development of SNS01-T. While a certain number of CpG motifs are necessary for proper silencing of the promoter in non-B cells, the investigators have minimized the number of CpG motifs in order to minimize any possible inflammatory response caused by the drug product. Senesco will include assessments of pro-inflammatory cytokines at 6 hours and 24 hours following the initiation of dosing in the clinical protocol in order to assess whether an inflammatory response is occurring.

The starting dose for the clinical study will be based on safety information from toxicology studies. Based on previous work and draft data from the mouse and dog toxicology studies performed under Good Laboratory Practices (GLP), the No Observable Adverse Effect Level (NOAEL) is expected to be 0.5 mg/kg, based on the dose level of the species most sensitive to the toxicological effects of SNS01-T. The proposed starting dose, 0.094 mg/kg, is approximately one-fifth the expected NOAEL. The starting dose may be adjusted once all data from the GLP toxicology studies have been received.

Efficacy of SNS01-T has not been assessed in dog models. The in-life phase of GLP toxicology studies in dogs has been completed and the analysis is being completed; the results will be included in the IND submission.

With respect to Senesco's statement in the RAC submission that human efficacy might be improved relative to that observed in the SCID mouse model, the investigators stated that SNS01-T contains human eIF5A siRNA, which is likely to be more efficacious in human MM patients than in the SCID mouse model.

The Mayo myeloma group meets on a weekly basis; all new clinical trials are reviewed in depth and current active trials are discussed. This continual review of trials minimizes physician variability in discussing current protocols. Other clinical trials in addition to this trial are likely to be discussed with a potential participant, depending on the number of open clinical trials at any one time.

Over-expression of DHS has been reported in multiple metastatic tumor types but has not been reported in MM. Levels of hypusinated eIF5A within tumor tissue are more relevant to understanding the biology of MM since the only known function of DHS is to hypusinate eIF5A. Given that no commercially available antibodies specific to the hypusine form of eIF5A exist, such an analysis in participant tumor samples is not feasible. Moreover, since SNS01-T also contains a plasmid expressing the hypusination-resistant mutant of eIF5A, which has been shown to induce apoptosis independently of the siRNA in MM cells (although not to the same degree), the investigators do not believe that success of the drug product would be dependent on targeting individuals with tumors expressing high levels of DHS or hypusinated eIF5A. Senesco would prefer not to exclude MM patients who might derive benefit from the experimental treatment.

The human eIF5A siRNA component of the SNS01-T drug product will not silence murine eIF5A due to lack of sequence homology. Preliminary toxicology experiments have not identified any toxicity related to the suppression of murine eIF5A using a mouse eIF5A-specific siRNA. Senesco has also included additional groups in the mouse pivotal toxicology study to further address the safety of nontargeted suppression of eIF5A. The safety of suppressing eIF5A is also being assessed in the dog pivotal toxicology study, since the human eIF5A siRNA has 100 percent homology to the dog eIF5A mRNA.

Although the primary objective of the proposed clinical trial is assessment of safety, Senesco will be performing analysis of participant bone marrow samples to determine if SNS01-T is reaching the bone marrow and will be looking for improvement of indices to determine whether the drug product is impacting myeloma cell growth and/or survival.

Since the biodistribution analysis was performed using unfixed bone marrow samples, it was not possible to determine which cell types in the bone marrow were taking up the siRNA.

The investigators agreed to modify the informed consent document to include the suggested clarifications and language modifications and to modify parallel portions of the protocol.

2. Responses to RAC Discussion Questions

Dr. Lust agreed to bring RAC comments regarding dose-escalation design to their statistician, who will be reviewing the protocol.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

<u>Preclinical Issues</u>

- In order to be effective, the nanoparticle-plasmid complex must reach the bone marrow or other sites of tumor. However, preclinical efficacy was demonstrated in a tumor xenograft model in which the nanoparticle was delivered to a subcutaneous tumor. Demonstration of delivery of the nanoparticle from the vascular compartment into a subcutaneous tumor does not establish that intravenous administration will result in delivery to a small tumor nodule in the bone marrow, which is the goal in humans. In fact, studies have shown that vectors that are very effective at treating subcutaneous MM tumors are often completely ineffective at treating orthotopic tumors. Although there are a host of factors that account for this difference, delivery is probably foremost. An orthotopic tumor model should be considered in support of the preclinical basis for this approach, as such models will better delineate the ability of the plasmid to reach the bone marrow and provide better insight into the appropriate doses for the administration route planned in this

trial. While information on biodistribution may be obtainable through the toxicology studies that are ongoing, these data were not available. Therefore, the RAC was not able to assess whether these studies would help answer the question of whether intravenous administration to patients with myeloma is effectively modeled by the xenograft tumor system. If further studies using an orthotopic model are not done, the rationale for this decision should be included in this protocol.

- The use of a B-cell-specific promoter is designed to prevent the gene modified eIF5A transgene from being expressed in normal cells where it could induce apoptosis. However, the bioavailability of the siRNA is defined by nanoparticle distribution. The presence of this siRNA in normal cells could result in downregulation of endogenous eIF5A, thereby preventing apoptosis. The investigators indicate that, based on preclinical data, any reduction in endogenous eIF5A will not prevent normal cell apoptosis except perhaps in cells under physiologic stress. Many immune system cells become activated, which is a type of cellular stress. These cells must undergo apoptosis to prevent unchecked immune responses, which can have adverse effects including autoimmunity. Studies to examine the growth pattern of immune cells, including T cells, after exposure to the siRNA under stress would be helpful to assess the safety of intravenous delivery and the effect of off-target expression of the siRNA.

- It is possible that the antitumor effect of this trial is not due solely to transduction of individual tumor cells; a paracrine effect may also play a role. This possibility should be investigated in preclinical models and could be done simultaneously with the clinical trial.

- The siRNA component of the study agent is likely to target eIFAL1 (which may be a pseudogene) because of sequence homology with the eIF5A. Little is known about the function of eIF5AL1 as compared to eIF5A. The potential effects of the siRNA on eIF5AL1 should be considered. Specifically, using the various test systems employed in other preclinical studies, it would be useful to confirm that there are no alterations in eIF5AL1 (if expressed) following transfection of the siRNA targeting eIF5A.

Clinical/Trial Design Issues

- This trial proposes to enroll four research participants per cohort, which differs from the usual three participants per cohort in a modified Fibonacci dose escalation trial design. While one design is not superior to the other, and there may be a number of reasonable designs, the rationale underlying the proposed design should be discussed in the protocol.

- Documented over-expression of DHS in a participant's tumor cells currently is not an inclusion criterion. However, every effort should be made to evaluate whether biological and clinical responses are related to tumor over-expression of DHS in that individual's tumor.

- The speculative statement in the protocol that SNS01-T may be more effective in humans than what was seen in the mouse models should be deleted.

- The ability of tumor cells to respond to this agent will be based on both the ability to deliver this agent effectively to the tumor and expression within the tumor tissue. However, the biodistribution will not be limited to tumor tissue and the promoter employed will not be 100 percent specific. It is critical, to the extent feasible, to understand the potential consequences of off-target expression using any biomarkers, including inflammatory markers, or other measures that could indicate increased apoptosis in normal cells.

- The protocol inclusion/exclusion criteria should state clearly that only those research participants who have failed to respond to all standard therapies will be eligible to enroll in this Phase I trial.

Ethical/Social/Legal Issues

- The informed consent document should specify any alternative treatments and clinical trials available to the participants.

- Regarding any injury to a participant that results from this intervention, the informed consent document indicates that Senesco will pay for appropriate medical treatment beyond that covered by the participant's health insurance or other third-party or government programs, in accordance with what is considered usual and customary. As this is an experimental agent, what is considered usual and customary might vary by insurance provider. If the intent is to cover all expenses not covered by health insurance, as was indicated at the RAC meeting, that intention should be stated clearly.

G. Committee Motion 4

Dr. Zaia summarized the comments and concerns of the RAC. It was moved that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 2 recusals (Drs. Bradley and Fong).

XIII. RAC Orientation: Ethics Rules for Special Government Employees and the Federal Advisory Committee Act

[New RAC members were oriented to the ethics rules and regulations that pertain to them as they serve on the RAC. No notes were taken of this session, and no transcript of it was prepared.]

XIV. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Federoff, Fong, Strome, Yankaskas, and Zaia

A. GTSAB Report

Dr. Yankaskas reported that of the 20 protocol submissions received by the OBA in the past 3 months, 11 protocols were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, nine were oncology protocols, one was for an autoimmune disease (diabetes), and one was for a monogenic disease (Leber's Congenital Amaurosis). The vectors used in these 11 protocols were four plasmids, three retroviruses, two adenoviruses, one vaccinia virus, and one adeno-associated virus.

Fourteen protocols submitted *Appendix M* followup information indicating their enrollment. Of trials that have initiated enrollment in the past 3 months, three protocols had been reviewed by the RAC at a previous public meeting. Dr. Yankaskas provided highlights of responses to the RAC recommendations from these four protocols.

- **Protocol #0509-727, Clinical Translation of a Mammaglobin-A DNA Vaccine for Breast Cancer Prevention and Therapy**, was reviewed by the RAC in December 2005. Data was submitted to support that mammaglobin-A was not associated with tumor development, in response to RAC concern regarding ectopic expression. The trial design was changed to a fixed dose rather than a dose escalation study, citing RAC member comment and an article that noted, "The initial clinical trial of many new vaccines will not be a toxicity or dose-ranging trial but rather will involve administration of a fixed dose of vaccine."

- **Protocol #0801-952, Phase Ib Study of Autologous Ad-ISF35-Transduced CLL B Cells and Fludarabine, Cyclophosphamide, and Rituximab (FCR) in Subjects with Fludarabine-Refractory and/or del(17p) Chronic Lymphocytic Leukemia (CLL)**, was reviewed by the RAC in June 2010. The investigators will be conducting studies recommended by the RAC to explore if the Ad-ISF35 vector can replicate in CLL cells and the mechanisms underlying the phenotypic

changes on unmodified, bystander CLL cells. The informed consent document will be amended to clarify an institutional conflict of interest (due to patent rights) and the use of future participant samples.

- **Protocol #0904-976, A Phase I Ascending Dose Trial of the Safety and Tolerability of Toca 511 in Patients with Recurrent Glioblastoma Multiforme**, was reviewed by the RAC in June 2009. To facilitate studies of the role of the immune response in efficacy and any unexpected AEs, peripheral blood mononuclear cells (PBMCs) will be collected before and after vaccine administration and stored for future analysis. Tumor samples will be analyzed for the vector. In the initial trial, individuals who are positive for xenotropic murine leukemia virus-related virus will be excluded to avoid inadvertent recombination.

