RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 16-17, 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]

June 16-17, 2010

The Recombinant DNA Advisory Committee (RAC) was convened for its 121st meeting at 8:00 a.m. on June 16, 2010, at the Hilton Hotel and Executive Center, Rockville, Maryland. Dr. Howard Federoff (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 4:35 p.m. on June 16 and from 8:30 a.m. until 2:40 p.m. on June 17. The following individuals were present for all or part of the June 2010 RAC meeting.

Committee Members

Jeffrey S. Bartlett, Nationwide Children's Hospital/The Ohio State University
Michael J. Buchmeier, University of California, Irvine
Hildegund C.J. Ertl, The Wistar Institute/University of Pennsylvania
Hung Y. Fan, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University
Jeffrey P. Kahn, University of Minnesota
Joseph A. Kanabrocki, The University of Chicago
Louis V. Kirchhoff, University of Iowa
Margaret Mallino, University Park, Maryland
Anna C. Mastroianni, University of Washington School of Law *(Day 1 only)*
Bernard Roizman, The University of Chicago
Susan R. Ross, University of Pennsylvania
Scott E. Strome, University of Maryland *(Day 1 only)*
Lee-Jen Wei, Harvard University *(Day 1 only)*
David A. Williams, Children's Hospital Boston/Harvard Medical School
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**

Denise Gangadhara, Ph.D., Centers for Disease Control and Prevention (CDC)
Craig C. Mello, Ph.D., Howard Hughes Medical Institute
Suman Mukhopadhyay, Ph.D., National Institute of Allergy and Infectious Diseases, NIH
Saraswathy V. Nochur, Ph.D., Alnylam Pharmaceuticals, Inc.
Olaf Schneewind, M.D., Ph.D., University of Chicago
Martin Schriefer, Ph.D., CDC
Phillip A. Sharp, Ph.D., Massachusetts Institute of Technology
Susan L. Welkos, Ph.D., U.S. Army Medical Research Institute for Infectious Diseases

Nonvoting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services

[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.

Mercedes A. Serabian, U.S. Food and Drug Administration (FDA), U.S. Department of Health and Human Services (DHHS)
Daniel M. Takefman, FDA

NIH/OD/OBA Staff Members

Chezelle George
Linda Gargiulo
Maureen Montgomery
Marina O'Reilly,
Gene Rosenthal,
Tom Shih, M.D.,
Mona Siddiqui

Attendees

There were 85 attendees at this 2-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. Federoff, RAC Chair, called the meeting to order at 8:00 a.m. on June 16, 2010. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on May 19, 2010 (75 FR 28029). 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 six protocols, discussion of attenuated strains of *Yersinia pestis* that are excluded from the CDC Select Agent Regulations – Evaluation under Section III-A-1 of the *NIH Guidelines*, discussion of a proposal to exempt the mating of certain biosafety level (BL) 1 transgenic rodents from the requirements of Section III-E-3 of the *NIH Guidelines*, discussion of proposed changes to Section III-E-1 the *NIH Guidelines*, and presentation of updates and discussion of proposed changes to Section III-C-1 of the *NIH Guidelines*.

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

II. Minutes of the March 10-11, 2010, RAC Meeting

RAC Reviewers: Drs. Bartlett and Kanabrocki

Dr. Kanabrocki stated that the March 2010 RAC minutes document was an accurate reflection of the proceedings. He noted that he had submitted some revisions for clarity and enhanced readability and, in the discussion of the final protocol, the vector was referred to as "replication competent" but should have been characterized as "replication incompetent." Dr. Barlett concurred.

A. Committee Motion 1

Approval of the March 2010 RAC meeting minutes was moved by Dr. Bartlett; no second was requested. The RAC voted unanimously by voice vote to approve the March 10-11, 2010, RAC meeting minutes.

III. Gene Transfer Safety Assessment Board Report

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

Dr. Zaia reported that, of the 16 protocol submissions received by the OBA in the past 3 months, 10 protocols were not selected for public review at this RAC meeting. Of the 10 protocols not selected for public review, six were oncology protocols, two were for infectious diseases, one was for diabetic peripheral neuropathy, and one was a nontherapeutic vaccine study. The vectors used in these 10 protocols were five plasmids, two adenoviruses, one fowlpox, one measles virus, and one herpes virus.

Eleven protocols submitted *Appendix M* followup information indicating their enrollment. Of trials that have initiated enrollment in the past 3 months, three protocols submitted M-I-C-1 responses to the OBA, all three of which had been reviewed by the RAC at a previous public meeting. Dr. Zaia provided highlights of responses to the RAC recommendations from these three protocols.

- **Protocol #0810-942, A Phase Ib, Multi-Center, Single Blinded, Placebo-Controlled, Sequential Dose Escalation Study To Assess the Safety of Topically Applied AGO13 in Subjects Receiving Induction Chemotherapy for the Treatment of Cancers of the Head and Neck**, was reviewed by the RAC in December 2008. Concerns were raised regarding the stability of the thymidine-minus *C. lactis* strain that is proposed to be used. Additional experiments were conducted to show that no thymidine A+ revertants could be detected in at least $2x10^{10}$ colony forming units (cfu) of the experimental agent. Additional studies were performed to evaluate the risk of disease from systemic circulation of the gene modified *L. lactis* in neutropenic patients; studies in both neutropenic hamsters and rodents demonstrated that the bacteria could not survive in systemic circulation.

- **Protocol #0907-988, A First-in-Human Safety and Dose-Finding Study of a New Type-16 Human Rhinovirus (RG-HRV16) Inoculum in Healthy Volunteers**, was reviewed by the RAC in September 2009. Since the transmission rate of this virus has not been investigated, the first five participants in each dosing group will not be living in a communal environment and household contacts will be encouraged to participate to monitor the spread of the virus. Spaced by at least 7 days to obtain adverse event data, one-by-one inoculations will occur whenever the dose is increased. Stopping rules have been implemented so that, for the first five participants in any new dose cohort, if a household contact develops cold symptoms the etiology will be established before the study will resume. If RG-HRV16 is the cause of those symptoms, an independent medical monitor and a National Institute of Allergy and Infectious Diseases (NIAID) medical officer will review the data on transmission and severity prior to proceeding.

- **Protocol #0910-1004, An Open Label Dose Escalation Study To Evaluate the Safety of a Single Escalating Dose of ACRX-100 Administered by Endomyocardial Injection to Cohorts of Adults with Ischemic Heart Failure**, was reviewed by the RAC in December 2009. As the preclinical model used porcine stromal cell derived factor (SDF) 1, a question was raised regarding whether the human SDF-1 binds the porcine receptor, the answer to which could inform the starting dose. Additional data was sought on transgene expression. The study sponsor has performed efficacy, biodistribution, and toxicology studies, and has submitted this information to the FDA. Given the high incidence of cardiac events, the stopping rules for the trial have been revised.

A total of 21 serious adverse events (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. Zaia drew RAC members' attention to a paper to be published on June 17, 2010, regarding the first use of a multivalent gene transfer vector in which expression occurred for up to 2 years and research participants are continuing to be followed, and a conference sponsored by OBA and the National Institute of Neurological Disorders and Stroke on June 30 and July 1, 2010, on the use of sham neurosurgical procedures in clinical trials, which is being co-chaired by Dr. Federoff.

Dr. Fan summarized a symposium scheduled for December 9 and 10, 2010, organized by the RAC in partnership with CliniGene titled "Integrating Vectors in Gene Transfer: Update on Insertional Mutagenesis and Vector and Study Design." The seven sessions planned for this symposium are:

- Overview of human gene transfer trials involving retroviral/lentiviral vectors
- Non-enhancer mediated mechanisms of insertional oncogenesis
- Lessons from oncogenic retroviruses
- Improving design and safety of gene transfer vectors
- Models for assessing safety in retroviral/lentiviral gene transfer experiments
- Monitoring for insertional mutagenesis and stopping rules
- Clinical and ethical issues in the design of gene transfer trials using integrating vectors in stem cells

IV. Discussion of Human Gene Transfer Protocol #1004-1034 entitled: Phase I Study of the Administration of EBV-CTLs Expressing CD30 Chimeric Receptors for Relapsed CD30+ Hodgkin's Lymphoma and CD30+ Non-Hodgkin's Lymphoma

Principal Investigator:	Helen Heslop, M.D., FRCP, Baylor College of Medicine, The Methodist Hospital and Texas Children's Hospital
Additional Presenters:	Gianpietro Dotti, M.D., Texas Children's Hospital; Carlos A. Ramos, M.D., Texas Children's Hospital; and Barbara Savoldo, M.D., Ph.D., Texas Children's Hospital
RAC Reviewers:	Ms. Mastroianni, Dr. Ross, and Dr. Yankaskas

A. Protocol Summary

Hodgkin's lymphoma (HL) represents about 11 percent of all lymphoma diagnoses. Approximately 8,000 cases of HL are detected each year, which represents less than 1 percent of all cancers. The National Cancer Institute (NCI) estimates that 65,980 new cases of non-Hodgkin's lymphoma (NHL) will be diagnosed in 2009, which represents 89 percent of all lymphomas and 4 percent of all cancers diagnosed. As the seventh most common cancer in the United States, NHL is more prevalent in males than females, with 55 percent of lymphoma diagnoses in male patients. The average age at diagnosis is 65 and, due to the aging of the population, the incidence of NHL is expected to rise in the coming years.

Patients with HL and NHL associated with infection with Epstein Barr virus (EBV) have had clinical responses to a nontoxic treatment based on the infusion of EBV-specific cytotoxic T lymphocytes (EBV-CTLs) that kills tumor cells infected with the virus. The investigators propose to extend this approach to all HL patients by targeting a different antigen (CD30), which is invariably expressed by HL tumor cells and by some NHL cells.

Using gene transfer technology, the investigators propose to transduce into the research participants' T cells a gene for an artificial receptor that will direct the T cells to tumor cells expressing the CD30 molecule, and will allow the T cells to kill the tumor cells. The gene will be placed into T cells that are preselected for their ability to recognize EBV, which is chronically present in most people. Because these

EBV-CTLs meet the virus positive cells as well as tumor cells, they receive extra stimulation. The gene for the artificial receptor will be transduced into research participants' EBV-CTLs using a Moloney retroviral vector. These modified T cells will then be infused into the bloodstream through a central line or a vein.

Research participants will be dosed in the clinic and will be monitored closely for several hours after infusion. Samples from peripheral blood will be collected at regular intervals. The investigators plan to examine the safety, persistence, and function of the cells infused into the research participants.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novelty of the CD30 target antigen that is expressed on normal cells and that may be involved in T-cell homeostasis. Targeting this antigen using modified T cells that have the ability to expand and persist raises safety concerns that are particularly important because the investigators propose to enroll pediatric research participants.

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

Ms. Mastroianni asked the investigators to justify the inclusion of pediatric research participants in this Phase 1 (safety/toxicity) study, and suggested that a separate assent document be included for them. In addition, she wondered whether children would be able to "lie relatively still" for "3-6 hours" during pheresis. She offered the following comments related to the "procurement" and "infusion" informed consent documents:

- The investigators should explain why they propose to use two separate informed consent documents, when the participant population for infusion appears to be the same as that for procurement.
- Language should be revised so that participants will not be confused concerning the potential for this Phase I trial to convey benefit; examples include "treatment" and "therapy." The sections titled "Purpose" in both consent forms should be reviewed to ensure that participants clearly understand that this is a Phase I safety trial.
- A simple schedule or table for the timing and requirements for infusion would assist understanding of participants' obligations. In addition, the escalating dose approach should be explained in the infusion consent document so it is clear that all participants will not be receiving the same dosage.
- The fact that participants will receive two doses of CTL given 2 weeks apart should be reflected in the informed consent document, and the requirement that gene-modified cells will not be administered within 4 weeks of chemotherapy or radiotherapy should be made clear to potential research participants.
- The additional risks and benefits of the referenced blood tests, scans, and/or bone marrow studies before, during, and after infusion should be explained in the infusion consent document.
- Ongoing followup obligations should be specified for participants who choose to withdraw from the study after infusion.

Ms. Mastroianni suggested that provisions to maintain the confidentiality of research participants should provide specific plans for maintaining confidentiality, especially of individually linked data. She asked the investigators whether they anticipate a greater desire to participate than the study can accommodate and, if so, how they would address that situation.

Dr. Ross noted that experiments using the original CD30 knockout mouse showed impaired negative selection of T cells in the thymus but adequate immune responses. Followup studies on these mice have indicated a role for CD30 in natural killer (NK) cell survival and control of cytomegalovirus infection, and that these mice have impaired germinal center and recall memory antibody responses. Given these results in mice, she asked the investigators about how they will ensure that other aspects of immunity are not impaired in the dosed research participants. She asked if the knockout mice showed evidence for

development of autoimmunity and, if so, how this development would be monitored in research participants. Because CD30 is upregulated on the T cells of HIV-infected and HTLV-1-infected individuals, Dr. Ross suggested that both patient populations be excluded from participation in this clinical trial. Given that the investigators have previous data showing that infusion of EBV-CTLs can ameliorate disease when the lymphomas express EBV antigens, Dr. Ross asked whether the tumors in this trial would be examined for expression of EBV proteins and how many patients with EBV+ and EBV- tumors are anticipated to be enrolled. She asked the investigators whether they have conducted experiments to show the added benefit to having CD30 CAR on the EBV-CTLs in the recognition of EBV+ cells.

Regarding the study design, Dr. Yankaskas asked the investigators to explain the rationale for excluding individuals who received rituximab within 4 months of blood collection for lymphoblastoid cell lines (LCL) initiation and for the proposed cutoff criterion of circulating CD19+ B cells. He also requested an explanation as to whether patients with relapsed refractory CD30+ HL or CD30+ NHL, who will receive high-dose therapy and autologous stem-cell transplantation, are expected to have higher or lower risks because of these treatments, including any associated immunosuppressive treatments, and he asked the investigators whether they plan additional monitoring for such individuals. Dr. Yankaskas noted that cytokine storm was a possible cause of death in one previous CTL trial and, therefore, the investigators should consider adding this risk to the informed consent document. He also noted that the data demonstrate a lack of detectable CD30 in lung cancer and lung fibroblast cell lines, and asked the investigators whether there are *in vivo* CD30 expression data from animals or humans in other CD30 studies and whether this trial would assess CD30 expression if there are adverse reactions in any organs.

C. RAC Discussion

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

- Dr. Kahn asked whether this protocol would be a “course of last resort” for this pediatric population. He noted that the FDA is planning a meeting in early November 2010 that will focus on this issue.

- Dr. Williams explained that he is not concerned about including children in this trial. He stated that 70 percent of the children in the United States who have cancer are participating in a clinical trial, many of which are Phase I trials. He noted that this is a terrible disease for which no good alternative therapies exist, and this experimental therapy has as much chance of working as other alternative therapies. He noted that current survival rates of 85 percent in acute lymphocytic leukemia are due to inclusion of children in Phase I studies 30 years ago.

- Noting that transfected T cells in adults are not likely to be exactly the same as T cells in children, Dr. Ertl suggested that the investigators consider conducting two separate trials. She expressed concern that results from the adults might not be the same as results from the children.

- Dr. Federoff suggested that the investigators first enroll adult participants and investigate the safety and tolerability of the experimental treatment before progressing to enrolling any pediatric participants.

- Dr. Strome suggested that, if different disease processes or different biologic behaviors occur in adults compared with children, it would be important to choose one population or the other so as to understand the biological efficacy of this experimental therapy and to garner the most information.

- Dr. Strome suggested that the investigators determine whether their vector does not bind the Fc receptor.

- Although the difference in the T-cell responses between children and adults is not known, Dr. Williams noted that the current trial is informed by the previous neuroblastoma trial that uses the

same approach and has had exciting results for the pediatric oncology field. This proposed approach has led to demonstrable and significant differences in the persistence of the chimeric T cell receptor expressing cells – and some clinical benefit – in neuroblastoma.