- **Protocol #0910-1002, A Multiple-Site, Phase 2, Safety and Efficacy Trial of a Recombinant Adeno-associated Virus Vector Expressing Alpha 1 Antitrypsin (rAAV1-CB-hAAT) in Patients with Alpha 1 Antitrypsin Deficiency**, was reviewed by the RAC in December 2009. Since the adeno-associated virus (AAV) vector is produced with an HSV helper, the investigators will re-examine serum samples saved from preclinical animal studies for anti-herpes simplex virus antibodies. Potential T-cell responses to AAV capsid will be evaluated by ELISPOT assays and, as appropriate, intracellular cytokine staining assays.

A total of 14 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.

Dr. Yankaskas also discussed a current study using a vector that has been or is being used in six clinical trials, a thymidine kinase inactivated vaccinia with granulocyte macrophage colony stimulating factor (GM-CSF) vector called JX-594. This oncolytic therapeutic vaccinia virus is modified by thymidine kinase gene deactivation, GM-CSF insertion, and Lac-Z gene insertion. At least four clinical trials have been completed and two additional clinical trials have been initiated and are ongoing. Four of those trials are intratumoral injection for metastatic melanoma, liver tumors, and hepatocellular carcinoma, and two are intravenous administration for refractory solid tumors and hepatocellular carcinoma.

Four participants enrolled in the clinical trials have developed small skin pustules after intravenous administration at $1x10^9$ plaque forming units. In each case the overlying pustule was intact and shedding was not documented, but aspiration of the fluid through the skin or removal of a scab detected the vector. Pustules were covered in accordance with CDC recommendations for routine vaccinia vaccination pustules. Only one or two pustules developed in the three participants who had CD4+ counts above 200, but 10 to 15 pustules developed in the fourth participant with a CD4+ count less than 50. Quantitative polymerase chain reaction (PCR) in the serum for vector genome was negative in all four participants. All pustules resolved within 2 to 3 weeks without any adverse effect and there was no evidence of toxicity or replication in other organs. There was no evidence of transmission to caregivers or family members.

The sponsor of this study noted that pustules are known to occur after vaccination with the Dryvax vaccine, which is the backbone vector for the agent that was used in the eradication campaign against smallpox. According to the CDC, a 1968 State survey calculated the risk of transmission of the vaccinia virus from a vaccinated person to a susceptible contact as 27 infections per million total vaccinations, with almost half of those contact infections being found in children 5 years old or younger. JX-594 is more attenuated than Dryvax and, therefore, may be less likely to transmit live vaccinia virus than the Dryvax vaccine.

All participants in this trial will receive information that is consistent with the CDC recommendations for preventing contact transmission of vaccinia virus after vaccination, and a picture of a typical vaccine pox lesion now is included in the participant training information.

B. RAC Discussion

Dr. Ertl recounted a situation in which a contact of the person who received the vaccinia virus died. She stated the importance of investigators being very careful about vaccinating individuals with immunocompromised contacts.

Dr. Buchmeier stated that it would be useful to know more about how the investigators are monitoring those responses in real time, including the evidence that there is no spread to other organs and information about what tests they are conducting and whether they could respond quickly enough if an AE were to occur. Dr. Yankaskas agreed to ask the GTSAB to pose that question to the investigators.

In response to Dr. Ertl's question about whether the investigators plan to stop dosing individuals with low CD4 counts, Dr. Kirn, Jennerex (via teleconference) responded that the DSMB and the principal investigators who treat these individuals believe that, given that these terminal cancer patients have no other available therapy, these acne-formed pustules are not a dose-limiting toxicity. Therefore, the investigators intend to continue to dose those individuals and monitor closely, including through the DSMB.

C. Update on Protocol #0808-936 entitled: A Phase I Trial of the Immunostimulant JVRS-100 for the Treatment of Patients with Relapsed or Refractory Leukemia

Presenters:	David Claxton, M.D., Penn State Milton S. Hershey Medical Center; Jeff Fairman, Ph.D., Juvaris BioTherapeutics, Inc.
Ad Hoc Reviewer:	Hildegund Ertl, M.D., Wistar Vaccine Institute University of Pennsylvania

1. Presentation

Drs. Claxton and Fairman discussed whether results of a Phase II clinical study should impact the ongoing study of JVRS-100 for the treatment of acute myelogenous leukemia (AML). Unexpectedly and contrary to all previous animal studies, JVRS-100 reduced antibodies—but not T-cell response—to seasonal flu in a Phase II study; however, no safety concerns were noted. They provided a brief review of the Phase I study of JVRS-100 plus Fluzone®, reviewed results to date in the Phase II study of JVRS-100 plus Fluzone®, and summarized the Phase I data in the AML study in which JVRS-100 was administered intravenously.

JVRS-100 is a cationic lipid DNA complex that is used either as an adjuvant when administered with an antigen by the intramuscular route or that can be administered intravenously to elicit a cytokine response that has been shown to exhibit antitumor effects in animal models and in dogs with naturally occurring tumors.

The Phase I study was a double-blind study of 128 healthy adults 18–49 years old, using a single IM injection of Fluzone® with or without JVRS-100. The primary endpoints were safety and tolerability and secondary endpoints were antibody responses and T-cell responses. The AE profile indicated most of the AEs were mild to moderate and were seen mostly at the highest dose. Results of this Phase I study indicated an inverse dose response as well as substantial T-cell response.

The Phase II trial is studying an adjuvanted trivalent inactivated influenza vaccine in the elderly and was initiated in November of 2009. Interim data analysis initiated in March 2010 in the Phase II trial showed a reduced antibody response to seasonal flu when dosed with JVRS-100, so the investigators voluntarily halted the Phase I AML trial in order to sort out what was happening. To date, the six enrolled participants in the Phase I AML trial have been investigated for markers of immune activation and a subset of participants in the Phase II trial were analyzed for T-cell responses. The Phase II study is ongoing and is currently still blinded. Preliminary safety data (unblinded through 28 days post vaccination) plus SAE information (through 4 months post vaccination) indicate no treatment-related SAEs or other safety signals of concern, and vaccination was well tolerated.

The summary interpretation is a definite suppression in the hemagglutinin-inhibiting antibody response in a dose-dependent manner, a statistically lower immune response in the highest dose group, and a trend

in the other dose groups. These observations held among multiple endpoints for analysis of the antibody response, obviously a discrepancy between the preclinical (including nonhuman primate) and clinical trials. The investigators posit that the immune suppression is associated with preexisting immunity. Modest data suggest that a cell-mediated stimulation was produced for these research participants.

No data indicate that the material has injured any participants, and the investigators would like to reopen the Phase I AML trial and accrue additional participants. The medical reviewer at the FDA agrees, noting that the serological findings, which have brought efficacy issues into question to deal with an adjuvant study, do not seem to be immediately relevant to the leukemia context. No safety concerns exist based on the clinical data collected to date.

2. *Ad Hoc* Review and RAC Comments/Discussion

Dr. Ertl generally agreed with the investigators. She stated her belief that the result in the Phase II trial was a reduction of antigen, not an immunosuppressive mechanism. An adjuvant can reduce antigen via several mechanisms, including increasing phagocytes and processing proteolysis. Antigen reduction would be consistent with the inverse dose response. Dr. Ertl suggested that the investigators look for impairment of other immune responses by testing cryopreserved PBMCs from flu patients before and after for T-cell responses to persistent viruses. Once a dramatic drop in T-cell response does not occur, immune suppression is not at issue and there would be no reason to keep the AML trial on hold.

Dr. Zaia asked whether the investigators would need to disclose to future participants that a negative effect on their immune response could make their disease worse. Dr. Ertl responded that disclosure would need to be specific; she suggested sample wording: "We have observed a reduction in titers upon immunization against influenza virus. This is not necessarily relevant to this trial where we are trying to elicit a different immune response, and we have no indication that this may impair your immune response."

XV. Discussion of Human Gene Transfer Protocol #1007-1061 entitled: A Phase I Dose Escalation Safety Study of Subretinally Injected RetinoStat®, a Lentiviral Vector Expressing Endostatin and Angiostatin in Patients with Advanced Neovascular Age-Related Macular Degeneration

Principal Investigator:	Peter A. Campochiaro, M.D., Johns Hopkins University School of Medicine
Additional Investigators:	Kyriacos Mitrophanous, Ph.D., Oxford Biomedica, Ltd.; Jan Peterson, Oxford Biomedica, Ltd.; Graham Price, MBBS, B.Sc., Oxford Biomedica, Ltd.; and Stewart Naylor, PhD., Oxford Biomedica, Ltd.
Sponsor:	Oxford Biomedica, Ltd.
RAC Reviewers:	Ms. Mastroianni, Dr. Ross, and Dr. Zaia

Dr. Zaia explained the special circumstances under which this protocol would be discussed at this RAC meeting. The sponsor of this protocol indicated that commercial confidentialities relate to intellectual property and patent issues; the RAC thus agreed not to disclose those issues at this presentation and the critical information was redacted from the documents that were submitted to the OBA. However, the RAC reviewers have seen this information under a confidential disclosure agreement. This September RAC meeting will not be closed to members of the public who attend in person; however, the portion of the meeting during which this submission is discussed will not be videocast over the Internet. Video recording was stopped and the discussion of the submission was archived, edited, and made available for future reviewing. In preparation for the public meeting, a pre-meeting of the reviewers was held with the members of the investigating team and the sponsor. At that meeting, which occurred 1 week before this RAC meeting, the reviewers were allowed to ask specific questions relating to the confidentially disclosed information. The RAC members agreed to phrase their questions at this public meeting by referring to doses as low, medium, and high; they also agreed not to mention the volume of the agent to be administered via the subretinal injection.

A. Protocol Summary

Age-related macular degeneration (AMD) is the second most common cause of vision loss among adults in developed countries. Approximately 1.7 million people in the United States have impaired vision due to AMD, a number that is expected to triple by 2030 as the population ages. Every year in the United States, more than 165,000 people develop AMD and 16,000 become legally blind with a visual acuity of less than 20/200, predominantly from the rapidly progressing "wet" form of the disease. Wet AMD is characterized by abnormal growth of blood vessels and their leakage in and around the retina. These abnormal blood vessels affect central vision, so that people with wet AMD are unable to read, recognize faces, and drive, and often lose their independence. Depression occurs commonly alongside advanced wet AMD.

Therapies using intravitreal injections of anti-Vascular Endothelial Growth Factor (VEGF) drugs such as ranibizumab (Lucentis) and bevacizumab (Avastin) have been efficacious in many patients with 68% of patients gaining some vision. However, these therapies require monthly or six-weekly intravitreal injections and continued monthly treatment provides superior outcomes to intermittent treatment based upon evidence of recurrent disease. Monthly visits and frequent injections are a substantial burden on patients and the greater the number of injections, the greater the risk of endophthalmitis. Consequently, there is a need for alternative longer-term therapies that avoid the risks associated with frequent and costly intravitreal injections.

The proposed therapeutic, RetinoStat®, is a minimal non-replicating, recombinant lentiviral vector derived from the non-primate lentivirus Equine Infectious Anaemia Virus (EIAV). RetinoStat® is designed to express human endostatin and angiostatin, two proteins with well-known broad-range anti-angiogenic actions. Whilst RetinoStat® is able to transduce many cell types, the anatomical separation of the eye from the rest of the body limits the ability of RetinoStat® injected subretinally to affect tissues other than the eye. The continual local secretion of endostatin and angiostatin from the RetinoStat® transduced retina is expected to result in chronic long-term suppression of the neovascular stimulus.

In a mouse model of AMD, significant inhibition (49 percent) of new blood vessel growth was demonstrated with a 6.2×10^5 transmission unit (TU)/eye dose of RetinoStat®. In safety studies performed in monkeys and rabbits, a dose of 1.1×10^5 TU/eye caused no adverse effects and was well tolerated with respect to the inflammatory response.

The proposed clinical investigation is an initial study of the safety of gradually increasing doses of RetinoStat® injected into one eye. Evidence for any effect of RetinoStat® on vision and other eye examinations will also be collected. Research participants will be 50 years old or older and will have severe wet AMD in at least one eye. Up to 18 participants will be included, and there will be three ascending dose groups with three participants in each group, followed by up to nine participants at the maximum tolerated dose.

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the use of the novel lentiviral vector derived from equine infectious anemia virus (EIAV) containing the transgenes, endostatin and angiostatin, for neovascular AMD.

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

Ms. Mastroianni noted that the informed consent document is excellent and she commended the researchers for their thoroughness and clarity in communication, and she also noted that the submission is missing responses to Appendix M-II-B-3-e through M-III-A-4. She requested clarification and justification of the differential treatment in inclusion criteria between women and men with regard to reproductive potential and activity, especially since participants must be at least 50 years old. Ms. Mastroianni noted a potential conflict in the informed consent document concerning retention of remaining

biological samples, which are explained as being destroyed on one page and as being stored for 10 years on another page; she requested clarification and rewording as appropriate. The provision in the protocol that the research participants will be advised of and be covered by the sponsor in the event of injury should be reflected in the informed consent document. Ms. Mastroianni suggested changing language in the protocol and the informed consent document that might imply therapeutic intent.

Because limited preliminary testing of this vector has been performed in the three animal models tested thus far (mouse, rabbit, rhesus), Dr. Ross suggested the investigators perform reverse-transcribed PCR to look at viral RNA in blood and urine and DNA PCR of blood cells at different times after administration of RetinoStat®, to ensure that infection is confined to the cells of the retina in the three models. This is particularly important because the investigators report a possible increased breakdown of the blood/ocular barrier in the RetinoStat®-administered eyes in rabbits and because EIAV-based vectors have not been used extensively in gene transfer models to date. Similar testing should be performed on study participants, and Dr. Ross suggested further that the investigators should plan to perform DNA PCR to look for integrated proviruses in PBMCs. She asked the investigators to detail how they will distinguish transgene from endogenous gene expression. Acknowledging that it is unlikely that RetinoStat® will cause insertional mutagenesis if infection is confined to non-dividing retinal cells, Dr. Ross inquired as to what clinical evaluations would be conducted to ensure that this does not occur in the research participants. The investigators stated that blood samples will be collected for immunology but Dr. Ross wondered whether additional parameters, other than antibodies to endostatin and angiostatin, would be measured.

Dr. Zaia noted that the rationale was well described, the study design appears suitable, and the justification for dose selection and injection volume appears appropriate. Noting that one of the safety features of this protocol is that the target cells are already differentiated and thus unlikely to replicate and mutate into malignant cells, he requested that the investigators present data to demonstrate that the EIAV vector actually integrates into these cells *in vivo*. Dr. Zaia requested additional details about the specific rule of dose escalation and the methods for assessing ocular direct toxicity events, especially as they will be used to define dose-limiting toxicity (DLT), in order to better appreciate the safety plan. Because the protocol notes that an EIAV-derived vector is being used in a study of Parkinson's disease, he requested presentation of information regarding safety and a summary of any new toxicology data. Dr. Zaia asked the investigators to reword some incorrect information about withdrawal from the study that appears in the informed consent document, and he suggested alternate wording. Additionally, he suggested that the potential for introducing infection into the eye, although a low risk, should be mentioned in the risk section of the informed consent document, as should the potential for an unknown side effect in this first-in-human experimental treatment.

C. RAC Discussion

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

- Dr. Fong asked whether the investigators would consider, in the proposed research population, injecting first in some place less potentially toxic than the retina.

- Dr. Federoff suggested that the investigators might want to study from a pharmacokinetic perspective, in the rabbit model, another process that could affect the intraocular levels of the transgene product.

- Dr. Sarzotti-Kelsoe asked whether the investigators had encountered any evidence of immunological tolerance being broken in their preclinical studies.

- Noting that inflammation in the eye could be due to a response to the vector or to the products of the transgenes, Dr. Zaia asked how the investigators would distinguish between the two, one of which might imply a breaking of tolerance. He also asked the investigators whether they are able to sample the cells and then determine an immune response to the peptides or proteins.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators reported that biodistribution and shedding studies were performed using the nonhuman primate and rabbit NOAEL dose. In the rabbit study, no vector shedding was detected in urine, saliva, and contralateral eye tear swabs. RetinoStat®-treated eye tear swabs were evaluated at days 2, 3, and 5 post-dosing; vector was not detected in the majority of samples, and when detected it was below the lower limit of quantification (LLOQ). By day 5, vector was detected in only one animal out of 18 (below the LLOQ). Vector DNA was detected in other sample types sporadically at early time points but only in a minority of animals and never above the LLOQ; signal was undetected in all of these sample types by day 29. In the nonhuman primate study, in-life sampling indicated that vector sequences were detected in a minority of animals at day 2 (white blood cells, saliva swabs, right eye tear swabs [all below the LLOQ]) but were not detected in plasma. Signal was undetected in all of these sample types at day 3, with the exception of white blood cells in which one out of six samples was positive (below the LLOQ). These results demonstrate that RetinoStat® remained within the ocular compartment at the tested dose level. The lack of evidence for vector escape from the subretinal compartment may explain why studies were unable to detect an immunological response to any vector components.

Vector testing will be performed on all study participants. Urine samples and blood plasma will be analyzed for vector RNA; in addition, PBMCs will be tested for integrated provirus. Results of these analyses will be provided to the DSMB on an ongoing basis.

Long-term followup is planned for all RetinoStat®-treated participants to monitor for potential long-term adverse effects of the experimental therapy, including recurrent or new malignancy at any site, autoimmune disease, reactivated infections, and causes of mortality. All participants will be encouraged to enter an open label, safety extension study for up to 5 years to ensure standardized followup. Following completion of the 5-year open-label followup study, all participants will be followed up on a yearly basis for a maximum of 15 years and thereafter according to any other regulatory requirements for the monitoring of participants who have received gene transfer.

Blood samples will be collected for immunology at initial screening and at weeks 1, 2, 12, 24, and 48 after RetinoStat® administration. Antibodies for all vector components including transgene products (endostatin and angiostatin) will be evaluated.

A RetinoStat® GLP biodistribution study in the rabbit indicated that greater than 95 percent of sequences derived from RetinoStat® were confined to the retina/choroid. EIAV-based lentiviral vectors similar to RetinoStat®, where the cDNAs for endostatin and angiostatin were replaced with reporter genes coding for either β-galactosidase (LacZ) or green fluorescent protein, were used to identify retinal cell types that are transduced by the vector. Following subretinal delivery, the lentiviral vector platform enables gene delivery and expression most evidently in retinal pigment epithelial cells but also rod and cone photoreceptors in both mice and cynomolgus macaques.

The RetinoStat® development program is supported by an independent DSMB, which will consist of a group of ophthalmologists and clinicians experienced in gene transfer clinical research, familiar with the indication, and possessing previous safety management experience. All ocular AEs or AEs related to study procedures will be reported to the DSMB on an ongoing and real-time basis. The DSMB will be able to modify the study design or suspend or terminate the study at any time. The decision to dose escalate will be made on the basis of the overall safety profile of RetinoStat®, which includes the ocular inflammation scores, ocular and non-ocular AEs, data from the ophthalmic and clinical safety monitoring, vector immunology, and the results of urine samples and blood plasma for vector RNA and integrated provirus. The draft DSMB charter was provided.

The same EIAV lentiviral platform as used in RetinoStat® is the basis of a gene transfer product, ProSavin®, for treatment of advanced, L-DOPA-resistant Parkinson's disease. In a study being conducted in Europe, this product has undergone full nonclinical evaluation and has now been

administered to humans with follow up for more than 2 years. Safety data on the administration of ProSavin® are available in eight participants for up to 2 years. To date, ProSavin® has been safe and well tolerated. All participants were ambulatory within 24 hours after surgery, no SAEs have been reported, and all other AEs have been mild and the majority have been determined as unlikely related to the study drug. Currently there is no evidence of any local immunological or inflammatory reaction to vector or ProSavin® as assessed by a number of techniques including direct imaging on MRI. No evidence exists of adverse immune responses or antibodies to any vector components or to the transgene products. Analysis of blood and urine has not detected any RNA or DNA from ProSavin®.

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

Mr. Price explained that the DSMB charter that was provided to the RAC is a draft and is still under review. He assured RAC members that the DSMB would have final decisionmaking authority as to characterization and continuance of this proposed clinical trial.

Dr. Campochiaro clarified that the key ethical question with regard to this Phase I trial is not the position of injection but is whether a potential participant should be asked to forego standard treatment of care that is beneficial in order to enter a trial in which benefit is unknown. He noted that numerous Phase I ophthalmology trials in neovascular AMD use the identical enrollment criteria to those enumerated for the current proposed trial. He further clarified that the patients who might benefit more from this experimental treatment have access to a standard of care that is better than what the investigators can offer in this Phase I trial, so that is the reason for targeting the proposed patient population.

Dr. Campochiaro stated that the investigators had not encountered any evidence of immunological tolerance being broken in their preclinical studies.

Regarding how the investigators might distinguish between a response to the vector and a response to the transgene product, Dr. Campochiaro explained that they would look for antibodies to the transgenes as well as antibodies to all of the vector components. For example, a finding of antibodies to vector, but not to endostatin or angiostatin, would suggest the vector as the inciting event.

E. Public Comment

Dr. Hildegund Ertl, a former RAC member from the Wistar Institute, asked whether the risk of participants getting an innate immune response or infection as a result of being dosed with this experimental treatment is commensurate with the benefit they might receive, which the investigators have stated is low or none. She noted that the eventual target population is individuals without fibrosis. Dr. Campochiaro explained that if safety is shown in this Phase I trial, a Phase II trial would target patients whose visual acuity might benefit from this experimental regimen.