- Dr. Zaia expressed concern about the heterogeneity in the sample population in that the eligibility criteria allow enrollment of newly diagnosed patients as well as enrollment of relapsed patients.

- Noting that financial resources are finite, Dr. Strome suggested that recommending a "two-trial system," with adults dosed first and followed by dosing of children would likely have the practical effect of children not getting this drug in the Phase I study.

- Dr. Mastroianni reiterated that the regulations require that a greater-than-minimal risk to children in Phase I trials must be justified by anticipated benefit to the pediatric research participants. She stated that this requirement is difficult to meet in a Phase I safety trial because the anticipated benefit is not known.

- Dr. Ertl suggested that the investigators alter their trial design from dosing two participants at each dose level to dosing a minimum of two or three adults before giving that same dose to a child.

- Dr. Kahn asked whether the justification for including children in this Phase I trial is to gather important pediatric data or whether the justification is potential benefit to pediatric participants – or whether both reasons exist simultaneously.

- Dr. Williams pointed out that the NIH regulations require clinical trial grant applications to justify not including children in trials. He also stated that investigators can petition the NIH Director's office to allow children to be included in trials.

- Noting that this patient population is significantly heterogeneous either because of the nature of the disease or the nature of the immune system, Dr. Zaia expressed concern that this safety study would be biased either by elderly participants or in some other way.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding justifying inclusion of pediatric research participants, the investigators noted that their team possesses more than 15 years of experience with adult and pediatric clinical studies of CTLs for treating malignant disorders in adults and children, and they have used CARs in clinical trials in adult and pediatric malignant disorders for more than 5 years. In many of those Phase I studies, between 20 percent and 60 percent of the adults and children dosed showed complete and sustained responses in otherwise resistant disease. Although CD30 is a new target antigen, the investigators stated their belief that the approach has the potential to produce clinical benefit for the targeted population. If any children are eligible for this study, they will be dosed and cared for at Texas Children's Hospital by staff with expertise in caring for pediatric research participants. The Baylor College of Medicine Institutional Review Board (IRB) allows for documentation of a child's assent through inclusion of a paragraph added to a regular adult informed consent document: "If your child is the one asked to take part in this study, you are signing to give your permission. Each child may agree to take part in a study at his or her own level of understanding. When you sign this, you also note that your child understands and agrees to take part in this study according to his or her own understanding. Child's name here: _____." In these situations, the child is not required to sign the consent form. Assent will be obtained for each child who is capable of providing assent based on age, maturity, and psychological state, and will be documented using the aforementioned "child clause" paragraph.

Two informed consent documents are proposed for this study because the risks and benefits of procurement are significantly different from those of infusion, and the investigators cannot guarantee to each participant that they will receive an infusion. Keeping the procurement and infusion documents separate gives the participant a longer time to carefully consider and discuss the risks and benefits of the infusion component before the full consent process is completed.

If a child requires pheresis, this will be done in the Pheresis Center at Texas Children's Hospital, which is accredited by the Foundation for Accreditation of Cellular Therapy as a collection center for children undergoing hemopoietic stem cell transplantation; the Center has extensive experience pheresing children of all ages.

Human cytomegalovirus (CMV) is unable to infect other hosts and does not infect rodents, and human and murine CMVs have developed different immune evasion strategies in human and murine cells; therefore, information gained from the murine model cannot be translated to humans. However, the investigators reported that research participants who received CD30 monoclonal antibody-based therapies showed no evidence for impaired control of CMV infection.

Regarding the impaired germinal center and recall memory responses observed in knockout mice, no defects have been reported in patients receiving CD30-Ab based therapies. However, research participants will be monitored for their immunoglobulin level and, if a significant decrease occurs after infusion, immunoglobulin will be administered. Since the investigators propose dosing participants who have otherwise intractable malignancies, they believe this risk is acceptable, given the hoped-for benefit. Effects on other aspects of antiviral immunity will be followed pre- and post-infusion. No evidence for autoimmune disease was observed in the knockout mice, but the investigators will monitor research participants for signs of autoimmune disease via physical exam and monitoring studies.

Most referred patients will have had EBV protein expression evaluated in their tumors as standard of care; for those who have not, the investigators will evaluate expression of a separate screening protocol to determine the studies for which they are eligible. From past experience, the investigators predict that 30 percent to 40 percent of referred patients will have EBV-positive tumors. However, even individuals with EBV-negative tumors will have EBV in their normal B cells, which should provide stimulation to the transduced EBV-CTLs.

Regarding the rationale for excluding individuals who received rituximab within 4 months of blood collection for LCL initiation, the investigators noted that, in previous studies, they consistently were unable to manufacture LCLs (which are required as antigen-presenting cells for EBV-CTL generation) within 4 months of rituximab therapy since this agent effectively depletes normal B cells. The earliest such individuals can manufacture LCLs is when the B-cell count rises above 2 percent.

The protocol in which a research participant developed a cytokine storm is significantly different from this proposed study; nevertheless, the investigators added information about this event to the informed consent document and mentioned the differences.

No reports of *in vivo* CD30 expression data exist in the published studies of CD30 monoclonal antibody administration. If any adverse events (AEs) occur and a biopsy has been performed, the investigators acknowledged that they would examine the affected organ(s) for CD30 expression.

Participants will receive only one infusion of CTLs; changes to the protocol description were made to reflect this correction.

The investigators stated that they do not anticipate that the number of individuals who wish to participate in this study will be significantly larger than the study can accommodate. However, if public interest in this disease is greater than anticipated, they will discuss other clinical trial options with these individuals.

The investigators modified the informed consent document to include the suggested clarifications and language modifications, and to decrease the chances of therapeutic misconception. They also explained how research participant confidentiality would be maintained.

The investigators made various changes to the full protocol and the informed consent documents at the request of the Protocol Review Committee at their institution. The specific changes were enumerated.

2. Responses to RAC Discussion Questions

Regarding the inclusion of children who have no good treatment options, Dr. Ramos explained that the investigators have not seen significant risks from previous trials and believe that these children should be given the option of participating in this trial, thereby receiving a “treatment” that could be effective. Dr. Ramos noted that most of the research participants will be adults because the frequency of the disease is higher in non-pediatric age groups.

Regarding the suggestion of conducting an adult trial and a trial for children, Dr. Savoldo explained that most of the research participants in this trial will be heavily immunosuppressed because of previously attempted treatment. Therefore, immunological function likely would not be informative in comparison with healthy controls of any age.

Dr. Savoldo explained that the possibility of treating both children and adults would help the investigators understand whether EBV responses are appropriate for maintaining cell persistence. It appears from their previous study that, in children as well as in adults, the reactivation levels of EBV do maintain persistence.

Dr. Ramos reiterated that the risks were minimal in the investigators’ first Phase I trial in which they used chimeric antigen receptors in a pediatric population; no significant adverse effects were seen from that experimental treatment.

Asked to estimate the number of adults and children expected in this trial, Dr. Ramos explained that the investigators extrapolate from their current lymphoma trials that 80 percent of participants in this proposed trial would be adults and 20 percent would be children. Given the expected enrollment of 10 to 18 research participants, those percentages would translate to two or three pediatric participants in this trial.

E. Public Comment

Dr. Borror offered several suggestions for clarifying the wording in the informed consent document.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The RAC spent most of its discussion on whether and when pediatric research participants should be enrolled. The Committee considered the HHS regulations regarding enrollment of pediatric patients in research. If the protocol procedures are greater than minimal risk, which is the case in the current research, the risk must be justified by the anticipated benefit to the subjects and the relation of the anticipated benefit to the risk must be at least as favorable to the subjects as that presented by available alternative approaches (45 CFR 46 Subpart D). The Committee members agreed that pediatric subjects who would be enrolled in this protocol would not have other proven therapeutic options. The Committee also recognized that because this trial uses EBV-specific T cells and EBV infection typically occurs in childhood, EBV-specific T cells in children might function differently from those in adults. This difference in function could have implications for the safety and efficacy of this approach; therefore, data from adult research participants might not completely be predictive of the experience in children with these same diseases.

In light of the potential differences between the effect of this experimental therapy in children and adults, and based on previous trials using this approach and the preclinical data supporting this protocol, half of

the RAC members concluded that children should be allowed to be enrolled and dosed simultaneously with adult participants and the data analyzed from both populations ("Option 2"). These RAC members noted that children with cancer are routinely enrolled in Phase I pediatric trials even though these are primarily safety trials with secondary efficacy endpoints. In addition to the possibility that potentially eligible children would be denied access to a potentially beneficial investigational agent pending data from adults, an additional risk is that this approach might not be efficacious in adults but might prove to be efficacious in children, perhaps because of differences in EBV-specific T cells in adults versus children. If no efficacy is seen in an adults-only trial, an incomplete assessment of potential efficacy might result.

The remaining RAC members believed that, provided the local IRB concludes that enrollment of children in this Phase I trial is consistent with regulatory requirements, children should be enrolled concomitantly with adults, but only after sufficient data is obtained from adults to justify the risk in children ("Option 1"). The sufficiency of the data should be determined in consultation with the appropriate experts and the IRB. One aspect of sequential enrollment is that were an unanticipated safety issue observed in the first few adults, then the children would be spared exposure to this unanticipated problem, which fulfills the intent of the HHS regulations.

Recognizing the compelling arguments on both sides, NIH OBA concurred with the recommendation for sequential enrollment, which does not preclude enrollment of children in this Phase I trial and appears to be most consistent with the current regulations. The threshold for enrolling children in Phase I, first-in-human trials is different than for adults. In this case, when children have a terminal disease with no approved effective alternative treatments, enrollment in experimental protocols may be appropriate; however, because data from adults may inform the risk/benefit ratio for children, the OBA believed it would be prudent to obtain that data, even if the data might be imperfect.)

On the following additional points made during the RAC's indepth review and public discussion there was concurrence by all 18 members present, with no abstentions or recusals.

<u>Preclinical Issues</u>

- The proposed CAR has an external immunoglobulin G (IgG) Fc spacer domain that potentially could bind with IgG Fc receptors on innate immune cells (e.g., Hombach, A., *et al*., 2010. Adoptive Immunotherapy with Genetically Engineered T cells: Modification of the IgG1 Fc 'Spacer' Domain in the Extracellular Moiety of Chimeric Antigen Receptors Avoids 'Off-Target' Activation and Unintended Initiation of an Innate Immune Response. *Gene Therapy*: 1-8). As this would result in the gene-modified cells being targeted for deletion, this should be explored in preclinical models.

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

- EBV infection usually occurs in childhood and, therefore, EBV-specific T cells in children may not be functionally equivalent to adult EBV-specific T cells. If pediatric patients are enrolled, analysis of their T cells should be undertaken to determine whether they have similar reactivity to EBV and whether this could alter the outcome.

- Even without enrollment of pediatric patients, the protocol proposes to enroll a potentially heterogeneous population of lymphoma patients, including patients with 1) recurrent HL or NHL, or 2) newly diagnosed patients unable to receive or complete standard therapy, or 3) those with relapsed or refractory HL or NHL whose treatment plan includes high-dose chemotherapy and stem-cell transplantation. Analysis of safety and efficacy data needs to recognize this heterogeneity. The investigators should consider establishing separate cohorts that would have a homogenous population in order to facilitate interpretation of the data.

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

- The clarity of the informed consent document would be enhanced by incorporating the following changes:
  - If the goal of requiring contraception for the first 3 months is to prevent exposure to the agent rather than just pregnancy, then barrier methods for men and women should be specified. In addition, the rationale for a 3-month time frame for contraception should be included.
  - The section on "Potential Benefits" currently states: "The benefits of participating in this study may be: that your immune system may begin to kill the cancer cells. This could make the cancer grow more slowly, or get smaller, or go away for a while" and then goes on to say that there may not be a benefit. The potential lack of benefit should be highlighted first to prevent therapeutic misconception.
  - The Procedure/Procurement section of the consent document would benefit from a simple schedule/table that outlines the timing of, and amount of time, each procedure will take.
  - The informed consent document should include the risks of any procedures that are part of the study and not just standard of care, for example, additional study-specific blood tests or bone marrow studies.

G. Committee Motion 2

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. The recommendations were divided into Option 1 – recommendations plus a suggestion that the investigators conduct their trial in separate cohorts with adults dosed first – and Option 2 – recommendations plus a suggestion that the investigators simultaneously enroll adults and children.

It was moved by that the RAC vote on these summarized recommendations with the two options. The vote was 10 in favor of the recommendations including Option 1, 8 in favor of the recommendations including Option 2, 0 abstentions, and 0 recusals; Dr. Roizman subsequently changed his vote from Option 1 to Option 2 to reflect fully the divided sentiment of the RAC members. The final vote tally was 9 RAC members voting for Option 1 and 9 RAC members voting for Option 2.

H. Followup FDA Presentation

For the September 2010 RAC meeting, Dr. Takefman offered to present and lead a discussion about the FDA perspectives on pediatric enrollment in Phase I trials and possibly in subsequent phase studies.

V. Attenuated Strains of *Yersinia pestis* that are Excluded from the Centers for Disease Control and Prevention Select Agent Regulations – Evaluation under Section III-A-1 of the *NIH Guidelines*

Presenters: Joseph A. Kanabrocki, Ph.D.; Olaf Schneewind, M.D., Ph.D., University of Chicago; Martin E. Schriefer, Ph.D., CDC (*via teleconference*)

Discussants: Denise Gangadharan, Ph.D., CDC (*via teleconference*); Suman Mukhopadhyay, Ph.D., NIH; and Susan L. Welkos, Ph.D., U.S. Army Medical Research Institute for Infectious Disease (*via teleconference*)

A. Introduction by Dr. Kanabrocki

Dr. Kanabrocki introduced this agenda item by stating that the OBA had received a request for an opinion on as to whether the transfer of resistance to chloramphenicol into an attenuated strain of *Yersinia pestis (Y. pestis)* required review by the RAC and approval by the NIH Director. This request led to a discussion among the members of the RAC's Biosafety Working Group (BSWG). Although the request was subsequently withdrawn, the BSWG believed it important for the RAC to discuss this issue and set a paradigm by which such future requests could be evaluated. He asked that this discussion be framed in the context of attenuated strains of all pathogens, with *Y. pestis* as a "case study." Dr. Kanabrocki discussed Section III-A-1 of the *NIH Guidelines* (experiments that must be reviewed by the RAC and approved by the NIH Director) and provided an overview of *Y. pestis*.

Section III-A-1-a deals with the deliberate transfer of a drug-resistance trait to microorganisms that are not known to acquire the trait naturally. The RAC reviews such experiments if the acquisition of an antibiotic resistance trait could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. The applicability of this section of the *NIH Guidelines* to attenuated strains of pathogens depends on the answers to these following questions:

- Does the deliberate transfer of antibiotic resistance, having the potential to compromise use of a therapeutic drug, extend to agents capable of causing any disease, or should it be limited to those agents that raise public health concerns?
- Does the proposed genetic manipulation of the organism change the assessment of whether it raises a public health concern?
- How is a public health pathogen defined? By pathogenicity? By the susceptible population? Should the route of transmissibility be a factor?

If an agent causes severe disease almost always requiring treatment, then it would be subject to review as a Major Action under the *NIH Guidelines*; if an agent rarely causes disease in humans, plants, or animals, then it would not be subject to review as a Major Action. Pathogens falling in between these two extremes are more difficult to classify as warranting Major Action review or not – for example, agents such as Chlamydia that cause mild disease and that may require treatment are of public health importance,.

Y. pestis has caused bubonic and pneumonic plagues throughout human history. It remains endemic in many parts of the world, mainly in Africa and China. In 2006, 13 cases and 2 deaths were reported in the United States – from New Mexico, Colorado, California, and Texas. It is a zoonotic disease with rodents being the primary reservoir, and fleas the transmitters of the agent. More recently, the focus is on its potential as a bioweapon. Documented antibiotic-resistant strains have been observed only in Madagascar. One strain that was isolated from a 16-year-old boy with bubonic plague was resistant to streptomycin, chloramphenicol, tetracycline, sulfonamide, ampicillin, kanamycin, and spectinomycin; a second case was a 14-year-old boy who was infected with a strain that was resistant to streptomycin.

The wildtype strain of *Y. pestis* is regulated as a Select Agent (SA) by the CDC. Strains excluded from the SA regulations are pigmentation deficient [$pgm^{(-)}$] strains and low calcium response [$lcr^{(-)}$] strains, both of which are considered to be attenuated, yet a fatal laboratory-acquired infection in 2009 involved experiments with a *Y. pestis* $pgm^{(-)}$ strain. Animal models demonstrate that if there is supplementation of serum iron at the time of inoculation, virulence of the $pgm^{(-)}$ strain can be rescued.

Questions to be addressed concerning the attenuated strains of *Y. pestis* include:

- Are attenuated strains of *Y. pestis* considered to be disease agents in humans?
- Is the evidence sufficient – or what additional evidence is needed – to establish that the $lcr^{(-)}$ strain is avirulent in humans such that it cannot cause human disease?
- Under the *NIH Guidelines* all strains of *Y. pestis* are classified as Risk Group (RG) 3 agents (list of agents may be found in Appendix B); therefore, most recombinant deoxyribonucleic acid (DNA) experiments with this agent should be performed at BL3 containment. Should attenuated recombinant strains of *Y. pestis* be generally contained at BL3 or at BL2?

- Should different containment and/or biosafety practices be specified for pgm$^{(-)}$ strains versus lcr$^{(-)}$ strains?

B. Presentation by Dr. Schneewind

Dr. Schneewind explained that three forms of plague disease exist: bubonic plague, which is transmitted by flea bite; secondary pneumonia from bubonic plague, which can be transmitted by aerosol and leads to pneumonic plague; and sylvatic plague, which is a form of bubonic plague that does not result in the appearance of buboes (*i.e* swollen lymph nodes). Rodents play an instrumental role as a zoonotic reservoir for the disease and in the United States; several different rodents, primarily the prairie dog, can harbor the pathogen. The primary virulence factors of *Y. pestis* are encoded by three plasmids, pCD1, pMT1 and pCP. Of these, pCD1 encodes the critical virulence elements of *Y. pestis*: The Type 3 secretion pathway which is composed of 26 protein components that are localized throughout the double membrane envelope of the bacterium. The 26 component complex forms a needle-like structure that is capped by the low-calcium response virulence (LcrV) protein (called LcrV). Using this needle structure and the LcrV cap, *Y. pestis* can recognize and inject the Type 3 secretion components into immune cells. The transport of these effectors (called Yersinia Outer Proteins or YOP) into the cytoplasm of immune cells will incapacitate their ability to phagocytose and kill *Yersinia* species. Plague is associated with a high mortality without therapy; pneumonic plague cases are invariably fatal and bubonic plague cases have an associated mortality of 50 percent.

In the 20th Century, two efforts were launched that led to large-scale vaccination against plague. The first effort used live attenuated vaccines derived from non-pigmented strains of *Y.pestis*. These vaccines did cause side effects but they were used to immunize millions of humans and are thought to have stemmed the plague epidemic in Asia. More recently during the Vietnam War, the United States immunized its soldiers with the whole-cell killed vaccine, which did not produce the severe side effects of the live attenuated vaccine but had the disadvantage of protecting against only bubonic plague but not against pneumonic plague. This vaccine, which was licensed by the FDA, is no longer available. Currently no FDA-licensed vaccine is available in the United States. Recent efforts from the military and academic researchers have focused on generating a recombinant plague vaccine that would confer protective immunity and be safer than either whole-cell killed vaccines or live-attenuated vaccines. Most of the protective immunity against plague is presumed to be based on the LcrV protein.

In contrast to the virulence factors that are encoded on plasmids, *Y. pestis* also contains a 102 Kb chromosomally-encoded virulence sequence composed of a pigmentation component and a “high pathogenicity island” (HPI). A little more thatn 80 genes are involved in this virulence island, but an essential component that is absolutely required for the growth of Yersinia in host tissues is the siderophore (or “yersiniabactin”) that removes iron from transferrin found in host cells.

Dr. Schneewind provided a summary of animal models and research that has been conducted for the past 100 years, noting that hundreds of wildtype strains have been evaluated in these animal models and that considerable heterogeneity exists among wildtype strains of *Y. pestis*. The general pattern of disease outcome observed with wild-type *Y. pestis* is that the highest virulence and lethatlity are observed when organisms (1-10 colony forming units - CFU) are injected subcutaneously or into the blood stream. Lethal outcomes for infections acquired through the respiratory tract (either by intranasal instillation or aerosolization) require a ten-fold higher dose of bacteria (~100 CFU). Strains from which the pCD1 plasmid has been removed (so as to be unable to catalyze the Type 3 secretion reaction) are considered avirulent – meaning the inoculating dose must be very high (>10^7 CFU) to cause death. By contrast, non-pigmented strains of *Y. pestis* inoculated through the respiratory tract or subcutaneously in the various animal models tested are generally avirulent. However, unlike the lcr$^{(-)}$ strains, inoculation into the bloodstream is associated with a high probability of lethality. Thus, for this reason, pgm$^{(-)}$ strains of *Y. pestis* are considered to be attenuated as opposed to avirulent.

The preferred therapy for plague infections is intramuscular injection of the aminoglycoside antibiotic, streptomycin. These aminoglycosides stop protein synthesis of the Type 3 effectors, which are made in extraordinary abundance in the pathogenesis of plague. Because the disease progression is so rapid,

killing the organism could take too long and might not be as effective as stopping the protein synthesis. If streptomycin cannot be given, then gentamicin (a related aminoglycoside antibiotic) is used as a therapy for plague. For post-exposure prophylaxis, the recommendation is to use an antimicrobial that can be administered by mouth; tetracycline is the drug of choice. Ciprofloxacin, a DNA gyrase inhibitor, has been used for plague therapy in experimental animals, and chloramphenicol, another oral antibiotic, is typically not used because of severe side effects.

C. Presentation by Dr. Schriefer

Dr. Schriefer's presentation focused on drug treatment for plague. Most of the natural disease encountered worldwide and in the United States is bubonic plague and is flea-transmitted, accounting for 78 percent to 85 percent of disease. The prevalence of septicemic plague, at 10 to 25 percent, has fluctuated but, in recent years, septicemic cases in the United States have been recognized more frequently. Pneumonic plague represents 2 to 5 percent of plague cases.

Few studies have focused on meningitic involvement in plague. A review of plague cases in New Mexico from 1970 to 1979 indicated 105 reported cases of plague and, among those, about 6 percent developed meningitic involvement. All of those meningitis cases were secondary to the initial plague presentation, and evolved between 9 and 14 days after the primary clinical onset. Among the six patients who developed meningitic involvement, three had primary bubonic plague and three had septicemic plague. A summary of plague cases reported between 1960 and 2006 in the United States indicates that, among the 446 cases, about 4 percent had meningitis reported as a secondary complication of disease. The number of cases of plague meningitis is decreasing, particularly in the United States, because of better case recognition and more prompt and appropriate antibiotic treatment. Fatal cases of plague in the United States, involving meningitis, do occur; one example was a wildlife biologist who developed plague with involvement of the meninges and died in 2008.

Dr. Schriefer presented some points to consider in deciding if chloramphenicol should continue to be considered as a treatment option for the two attenuated strains of *Y. pestis*. He noted that there exists little or no evidence that $lcr^{(-)}$ strains are virulent in animal systems by a variety of inoculation routes. However, he advised that the $pgm^{(-)}$ strains should be considered separately because they can result in lethality in nonhuman primates and in rodents, depending on the route and dose of inoculation.

D. Presentation by Dr. Gangadharan

Dr. Gangadharan discussed the assessment and decision to exempt the $pgm^{(-)}$ and $lcr^{(-)}$ strains from the Select Agent Regulations. She also reviewed the exclusion process for attenuated strains. Requests for exclusion from the HHS Select Agent Regulations are submitted to the CDC Division of Select Agents and Toxins (DSAT). DSAT seeks input from an intra-governmental advisory group--the Intra-Governmental Select Agents and Toxins Technical Advisory Committee (ISATTAC). ISATTAC is composed of Federal Government employees from Agencies such as the CDC, the NIH, the FDA, the U.S. Department of Agriculture, and the Department of Defense.

Five criteria are used to evaluate the strains requested for exclusion:

- A documented history of not causing disease in humans or relevant animal models
- Defined genetic mutations or alterations known to attenuate virulence in humans or relevant animal models
- Mutations have low frequency of reversion to wild-type virulence
- Difficulty in engineering the attenuated strain to restore wild-type virulence (the animal model used to test virulence for each pathogen is important)
- Quantitative measures may be required, such as demonstration of at least a 4-log to 6-log reduction in virulence in an appropriate animal model

Dr. Gangadharan presented detailed information submitted by those entities seeking exclusion of the $pgm^{(-)}$ and $lcr^{(-)}$ *Y. pestis* strains in response to the criteria listed above.

The ISATTAC discussed and reviewed the data provided by the requesters and concluded that the pgm$^{(-)}$ *Y. pestis* strains do not pose a public health threat and should be excluded from the Select Agent Regulations. Because several mutations can give rise to the pgm$^{(-)}$ phenotype, the ISATTAC restricted the exclusion to strains with a confirmed deletion of the 102 Kb chromosomal region.

The ISATTAC discussed and reviewed the data provided by the requesters and concluded that the lcr$^{(-)}$ *Y. pestis* strains do not pose a significant threat to the public health. These strains were irreversibly attenuated through the loss of the virulence plasmid and, therefore, also should be excluded from the Select Agent Regulations.

E. RAC Discussion

Dr. Welkos opined that the virulence characteristics of the two attenuated *Y. pestis* strains are sufficiently distinct such that pgm$^{(-)}$ and lcr$^{(-)}$ strains should not be considered as equally impaired. However, both are sufficiently attenuated with respect to wild-type *Y. pestis* that they should also not be considered RG3 organisms. Dr. Welkos indicated that both strains could be manipulated safely under BL2 containment, though greater attention or perhaps more stringent practices should be applied to work involving the pgm$^{(-)}$ strain. From a biosafety perspective, the reversion of attenuation by recovery of a 102 Kb deletion in the case of pgm$^{(-)}$ or reacquisition of the pCD1 plasmid in the case of lcr(-) are not processes which occur readily in nature and are likely to require deliberate human intervention to facilitate the recovery of these traits.

Dr. Mukhopadhyay stated that no vaccines currently exist for plague. He asked the RAC to keep in mind that *Y. pestis* is an important world-wide public health pathogen for which remedies and cures are needed, and that the science must be allowed to move forward in order to do so.

Dr. Corrigan-Curay summarized written comments received from a group of investigators that both strains are attenuated and do not cause disease, and that they acquire antibiotic resistance naturally. She framed the central question for consideration and discussion by the RAC as to (1) whether the lcr$^{(-)}$ strain is so attenuated that the introduction of antibiotic resistance in this organism cannot rise to the level of a Major Action because it does not cause disease in humans and (2) whether the pgm$^{(-)}$ is identical in this respect and, if not, under what circumstances should the transfer of antibiotic resistance into this strain be considered a Major Action.

Dr. Kanabrocki discussed the experience of the University of Chicago (his institution) Institutional Biosafety Committee (IBC) in its evaluation of pgm$^{(-)}$ strains. These strains have been worked with for many years at BL2 management. The basis of that decision has been the evaluation of the virulence data, whether this agent causes disease in healthy adults, the fact that these strains have been used in parts of the world as vaccine strains, and that these strains have been exempted from oversight by the Select Agent Program because they are not viewed as a threat to public health. Until the one case in 2009, there were no cases of human disease caused by pgm$^{(-)}$ strains, and the *Y. pestis* research community has worked with pgm$^{(-)}$ strains routinely at BL2 for many years with no adverse events or laboratory worker infections. The IBC decided that the pgm$^{(-)}$ strains were sufficiently attenuated and not a risk to public health; therefore, it was the IBC's opinion that transfer of antibiotic resistance to pgm$^{(-)}$ would not rise to the level of a Major Action.

Dr. Zaia wondered about the history of the laboratory worker who became infected with pgm$^{(-)}$ *Y. pestis* and subsequently died – and whether that individual had hemochromatosis. According to Dr. Corrigan-Curay, there is no publicly available medical history documenting that the condition of hemochromatosis was present.

Dr. Ertl summarized her concern by stating that an individual had died as a result of exposure to an attenuated [pgm(-)] strain of Y. pestis and it was unknown whether or not he had an underlying disease that made him uniquely susceptible to that organism. If the introduction of antibiotic resistance into pgm$^{(-)}$ *Y. pestis* does not qualify as a Major Action, then any investigator could take this attenuated pathogen

and add antibiotic resistance which could preclude ever treating that strain with any therapeutic drugs currently available.

Dr. Schneewind pointed out that, for decades, laboratory researchers have worked with non-pigmented plague strains without any laboratory incident and that this is a reflection of the fact that the strain is severely attenuated and that it typically does not cause disease in a broad spectrum of the population.

F. Public Comment

No comments from the public were made.

G. Committee Motion 3

Dr. Corrigan-Curay summarized the recommendation from the BSWG and the discussion at today's RAC meeting:

> Both lcr$^{(-)}$ and pgm$^{(-)}$ can be worked at BL2; however, for pgm$^{(-)}$ there may be important considerations about using a hood or other specific containment. Lcr$^{(-)}$ is avirulent and transfer of drug resistance to this strain should not be considered under Section III-A-1 of the *NIH Guidelines*. Pgm$^{(-)}$ is attenuated and should be considered under III-A-1 for certain experiments involving the introduction of antibiotic resistance. The transfer of chloramphenicol resistance alone in a pgm$^{(-)}$ strain would not rise to the level of a Major Action. However, resistance to other antibiotics or the introduction of multiple antibiotic resistance markers might rise to the level of a Major Action.

Dr. Federoff suggested that these recommendations might warrant reconsideration when all of the information pertaining to the single case of the infected laboratory worker comes to light.

Approval of the above recommendation was moved by Dr. Flint and seconded by Dr. Ertl. The RAC voted by voice vote to approve this recommendation, with one opposed (Dr. Kanabrocki) and one abstention (Ms. Mallino).

VI. Proposal to Exempt the Mating of Certain Biosafety Level 1 Transgenic Rodents from the Requirements of Section III-E-3 of the *NIH Guidelines*

Presenter: Dr. Flint

A. Presentation by Dr. Flint

Dr. Flint reviewed the current *NIH Guidelines* requirements for the generation of transgenic rodents that may be housed at BL1, summarized the language discussed at the March 2010 RAC meeting, and reviewed the proposed revisions based on further discussions with various experts at a BWG meeting.

The current language states that Section III-E of the *NIH Guidelines* covers experiments that are of low biosafety risk that may be conducted at BL1 and may be initiated upon registration with the IBC. Experiments that involve generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA or DNA derived therefrom into the germline may be initiated upon registration with the IBC provided the transgenic rodent would be housed at BL1 containment. The generation of the transgenic rodent includes mating between rodents with two different transgenes or mating of a transgenic rodent with a nontransgenic rodent. However, breeding within a strain to maintain that strain of transgenic rodent is not subject to this section of the *NIH Guidelines*.

The overwhelming majority of matings of rodents that require BL1 containment will produce offspring that would not pose an appreciable risk to human health and, therefore, can also be housed at BL1. The impetus for considering this exemption for mating of certain transgenic rodents stems from the registration requirement; while each registration is not a significant burden, the total number of registrations required

leads to an administrative burden for the IBC and for researchers but does not appear to be commensurate with the biosafety risk.