Dr. Borror suggested a wording change in the informed consent document regarding anesthetic drops “freezing” the eye.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

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

- According to the draft DSMB charter submitted, the sponsor is not required to accept a recommendation by the DSMB to suspend, terminate, or substantially modify the study. If the sponsor does not accept the DSMB recommendations, the DSMB is given the option of

discontinuing monitoring and disbanding. To be an effective, independent oversight body, the DSMB should be the final arbiter of such decisions.

- The protocol proposes ophthalmological assessments to monitor for inflammation in the treated eye. Inflammation potentially could be the result of an immune response to the vector or breaking of immune tolerance to the transgene products. The protocol should include appropriate studies to distinguish between these two etiologies.

Ethical/Social/Legal Issues

- As stated in *NIH Guidelines,* to obtain vital information about the safety and efficacy of gene transfer, research participants should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Participants should be asked to advise their families of the request and of its scientific and medical importance.

- An inclusion criterion of the protocol states that women must be surgically sterile or postmenopausal, defined as no menstruation for more than 2 years. However, men have the options of being "surgically sterile or agreeing to use two forms of contraception including one barrier method for at least 3 months following RetinoStat® administration if their partner is of childbearing capacity." While most women enrolling in the trial may be postmenopausal because they must be at least 50 years old to enroll, there does not appear to be a compelling reason to not offer female participants the option of preventing pregnancy through the use of contraception methods that are deemed equally effective as those required of male participants.

- The revised informed consent document states: "Oxford BioMedica (UK) Ltd. may use your biological samples collected during this study for additional analyses other than those listed in this informed consent, such as retesting the levels of RetinoStat® antibodies. Oxford BioMedica (UK) Ltd. may store your samples for 10 years, without your identity, for general research or quality purposes." Participants should be provided more details regarding the potential uses of their samples for research that are not directly related to the development of RetinoStat®.

- The informed consent document should be reviewed for language that may not be clear to potential participants. For example, the use of the term "freeze the eye…" to explain the effect of a topical anesthetic could be clarified.

In addition, as you note, in preclinical models, the levels of angiostatin and endostatin reach steady state at six months. Clearance of these proteins is by efflux pumps. The RAC queried whether there are physiologic conditions or medications that could alter this efflux mechanism leading to unexpectedly higher concentrations of these antiangiogenic proteins and whether such increases could potentially have an adverse effect on the treated eye. You stated that there are no known physiologic conditions or drugs that will affect these efflux pumps. Should new data arise regarding drugs or other diseases that can alter the activity of these efflux pumps, it may be important to investigate whether this could result in a significant change in subretinal concentrations of angiostatin and endostatin and the potential effect on the treated eye.

G. Committee Motion 5

Dr. Zaia orally summarized the RAC recommendations to include a variety of preclinical and clinical concerns that will be included in the letter to the investigators expressing the comments and concerns of the RAC. It was moved by Dr. Yankaskas that the RAC approve these summarized recommendations. The vote was 19 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Fost).

XVI. Discussion of Human Gene Transfer Protocol #1007-1053 entitled: A Phase 1/2 Randomized, Blinded, Placebo-Controlled, Sequential Dose Escalation Study of the Safety and Pharmacodynamics of BHT-3034, an Acetylcholine Receptor Tolerizing Plasmid

Principal Investigator:	Gil Wolfe, M.D., University of Texas Southwestern Medical School
Additional Investigators:	Joann Quan, M.D., Bayhill Therapeutics, Inc.; Michael Leviten, Ph.D., Bayhill Therapeutics, Inc.
Sponsor:	Bayhill Therapeutics, Inc.
RAC Reviewers:	Drs. Fost, Koch, and Ornelles
Ad Hoc Reviewer:	Audrey S. Penn, M.D., National Institute of Neurological Disorders and Stroke, NIH

A. Protocol Summary

Myasthenia gravis (MG) is an autoimmune disease in which the immune system attacks normal proteins located at the junction of the muscle and the nerves that stimulate the muscle to contract. This autoimmune response damages the neuromuscular junction (NMJ) and inhibits the ability of the muscles to contract, causing a condition of chronic muscle fatigue and weakness. The acetylcholine receptor (AChR) is the primary muscle antigen targeted by the immune system with a majority of the immune response directed at the α subunit. BHT-3034 is a product designed to reduce or stop the harmful response of the patient's immune system to his/her own AChR and, therefore, to preserve the ability of the muscles to respond to the stimulating nerves, thereby improving the patient's disease.

BHT-3034 is an antigen-specific immunotherapy agent expected to stop autoimmune destruction of the neuromuscular junction (NMJ) and stabilize or reduce the muscle weakness that characterizes this disease. BHT-3034 is a plasmid expression vector encoding the gene for the α-chain (Chrna1) of the human nicotinic acetylcholine receptor (AChR) under the control of a mammalian promoter. Preclinical studies conducted at Bayhill using rodent homologs of BHT-3034 demonstrate efficacy in reducing the severity of symptoms in rat and mouse experimental autoimmune myasthenia gravis (EAMG) studies. Specifically, BHT-3034 plasmids significantly reduced the severity of muscle weakness symptoms and suppressed the mortality associated with the rodent MG models. Weekly intramuscular injections were an effective dose regimen and symptoms remained suppressed throughout the dosing period which extended for several months in some studies. Mechanism of action studies showed that the rat homolog of BHT-3034 decreased the immune response to AChR.

This is a multi-center, randomized, blinded placebo-controlled sequential dose escalation phase 1 trial in patients with MG. Subjects with MG will be screened for eligibility. Key eligibility criteria include a diagnosis of Class I (ocular) or Class II myasthenia gravis (MGFA Clinical Classification), onset of MG symptoms within 18 months, age 18-75 inclusive, no concurrent treatment with immunosuppressive medications, and a positive test for anti-AChR antibodies. Up to four dose levels of BHT-3034 will be enrolled and up to 40 subjects will be randomized. A cohort of 9 subjects will be treated at each dose level. Subjects will be randomized to BHT-3034 or placebo in a 2:1 ratio. BHT-3034 or BHT-placebo will be administered intramuscularly weekly for 12 weeks (Weeks 1 to 12).

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the use of a novel transgene—the AChR expressed from a plasmid as a DNA vaccine. The risk of this approach is that the autoimmune response to the AChR could be enhanced rather than decreased, leading to progression of disease or generalized disease for those with ocular symptoms only.

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

With regard to entry criteria, Dr. Fost requested that the investigators discuss the rationale for using, in this Phase I study, research participants who are in the early phase of the disease, some of whom may do well and might not require additional therapy; he suggested enrolling patients who are more likely to benefit from this agent because they have more serious disease or have not responded to first-line therapy. He suggested altering the time frame and thus the wording of information in the protocol related to the sponsor's right to limit disclosure of information (results) deemed confidential. Noting that the

informed consent document is clear, Dr. Fost offered a variety of wording changes that would clarify the document for potential participants.

Dr. Koch asked the investigators to provide the statistical information for the preclinical studies in mice and rats and to discuss possible dose responses in more detail in these studies. In addition, data from preclinical models regarding expression of transgene in muscle and the expected overall duration of expression was requested; or in lieu of available data, potential expression patterns from the literature were requested. In light of the multiple sclerosis results with BHT-3009 in which no apparent dose response was seen in humans, concern exists that the dose range studied is not broad enough or expression of the transgene is generally ineffective with low expression. Dr. Koch requested discussion of this issue. He suggested that the investigators comment about considering muscle biopsies to measure transgene expression in dosed participants, more thorough toxicology studies since no data from rats is given, exacerbation of disease in animal models, and whether spontaneous remission is possible and could cloud trial results. Since the proposed research participants will be in early stages, he asked whether animal data supports the possibility that this experimental treatment could block disease progression, and thus whether a real benefit could exist that this experimental therapy might decrease the early stages of disease.

Noting that the study was clearly presented and well supported, Dr. Ornelles asked the investigators two questions regarding this protocol. He asked for clarification about the exception to the exclusion criteria of a history of malignant neoplasm—whether patients with malignant *in situ* cervical carcinoma are excluded or whether the exclusion is for patients with nonmalignant *in situ* cervical carcinoma with curable intent. Dr. Ornelles requested clarification about how participants who need corticosteroid therapy would be treated on this protocol; specifically, whether a standard procedure is in place for these participants, how attending physicians would be dissuaded from increasing the dose from the stated limit, and whether clear clinical guidelines exist for administering increased levels of the steroid or alternative therapies.

Dr. Penn noted that issues to be considered include the presence of epitopes reactive with TH2 and regulatory T cells capable of initiating suppression. She asked whether the product of this vector has the conformation of the alpha chain with its conformational epitope, which is important for cell production of anti-AChR antibodies. Dr. Penn asked as to whether the investigators have conducted passive transfer studies with rodents using the plasmid expression vector, particularly because of the unsettled question of when to perform these studies in the course of ocular or mild generalized MG. She explained that ocular MG may exist as a form of MG that does not progress and in which greater than 50 percent of patients do not have detectable anti-AChR; therefore, the goal would be to prevent further generalization within 18 months of onset in individuals with antibodies.

C. RAC Discussion

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

- Dr. Zaia asked whether the control group would be stratified for corticosteroid use.
- Dr. Rogatko requested discussion about why the investigators chose the sample size of 40, and he suggested that a smaller sample size might achieve the same degree of precision.
- Dr. Badley asked whether the investigators had looked at immune complexes and kidney function in their preclinical animal models.
- Noting that the investigators are proposing to use 6 milligrams at weekly injections, Dr. Sarzotti-Kelsoe asked what pain level the research participants are likely to experience.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators stated that significant unmet medical needs exist in the treatment of MG, for both ocular and generalized disease. Most patients with either ocular or generalized disease do not respond adequately to anticholinesterase therapy alone, and then are confronted with the choice of more aggressive, invasive, and potentially risky therapies including corticosteroids, immunosuppressive agents, thymectomy, and plasma exchange. Most therapies for MG have broad, nonspecific immune effects and require chronic use, and therefore the benefits of these therapies must be weighed against the risks of untoward immune and other adverse systemic effects. A safe, well tolerated therapy to reduce the symptoms of ocular and/or generalized MG would have a major impact in improving the overall treatment of MG, for patients in early as well as more advanced stages of the disease.

Between 50 percent and 60 percent of patients who present with ocular symptoms will progress to generalized MG, usually within the first 1 to 2 years. Most patients with generalized disease will require treatment with immune therapies, including corticosteroids, azathioprine, mycophenolate mofetil, cyclosporine, or tacrolimus. While corticosteroids are effective, substantial doses may be required for prolonged duration, increasing the risk of toxicities that include hypertension, impaired glucose tolerance, weight gain, osteoporosis, and cataracts. The immune therapies have broad effects on the immune system and are associated with a narrow therapeutic index. None of the current therapies for MG is known to reduce the risk of progression to generalized disease; thus a clear unmet need exists for an agent to reduce progression of disease.