At the March 2010 RAC meeting, the proposed wording noted that each parental transgenic rodent (and the transgenic rodent that results from this mating) cannot contain any one of the following genetic modifications: a transgene that codes for an amyloid or prion, more than 50 percent of the genome of an exogenous virus from a single viral family, or the expression of the transgene under the control of a retroviral long terminal repeat (LTR). The BWG proposed that the clauses relating to transgenes that encode amyloid or prions be deleted from those original recommendations and that the LTR clause be limited to gamma retroviral LTRs: "the expression of the transgene is under the control of a gamma retroviral long terminal repeat in each parental transgenic rodent and the transgenic rodent that results from the mating."

In summary, the new language proposed by the BWG is:

> The mating of two different transgenic rodents or the mating of a transgenic rodent with a nontransgenic rodent with the intent of creating another transgenic rodent that can be housed at BL1 containment will be exempt from the *NIH Guidelines* if
>
> - Both parental rodents can be housed under BL1 containment AND
> - Each parental transgenic rodent does not contain any one of the following genetic modifications: More than 50 percent of the genome of an exogenous virus from a single family or expression of the transgene is under the control of a gamma retroviral long-terminal repeat AND
> - It is anticipated that the transgenic rodent that results from the mating will not contain more than 50 percent of the exogenous viral genome from a single family.

B. RAC Discussion

Dr. Williams asked whether there had been documentation of retroviral recombination. Dr. Fan responded that recombination had been observed in tissue culture, and that the recommendations proposed represent a middle ground of opinion regarding the relative risk of mobilization of a retrovirus from an LTR-driven transgene in a transgenic animal. Dr. Corrigan-Curay clarified that these recommendations are not adding a new requirement for registration.

C. Committee Motion 4

Dr. Ertl moved and Dr. Roizman seconded that the recommendations of the RAC Biosafety Working Group, as presented by Flint, be approved. The RAC voted unanimously by voice vote to approve those recommendations.

VII. Discussion of Human Gene Transfer Protocol #1004-1036 entitled: Phase I/II Study of Metastatic Cancer Using Lymphodepleting Conditioning followed by Infusion of Anti-VEGFR2 Gene Engineered CD8+ Lymphocytes

Principal Investigator:	Steven A. Rosenberg, M.D., Ph.D., NCI, NIH
Additional Presenters:	Dhana Chinnasamy, Ph.D., NCI; Stephen Gottschalk, M.D., Texas Children's Hospital; Richard A. Morgan, Ph.D., NCI; Lisa Wang, M.D., Texas Children's Hospital
RAC Reviewers:	Ms. Mallino, Dr. Strome, and Dr. Zaia

A. Protocol Summary

This study will be performed in research participants who have metastatic cancer with no effective treatment options available. Overexpression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR) are strongly associated with tumor angiogenesis, survival, invasion, metastasis,

recurrence, and poor prognosis in human cancers. Therefore, the investigators have developed an experimental procedure for dosing cancer patients that uses their own blood cells – genetically modified in the laboratory with anti-VEGFR2 genes and T-cell receptor genes – that are then re-infused. The investigators have shown in the laboratory that T cells transduced with the VEGFR2 chimeric antibody receptor (CAR) recognize human endothelial cells.

The primary objectives of this study are to determine the side effects of this experimental treatment and to determine whether the patient's tumor decreases in size after dosing on this study. The secondary objective of this study is to determine the survival of the infused cells in the body. This study is proposed to be conducted in two phases: the first phase will determine a dose that can be given safely to research participants and the second phase will evaluate if the experimental treatment can shrink tumors in humans. Up to 118 individuals could participate in this Phase I/II study.

Initially, research participants will have lymphocytes collected through leukapheresis. The lymphocytes will be grown in the laboratory, during which time anti-VEGFR2 genes and T-cell receptor genes will be put into the cells using retroviral transduction. The retrovirus will serve as the vehicle to deliver the genes into the cells.

Once the cells are grown in the laboratory and the gene inserted, participants will be given chemotherapy (cyclophosphamide and fludarabine) for 7 days to suppress the immune system. One to 4 days after the chemotherapy, all participants will be given the cells intravenously (IV) during 20 to 30 minutes, followed within 24 hours with IV interleukin 2 (IL-2), a hormone that stimulates lymphocyte growth, every 8 hours for up to 15 doses (depending on individual tolerance). Participants will be given appropriate medications to treat the side effects of this dosing regimen and to prevent infection secondary to the immune suppression caused by the chemotherapy.

Research participants will return to the NIH after 4 to 6 weeks to have their tumor(s) evaluated. If the tumor shrinks, the participants will continue to return to the NIH to have their tumors evaluated. If a partial response to the dosing occurs or stable disease subsequently progresses, research participants could be re-treated. If there is no response, participants will be taken off this study.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of the protocol. Key issues included that this is the first time VEGFR2 would be used as a target for a CAR. It was noted that, while it is overexpressed in tumor cells, VEGFR2 regulates angiogenesis in normal cells. Further discussion was deemed to be needed regarding the dose-dependent toxicity noted in one preclinical model and the safety of targeting this antigen with cytotoxic T cells that have the potential to persist.

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

Ms. Mallino noted that the protocol presentation is well organized and easy to read, as is the response to Appendix M. She stated that the informed consent document is concise in its presentation, and that it appropriately informs the participant about the impact the clinical trial will/might have on the participant as well as the knowledge that might be gained from proceeding with this protocol.

Noting that this protocol is well written and innovative, Dr. Strome wondered what evidence exists in humans that the non-myeloablative chemotherapy regimen, suggested in this protocol, has the potential for therapeutic benefit in the absence of T-cell transfer. He asked the investigators to justify the use of the 4-1BB ligand in a setting in which the toxicity of the reagent is unknown, and he requested information about the anticipated side effects of the longterm presence of T cells expressing a CAR against VEGFR2, assuming a complete response, and the expression patterns of VEGFR2 in humans. Dr. Strome suggested adding another group to the Phase I trial to evaluate total body irradiation (TBI) at the maximum tolerated cell dose and then including TBI in the Phase II study, especially since the use of TBI was associated with improved outcome in the investigators' other trials of adoptive cell transfer. Dr. Strome asked the investigators how they arrived at the starting cell dose, noting his ethical concern about

starting a research participant with a dose lower than the effective dose in a murine tumor model. Regarding the hearing losses that the investigators are observing, Dr. Strome asked whether hearing loss has been observed in murine models, whether the investigators believe it is associated with the preconditioning regimen or the cell transfer, what the characteristics of the hearing loss are, and whether baseline audiograms with otoacoustic emissions would be performed as part of this protocol.

Dr. Zaia requested further data about the observation of a lethal effect of the KDR-specific CAR cells when initially evaluated in mice, especially whether this effect was purely a CD4 effect or whether additional CD8 cells would reproduce this potential lethal event. This effect was explained as due to cytokine-induced pathology and a CD8 subset of these T cells did not induce lethal effect, leading to the current study design in which only CD8 cells are proposed to be infused. He asked the investigators whether they had considered a CD4 add-back component to their study design, in light of potential benefit from CD4 cells in transferring anti-tumor effects. Dr. Zaia expressed concern that the exclusion of IL-2-related AEs as reportable events could mask those events attributable to the research agent, especially when they occur within the first few days after the CD8 infusion. He asked the investigators to describe how they would recognize a dose-limiting toxicity (DLT) during the early days post-infusion. Dr. Zaia asked the investigators to clarify their intent to maintain any bronchospasm/urticaria grade >2 as a DLT; as this definition might be too stringent and could interfere with completion of the study, he suggested that the investigators consider using grade >3 bronchospasm/urticaria as a DLT. With regard to the informed consent document, Dr. Zaia noted that its language is quite technical and might need to be revised. He suggested that the benefits paragraph indicate the likelihood that no longterm benefit would accrue for participants in this study but that the information derived from this study could benefit future patients, and he requested that the investigators not use the term “gene therapy” in accordance with NIH recommendations for describing gene transfer research.

C. RAC Discussion

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

- Dr. Ertl considered the starting dose of $1x10^6$ cells to be a prudent dose. However, she expressed misgivings about increasing the dose dramatically before the lower doses have been tested. She noted a potential risk for an off-target reactivity that would be more severe when more cells are given.

- Dr. Bartlett asked whether the CAR-positive T cells are activated by soluble receptor. He noted that soluble VEGFR2 is seen in many cancers and has been suggested as a marker for some cancers. He noted having observed high levels with chronic lymphocytic leukemia (CLL) and the possibility of elevated levels of this receptor in the circulation in some of these patients. He wondered whether the investigators had modeled any of these possibilities in preclinical studies.

- Dr. Strome asked if the investigators have seen or have looked for thrombotic events. He suspected that targeting the tumor vasculature might result in a higher incidence of such events.

- Dr. Federoff asked whether screening and exclusion should be conducted for those individuals who might be prone to toxicity, for example, those who have proliferative diabetic retinopathy or physiological angiogenesis in the setting of subchronic ischemia.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators explained that it is possible that the nonmyeloablative chemotherapy regimen could have some therapeutic benefit in the absence of T-cell transfer; however, such benefit would be limited with only one dosing. The study has been designed so that research participants will be evaluated 4 weeks to 6 weeks after the initial dosing cycle. If tumor shrinkage is observed, a confirmatory evaluation will be conducted in 1 month. The investigators do not expect to see a confirmation of response at 2

months to 3 months after the nonmyeloablative chemotherapy regimen is administered if the response was due solely to the single cycle of chemotherapy. In addition, for participants with chemotherapy-sensitive tumors (i.e., sarcoma), only responses seen at day 28 and maintained at 4 months will be considered by the investigators to be a positive response for accrual to the second phase of this study.

The investigators report comparing the survival of cells transduced with vectors containing 4-1BB with those lacking 4-1BB and observing an increase in survival with the cells transduced with 4-1BB-containing vectors. In mouse models, cells transduced with 4-1BB survived longer but all cells become quiescent, and this finding with 4-1BB vectors is consistent in different mouse models. Because of these results, the investigators have decided to use a 4-1BB-containing vector, which appears to be the optimal agent, and they plan to start the dose escalation scheme with a low dose of cells in order to evaluate this agent safely.

In prior cell therapy protocols, cells become quiescent after several weeks and lose their activity; therefore, the investigators do not anticipate any longterm side effects of the CARs against VEGFR2. The slow dose escalation design of the proposed study is expected to allow safe determination of the toxicity profile of the anti-VEGFR2 CAR-transduced cells. VEGFR2 is highly over-expressed in normal human vasculature and is expressed in low levels in normal tissue; however, it is also over-expressed in tumor endothelial cells and on some tumor cells.

Because TBI adds some toxicity to the treatment regimen, the investigators have decided not to use TBI in this first study of the anti-VEGFR2 CAR-transduced cells. If the study proposed here without TBI is successful, the investigators will consider conducting a subsequent study to include TBI.

In deciding on the starting dose of $1x10^6$ anti-VEGFR2 CAR-transduced cells, the investigators considered the recent concerns about the safety of CAR containing gene transfer products along with the prospect of direct treatment benefit. Results of preclinical studies conducted in C57BL/6 mice treated with the VEGFR2 CAR demonstrated no toxicity at a dose of $2x10^7$ DC101-CAR transduced syngeneic T cells. However, in BALB/c mice the transfer of $2x10^7$ CAR transduced cells was lethal but a tumor inhibitory effect was achieved with minimal morbidity when the number of administered T cells was reduced to $5x10^6$. When CD8-purified CAR-transduced cells were administered, no toxicity occurred in BALB/c mice given $2x10^7$ cells, with no diminution of the therapeutic effect. Histopathologic analysis of BALB/c mice treated with $2x10^7$ DC101-CAR transduced T cells and IL-2 revealed findings characteristic of cytokine-induced hypotension, including multifocal mild coagulation necrosis in the liver and mild hepatic pericholangitis and pulmonary perivasculitis, villous atrophy, villous blunting, and crypt epithelial hyperplasia of the small intestine and colon. No abnormalities were seen in the gross appearance of the heart, lungs, liver, kidneys, spleen, pancreas, uterus, ovaries, or brain. Both C57BL/6 and BALB/c mice tolerated $2x10^7$ CD8-purified CAR-transduced cells without toxicity. Therefore, in the current protocol the investigators propose to administer CD8-purified CAR-transduced cells, and the starting cell dose is proposed to be $1x10^6$ cells or a dose of 20-fold fewer cells than was tolerated by the mice. Since a 70 kg human is 3,000 times heavier than a mouse, the starting cell dose is estimated at 60,000-fold lower than that safely tolerated by the mice. The investigators expressed a willingness to consider a higher initial starting dose as recommended by the RAC.

Hearing loss was observed only in patients treated in clinical studies using T-cell-receptor transduced cells that target melanocyte receptors (gp100 and MART-1) because of the expression of melanocytes in stria vascularis of the cochlea. The hearing loss is attributed to the cell therapy in these studies. Hearing loss resolved completely in nearly all of the research participants. Since the anti-VEGFR2 CAR does not target melanocyte receptors, the investigators do not plan to conduct baseline audiograms in this clinical trial.

The toxicity of the VEGFR-2 CAR-transduced T cells documented in tumor-bearing BALB/c mice was attributed to the presence of large numbers of CD4+ T cells in the infusion product. Tumor treatment with no toxicity was secured in BALB/c mice (in three independent experiments involving 15 tumor-bearing mice) treated with $2x10^7$ purified CAR transduced CD8+ T cells, while mice receiving $2x10^7$ VEGFR-2 CAR-modified T cells containing both CD8+ T cells and CD4+ T cells showed morbidity and mortality in

the same experiment. In multiple experiments, treatment of non-tumor-bearing BALB/c or C57BL/6 mice with $2x10^7$ CAR transduced T cells containing equal numbers of CD4+ and CD8+ T cells was well tolerated with no adverse effect or toxicities. Taken together, the investigators concluded that these findings suggest that the cause of the toxicities observed with the adoptively transferred VEGFR-2 CAR-transduced T cells was restricted to the CD4+ T-cell recognition of the target antigen VEGFR-2 in the tumor vascular network and subsequent downstream molecular events, rather than their off-target cell-mediated cytotoxicities to normal vessels or tissues.

The investigators have experience with treating more than 3,000 patients with high-dose IL-2, and are familiar with the toxicity profile of this agent. They plan to compare the toxicities observed in this clinical trial with those seen in individuals administered high-dose IL-2 alone in order to determine if toxicities are observed at a higher incidence in the presence of the anti-VEGFR2 CAR-transduced cells.

While the investigators are willing to use grade >3 bronchospasm/urticaria as a DLT, they do not expect these toxicities will be encountered.

The investigators agreed to modify the informed consent document and protocol description to include the suggested clarifications and language modifications, and to reword where necessary in order to decrease the chances of therapeutic misconception.

2. Responses to RAC Discussion Questions

Dr. Rosenberg stated that the investigators have given IL-2 to more than 2,000 patients and they have given IL-2 in conjunction with cells to approximately 400 patients. Their process is to give the cells and then wait a minimum of 3 or 4 hours before starting the IL-2 to be sure there is no acute toxicity; with one exception, no toxicities have been seen. The investigators are familiar with how to give IL-2 to cancer patients, having treated more than 800 consecutive metastatic cancer patients with IL-2 without a single treatment-related death. They keep a close tally of the toxicities and continually compare toxicities to their known set of IL-2 toxicities to determine if anything unusual occurs.

In response to concerns about the low starting dose of $1x10^6$, Dr. Rosenberg acknowledged that it would be reasonable to start at $1x10^7$ cells, which is 5,000-fold lower than the mouse MTD on a per-kilogram basis. Since toxicity, if it occurs, is likely to be immediate, he suggested that the investigators could give a test dose of $1x10^6$ or $2x10^6$ and, if that dose is tolerated, the next day give $1x10^7$ and start IL-2.

In terms of circulating VEGFR2, Dr. Rosenberg explained that the fact that this experimental regimen worked in all five of the animal models despite the presence of VEGFR2 leads to hope that the level of circulating VEGFR2 would not be a problem in this clinical study.

Regarding the possibility of thrombotic events, Dr. Rosenberg stated that pathology analyses at autopsy of the BALB/c mice that had exhibited toxicity showed surprisingly little organ toxicity and no thrombotic events.

Dr. Rosenberg explained that even if $5x10^{10}$ cells turns out to be a safe dose, the investigators are not planning to go beyond that dose due to technical limitations in growing more than 10^{10} cells.

In the mouse model, the investigators have seen a direct correlation between the number of cells given and the likelihood of seeing a response; there does not appear to be a maximum biologic dose.

Other designs have been proposed, for example having one participant in a cohort and then increase the dose; as soon as any toxicity of any kind is seen, the trial would stop, the investigators would drop back one cohort, and then re-start the trial using three research participants per cohort. Dr. Rosenberg explained that the investigators elected not to do that because, given the biologic variability among individuals, three participants per cohort would be necessary from the beginning.

Dr. Rosenberg agreed to include diabetes on the list of exclusion criteria.

While there is a correlation between T-cell persistence and the likelihood of seeing an antitumor effect, Dr. Rosenberg explained that that relationship is not necessarily causal and the animal data suggest that the therapeutic effect happens very quickly with these T cells.

E. Public Comment

Dr. Borror requested that the informed consent document be modified to remove references to "treatment" and "therapy." She also suggested modifying the statement about returning annually to the NIH in order to assure participants that they would be allowed to withdraw from the study if they so desire.

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:

Preclinical Issues

- Because VEGF is expressed on normal as well as malignant tissue, there is a risk of "on-target" toxicity (i.e., activity by cytotoxic T cells against VEGFR-2 on normal tissue). Although preclinical studies conducted to date have not shown toxicities in normal tissue, it would be prudent to consider additional preclinical studies in models in which VEGFR-2 needs to be upregulated under physiological conditions, e.g., in wound healing.

Clinical/Trial Design Issues

- Certain individuals (e.g., those with ongoing silent ischemia and resultant angiogenesis) may be more at risk for on-target toxicities due to overexpression of VEGFR-2. The issue is addressed in the eligibility criteria by exclusion of certain patients who may be at increased risk (e.g., those with a history of coronary revascularization or ischemic symptoms). Another condition to screen for and exclude would be diabetic proliferative retinopathy. In addition, as diabetics often have silent vascular disease, the investigators should consider an exclusion criterion that would capture those potential participants who may be most at risk, for example individuals with longstanding or uncontrolled disease.

Ethical/Social/Legal Issues

- The informed consent document should be revised as follows: 1) avoid using words such as "treatment" and "therapy" that can mislead prospective participants about the therapeutic potential of the study agent, and 2) clarify that although participants are expected to return to the NIH each year for 5 years for a physical examination, both for their benefit and for the benefit of the study, participants may withdraw from the study at any point.

G. Committee Motion 5

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved that the RAC approve these summarized recommendations. The vote was 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VIII. Discussion of Human Gene Transfer Protocol #1004-1037 entitled: Phase I/II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of CD8 Enriched Tumor Infiltrating Lymphocytes Genetically Engineered to Express IL-12

Principal Investigator: Steven Rosenberg, M.D., Ph.D., NCI, NIH
Additional Investigators: Richard A. Morgan, Ph.D., NCI

RAC Reviewers: Drs. Fan, Federoff, and Yankaskas

A. Protocol Summary

This study will be performed in research participants who have metastatic melanoma. Tumor-infiltrating lymphocytes (TILs) generated from the research participant's tumor, grown in the laboratory, will be transduced with a retroviral vector expressing interleukin 12 (IL-12) genes. IL-12 has been shown to stimulate the immune system, and TIL expressing IL-12 will deliver IL-2 to the tumor environment. The investigators have shown in mice that T cells transduced with IL-12 genes cause tumors to shrink.

The primary objectives of this study are to determine the side effects of this experimental treatment and to determine whether the research participant's tumor decreases in size after dosing on this study. The secondary objective of this study is to determine the survival of the infused cells in the body. This study is proposed to be conducted in two phases: the first phase will determine a dose that can be given safely to research participants and the second phase will evaluate if the experimental treatment can shrink tumors in humans. Up to 77 individuals could participate in this Phase I/II study.

Initially, research participants will have a biopsy of their tumor so that TILs can be isolated from the tumor. During the procedure to grow the TILs in the laboratory, IL-12 genes will be put into the cells using retroviral transduction. The retrovirus will serve as the vehicle to deliver the genes into the cells.

Once the cells are grown in the laboratory and the genes inserted, participants will be given chemotherapy (cyclophosphamide and fludarabine) for 5 days to suppress the immune system. One to 4 days after the chemotherapy, participants will be given the cells IV during 20 to 30 minutes. Participants will be given appropriate medications to treat the side effects of this dosing regimen and to prevent infection secondary to the immune suppression caused by the chemotherapy.

Research participants will return after 4 to 6 weeks to have their tumor(s) evaluated. If the tumor shrinks, the participants will continue to return to have their tumors evaluated. If a partial response to the dosing occurs or stable disease subsequently progresses, research participants could be re-treated. If there is no response, participants will be taken off this study.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of the protocol. Key issues included that, although the use of TILs for malignant melanoma is not new, the inclusion of IL-12 is novel. The potent biological activity of IL-12 and the potential for persistence of the transduced cells were deemed to deserve further discussion.

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

Dr. Fan asked the investigators to discuss the rationale for the final vector design and whether the vectors driven by the MSGV LTR had been tested. He noted that, in the preclinical data with murine IL-12 vectors, the two nuclear factor of activated T cells (NFAT) driven vectors are of the self-inactivating LTR design but the proposed human IL-12 vector has an active MSGV LTR. In addition, Dr. Fan noted that the murine IL-12 NFAT vector with the transgene in the forward orientation appeared to be more effective than the murine IL-12 vector with the transgene in the reverse orientation, and that the proposed human IL-12 vector would have the transgene in the reverse orientation. He stated that recent data had shown that endogenous xenotropic murine leukemia viruses (MuLVs) might be able to infect humans and might potentially cause disease. Toward that end, he asked whether the proposed packaging cell line expresses endogenous xenotropic MuLV sequences. Dr. Fan suggested that the investigators add a polymerase chain reaction (PCR) test for xenotropic MuLV prior to administering the vectored cells to research participants.

Dr. Federoff asked the investigators to discuss the following issues:

- Whether the NFAT promoter would be subject to transcriptional silencing, and whether other signaling pathways could transcriptionally activate NFAT and the related safety considerations
- The clinical parameters that influenced the investigators' decision to propose giving the cell infusions 1 to 4 days after nonmyeloablative conditioning
- The safety concerns with regard to re-treating research participants, since those with a partial response and stable disease could be re-treated, and the clinical and or other rationale for selecting 2 months as the basis for re-treatment
- Provided evidence of stable disease or partial response, whether research participants should be treated with banked transduced cells if a dominant clone has been detected
- What other signaling pathways can transcriptionally activate NFAT?

Regarding the study design, Dr. Yankaskas requested that the investigators describe the criteria and rationale for selecting the time (1 to 4 days) between completion of the nonmyeloablative (but lymphocyte depleting) preparative regimen and the infusion of gene-transduced TILs. He asked for a description of the time course of lymphocyte recovery from the nonmyeloablative regimen, the variability of that recovery time, and whether recovery time could influence the effectiveness of the IL-12 transduced TILs. Given that previous studies suggest increased survival in patients with metastatic melanoma treated with TILs, IL-2, and increasing doses of TBI, Dr. Yankaskas asked for an explanation of whether TBI would be likely to augment this proposed experimental therapy and whether the investigators plan to evaluate it. With regard to the informed consent document, he requested that the investigators change "gene therapy" to "gene transfer" to comply with standard RAC nomenclature.

C. RAC Discussion

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

- Noting that the investigators have a complete response against a 1 cubic centimeter tumor with only $1x10^4$ cells, Dr. Ertl asked Dr. Rosenberg to explain the likely mechanism by which such a small number of cells would be effective.

D. Investigator Response

1. Written Responses to RAC Reviews

The rationale for the final human IL-12 vector design was based on two primary criteria. First, the resultant vector should be able to be produced at clinical scale and be of sufficient titer to transduce human lymphocytes. (Self-inactivating vectors are difficult to produce at clinical scale and thus were not a feasible alternative.) Second, the vector should have low background synthesis of human IL-12 in unactivated lymphocytes. When the NFAT-responsive human IL-12 gene expression cassette is oriented in the opposite transcriptional direction (relative to the LTR), background synthesis of human IL-12 is minimal.

A murine IL-12 vector using the LTR to drive murine IL-12 was produced and tested *in vitro* and *in vivo*; this vector constitutively produces murine IL-12 and is designated MSGVl-mflexiIL12.

After consulting with Dr. Maribeth Eiden (retrovirus expert and a chair of the NIH IBC) on this issue, the investigators determined that xenotropic MuLVs are only induced when human cells are put into a mouse and then xenotropic MLVs are rescued. Since xenotropic viruses have not been induced from the proposed PG13 cells without the expression plasmids in the genome, the investigators believe that xenotropic MuLVs do not pose a safety issue in this application.

No data exists on the transcription silencing of the NFAT inducible promoter. As it is a hybrid promoter composed of two endogenous human sequences (the NFAT response element and a minimal IL-2 promoter), it may be regulated similarly to endogenous genes. Although the investigators recently published data indicating that transcriptional silencing in mature T cells is not observed in humans treated

with LTR-driven transgenes, they could not state how this observation would translate to the NFAT-responsive promoter.

The investigators explained that they plan to infuse cells the day after the nonmyeloablative conditioning regimen unless there is not a sufficient amount of cells on that day, which happens occasionally. In that case, the cells will remain in culture and will be administered on days 2, 3, or 4 when a sufficient number of cells are available.

Regarding re-treating research participants who exhibit partial response and have stable disease, the investigators responded that no additional safety concerns exist. Such individuals must continue to meet the original eligibility criteria to be considered for re-treatment, and toxicity related to cyclophosphamide or fludarabine should be stable and resolved to less than Grade 1 prior to re-treatment.

The duration of a partial response or stable disease relative to dosing must be greater than or equal to 2 months, with a confirmatory measurement being required for response determination. If research participants partially respond to experimental treatment or if they have stable disease that subsequently progresses, they may be re-treated, when progression is documented, with the same schedule that they had been given safely. Research participants who experience Grade 3 toxicity due to cell infusion – which is reversible within 24 hours with supportive measures – may be re-treated, but individuals who develop Grade 4 toxicity due to cell infusion will not be re-treated.

Cells used for re-treatment will consist of cells from the first dosing that have been cryopreserved. If cells from the first dosing are not available, cells for re-treatment will consist of a new lot of transduced TILs.

Regarding the signaling pathways that could transcriptionally activate NFAT, the investigators explained that any pathway that increases intracellular Ca2+ would activate the NFAT responsive promoter. However, because this mechanism in T cells is through antigen-mediated TCR activation, the investigators are not concerned about additional safety considerations.

Regarding the time course of lymphocyte recovery from the nonmyeloablative but lymphocyte-depleting preparative regimen, the investigators referred to their publication (Dudley, M.E., et al., J Clin Oncol 23:2346-2357, 2005) that describes the dosing of 35 metastatic melanoma patients with nonmyeloablative chemotherapy regimen and TIL followed by high dose IL-2. In the analysis of PBL subsets in research participants from 1 month to more than 1 year after cell transfer, the average pretreatment CD4+, CD8+, and B cell counts were within normal limits. However, the investigators noted that by 30 days to 60 days after dosing, the average CD4+ count fell and remained below 200 cells/mm^3 for the duration of the study. CD8+ cell counts transiently rose to a maximum of 835 cells/mm^3 60 days to 90 days after cell transfer, but decayed to normal levels over time. Like T cells, the B-cell compartment was transiently depleted by the chemotherapy, but B-cell levels returned to the normal range with a few months of dosing in most individuals. Platelet recovery to more than 30,000/mm^3 and absolute neutrophil counts (ANC) values at normal levels were achieved typically within 2 weeks of cell transfer in the first course of dosing.

If this proposed study is successful, the investigators intend to consider conducting a subsequent study that would include TBI.

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

2. Responses to RAC Discussion Questions

Dr. Rosenberg noted that one of the major impacts of IL-12 is to stimulate gamma interferon from natural killer cells, as well as from T cells. The investigators were surprised to see that it was not necessary for the cell they were giving to be able to react with IL-12. The investigators theorize that being a central cytokine is the explanation for why only a small number of cells is needed to produce an effect. The investigators suspect that these cells expand inside the body, which is why they are able to administer many thousands of cells below the predicted safe dose.

Dr. Rosenberg explained that the investigators looked inside the mouse tumors and noted the presence of more cells when IL-12 was given than when it was not, but the exact number of cells is difficult to quantitate in an intact mouse. *In vitro* the cells proliferate, but whether that would be an indication of how much they would proliferate *in vivo* is difficult to determine. These are reasons why the investigators have chosen to start with a cautious dose.

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:

Preclinical Issues

- The IL-12 gene is designed to express an IL-12 fusion protein composed of two subunits linked using a G_6S peptide. Since the peptide can create two open reading frames, it is possible that a novel epitope could be produced that will elicit an immune response. To address this safety question, preclinical mouse model studies should be carried out to determine whether the novel epitope is produced and, if so, whether it causes an immune response.

Clinical/Trial Design Issues

- Since the NFAT responsive promoter will be used to regulate IL-12 expression, a literature review should be conducted to determine whether any medications could also lead to activation of the NFAT promoter and increase production of IL-12. If any medications having such effects are found, they should be an exclusion criterion, and prospective participants should be screened for their use.

- The protocol proposes to use a retrovirus vector based on a derivative of the murine stem cell virus, MSGV1. Recent data have indicated that endogenous xenotropic murine retroviruses may be able to infect humans and potentially cause disease, for example the xenotropic murine leukemia virus-related virus (XMRV). Therefore, when the cells are tested for replication-competent retrovirus prior to administration, they should also be tested by rtPCR for xenotropic murine retroviral envelope.

G. Committee Motion 6

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Fan and seconded by Dr. Kirchhoff that the RAC approve these summarized recommendations. The vote was 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IX. Proposed Changes to Section III-E-1 the *NIH Guidelines*

Presenter: Dr. Roizman

A. Presentation by Dr. Roizman

Dr. Roizman reviewed the current Section III-E-1 of the *NIH Guidelines*, the reasons for concern, and the proposed changes to the wording of that section.

The current regulations concern the expression or propagation of viruses in culture under BL1 containment. In the current rules, recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated and maintained in tissue culture using BL1 containment if it is demonstrated that the cells lack helper virus for the specific families of defective viruses being used. The DNA may contain fragments of the genome of viruses from more than one family, but each fragment must be less than two-thirds of a genome.

The concern about this rule is that it might be possible to construct, by genetic recombination or synthetic manipulation, viral agents derived from multiple sources that could function with less than two-thirds of the genome present. Therefore, it was proposed that "two-thirds of the genome" be changed to "one-half of the genome." In addition, the generation of replication-competent virus could derive from mechanisms other than the presence of a helper virus; therefore, it was proposed to amend this section to require a demonstration that the preparations being transferred in cell culture are free of replication-competent virus.

In March 2009, the OBA published a proposed revision of Section III-E-1. Comments from that publication resulted in new wording being drafted and the proposed language was published for further public comment in the *Federal Register* on April 22, 2010. A final Notice of Action will be published after the public discussion at the current RAC meeting.