Patients at earlier phases of disease are more likely to benefit from an agent such as BHT-3034, which is designed specifically to decrease the autoimmune response to the AChR. Those individuals with early disease, suggesting an earlier stage of autoimmune dysregulation, may be more susceptible to "correction" as compared to those who have longstanding, established disease in which additional, secondary effects may be manifest. Treatment at earlier phases of disease is also supported by animal data: a greater benefit was consistently seen in mice and rats with mild disease, suggesting that BHT-3034 may be particularly effective for treatment at earlier phases of disease.

With respect to dose range, the starting dose will be based on a calculated human equivalent dose, using allometric scaling from animal studies of the efficacious dose and incorporating an appropriate safety margin based on results from the ongoing toxicology study.

The investigators acknowledged the existence of only limited information on the dose-response relationship for DNA plasmids. The literature on other DNA plasmids largely refers to use of this approach for increasing, not decreasing, immunity and therefore is of limited relevance for BHT-3034. Bayhill's experience with two other DNA plasmids with similar constructs, BHT-3009 for multiple sclerosis and BHT-3021 for type 1 diabetes, suggests that the likely dose range proposed will bracket an effective dose level. The starting doses for these compounds were selected in a manner similar to that proposed for BHT-3034.

Expression of BHT-3034 was examined in mouse muscle following intramuscular injection. Expression above background was detected at the 6-hour time point and expression increased to a peak at 4 days. Expression gradually declined through day 28, which had a median relative expression level approximately 20-fold lower than the peak at day 4. The efficacy seen with weekly BHT-3034 dosing in animal studies is consistent with maintaining peak levels of RNA expression; similar results for peak expression and dose regimen were observed for the BHT-3021 program.

Bayhill has considered performing muscle biopsies to look for expression in participants being dosed within the clinical trials of BHT-DNA plasmids. However, for this trial in participants with MG as well as for the previous trials in participants with multiple sclerosis and type 1 diabetes, the investigators and sponsor believed that muscle biopsy would be unduly onerous and would not likely provide information

that could be interpreted clearly. Although muscle biopsy will not be performed in this study, the investigators will continue to assess possible approaches to evaluate expression in study participants.

The primary toxicology study supporting the Phase I/II study is a 13-week repeat dose GLP study in Lewis rats. The frequency, duration of dosing, and endpoint evaluations in this study were designed to provide sufficient safety data for the proposed clinical trial. The plasmid has been manufactured using the same process used to produce BHT-3034 for the clinical trial. Lewis rats were chosen because this strain was used for the experimental autoimmune MG (EAMG) efficacy studies and is genetically susceptible to developing an immune response to the AChR protein encoded by BHT-3034. The sponsor has received interim data through 10 weeks of dosing, and no deaths and no adverse clinical observations have been associated to date with the drug. In addition, no significant differences in body weights or food consumption have occurred between the treatment groups.

In the four EAMG studies in which antibody levels were evaluated by either a radioimmune assay detecting total anti-AChR antibodies or an ELISA assay that also evaluated specific antibody subsets, no evidence of increased antibody production in response to drug was observed. The investigators hypothesized that the absence of antibody induction by the BHT-3034 plasmids may be due to the fact that the encoded antigen has been engineered to be a non-secreted protein that is unlikely to contact B-cell receptors directly and to activate antibody production. Ongoing studies will evaluate dose response and effect of dosing interval in EAMG models.

While MG typically has a variable disease course, spontaneous remission is rare. Even with aggressive therapy, complete stable remission occurs only in about 20 percent of cases. The variation in symptoms combined with the narrow therapeutic index of drugs used for treating MG creates challenges for management of this complex disease. In order to address the issue of natural variation of disease, the investigators have incorporated measurement of outcomes at multiple time points during the study. In addition, the study design proposes two measurements at baseline prior to study drug initiation so that this variability can be characterized.

The sponsor's intent is to allow enrollment of research participants who have had adequate treatment of *in situ* cervical carcinoma. Individuals with a history of cervical carcinoma *in situ* are eligible for this study as long as the condition has been treated appropriately. The investigators pledged to revise the wording in the exclusion criteria to improve clarity with regard to this issue.

The guidance on corticosteroid use within the clinical study is written to acknowledge some range in clinical practice and to provide a boundary to ensure the safety of study participants. Current animal data suggests no major safety concerns associated with concurrent dosing of BHT-3034 and moderate doses of corticosteroids. As no animal data support concurrent dosing of BHT-3034 and high doses of corticosteroids, participants who require 60 mg per day of prednisone (or its equivalent) or higher for 7 or more consecutive days should not receive further dosing with BHT-3034. Careful attention to administration of corticosteroids will be required in further studies to allow assessment of efficacy of an investigational agent such as BHT-3034.

As requested, the investigators provided amended Figures 2, 3, and 4 for mouse and rat EAMG studies, including information on sample sizes and statistical tests.

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. Quan acknowledged that the investigators had discussed whether to stratify for corticosteroid use within the study and decided not to do so partly because this protocol is primarily a safety study and partly because all of the different ways to stratify would make it difficult to complete enrollment within one cohort. They do intend to keep track of the types of participants, particularly ocular versus generalized,

and would consider closing enrollment at some point to one or the other group to balance the trial between active and placebo arms.

The sample size of 40 was chosen as a large-enough number to provide indication of any major safety issues. Because they believed a placebo group was needed, the investigators decided that a 2:1 randomization would result in six research participants for each active group and three for each placebo group within each dose cohort.

Regarding whether the investigators had looked at immune complexes and kidney function in their preclinical animal models, Dr. Leviten stated that they have not looked at immune complex function. However, from previous programs in terms of toxicology in healthy animals, the investigators have never seen any toxicity related to kidney function and no DNA has been detected in the kidney; in addition, in the clinic, no DNA has been detected in the urine.

Regarding the pain level from the weekly injections, Dr. Quan explained that other compounds that the investigators have tested in the clinic weekly for 12 weeks at 6 milligrams have been associated with very little adverse effects related to the injections. The injections for this proposed trial will total 6 milligrams but will be divided into two doses of 3 milligrams each.

E. Public Comment

Dr. Ertl asked whether the investigators had contemplated doing lymph node biopsies in some of the research participants to obtain a careful analysis of T-cell responses as well as dendritic cell activation, which can be detected in lymph nodes but not in blood. Dr. Leviten responded that, to date, the investigators have limited their analyses to blood. While the possibility of doing lymph node biopsies is scientifically interesting and would advance the knowledge, Dr. Leviten acknowledged that lymph node biopsies might not be tolerable for potential participants, and therefore might produce recruitment difficulties.

Dr. Borror noted that the section entitled “Why Is This Study Being Done” contained particularly complex language, and she suggested that the investigators reword that section so it could be understood by a layperson.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

<u>Preclinical Issues</u>

- A central hypothesis of how delivery of this antigen promotes tolerance is that the alpha chain of the human nicotinic AChR expressed by the plasmid remains intracellular and is only presented extracellularly by antigen-presenting cells as an MHC-associated epitope. However, an alternative hypothesis is that the extracellular portion is expressed and that this leads to the formation of immune complexes. While immune complexes have not been observed in the trial for diabetes that employs this general strategy (administration of a plasmid expressing an antigen for tolerizing studies), preclinical studies should be done to look for immune complexes.

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

- In addition to screening for potential immune complexes in preclinical studies, appearance of these complexes should be monitored for in the clinical trial. Renal functions, including proteinuria, should be closely monitored as these may be an indication of immune complex deposition.

- While preclinical studies support that the vaccine can result in tolerization, it is not yet known whether the down-regulation of the immune response seen in animals is due to tolerance or an alternative mechanism; for example, continued activation of T cells by antigen may lead to activation induced T-cell death. As the potential risks may be related to the underlying mechanism, it is important to consider the feasibility of additional immunological studies that could further elucidate the underlying mechanism(s).

- The study proposes to enroll 40 participants, incorporating a placebo arm with 2:1 randomization, but the statistical rationale for enrolling this number of participants as opposed a different number of participants is not articulated. Justification for the number of participants in each arm should include a discussion of projected differences in endpoints that would be considered a positive study. On a related point, the protocol proposes to allow up to 20 mg/day of prednisone at the discretion of the treating physicians. As the investigators acknowledge, administration of steroids may impact the efficacy of this approach. Since this is a randomized control study designed to discern host specific effects, the potential effect of steroids should be controlled in the statistical analysis.

Ethical/Social/Legal Issues

- The protocol includes a statement that the "investigator shall have the right, consistent with high academic standards and the contract with the sponsor, to publish or present the results of research hereunder 1 year after completion of the trial." Ideally there should be no restrictions on publication beyond the 1-year time period after the trial has concluded, which should allow for complete analysis of the data. Any such restrictions, for example to protect commercial confidential data, should be outlined specifically and stated clearly in the protocol to enhance transparency.

- The informed consent document contains complex language, for example in the section "Why Is This Study Being Done." A careful review should be conducted to make the document more understandable to potential research participants.

G. Committee Motion 6

Dr. Zaia summarized the comments and concerns of the RAC. It was moved by Dr. Koch and seconded by Dr. Roizman that the RAC approve these summarized recommendations. The vote was 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XVII. Day 2 Adjournment

Dr. Zaia, Acting RAC Chair, adjourned Day 2 of the September 2010 RAC meeting at 5:30 p.m. on September 16, 2010.

XVIII. Day 3 Call to Order and Opening Remarks

Dr. Federoff, RAC Chair, called Day 3 of the September 2010 RAC meeting to order at 8:30 a.m. on September 17, 2010.

XIX. Presentation of Certificate of Appreciation to Departing RAC Member

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

Dr. Patterson presented a certificate of appreciation from the NIH to the one RAC member whose service to the NIH, the research community, and the public was ending at this RAC meeting: Dr. Federoff. She

noted that Dr. Federoff has chaired the RAC for the past 4 years. Dr. Federoff thanked the OBA staff for all their hard work.

XX. Discussion of Human Gene Transfer Protocol #1007-1056 entitled: A Phase I, Dual Cohort, Two Site, Clinical Trial Evaluating the Safety and Activity of Redirected Autologous T Cell Expressing a High Affinity TCR Specific for MAGE-A 3/6 or NYESO-1 Administered Post ASCT In Patients With Advanced Myeloma

Principal Investigator: Aaron P. Rapoport, M.D., University of Maryland School of Medicine

and

Discussion of Human Gene Transfer Protocol #1007-1057 entitled: Phase I Study to Assess the Safety and Activity of Enhanced TCR Transduced Autologous T Cells Against Cancer-Testis Antigens In Metastatic Melanoma

Principal Investigator: Gerald P. Linette, M.D., Ph.D., Washington University School of Medicine

For both protocols:
Sponsor: Carl June, M.D., University of Pennsylvania
Financial Sponsor: AdaptImmune, Inc., Bent Jakobsen, Ph.D.
RAC Reviewers: Drs. Federoff, Kahn, and Yankaskas

A. Protocol Summary—*Protocol #1007-1056*

Multiple myeloma is the second most common hematologic cancer worldwide, with an estimated annual incidence of 16,000 and mortality of 11,000 per year. MM patients have only a 20 percent chance of surviving longer than 10 years. Although significant improvement in therapy has occurred during the past decade with the development of new drug regimens, there remains no cure for MM and new approaches are desperately needed. The purpose of this proposed trial is to evaluate genetic enhancement of the T cells of patients with advanced MM, so as to target their tumors.