The April 2010 proposal published in the *Federal Register* read as follows:

> Recombinant nucleic acids from a eukaryotic virus (excluding Variola major and Variola minor) and/or synthetic nucleic acids molecules based on a sequence from a eukaryotic virus (excluding Variola major and Variola minor) may be propagated and maintained in cells in tissue culture using Biosafety Level 1 containment if:
>
> - There is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids OR
> - For Risk Group 3 or 4 viruses, no more than half of the genome is present, all viruses from a single family being considered identical. These nucleic acids may contain fragments of the genome of viruses from more than one family but each fragment shall be less than one-half of a genome.
>
> In addition, there must be evidence that the resulting nucleic acids are not capable of producing a replication-competent virus in a cell line that would normally support replication of the wildtype virus.
>
> If a gene deletion is the basis for a reduction in containment, evidence should be presented to the IBC that the cell culture cannot complement the missing genes or reconstitute the infectivity of the virus by homologous recombination between genes resident in the cells and the viral nucleic acid sequences introduced into the cell culture. Such evidence could consist of a demonstration that the cell culture does not contain a contiguous copy of the nucleic acid sequences deleted from the virus.
>
> It must be demonstrated that the cells lack helper virus for specific families of defective viruses being used. If helper virus is present, Section III-D-3 applies and IBC review is required prior to initiation.
>
> A minimum of BL2 containment is required for experiments with retroviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Two public comments were received, one from the American Biological Safety Association (ABSA) and one from the Division of Select Agents and Toxins (DSAT), CDC. As a result of the ABSA comments, the OBA will develop a guidance document for IBCs and investigators in addition to the final *Federal Register* notice. The response to the DSAT comment regarding possibility of generation of infectious virus from multiple constructs each containing less that ½ of the viral genome was that the likelihood of such an

event is extremely remote and the changed language actually reduces the amount of virus present. Requiring spatial and temporal separation to avoid accidental reconstitution has the potential of creating a burden on the investigator and was not required previously under Section III-E-1. The final *Federal Register* notice will emphasize the need for good biosafety practices, including labeling of specimens and separation of reagents, and temporal and spatial separation will continue to be required only for work with RG3 influenza viruses due to the propensity for these viruses to reassort and their ability to spread easily from person to person.

B. Public Comment

No public comments were requested.

C. Committee Motion 7

It was moved by that the recommendations of the RAC Biosafety Working Group, as presented by Dr. Roizman, be approved. The RAC voted unanimously by voice vote to approve the recommendations.

X. Day 1 Adjournment/Dr. Federoff

Dr. Federoff, RAC Chair, adjourned Day 1 of the June 2010 RAC meeting at 4:35 p.m. on June 16, 2010.

XI. Day 2 Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff, RAC Chair, called Day 2 of the June 2010 RAC meeting to order at 8:30 a.m. on June 17, 2010.

XII. Presentation of Certificates of Appreciation

Presenter: Amy P. Patterson, M.D., Office of the Director, NIH

Dr. Patterson presented certificates of appreciation from the NIH to the seven RAC members whose service to the NIH, the research community, and the public was ending at this RAC meeting: Drs. Ertl, Flint, Kirchhoff, Kodish (*not present*), Strome (*not present*), Wei (*not present*), and Williams. The departing RAC members thanked the OBA staff for their work and Dr. Federoff for his chairing of the RAC. Dr. Federoff added his thanks to these seven RAC members.

XIII. Update on Proposed Changes to Section III-C-1 of the *NIH Guidelines* on Human Gene Transfer

Presenters: Dr. Corrigan-Curay; Craig C. Mello, Ph.D., University of Massachusetts Medical School (*via teleconference*); Saraswathy Nochur, Ph.D., Alnylam Pharmaceuticals, Inc.; and Phillip A. Sharp, Ph.D., Massachusetts Institute of Technology

A. Introduction by Dr. Corrigan-Curay

Dr. Corrigan-Curay summarized the genesis and history of these proposed changes. The RAC was asked to consider the application of the *NIH Guidelines* to synthetic biology, focusing on the degree to which this technology was currently covered including whether the scope needed to be modified to capture synthetic biology research, and to develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic biology.

The approach followed throughout this process was to base the level of review on risk, not technique, thereby capturing products made by synthetic techniques that are currently covered under the *NIH Guidelines* (provided the same biosafety risk concerns are raised) and recognizing that all future scientific developments cannot be anticipated. A definition of synthetic nucleic acids was crafted for this purpose: Section I-B, "Definition of Recombinant and Synthetic Nucleic Acids," is proposed to read:

(i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell,
(ii) Synthetic nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, or
(iii) Molecules that result from the replication of those described in (i) or (ii) above.

Under the *NIH Guidelines*, the definition of recombinant nucleic acids is limited to those "that can replicate in a living cell." To exempt similar non-clinical research, the following exemption was proposed for synthetic nucleic acids that:

- Can neither replicate nor generate nucleic acids that can replicate in any living cell, and
- Are not designed to integrate into DNA, and
- Do not produce a toxin that is lethal for vertebrates at an LD50 < 100 nanograms/kg, and
- Are not deliberately transferred into one or more human research participants.

The public comments regarding the proposed language published in the Federal Register, which included some from the Oligonucleotide Safety Working Group (OSWG) representing a number of pharmaceutical companies, urged the RAC and the OBA to differentiate synthetic ribonucleic acid (RNA) and DNA agents from gene transfer. This request was based on characteristics that include their short half-life, more predictable pharmacokinetics, lack of ability to integrate into the genome, lack of replication or potential for inadvertent replication, and lack of a transgene for coding a protein. The comments stated that these characteristics made these synthetic agents more similar to small molecule drugs than vector-mediated gene transfer.

In December 2009, the RAC voted to recommend that the human research involving DNA oligonucleotides be exempt from further review under the *NIH Guidelines*, since the mechanisms of action are well characterized. However, the RAC continued to consider that human research with RNA oligonucleotides be covered by the NIH Guidelines because research with RNA oligonucleotides is a relatively new field. The RAC considered the emerging preclinical data and whether short-interfering RNA (siRNA) and micro-RNA (miRNA) could lead to longterm gene silencing and, if so, the clinical implications of that silencing.

Public comment regarding the December 2009 proposed wording was received from the OSWG, stating that RNA oligonucleotides also have transient "drug-like" pharmacologic effects that reverse within days or weeks when administration is stopped, which is much like small molecule drugs that also have the potential for off-target effects but do not require RAC review. Unlike gene transfer, there is no potential for germline transmission, integration, gene expression, replication, shedding, or longterm persistence. Off-target effects are not substantially different for RNA oligonucleotides and DNA oligonucleotides, in that siRNAs and antisense DNA oligonucleotides both target specific messenger RNA (mRNA) sequences and it is possible to screen for off-target effects using genome homology databases. Gene transcription modulation can occur with small molecule drugs and is not unique to RNA therapeutics.

B. Presentation by Dr. Sharp

Dr. Sharp briefly discussed the mechanism of RNA interference, differences between synthetic DNA and RNA oligonucleotides, clinical experience with DNA and RNA oligonucleotides, and issues of concern for synthetic RNA oligonucleotides mentioned by the RAC, including potential off-target effects and longterm effects.

Comparing DNA oligonucleotides with RNA oligonucleotides, Dr. Sharp stated that both:

- Are typically less than 40 nucleotides (nt), with ~20 nt being the optimal size
- Regulate activity at the mRNA level through either binding to the RNA or directing the cleavage of the RNA
- Have been observed *in vitro* to cause nonspecific sequence effects, including immunostimulatory effects
- Do not have characteristics common to recombinant DNA integration and replication
- Have reversible effects, as observed in animal trials
- Have been studied in clinical trials (although DNA oligonucleotides have been studied more extensively)

Off-target effects are not unusual in drugs, and can be due, for example in small molecules, to nonspecific binding, interacting with unknown targets, and downstream effects of metabolites. Knowing the sequence for specific intended targets enables minimization of potential off-target effects. Dr. Sharp opined that the bar should not be set higher for synthetic oligonucleotides because of theoretical concerns. He described potential strategies for minimizing non-specific immune stimulation by siRNAs. While siRNA induced epigenetic effects, proposed to involve changes to chromatin, have been observed, the reported changes have been transient.

He concluded that short, synthetic DNA and RNA oligonucleotides that do not use viral vectors for delivery do not pose concerns similar to those of recombinant DNA constructs, thus DNA and RNA oligonucleotide research should be reviewed by the same processes and agencies. Off-target effects (whether sequence-mediated or not) are not unique to oligonucleotides and are observed with all classes of drugs. The safety of these synthetic oligonucleotides is well supported by toxicologic animal studies prior to studies in humans, and clinical data to date support similar review of RNA and DNA oligonucleotide therapeutics. Dr. Sharp added that synthetic RNA oligonucleotides should be exempt in Section III-F-1 of the *NIH Guidelines*, along with synthetic DNA oligonucleotides.

C. Presentation by Dr. Nochur

Dr. Nochur provided background regarding the OSWG, a group of ~70 pharmaceutical companies and representatives from regulatory agencies. The focus of the OSWG is to look at developmental aspects of short synthetic oligonucleotides, including antisense oligonucleotides, siRNAs, aptamers, and immunostimulatory oligonucleotides. Its objectives are to share information, facilitate dialogue, and enable discussion of the safety issues either observed or anticipated. The OSWG keeps track of all publications related to the development of short synthetic oligonucleotides as drugs; multiple subcommittees scan the literature and share information with each other.

The OSWG believes that synthetic DNA or RNA oligonucleotide molecules should be exempt from NIH/RAC review if they have all of the following characteristics:

- Less than 100 base pairs
- Non-integrative and non-replicative
- Cannot function as a gene
- Cannot be translated
- Have a transient effect

The rationale for this belief is that synthetic RNA oligonucleotides do not have the liabilities of classic recombinant molecules and synthetic oligonucleotides have been used in clinical trials for more than 20 years under the jurisdiction of the FDA, with no significant or unexpected safety issues. There is no scientific basis for exempting DNA and not RNA oligonucleotides; both DNA and RNA oligonucleotides have a similar mechanism of action (i.e., they target mRNA), they do not impact the genome, most antisense drugs in current development are DNA-RNA hybrid molecules, modifications used with synthetic DNA oligonucleotides blur clear lines of distinction between DNA and RNA oligonucleotides, and effects are transient and reversible upon cessation of dosing with DNA and RNA oligonucleotides. Synthetic oligonucleotides are not unlike small molecules in their overall impact on cell systems; nonspecific effects are known to occur. However, synthetic DNA or RNA oligonucleotides delivered

and/or expressed with the use of viral vectors (e.g., short-hairpin RNAs) are not sought to be exempt from RAC review.

Given this analysis, the OSWG recommends exemption of synthetic RNA oligonucleotides from RAC review of clinical trial protocols and not requiring additional oversight from the NIH/RAC, similar to what has been done for DNA oligonucleotides. In response to the NIH/RAC request for sharing of clinical safety data of synthetic RNA oligonucleotides, the OSWG requests active NIH/RAC participation at conferences such as the Drug Information Association's Oligonucleotide-Based Therapeutics Conference, TIDES meetings, and Oligonucleotide Therapeutic Society meetings, in order to keep apprised of what is occurring in the field of synthetic oligonucleotide development. The OSWG will provide the NIH/RAC with "white papers" generated by OSWG subcommittees that reflect the current thinking on safety-related issues in the development of synthetic oligonucleotides as therapeutics. In addition, several companies developing synthetic RNA-based oligonucleotides have agreed to share safety data from completed clinical studies, possibly at an annual meeting to be organized by the NIH/RAC.

D. RAC and Public Discussion

In response to Dr. Ertl's query, Dr. Nochur stated that academia is not represented directly within the OSWG. The OSWG is focused on development aspects; although academic publications help to focus on the issues that might come up, the OSWG works on the practical aspects of how to develop drugs. James D. Thompson, Ph.D. (Quark Pharmaceuticals), explained that the OSWG obtains information from academia through the Oligonucleotide Therapeutics Society, to which a large number of OSWG members belong. Dr. Sharp stated that this field is not primarily academic but is a field of therapeutics that will be driven primarily by organizations with the integrative capacity to meet the chemistry, formulation, and delivery aspects of clinical trials.

Dr. Flint asked whether the future of this field is likely to focus on siRNAs that have been designed to target a particular, specific mRNA or whether the focus would be on miRNA sequences. She noted that some RAC members were concerned about the differences between the function of miRNAs, which can have a broad spectrum of targets, and what it appears that OSWG members are trying to do, which is to go after particular cellular or viral gene products. Dr. Nochur responded that the goal and promise of this field is to have the potential to go after classic targets that are non-druggable by monoclonal antibodies or by small molecules; specificity is very important in terms of being able to take the advantage of targeting a particular sequence.

Dr. Roizman expressed his concerns as: targeting miRNAs simply because they target and control a large number of genes, targeting genes that control miRNA pathways, and targeting a gene that would have irreversible downstream effects.

Akshay Vaishnaw, M.D., Ph.D. (Alnyam Pharmaceuticals), explained that the siRNA conforms to an orthodox model of drug development whereby pharmacodynamic effects are seen as long as the siRNA is present. A detailed understanding is emerging of the mechanism of action and the properties of siRNAs and, as more is learned, it appears that siRNA conforms to the current understanding of how small-molecule drugs are meant to work.

Dr. Ertl expressed concern about epigenetic changes, for example in stem cells that might initially not be obvious but then become obvious years later. She asked whether there was a risk that subtle epigenetic changes could be dangerous. Dr. Sharp responded that, to date, extensive studies looking for epigenetic-type changes have shown no irreversible effects. Dr. Mello explained that the long-lasting epigenetic effects seen in *C. elegans* are induced only when either a transgene is used to trigger the silencing, a co-suppression-like phenomenon, or when long double-stranded RNA is used; siRNA does not induce transient silencing or evidence of any epigenetic silencing. The silencing that might last for several generations is only observed in the germline and it is dependent on a family of RNA-dependent RNA polymerases that do not exist in a human.

Dr. Mello stated that, in many diseases (cancer is one example), epigenetic effects are involved in the disease process. Therefore, being able to reverse or interfere with an epigenetic effect could be therapeutically relevant in the future. Interfering with epigenetic effects is not likely to be an off-target problem with siRNA, given what is known at present.

E. Conclusions

Dr. Corrigan-Curay suggested that, in light of continued debate and emerging data, some of which may not have been available during earlier deliberations, the RAC should consider deferring its final recommendation regarding synthetic nucleic acids that are not delivered in a vector until a more complete dataset is developed.

The proposed wording at this point in RAC discussion was presented as:

Section III-C-1: Human Gene Transfer

Human gene transfer includes all experiments involving the deliberate transfer of either:

1. Recombinant DNA, or DNA or RNA derived from recombinant DNA; or
2. Synthetic DNA or RNA that
    - Contains more than 100 nucleotides or base pairs in total; or
    - biological properties that enable integration into the genome; or
    - Have the potential to replicate in a cell; or
    - Can be transcribed or translated

After much discussion by RAC members and guest-experts, it was decided that the OBA would finalize the wording and email the proposed final wording to RAC members for their approval.

F. Committee Motion 8

It was moved by Dr. Flint that the RAC accept this proposal with any minor modifications made in consultation with the RAC after this June 2010 RAC meeting.

G. Next Steps

Dr. Corrigan-Curay announced that the OBA would organize a state-of-the-science conference to bring academic and industry experts together in a public forum to review existing preclinical and clinical data on safety and efficacy, examine whether preclinical models have been predictive to date, review the data on mechanisms of action and potential for epigenetic and longterm phenotypic changes, and identify opportunities for research that would advance the understanding of this field and the safety for clinical applications including potential monitoring strategies. She welcomed RAC members on a steering committee to plan this conference, along with representatives of the FDA and the OSWG.

XIV. Discussion of Human Gene Transfer Protocol #0810-952 entitled: 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)

Principal Investigator:	Januario E. Castro, M.D., University of California San Diego (UCSD)
Sponsor:	Memgen LLC
RAC Reviewers:	Drs. Ertl, Williams, and Zaia

A. Protocol Summary

Chronic lymphocytic leukemia (CLL) is an accumulative disease of slowly-dividing mature-appearing monoclonal B cells in the blood, marrow, and lymphoid tissues. There is no cure for this disease, which is the most common form of adult leukemia in Western societies, accounting for approximately 30 percent of all leukemias. While most CLL patients respond to chemotherapeutic regimens, approximately 10-20% of patients will have limited or no response to cytotoxic agents. Furthermore, many patients that fail these chemotherapy regimens will eventually develop therapy resistant disease.

The protocol proposes gene transfer for research participants with chronic lymphocytic leukemia (CLL) by adenoviral vector transfer into CLL B cells of ISF35, the human CD40 ligand, CD154. Relapsed or refractory CLL is frequently associated with cytogenetic changes in the leukemic cells often associated with deletions in the short arm of chromosome 17 (del(17p)) and loss of functional p53, a tumor suppressor gene. The expression of ISF35 is proposed to upregulate pro-apoptotic factors, enhance expression of p73, a surrogate for p53, and sensitize the del(17p) cells to chemotherapy. The primary objectives of the protocol are to assess the toxicity, tolerability, and safety of repeat administration of three infusions of $3x10^8$ autologous Ad-ISF35 transduced CLL B cells given intravenously in combination with standard chemotherapeutic regimen consisting of fludarabine, cyclophosphamide, and rituximab (FCR) in up to 12 subjects with CLL that is fludarabine-refractory and/or del(17p).

B. Written Reviews by RAC Members

Nine RAC members voted to review for in-depth review and public discussion of this protocol. Key issues included that the infusion of lymphoid cells modified by a co-stimulatory molecule might cause a prolonged inflammatory response secondary to cytokines. A serious adverse event in one participant (hypotension, dyspnea, acute renal failure, anemia, and elevated liver enzymes) highlights this potential safety risk. In addition, the possibility that the elevation in liver enzyme blood tests in this case was due to infusion of the adenoviral vector is unexpected and was deemed to deserve public discussion.

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

Dr. Ertl asked why the investigators chose not to irradiate the CLL cells that will be transduced and then transferred back into the research participant. She requested further discussion of the mechanisms by which the investigators believe the untransduced CLL cells will undergo phenotypic changes, will upregulate expression of costimulators and apoptosis-related molecules, and thereby will become more susceptible to the immune system and to chemotherapy. Noting that the CD40 ligand encoded by the adenovirus vector is derived, in part, from a mouse sequence, Dr. Ertl asked whether this protein would induce an immune response in humans that could cross-react with human CD40 ligand, whether this potential cross-reaction has been assessed in nonhuman primates, and whether it will be monitored in research participants. She also suggested that participants be monitored for antibody responses to CD40 ligand. In addition, Dr. Ertl indicated that, although mouse toxicity studies showed the Ad-ISF35 vector to be well tolerated, adenovirus vectors are known to be well tolerated by mice (even at excessive doses) while humans are far more sensitive to these vectors.

Dr. Williams asked the investigators to clarify and update the data on the research participants who were already enrolled prior to the protocol being placed on clinical hold. He requested evidence as to whether ISF35 induces a humoral (antibody) response in murine studies, asked whether the murine studies conducted to date used intravenous injections, and requested information about the dosage and route of administration of ISF35 leading to the noted side effects in humans. Dr. Williams noted that a Phase I clinical trial at M.D. Anderson Cancer Center evaluated a single intravenous infusion of autologous Ad-ISF35-transduced CLL cells at three dose levels ($1x10^8$, $3x10^8$, and $1x10^9$ transduced cells) in nine individuals with CLL; no DLTs were experienced at any of those dose levels and no MTD was identified. While Grade 3 toxicities included transient thrombocytopenia and were observed in four research participants, interim data was provided on only two of three participants.

Dr. Zaia asked the investigators to provide additional information to support their approach in this study, in which the application of a lymphodepleting chemotherapy following the experimental treatment regimen is predicted to induce an apoptosis-based clearing of CLL cells. He requested clearer definitions of the

eligibility for continued infusion of cells and for repeat cycles of FCR. Regarding the informed consent document, Dr. Zaia asked whether a conflict of interest determination had been made. He also noted that the risk section is inclusive of potential problems but does not characterize these as likely to be temporary AEs that usually respond to treatment, and omits the possibility that a research participant could die from a severe anaphylactic reaction.

C. RAC Discussion

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

- The UCSD owns the patent for Ad-ISF35 and intends to license the agent to Memgen LLC. Regarding the possibility of conflict of interest on the part of the UCSD, Dr. Zaia suggested informing potential participants about the likelihood that the medical center at which they are receiving experimental treatment could benefit from the results of this clinical trial.

- Dr. Takefman clarified that the FDA does not require inclusion in the informed consent document any risk of cancer induced by adenovirus, because the FDA is not aware of any such risk.

- Dr. Kahn asked the investigators to send to the RAC a description of how the conflict of interest process is handled at the UCSD – which bodies are involved and how they interact with the administration, the investigators, and other stakeholders at the university.

- Dr. Kahn asked the investigators to indicate how much identifying information would be included with the banked samples from research participants. He also asked for clarification as to what the future uses of those samples might be.

D. Investigator Response

1. Written Responses to RAC Reviews

The transduced CLL cells that are transferred back into a research participant comprise less than 1 percent of the total leukemic burden, and the investigators determined that, for this disease, the benefits of not irradiating cells would outweigh the perceived risks. Since CLL cells are known to be slowly dividing and nonmetastatic, the use of nonirradiated cells was not considered a high risk for CLL patients, and it was possible that irradiation could limit the survival of cells and mitigate their functional activity. Ad-ISF35-transduced CLL cells are frozen prior to readministration, so longterm storage of irradiated cells could negatively impact their quality and viability upon thawing and infusion. Non-irradiated cells have been used for all the *ex vivo* clinical trials conducted to date, and there has been no evidence of uncontrolled proliferation, longterm survival or persistence of transduced cells, or conversion of the transduced cells into a more aggressive leukemic population *in vivo*.

The interaction of CD40 receptor with its ligand, CD154 (normally expressed on T cells), induces expression of immune costimulatory molecules and enhances the antigen-presenting capacity of normal B cells as well as neoplastic CLL cells both in mouse and in man. CD40 triggering by CD154 increased the expression of a variety of immune accessory molecules, such as adhesion molecules (CD54) and costimulatory molecules (CD80, CD86, CD70). Thus, CD40-activated CLL B cells could induce proliferation of allogeneic or autologous CD4+ and CD8+ T cells. Directly expressing the CD154 transgene on the readily accessible CLL cells may mimic the function of an activated T cell that expresses surface CD154 to generate a targeted immune response. The most efficient strategy for a cellular immune therapy for CLL appeared to be to express CD40 ligand (CD40L) directly on the CLL cells, since the CLL cells are readily accessible from the blood and do not require cellular purification or expansion methods. Using a replication-incompetent adenovirus to express CD40L on CLL cells resulted in stable expression of CD40L on the CLL cell surface. In turn, this surface expression of CD40L resulted in the same phenotypic activation of the CLL cells as seen with activation of CLL cells co-cultured with activated T cells or agonistic antibodies for CD40. These transduced and activated CD40L CLL cells induced allogeneic and autologous T-cell responses.

Adaptive cellular immune effector mechanisms are one explanation for the therapeutic activity seen in clinical studies to date. A role for the innate immune system in the effects of CD154 gene transfer was suspected by the rapid reductions in leukemia cell counts and lymph node size observed in patients several days after intravenous infusion of CD40L-expressing autologous leukemia cells. The rapidity of this response makes it appear unlikely that the early clinical effects of this experimental treatment reflect an adaptive immune response.

While adaptive and innate anti-leukemic immune responses are one set of mechanisms of action of the CD40L-active cellular therapy strategy, pro-apoptotic regulation of CLL cells is another mechanism. Cytotoxic activity of various anticancer drugs, such as F-ara-A, was enhanced for CLL cells following CD40 ligation, particularly for CLL cells that lacked functional p53. These results indicate that CD40 activation can sensitize leukemia cells to apoptosis via induction of TAp73 and restore the sensitivity of p53-deficient CLL cells to anticancer drugs that ordinarily require functional p53 for their cytotoxic activity, such as purine analogues (fludarabine) and alkylating agents (cyclophosphamide).

Analysis for antibodies cross-reactive to human CD40L will be conducted on participant samples as part of the pharmacodynamic studies. Analysis of the four participants dosed with ISF35 from this protocol (prior to clinical hold) did not generate cross-reactive antibodies to human CD40L. In contrast, antibodies reactive against the adenovirus vector have been induced following dosing, indicative of a host anti-adenovirus response and the ability to mount an effective humoral immune response. While this process has not been assessed in nonhuman primates, it will be monitored in research participants.

The proposed doses of Ad-ISF35 vector that research participants will receive as part of the clinical development program have been refined based on the published literature on Ad5 vectors in humans and in nonhuman primates, as well as the results of preclinical toxicology studies in mice and clinical trials conducted by the investigators.

Assessment of anti-CD154 antibody generation was examined in the mouse repeat-dose toxicity study. The investigators stated that mice intratumorally injected three times with Ad-ISF35 showed no evidence of IgG antibodies generated against the mouse CD154 molecule. All murine toxicology and pharmacology studies using ISF35 adenovirus have used intratumoral injection of Ad-ISF35; no intravenous studies with Ad-ISF35 have been conducted. Intravenous toxicity studies were originally conducted using the fully murine CD154 adenovirus vector; these studies supported initiation of the first two gene transfer protocols investigating the CD154 cellular vaccine strategy.

Dr. Castro has no conflict of interest with regard to this clinical trial. While Dr. Castro is the principal investigator at the UCSD and the Ad-ISF35 technology was licensed from the UCSD by Memgen LLC, Dr. Castro has no financial ownership of the Ad-ISF35 technology, the patents, or Memgen. Memgen LLC has determined that no conflict of interest exists for the additional clinical trial sites and personnel proposed for this multicenter study.

The investigators explained that the conclusion that few AEs would be permanent is supported by the clinical course of AEs seen to date in the trials using Ad-ISF35 and Ad-murine CD154 vector. While all AEs associated with the investigational agent have been transient and reversible in the research participants, it is possible that a participant could die from a severe anaphylactic reaction and the investigators offered to amend the informed consent document to so indicate. The risk of developing a new cancer from Ad-ISF35 is low, and a genotoxic drug combination such as FCR would have a higher risk of carcinogenicity. However, since Ad-ISF35 is still an investigational drug without carcinogenicity studies, the investigators noted that the possibility for developing a new cancer from Ad-ISF35 cannot be completely ruled out, and this risk-factor language should continue to be included in the informed consent document.

The investigators updated the data on the research participants who had been enrolled in the protocol prior to clinical hold. While seven participants had been enrolled, only four participants received the investigational agent. The four participants who received ISF35 followed by FCR were participants 1, 3,

5, and 6; participants 2, 4, and 7 underwent leukapheresis but did not receive the experimental treatment. Participant 2 tested positive for hepatitis B infection, a protocol exclusion criterion; participant 4 decided against participation in the study after enrollment; and participant 7 did not receive the experimental treatment due to the study being placed on clinical hold.

The investigators explained that the route of administration for all noted side effects in humans was intravenous infusion of autologous Ad-ISF35 CLL cells. In the Phase I dose escalation study using ISF35, toxicities were primarily grades 1 or 2, consisting mostly of flu-like symptoms, with the exception of one participant in the high-dose cohort ($1x10^9$ cells) with Grade 3 headache on day 3 that resolved. The individual who experienced Grade 3 headache was considered to have had a DLT. The investigators also noted that participants who received the highest dose of autologous Ad-ISF35-transduced cells generally experienced a higher incidence of Grade 2 versus Grade 1 events. Given the DLT, a dose of $3x10^8$ cells is being proposed for the current repeat-dose Phase 1b study.

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

2. Responses to RAC Discussion Questions

Dr. Castro explained that hypophosphatemia has been seen by the investigators in both the *ex vivo* protocol and the intranodal protocol. In reviewing the literature, some evidence exists that, in patients with melanoma treated with high dose IL-2, one of the complications of high doses of IL-2 and/or cytokines may be regulation of hormones that regulate the calcium and phosphorus pathway. As a result, the investigators recently have asked the research participants to drink extra milk on the day of infusion and for two days thereafter. Doing so has resulted in a substantial reduction in the presentation of hypophosphatemia.

While research participant number 6 experienced a serious adverse event, Dr. Castro explained that, in the setting of CLL and high white blood cell counts, the response experienced by that individual was encouraging. After the FCR regimen, the bone marrow suppression recovered gradually and positively after initiation of biotherapy with a tumor marker and high-dose methylprednisolone. Therefore, whatever was responsible for those adverse events and the cytopenias was neither durable nor persistent in this research participant.

Dr. Castro noted that the first research participants infused with adenovirus CD154 (the mouse construct) now have been followed up for about 10 years. The investigators have not observed an increased rate of secondary malignancies or any development of autoimmune diseases.

Acknowledging that the investigators do not yet have cytokine data for the research participant who experienced an SAE to compare to the participants who better tolerated this experimental treatment, Dr. Castro stated that they have banked specimens for all participants.

Dr. Castro agreed to provide to the RAC documents that outline UCSD's conflict of interest process.

E. Public Comment

Dr. Borror suggested revising the informed consent document due to some complex and technical language. In addition, she expressed concern about the wording in the informed consent document about specimens and DNA from participants being stored for future research, and that participants are being asked in that document to consent to those uses even though they have no idea what those uses are. Although it is likely that most participants will not be deterred by this request for consent, Dr. Borror noted that some participants might not want to consent to future use of their specimens but would still want to participate in this trial. In addition, she noted that the wording for future use of the specimens was not specific to leukemia research.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

It was noted that, contrary to the *NIH Guidelines*, the investigators initiated this study and enrolled four participants before the protocol had been submitted to the NIH for registration and RAC review. The investigators' institution discovered the oversight after the study was put on hold due to concerns raised by the Data and Safety Monitoring Board regarding major deficiencies in the management of two other studies using the same vector. OBA had contacted the IBC and the Vice Chancellor for Research regarding the implementation of a corrective action plan to address these violations. In addition to the belated review of this protocol, the RAC assessed the initial data submitted on the four participants who were dosed with the study agent. Those data indicate that three participants experienced some improvement in their condition, one of which was a complete response. The fourth participant had an SAE event – hypotension, dizziness and syncope – within 24 hours of the first infusion and, after the second infusion, a second AE involving nausea, vomiting, and hypotension that required hospitalization. That participant developed acute renal failure and elevated liver enzymes, and was withdrawn from the study.

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

<u>Preclinical Issues</u>

- The histogram included with the protocol showed the expression of the immune accessory molecules CD54 and CD95 that promote T-cell activation in cells transduced with the Ad-ISF135 vector compared to cells transduced with an AdLacZ vector. These data do not demonstrate a significant difference between the two vectors. A dot plot comparing expression of CD54 and CD95 in cells transduced with Ad-ISF35 and non-transduced cells also does not provide evidence that T-cell activation was achieved because of the transgene. Data in a dot plot comparing expression in cells transduced with the Ad-ISF135 vector to cells transduced with an AdLacZ vector (i.e., the data in the histogram) should be submitted.

- The adenoviral vector (inactivated E1 and E3 genes) is replication defective, i.e., its capacity to replicate in a cell-type dependent manner is reduced. However, because inadequate preclinical data were provided (the data were from studies conducted in a mouse model that does not support adenovirus replication), studies should be performed to determine whether the vector replicates in CLL cells. If the vector does replicate, the risk of vector dissemination is increased. These studies may also shed light on the vector's apparent antitumor effects in the three research participants who received all of the planned doses. These studies may be performed concurrently with the clinical trial.

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

- Given the estimate of approximately one gene modified CLL cell to 10,000 unmodified CLL cells, the significant change in the expression of cell signals on the surface of the unmodified CLL cells is unlikely to be due to cell-to-cell interactions as proposed. Additional studies, including analysis of cytokines and gene expression by PCR, should be conducted to understand the cytokine or other mechanisms underlying these changes. Broader analysis (e.g., microarrays) of participant specimens, the collection and banking of which is already part of the protocol, should also be considered.

- The murine sequences in ISF35 could lead to an antibody response to human CD154. Although such a response was not seen in any of the four enrolled participants, there is uncertainty about the specific sensitivity (nanograms/ml) of the screening assay that was used, and it is important that the assay be validated before conclusions are drawn about this risk.