It is now well established that human tumors have antigens, and that natural immune responses are associated with improved survival in a variety of cancers. The central problem is tolerance: the repertoire of TCRs is generally of too low avidity to efficiently recognize tumor antigens, and this explains the failure of most cancer vaccines. Iincorporation of high affinity T cell receptors (TCRs) specific for cancer testes antigens, which have been shown to enhanced efficacy against antigen expressing tumor cells, into autologous CD4 and CD8 T cells of patients with Stage III/IV melanoma may delay progression or promote regression of disease. To test this hypothesis, the investigators first isolated TCRs specific for the MAGE-A3 and NY-ESO-1 cancer testes antigens. Briefly, TCR gene cDNA sequences were isolated from eight MAGE-A3 *HLA-A*0101-EVDPIGHLY* restricted T cell clones, and one clone was selected based on optimal binding kinetics. Mutations were made in each of the three α and β chain complementary determining regions (CDRs). TCR gene cDNA sequences were also isolated from the NY-ESO *HLA-A*0201-SLLMWITQC* restricted T cell clone 1G4, and mutations were made in the CDRs of the β chain. High affinity Mage-A3 and NY-ESO-1 clones were selected by screening of phage display libraries for each of the TCR clones in three rounds of selection/enrichment. A series of candidates were further screened based on binding kinetics. Additional back mutations to the wild type TCR were required for the MAGE TCR in order to lower affinity to levels considered physiologically relevant. Additional mutations to the α chain of the NY-ESO TCR and then the CDR2 region of both chains were required for the NY-ESO-1 TCR to achieve optimal high affinity clones. Two high affinity MAGE-A3 (a3a and b2a) and NYESO-1 (c259 and c12/c2) candidates were evaluated in preclinical studies. Preclinical studies indicated that the high affinity TCRs exhibited enhanced degranulation and cytokine release (IFN-γ, Mip1β, IL-2, GMCSF and TNF-α) in response to antigen compared to each corresponding WT, which showed minimal response. The a3a and c259 high affinity clones were selected based on specificity for

target antigen and non reactivity to normal human cells. Effectiveness of NYESO-1 and MAGE A3 TCR transduced T cells was tested in the immunodeficient NSG (NOD/*scid*/γc null) mouse model using the aggressive human B-cell precursor acute lymphoblastic leukemia cell line (NALM-6), which expresses HLA-A1 and A2 and was engineered for expression of NY-ESO-1 and higher expression of Mage-A3 which is expressed naturally at low levels. The data from this study showed the WT-NY-ESO-1 and c259 TCRs both conferred enhanced survival, and the high affinity MAGE-A3 TCR was significantly more effective than the wt TCR. Similar results were observed for the a3a TCR in a solid tumor model, utilizing the ovarian tumor cell line OV79. To test the hypothesis using the candidate TCRs, the investigators designed a Phase I, open label, dual cohort study to evaluate the safety, bioactivity and anti tumor effects of autologous CD4 and CD8 T cells expressing the a3a or c259 TCRs in subjects with advanced myeloma. Eligible patients will be assigned to the a3a or c259 cohort based on their HLA type. T cells will be isolated from autologous apheresis product, and enriched by elutriation. T cells will be modified *ex vivo* by a lentiviral vector expressing either the a3a or c259 TCR, and then the cells will be further expanded using an artificial APC based T cell stimulation protocol that employs anti-CD3 and anti-CD28 monoclonal antibodies. Cell product will be administered as a single infusion of 10 billion cells. Subjects will be followed until they progress or up to 5 years post infusion. After this point, they will be monitored under a separate roll over protocol for up to 15 years in accordance with FDA Guidance for monitoring for delayed adverse events for retroviral vectors.

B. Written Reviews by RAC Members—*Protocol #1007-1056*

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential safety issues raised by infusion of a single dose of 10 billion cells expressing a high-affinity TCR that will lead to enhanced cytokine release. Given the high affinity of these TCRs, further discussion of potential on-target, off-tissue toxicity was deemed to be warranted.

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

Dr. Federoff asked the investigators to discuss the rationale for the $1x10^{10}$ T-cell dose in the context of prior trials, the increased affinity of the TCRs, and the data suggesting modest off-target effects. He requested information about what criteria would determine whether Bortezomib is used and what multiplicity of infection of the vectors would be used, given the stated interest in achieving greater than 60 percent transduction of T cells. Because MAGE3A demonstrated, in a variety of *in vitro* assays, that it has off-target effects, Dr. Federoff asked whether any cell or tissue types might be targeted inadvertently and might warrant additional clinical surveillance. He requested comment on whether recognition of apparent off-target antigens is due to a reduction of specificity rather than increased avidity and asked the investigators to elaborate on the implications of HLA restriction in terms of participant enrollment in and completion of the study.

Dr. Kahn suggested that the informed consent document be clearer about which parts of participation in this trial are research and which are standard therapy, since both are interspersed throughout the descriptions. He noted that the graphic representation of the study includes terms that are not in the glossary and that a heading labeled “Treatment Phase” might result in an overly optimistic view of the potential therapeutic benefits of participation in this trial. The section entitled “Pregnancy Risks” notes the requirement for participation in the Celgene Corporation RevAssist program as a condition of receiving lenalidomide; Dr. Kahn asked whether there is a separate informed consent document for participation in this program and/or additional information from the program. He reminded the investigators to include information in the informed consent document noting a request for autopsy in the event of death of participant— a request that is required for all gene transfer trials.

Dr. Yankaskas requested an explanation of the basis for selecting the anti-CD3 and anti-CD28 stimulation duration, a description of the expected duration of effects on the target T cells, and whether the genetic modification of the T cells would affect this stimulation. He asked whether all patients who meet the entry criteria would receive lenalidomide and how the effects of the T-cell infusions would be distinguished from those of lenalidomide. While acknowledging that the risks specified in the informed consent document and the methods used to minimize those risks seem appropriate, Dr. Yankaskas queried as to whether it

might be feasible to conduct *in vitro* studies to assess the risks of the gene-modified cells and how risks to participants would be minimized.

C. RAC Discussion—*Protocol #1007-1056*

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

- Dr. Buchmeier asked about the duration of persistence of the target T cells, including what happens to them, what is their behavior in terms of secondary responses, what fraction of the cells remains, and how would they respond if the tumor recurred.

D. Investigator Response—*Protocol #1007-1056*

1. Written Responses to RAC Reviews

The investigators explained that the single infusion dose of $1x10^{10}$ T cells was chosen based on published data using T cells engineered in a similar fashion as is proposed for this trial, as well as preliminary data presented at the April 2010 meeting of the American Association for Cancer Research (AACR) in April 2010. In general, adoptive transfer of autologous T cells is safe and well tolerated. The NIH Surgery Branch has published the only reports using TCR-transduced T cells in humans with cancer. Based on three key studies, the investigators chose to administer a flat dose of $1x10^{10}$ T cells as this dose appears to be safe and potentially efficacious. This dose is just above the minimum anticipated biological effect level.

The clinical TCRs proposed for evaluation have been chosen from panels of TCRs containing a range of affinities and reactivities, and have been selected based on a maximal on-target response while maintaining minimal off-target responses. With only occasional minor responses to some melanocytes, hepatocytes, and endothelial cells, MAGE-3A-transduced T cells have a clean on-target profile of response to a variety of normal human tissues.

Bortezomib is included as an option for pretransplant therapy because it gives the treating physician more discretion for pretransplant chemotherapy, since some patients may come to the protocol with refractory disease. Additional chemotherapy with bortezomib may be desirable to reduce disease burden and subsequent contamination of the stem-cell product with tumor.

The investigators will choose the multiplicity of infection that balances expression of at least 50 percent TCR and a copy number of 0.5 to 5 copies per cell. Based on the data from this experiment, the copy number range could be reduced to 0.5 to 4 or lower, depending on the data. This copy number range has been acceptable in the investigators' previous lentiviral vector clinical trials.

Research participants in the clinical trial will undergo rigorous monitoring on the day of infusion and daily to day 3, then on day 5 and day 7, and weekly until 1 month. This monitoring includes regular physical exams, complete metabolic and hepatic panels, and complete blood counts. Serum is collected on the day of T-cell infusion before the participant receives cells and 1 to 2 hours post infusion so cytokine levels can be monitored. The data does not indicate a requirement of additional clinical surveillance to monitor anticipated targeting of normal cells, and the investigators believe the current monitoring plan is sufficient to capture unanticipated toxicities.

Prior to deciding to proceed with this trial, the investigators performed an analysis of the number of patients seen at the University of Maryland, with similar numbers assumed for the University of Pennsylvania. They found that the combined restriction on patient selection based on HLA type, antigen expression, and the general acceptance rate for participation in these types of adoptive transfer trials (50 percent, based on their prior experience in myeloma trials of similar structure) would result in rapid completion of the NYESO-1 cohort within the first year, with slower accrual in the MAGE cohort, which could be completed in 18 months between the two sites.

The RevAssist program is a restricted distribution program under which lenalidomide is provided by Celgene. A prescriber of lenalidomide is required to be registered with the program, and research participants also must be enrolled in the program and must agree to comply with its requirements. In addition, only RevAssist contract pharmacies are able to provide lenalidomide. Additional details and information packets regarding lenalidomide and the RevAssist program can be found at http://www.revlimid.com. The reason for this program is to track the use of lenalidomide as it is a derivative of thalidomide and therefore there are serious reproductive safety concerns. The purpose of the program is to ensure that patients receiving, and physicians administering, lenalidomide are aware of these concerns and take precautions to avoid taking lenalidomide while pregnant, and that they take necessary precautions to avoid pregnancy while on lenalidomide. The RevAssist program has a separate informed consent document, and the language in the informed consent document for this protocol is provided to inform the participant at the outset of the study.

In general, the investigators have observed that T-cell stimulation with CD3/CD28 antibody coated beads results in expansion of T cells for 12 to 20 days depending on the donor cells, at which time restimulation is required or the cell viability begins to decline. Activation of the cells and their ability to respond to subsequent stimulations is reflected in cell volume, which peaks at about day 5 post stimulation and then decreases thereafter. T cells are targeted for harvest around day 9 to day 12 so that the cells are infused while they are still active and before the viability starts to decline, and also at the point where they are sensitive to restimulation upon infusion *in vivo*. The investigators have previously conducted several studies evaluating T-cell infusion post stem cell transplantation, in the setting of lymphoma and myeloma using this method of T-cell expansion. The data demonstrate the quantitative and functional restoration of immunity *in vivo*, supporting the success of the *ex vivo* expansion method.

Treatment of patients with lenalidomide as maintenance therapy is generally accepted as the standard of care, and therefore the investigators believe it is important to offer that treatment to participants in this clinical trial. Lenalidomide treatment begins at day 100 post T-cell infusion. Acute AEs associated with the MAGE-3A or NYESO-1 products will be distinguished easily from those associated with lenalidomide because AEs associated with T-cell infusion should occur within the first few weeks of infusion and lenalidomide has nonoverlapping toxicity with T-cell infusion-related toxicity, and the dose administered is a low dose (10 mg per day). The expected AEs associated with lenalidomide are thrombocytopenia and myelosuppression, which are not known or anticipated AEs from the T-cell infusions.

The investigators stated that five major risks are potentially associated with the MAGE or NYESO lentiviral vector gene-modified T cells: 1) off-target effects of the TCR due to the affinity enhancement process, 2) systemic cytokine release leading to cytokine storm, 3) insertional oncogenesis mediated by vector integration, 4) exposure of the participant to a replication-competent lentivirus, and 5) autologous engraftment syndrome. The investigators have addressed these risks *in vitro* to the extent possible using available *in vitro* and animal models.

The investigators agreed to modify the informed consent document to include the suggested clarifications and language modifications.

2. Responses to RAC Discussion Questions

Dr. June explained that in the investigators' ongoing 15-year follow-up of a clinical trial using a CD4/CD3-zeta "zetakine" chimeric antigen receptor in HIV-1-infectd subjects, 15 out of 17 study participants from 9 and 10 years ago have the same gene-marked cell frequency as at 6 months. The half-life of these cells in the participants appears to be greater than 17 years, and sequencing studies are under way; the participants are all well and are all stable engrafted. The investigators have encountered no incidence of insertional activation. Because the approach looks very safe so far, the investigators are not yet considering the use of suicide genes.

E. Public Comment—*Protocol #1007-1056*

Dr. Borror noted that the informed consent document used the term “gene therapy,” which should be changed to the more-appropriate “gene transfer.” She also requested clarification of the amount of marrow that would be collected during the early post-transplant blood and bone marrow tests.

F. Protocol Summary—*Protocol #1007-1057*

In 2010, it is estimated that 68,130 new cases of cutaneous melanoma will be diagnosed in the United States. Although many individuals will be detected early and cured with surgery, approximately 8,700 patients will die from metastatic melanoma. Patients with advanced metastatic melanoma suffer an especially poor prognosis, with a median survival of 6 to 10 months. Despite extensive clinical investigation, chemotherapy has no impact on survival. Melanoma is an inherently immunogenic cancer, and therefore approaches that harness a patient’s own immune response to his/her tumor may offer an effective alternative to chemotherapy. Recent advances now provide compelling evidence that T cells can mediate rejection of melanoma in humans. The purpose of this proposed trial is to evaluate genetic enhancement of the T cells of patients with metastatic melanoma, so as to target their tumors.

It is well established that human tumors have antigens that make them recognizable to the immune system, and that naturally occurring tumor immune responses are associated with improved survival in a variety of cancers. The central problem in harnessing a patient’s immune system to fight their tumor is that the tumor can induce tolerance to recognition by the immune system. This is a major issue with cancer vaccines, and natural T-cell responses are often not strong enough to fight the tumor. One way to avoid this problem is to generate high-affinity T cells capable of tumor recognition. Recent advances in T-cell culture and genetic modification technologies have made this approach possible.

The hypothesis driving the proposed clinical study is that autologous T cells can be altered genetically to target melanoma tumor cells, thus delaying progression or reversing disease. To do this, the investigators isolated T cells specific for a type of antigen present on melanoma tumors, called cancer testis antigens. High-affinity T-cell receptors (TCRs) are then generated using a laboratory method developed by Adaptimmune Ltd. These TCRs can be genetically inserted into a patient’s T cells to generate tumor-specific T cells. Two types of cancer testis antigen TCRs are being evaluated, each of which works in patients with the HLA-A1 or the HLA-A2 gene phenotypes. To test their hypothesis using the candidate TCRs, the investigators designed a Phase I, open label, dual cohort study to evaluate the safety, bioactivity, and antitumor effects of autologous CD4 and CD8 T cells expressing the a3a or the c259 TCRs in patients with metastatic melanoma.

Eligible research participants will be assigned to the MAGE-3A a3a or the NYESO-1 c259 cohort based on their HLA type. T cells will be isolated from participants using apheresis, which allows for collection of white blood cells without removal of other blood components. T cells then will be genetically modified in the laboratory using a gene delivery system derived from a lentiviral vector, which permanently modifies the T cells to express either the MAGE-3A a3a or the NYESO-1 c259 TCR. The cells then will be further expanded in the laboratory.

The vector to deliver the TCRs will be manufactured according to GMP at the Center for Biomedicine and Genetics in the City of Hope, Duarte, California. The cells will be manufactured at the GMP-compliant Clinical Cell and Vaccine Production Facility at the University of Pennsylvania, which has safely produced 377 T-cell products for 261 patients to date. Research participants will be dosed at the Siteman Cancer Center at Washington University (Barnes-Jewish Hospital) in St. Louis, Missouri. To enhance safety, cell product will be administered as a 30 percent/70 percent split infusion on consecutive days. Participants will be followed for up to 1 year post infusion, and then for as long as 15 years in accordance with FDA guidance for monitoring for delayed AEs for lentiviral vectors. This clinical trial is designed to demonstrate safety and indications of efficacy, to support development of a larger, controlled study in which the effectiveness of this approach will be tested.

G. Written Reviews by RAC Members – *Protocol #1007-1057*

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included the potential safety issues raised by the proposed infusion of a single dose of 10 billion cells expressing a high-affinity TCR that will lead to enhanced cytokine release. Given the high affinity of these TCRs, further discussion of potential on-target, off-tissue toxicity is warranted.

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

Dr. Federoff asked the investigators to discuss the rationale for the $1x10^{10}$ T-cell dose in the context of prior trials, the increased affinity of the TCRs, and the data suggesting modest off-target effects. He requested further information and discussion regarding the rationale for dose partitioning of 30 percent on day 5 and 70 percent on day 6, and why they did not propose a dose-escalating approach. He also asked what fraction of melanoma patients will express either of the cancer testis antigens and the appropriate major histocompatibility complex, and whether the kinetics of engineered T-cell loss would influence the 4-week and 8-week timepoints for cellular functional analyses. Regarding TCR signaling and given that chimeric antigen receptors (CARs) have enhanced signaling and in at least one case caused death, Dr. Federoff asked the investigators to expand on the intracellular signaling properties of T cells expressing a higher affinity TCR. He requested comment on the stopping rules, particularly why two DLTs would be required, and he asked the investigators to discuss the possibility of beginning at a lower dose and escalating across the trial.

Dr. Kahn noted that the language in the informed consent document includes multiple uses of the term “treatment” in ways that are likely to be misleading for potential participants; since this is a Phase I trial, he suggested replacing “treatment” with “modified T-cell infusion” or similar wording. He also reminded the investigators of the importance of informing potential participants that this is a Phase I trial and the implications of participating in early-phase research, noting that the language in the introductory paragraphs overstates the potential for benefit in a Phase I trial. Dr. Kahn asked the investigators to explain the optional research-related tissue biopsy, why a separate and additional agreement to rules regarding reproductive risks is required, and why participants are being directed to sign the form specifically in relation to contact with family for permission to autopsy.

Dr. Yankaskas asked the investigators to describe the detailed processes to generate and select the clones, since the methods and selection criteria in the Summary were not clear. He asked for details of the preclinical safety data and a description of the reasons for selecting the single infusion dose, including the data that support its safety. Noting that body CT or positron emission tomography (PET)/CT scans are planned at screening and weeks 4, 8, 12, and 16 and that recent medical and lay reports have documented radiation risks from such scans, Dr. Yankaskas suggested that the investigators consider addressing the radiation exposure in the informed consent document.

H. RAC Discussion—*Protocol #1007-1057*

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

- Dr. Rogatko asked the investigators how long it takes to observe a DLT. He suggested that the investigators include in the protocol a description of the amount of time that participants would be observed for DLTs before the next participant would be dosed.

- With regard to statistical considerations, Dr. Rogatko suggested that the investigators use the statistics provided in Protocol #1056 as a template for the current protocol. He requested that the calculations be presented based on the sample size using safety concerns, efficacy, and stopping rules, and that probabilities be assigned to these events.

- Dr. Takefman asked the investigators why they chose to use lentivirus for this study.

I. Investigator Response—*Protocol #1007-1057*

1. Written Responses to RAC Reviews

Regarding dose partitioning of 30 percent on day 5 and 70 percent on day 6, the investigators explained that they chose this schedule based on recommendations made at the RAC safety symposium on Gene-modified T cells in June 2010. The purpose of split dosing is to reduce the risk of an acute cytokine release syndrome. Each participant's serum will be monitored for cytokine levels before and 20 minutes and 4 hours after the T-cell infusion; in the event of any clinical or laboratory evidence of cytokine release syndrome, the following T-cell dose will be withheld.

As to why a dose-escalating approach was not proposed, the investigators responded that no dose escalation was performed in previous TCR gene transfer trials performed at the NIH. In three key clinical trials, no treatment-related deaths occurred at any dose and no SAEs occurred among the 18 participants dosed with the same TCR to be employed in this proposed trial. The highest dose administered in the NIH trials was more than one log higher than the dose proposed for this trial. Thus the currently proposed protocol has three important safety features compared with previous TCR trials in melanoma: the investigators propose using a dose that is one log lower than has previously been tested and found to be safe, a split-dose infusion schedule is proposed, and high-dose IL-2 therapy will not be used.

The investigators' review of the data supports the conclusion that the proposed dose of $1x10^{10}$ transduced T cells is safe and potentially efficacious in patients with metastatic melanoma after non-myeloablative chemotherapy. High-dose IL-2 will not be used because it is toxic and must be administered in an inpatient setting and no randomized data support its use after adoptive T-cell therapy in humans.

The investigators expect that a sufficient number of patients will be eligible and willing to participate in this trial. In their clinic, the investigators see 150 new patients with advanced melanoma each year. Because the frequency of MAGE expression in melanoma is 65 percent, they expect to see 24 patients per year who would be eligible for the MAGE-specific TCR cohort. Because the frequency of NYESO expression in melanoma is 30 percent, they expect to see 20 patients per year who would be eligible for the NYESO-specific TCR cohort. Approximately 50 percent of those patients would not be eligible for a variety of reasons including poor performance status, medical comorbidities, and no measurable disease.

The investigators believe the proposed timepoints for the research correlative studies for cellular functional analysis are feasible and realistic. In prior lentiviral vector T-cell gene transfer trials in which $1x10^{10}$ vector modified cells have been administered in the absence of lymphodepleting chemotherapy, engraftment post infusion was observed to persist beyond 8 weeks ranging from 1% to approximately 0.05% of total PBMCs. Based on published data demonstrating robust reconstitution of the T-cell compartment post T-cell infusion, and taking into account the at least 50 percent transduction release criteria and homeostatic proliferation of the engineered cells, the investigators expect that a significantly higher proportion of the T cells will contain the transgene at these early post infusion timepoints. Over time as the T-cell compartment is reconstituted from endogenous sources, the percentage of transduced T cells is expected to decrease, which is anticipated to occur after week 8 based on the well-characterized kinetics of T-cell reconstitution chemotherapy in melanoma patients.

With regard to TCR signalling in this trial, the transmembrane and cytoplasmic domains of the enhanced TCRs will not have been modified, and thus the investigators do not expect to find any significant alteration in the transcription activity of TNF-α, IL-10, INF-γ, or IL-6. In trials with engineered T cells, the National Cancer Institute has observed toxicity with CARs but not with TCRs in melanoma patients.

Regarding the stopping rules, in the event of a DLT, the data will be reviewed first by the DSMB and then forwarded to the IRB before the next participant is enrolled. If evidence suggests that the true DLT rate exceeds 30 percent, the study will be stopped for safety reasons and the data will be reviewed by the IRB, the regulatory sponsor, and the FDA. In any event, the DSMB will review all study data every 6 months.

The overall intent of the investigators in conducting this trial is to balance safety and potential therapeutic benefit in patients with an incurable illness. They explained that they selected a split dose schedule to minimize the risks associated with an acute cytokine release syndrome, as suggested by the RAC advisory committee. Although they expect to see a rise in serum cytokines, the risk of a clinically significant toxicity using enhanced TCRs is small compared to the risk with high-affinity, enhanced CAR constructs.

The investigators explained that the optional research-related biopsy is intended only for participants who do not respond (defined as less than 30 percent tumor reduction or progressive disease) as proof of principle to investigate mechanisms of resistance. The purpose would be to study the degree of T-cell infiltration into tumor sites and to assess molecular mechanisms associated with evasion of T-cell therapy. These mechanisms are important because targeted therapeutics currently in development could be administered to nonresponders to augment the T-cell therapy. The surgical co-investigator will perform this biopsy only if an accessible lesion is identified.

The investigators explained that the separate and additional agreement regarding reproductive risks is an institutional requirement from the Washington University School of Medicine IRB. It serves to highlight the need for strict adherence to birth control or abstinence practices as well as proper notification to the study physician in the event the research participant or partner becomes pregnant.

A detailed description of the generation and selection of the clones and details of the preclinical safety data was provided in the investigators' responses to Appendix M questions.

The investigators explained that the single infusion dose of $1x10^{10}$ T cells was chosen based on published data using T cells engineered in a similar fashion as is proposed for this trial, as well as preliminary data presented at AACR in April 2010. In general, adoptive transfer of autologous T cells is safe and well tolerated. The NIH Surgery Branch has published the only reports using TCR transduced T cells in humans with cancer. Based on three key studies, the investigators chose to administer a flat dose of $1x10^{10}$ T cells since this dose appears to be safe and potentially efficacious; this dose is just above the minimum anticipated biological effect level. In addition to omitting IL-2 to mitigate toxicities, the investigators chose to administer the T cells as a split dose infusion over 2 days to further minimize the risk of a cytokine release syndrome. They plan to monitor serum at frequent timepoints in order to document cytokine serum levels after the T-cell infusions. Daily visits for the first 2 weeks (except weekends) are required, thus ensuring close clinical monitoring in each participant in this trial.

The risks associated with radiation exposure during PET/CT imaging have received national attention. Being aware of the concerns related to participant safety, the investigators explained that they chose to obtain CT imaging every 4 weeks in order to document the kinetics of response to guide future clinical trial endpoints. The current accepted standard imaging is every 6 weeks for study participants with metastatic melanoma, but the investigators believe that obtaining two additional scans within the timeline is acceptable from a risk standpoint and those additional scans will provide useful information. The informed consent document contains the currently accepted language pertaining to the risks for cancer patients with a life expectancy of shorter than 5 years as recommended by the Washington University School of Medicine IRB, and the investigators have inserted language that clearly states that CT imaging every 4 weeks is more frequent than the standard 6-week schedule and that an increased risk from this additional radiation exposure is possible.

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

Regarding DLTs, Dr. June explained that it is common for research participants to have some transfusion-like reactions that include Grade 1 or Grade 2 chills and fever, usually within the first hours after dosing, independent of the transgene expression; these reactions are easily managed. The only other toxicities

seen were an autologous graft-versus-host–like syndrome that occurred in one myeloma participant in a prior study—a colitis-like syndrome that included diarrhea, with an onset at 3 weeks after T-cell infusion that likely was due to autologous T cells that were diminished of their amount of regulatory T cells. This syndrome has not been seen in hundreds of other dosed melanoma study participants.

Dr. Linette explained that because of the intensity required to monitor these participants, the investigators will only be able to dose one individual at a time during a 30-day period, which results in a natural staggering period for dosing.

Regarding the use of lentivirus for this study, Dr. June explained that the investigators ideally would like to conduct a head-to-head comparison to study silencing in humans using retrovirus and lentivirus vectors. In addition, it is possible to get slightly better packaging of larger inserts with lentivirus, efficiency is slightly higher with lentivirus, but cost is currently higher for lentivirus. The investigators have significant experience using lentivirus, and safety data on integration sites looks favorable using lentivirus in trials.

J. Public Comment—*Protocol #1007-1057*

No public comments were offered.

K. Synopsis of RAC Discussion and RAC Observations and Recommendations

Dr. Federoff noted that the RAC offered no observations or recommendations regarding Protocol #1007-1056. The following observations and recommendations were made during the RAC's in-depth review and public discussion of Protocol #1007-1057.

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

- The ongoing work to address possible changes in signaling due to the prolonged T-cell binding with the higher affinity modified TCR may be important for further development of this approach. The protocol would benefit from further discussion of the proposed studies that will be undertaken to explore these questions.

- The protocol stopping rules state that the protocol will be halted if there are two DLTs. The decision to use two rather than one DLT is based on recognition that there may be Grade 3 SAEs (as defined by the Common Terminology Criteria for Adverse Reactions [CTCAE]) from the chemotherapy or other interventions that are unrelated to the cells. However, the investigators note that there will be a pause to assess each DLT prior to moving forward with dosing of the next participant. The protocol should be specific in its definition of a DLT, not only with respect to toxicity type and grade based on CTCAE level but also by designating a time interval between agent administration and the onset of the event.

- This protocol was reviewed together with OBA protocol 1056, *A Phase I, Dual-Cohort, Two-Site, Clinical Trial Evaluating the Safety and Activity of Redirected Autologous T Cells Expressing a High Affinity TCR Specific for MAGE-A3/6 or NY-ESO-1 Administered Post ASCT in Patients with Advanced Myeloma,* which has the same sponsor and uses the same agent and dose. However, in Protocol 1056, the rationale for the number of participants to be enrolled is well developed from a statistical perspective. This rationale should likewise be developed in this protocol, which currently lacks a discussion of the statistical considerations for the proposed enrollment size based on toxicity, stopping rules, and potential efficacy.

<u>Ethical/Social/Legal Issues</u>

- The protocol incorporates an optional biopsy for research-related tests. The informed consent document should contain further detail on the studies to be done on these biopsies and whether there will be any future uses of this tissue beyond the protocol.

- The informed consent document contains a boxed agreement requiring an additional signature. As written, it appears to be a separate contract regarding limits on reproductive activity. The nature of the language used and this contractual format appears overly prescriptive and not consistent with the goal of informed consent, in which potential participants are informed of the risks and are requested to take appropriate precautions to avoid reproduction during the trial.

L. Committee Motion 7

Dr. Federoff summarized the RAC recommendations that will be included in the letter to the investigators expressing the comments and concerns of the RAC. It was moved by Dr. Kahn and seconded by Dr. Yankaskas that the RAC approve these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 4 recusals (Drs. Fost, Ross, Strome, and Zaia).

XXI. Closing Remarks and Adjournment

Dr. Federoff thanked the RAC members and the OBA staff, and adjourned Day 3 of the September 2010 RAC meeting at 11:00 a.m. on September 17, 2010.

[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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Andrew Byrnes, FDA
Ronald Crystal, Weill Cornell
Sharon Daily, Cato Research
Richard Dondero, Cenesco
Ann Galy, Genethon
Shane Grimes, Cato Research
Caitlin Hamill, FDA
John Hyde, FDA
Carl June, University of Pennsylvania
Larry Kwak, MD Anderson Cancer Center
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Michael Leviton, Bayhill Therapeutics
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Maritza McIntyre, RegenX Bio
Luigi Notorangelo, Children's Hospital Boston
Sean O'Connor, MD Anderson Cancer Center
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Attachment III
Abbreviations and Acronyms

AACR	American Association for Cancer Research
AChR	acetylcholine receptor
AE	adverse event
AMD	age-related macular degeneration
AML	acute myelogenous leukemia
BMT	bone marrow transplantation
CARs	chimeric antigen receptors
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
CSF	cerebrospinal fluid
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Reactions
DHHS	U.S. Department of Health and Human Services
DHS	deoxyhypusine synthase
DLT	dose-limiting toxicity
DSMB	data and safety monitoring board
EAMG	experimental autoimmune MG
EIAV	equine infectious anemia virus
eIF5A	eukaryotic translation initiation factor 5A protein
FBS	fetal bovine serum
FDA	Food and Drug Administration, DHHS
GLP	good laboratory practices
GM-CSF	granulocyte macrophage colony stimulating factor
GMP	good manufacturing practices
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HLA	human leukocyte antigen
HTLV	human T-cell leukemia virus
IND	investigational new drug
IRB	institutional review board
LINCL	late infantile neuronal ceroid lipofuscinosis
LLOQ	lower limit of quantification
LPL	lymphoplasmacytic lymphoma
LTR	long terminal repeat
MG	myasthenia gravis
MM	multiple myeloma
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
National Commission	National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NMJ	neuromuscular junction
NOAEL	no observable adverse effect level
NSAIDS	nonsteroidal anti-inflammatory drugs
OBA	Office of Biotechnology Activities, NIH
OCTGT	Office of Cellular Tissue and Gene Therapies, FDA
OD	Office of the Director, NIH
PBMCs	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PET	positron emission tomography
RAC	Recombinant DNA Advisory Committee

SAE	serious adverse event
siRNA	small inhibitory RNA
TCRs	T-cell receptors
TU	transmission unit
WAS	Wiskott-Aldrich syndrome