- The proportion of circulating anti-adenoviral T cells may be highly variable among research participants. In participants who have a significant proportion of circulating T cells against

adenovirus, adenoviral vector transduced cells may be cleared rapidly. The protocol should include monitoring of anti-adenoviral specific T cells before and after administration of the adenoviral vector to determine whether there is a correlation between the preexisting levels of these T cells and efficacy.

Ethical/Social/Legal Issues

- The University of California, San Diego, holds the patent for Ad-ISF135, which is being licensed to the sponsor, Memgen LLC. The informed consent document should inform participants of this financial conflict of interest and what steps have been taken to prevent it from affecting the objectivity of the investigator and the institution. In addition, the University's institutional policies and mechanisms for addressing individual and institutional conflicts in research should be submitted.

- The informed consent document does not provide participants with sufficient information about how their biological samples will be used in the protocol and whether those samples will be used for other research purposes in the future. While it may not be possible to describe all possible future uses, additional information should be provided regarding the general purpose(s) for which the optional blood samples will be collected, who will have access to the samples, whether the samples will remain identifiable, and where they will be stored (e.g., at UCSD or as part of a larger biobank). From an ethical standpoint, the consent should also enable the participants to decline to permit the future use of their specimens, e.g., through an opt-out provision.

- Until definitive data have excluded the possibility of vector replication, barrier contraception methods should be recommended for a time period similar to that used in gene transfer trials involving replication-competent oncolytic vectors.

- The informed consent document should be simplified.

H. Committee Motion 9

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Williams and seconded by Dr. Kahn that the RAC approve these summarized recommendations. The vote was 12 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XV. Discussion of Human Gene Transfer Protocol #1004-1028 entitled: A Phase II Study of Repeat Intranodal Injections of Adenovirus-CD154 (Ad-ISF35) in Patients with Chronic Lymphocytic Leukemia / Small Lymphocytic Lymphoma

and

Discussion of Human Gene Transfer Protocol # 1002-1029 entitled: A Phase II Study of Repeat Intranodal Injections of Adenovirus-CD154 (Ad-ISF35) in Subjects with Non-Hodgkin's Lymphoma (Follicular, Diffuse Large Cell, Mantle Cell, and Small lymphocytic Lymphoma/Chronic Lymphocytic Leukemia)

Principal Investigator: Januario E. Castro, M.D., University of California San Diego
RAC Reviewers: Drs. Ertl, Flint, and Kahn

A. Protocol Summaries

Protocol #1004-1028

CLL is the most common leukemia in western societies. Despite multiple scientific advances in this field, there is no cure for CLL, mandating development of novel therapeutic strategies. In this study, the investigators propose to stimulate the immune system by directly injecting an adenoviral vector, Ad-

ISF35, into enlarged lymph nodes. Ad-ISF35 may enhance the activation of immune cells against the leukemia cells and, with manageable low-grade toxicities, may induce an effective treatment for CLL.

This clinical protocol is the continuation of a previous study in which research participants received a single injection into the lymph nodes. The prior clinical study showed that injection of Ad-ISF35 is well tolerated by research participants and that it is capable of reducing the size of lymph nodes and spleen and decreasing the leukemia cell counts in peripheral blood. The proposed experimental treatment schema includes as many as six injections of Ad-ISF35 that will be administered 2 weeks to 4 weeks apart. The investigators hypothesize that lymph node injection of Ad-ISF35 will lead to activation of the immune system against leukemia cells.

The primary study objective is to determine and monitor clinical and biological responses in research participants dosed with repeat intranodal injections of Ad-ISF35. The secondary objectives of this study are to determine the safety of repeat administration of Ad-ISF35 injected directly into the lymph nodes of research participants with CLL and to determine pharmacodynamic parameters in research participants administered repeat intranodal injections of Ad-ISF35.

Protocol # 1002-1029

Non-Hodgkin's lymphoma (NHL) is the fifth most common form of cancer in the United States, excluding nonmelanoma skin cancers. In 2009, an estimated 53,400 new cases of NHL were diagnosed and 23,400 NHL deaths occurred in the United States. Although some advances have been made in the treatment of NHL, there is no cure for NHL, mandating development of novel therapeutic strategies.

In this study, the investigators propose to stimulate the immune system by directly injecting an adenoviral vector, Ad-ISF35, into enlarged lymph nodes. Ad-ISF35 can enhance the activation of immune cells against the lymphoma cells and, with manageable low-grade toxicities, could induce an effective treatment for lymphoma.

This clinical protocol is the continuation of a previous study in which research participants received a single injection into the lymph nodes. This early clinical study showed that injection of Ad-ISF35 is well tolerated by research participants and that it is capable of reducing the size of lymph nodes and spleen and decreasing the leukemia cell counts in peripheral blood. The proposed experimental treatment schema includes as many as six injections of Ad-ISF35 that will be administered 2 weeks to 4 weeks apart. The investigators hypothesize that lymph node injection of Ad-ISF35 will lead to activation of the immune system against lymphoma cells.

The primary objective of this study is to determine and monitor the biological responses in research participants with NHL (restricted to follicular lymphoma, diffuse large-cell lymphoma, mantle-cell lymphoma, and small lymphocytic lymphoma/CLL) treated with repeat intranodal injections of Ad-ISF35. Secondary objectives of this study are to determine the safety of repeat administration of Ad-ISF35 injected directly into lymph nodes of research participants with NHL and to determine pharmacodynamic parameters in research participants treated with repeat intranodal injections of Ad-ISF35.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of these two protocols. Key issues included that the DLTs seen at $1x10^{11}$ viral particles (vp) in the Phase I study indicate the potential for systemic inflammatory responses, the Grade 3 elevation in liver tests in a protocol using an adenoviral vector, and the decision to simultaneously extend this approach to NHL and CLL.

Three RAC members provided written reviews of these proposed Phase II trials. Since the only difference between the two protocols is in the participant populations, all three reviewers listed issues and posed questions pertaining to both protocols.

Dr. Ertl requested clarification as to why these proposed trials should be classified as Phase II, especially when a Phase I trial using repeated intratumoral injection of the vector has not been conducted. She asked whether the investigators have demonstrated that injection into a tumor mass causes transduction of CLL cells, and wondered whether CLL cells express the CAR that would allow for highly efficient transduction or whether they are CAR-negative and thus unlikely targets for adenovirus transduction. Dr. Ertl pointed out that the research participants should be informed about the potential uses of their leukemia cells, which are proposed to be stored, and she asked the investigators to rewrite the description of adenovirus vectors that appears on page 6 of the informed consent document. Noting a paucity of preclinical data, Dr. Ertl requested data regarding the effects on bystander cells, a dose titration experiment with CLL cells to offer a clearer understanding of the likelihood that the CLL cells will be transduced *in situ*, and a more comprehensive analysis of the mouse studies. She encouraged the investigators to test and cryopreserve pretreatment peripheral blood mononuclear cells (PBMCs), and she requested information regarding the investigators' plans for autopsies and specific postmortem tests, if a study-related death should occur.

Dr. Flint requested that the investigators indicate the human dose that is equivalent to the dose used in the preclinical studies that demonstrated decreases in A20 cell tumor mass in mice, and the relationship of that dose to the dose proposed in these two protocols. Noting that the Ad-ISF35 vector carries a transgene that contains a significant number of murine sequences and therefore might carry epitopes recognized as foreign by the human immune system, Dr. Flint asked the investigators to indicate how potential immunological responses to the transgene product would be assessed in research participants. She requested that the investigators discuss plans to monitor immunological responses, and asked for discussion of possible effects of entry of Ad-ISF35 into nonleukemic cells in injected lymph nodes. Rather than continuing with a dose of $3x10^{10}$ vp that appears to be ineffective, based on data from Phase II studies completed to date, Dr. Flint suggested that the investigators consider amending their protocol design to test one or more additional doses between $3x10^{10}$vp and $1x10^{11}$ vp in Phase I before accruing additional research participants in Phase II. She asked for clarification as to how tumor responses could be assessed in a meaningful way if some participants receive multiple injections into a single node but others do not.

While the informed consent documents have been modified from the versions of September 2009, Dr. Kahn noted that they remain overly complex and confusing, with ambiguous, potentially misleading, and/or duplicative information, all of which undermine the likelihood of adequate informed consent. Since the first three participants will receive all or some injections as inpatients while subsequent participants will receive injections as outpatients, he suggested that two separate consent forms be provided for these two groups. He noted that the description and consent for optional blood draws and optional release of excess tissue samples need to provide more specific information so that potential research participants can make a fully informed decision to participate. Dr. Kahn requested that the investigators specify Dr. Kipps' role in the research, given that he holds the patent for Ad-ISF35, and asked whether UCSD has a conflict of interest management plan in place for the conduct of these trials. He provided a list of revisions needed for the informed consent documents, and suggested that one possible approach for addressing these issues is for the investigators to work proactively with the IRB staff to craft a less complex, more understandable consent document and process.

C. RAC Discussion

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

- Dr. Ertl suggested that, when they are looking into the transduction mechanism in mouse models, the investigators should consider using a mouse with a lower tumor burden or using a naïve mouse model in order to obtain more useful information.

- Dr. Flint asked whether antibodies are produced against the chimeric protein.

- Dr. Flint noted the apparent discrepancy between the number of cells that receive the vector and the cells in which the investigators see a response. She asked the investigators to explain why it

is not possible, using direct intranodal injections, to determine how many and the nature of the cells that receive the vector.

- With regard to the putative bystander effect, Dr. Flint asked whether the vector could be replicating within any type of cell within the lymph node.

- Dr. Kahn requested clarification about the source of funding for these trials.

- Dr. Ertl expressed concern about the replication potential of the adenoviral vector proposed for this trial. Using the proposed high dose of adenovirus, if there is replication (which might differ among individuals), the research participants might be put at risk, as might their family members due to the possibility of prolonged shedding. She noted that this possibility cannot be assessed in the A20 mouse model because adenoviruses are species-specific and because a mouse tumor might not provide data relevant to human tumors.

- Both Dr. Flint and Dr. Ertl suggested that the investigators conduct an assay to detect replication. They stated that either an *in vitro* assay could use the cell line or a human cell transplant model could be used. Using the cells of the lymph nodes, if an increase in copy numbers is not evident within some number of hours, the investigators would be able to confirm that no replication had occurred.

- Dr. Flint explained that these vectors are not "replication incompetent" but are "replication defective." The degree to which these vectors replicate is cell-type dependent.

- Dr. Federoff reiterated that studies designed to explain the mechanism of the investigators' finding should also exclude whether replication occurred.

- Noting a high degree of interest in the robust biological findings presented in this study as well as the study discussed earlier in this RAC meeting, Dr. Federoff summarized the RAC sentiment. Further advancing this gene transfer methodology, which has the potential to become a real therapeutic, requires understanding it to the fullest extent, from a safety standpoint as well as to optimize its efficacy. The RAC believes that the research undertaken to date is important to continue but it may require some additional work in parallel. The focus of that additional work would be to develop further a detailed understanding of whether one or several mechanisms might be at play, and to help optimize and then be able to discuss the safety considerations as the investigators expand the number of participants in this and subsequent trials.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding whether these studies should be classified as Phase I or Phase II, the investigators agreed that these studies could be "Phase IIa" clinical trials. The trials constitute a continuation of a Phase I dose escalation study in which the investigators determined a maximum tolerated single intranodal dose as 3.3×10^{10} vp in research participants with CLL. Based on a preclinical study of safety in a mouse model of intratumoral injection of Ad-ISF35, the FDA approved a Phase II repeated dose intranodal administration study in December 2008. Because this was the first time that repeated dose administration was used in humans, the protocol incorporates toxicity assessment guidelines to identify DLTs and a proposed dose de-escalation to be used for research participants who develop DLTs. In addition, the study design uses a two-stage enrollment strategy that includes statistical endpoints required at the end of stage I to continue participant enrollment. This design prevents unnecessary exposure of human participants to medications or interventions that are clinically futile or suboptimal during the conduct of Phase II clinical studies.

The investigators hypothesize that intranodal/intratumoral injection of Ad-ISF35 induces phenotypic changes in transduced and nontransduced (bystander) cancer cells and likely in other cells that are part of the tumor microenvironment, such as fibroblasts, dendritic cells, and monocytes. These phenotypic changes create a cascade of events leading to immunological activation that contributes to antitumoral responses with measurable clinical effect. The investigators have not evaluated transduction of CLL cells *in vivo*, mostly because their data strongly suggest that the activity of intranodal/intratumoral injection is mediated by transcellular activation of nontransduced bystander cells capable of inducing immune-mediated antitumoral responses that lead to a measurable clinical benefit.

Studies in humans conducted by the investigators suggest that the effect of CD154 (ISF35) in CLL patients is potentially due to a bystander effect, which has been observed in individuals treated with infusion of autologous CLL cells transduced with Ad-ISF35 as well as after intranodal injection of Ad-ISF35.

While CLL cells do not express CAR, they do express integrins that allow transduction with adenovirus vectors using high multiplicity of infection *in vitro*.

The protocol design includes a comprehensive collection of plasma, serum, and PBMCs from participants in the Ad-ISF35 clinical trials. These samples will be collected at different time points and will include pretreatment samples, and studies will be performed in freshly isolated samples as well as samples that are cryopreserved.

The issue of property rights for body parts (which includes tissue and samples) is typically addressed via the "Moore Clause," which stems from the *Moore v. Regents* litigation that involved the commercialization of a cell line that was developed from Mr. Moore's cancer cells. Guidance language drafted to address this issue provides notice to the research participant that specimens taken during the study and products developed from those specimens are the property of the University. The IRB requires that informed consent documents include a Moore Clause when tissues will be banked. The Moore Clause for this protocol will read: "Dr. Januario Castro will be responsible for deciding how [the leftover specimens] will be used. The specimens collected from you and the DNA that they contain may also be used in additional research to be conducted by the University of California personnel collaborating in this research. These specimens, DNA, and their derivatives may have significant therapeutic or commercial value. You consent to such uses."

Regarding autopsy, the investigators explained that they intend to encourage family members to consent to autopsies of any research participant who dies in the course of the clinical studies. In addition to standard tissue and organ macroscopic and microscopic analyses, the investigators propose to evaluate Ad-ISF35 DNA and ISF35 gene expression by quantitative PCR analysis.

The equivalent mouse-dose in humans is $2x10^{13}$ vp calculated based on weight. Taking into account that mice are known to tolerate adenovirus vectors and that humans are more sensitive, the investigators conducted a Phase I study in humans using doses ranging from $3.3x10^{10}$ vp to $33x10^{10}$ vp. The results of the Phase I study showed that the MTD was $3.3x10^{10}$ vp per injection, which is the dose chosen to conduct these Phase II clinical trials.

The investigators are conducting studies to evaluate the production of antibodies against adenovirus vector and cross-reactive antibodies against human CD154. These studies show that, even though some individuals generated antibodies against Ad-ISF35, none of the research participants enrolled in the two intranodal studies or the *ex vivo* protocol using Ad-ISF35 transduced CLL cells in combination with FCR have shown measurable antibody reactivity against human CD154.

Regarding the possibility of immunological responses, the investigators plan to conduct studies of antibody reactivity against the viral vector (human CD154) and anti-adenovirus neutralizing antibodies.

Transduction of non-leukemia/lymphoma cells could be beneficial as these cells may serve as activation primers on bystander cells. However, this beneficial effect also could produce unexpected side effects,

such as immunological reactions against those cells with the possibility of autoimmune disease. Regarding this potential side effect, the investigators will conduct longterm followup to monitor the development of autoimmune diseases in research participants who receive Ad-ISF35 intranodal injections. To date, none of the 26 research participants who have received Ad-ISF35 intranodal injections has developed autoimmune disorders related to this intervention.

With regard to assessment of tumor responses when only some participants receive multiple injections into a single node, the investigators explained that they would continue to inject a single lymph node as many times as technically possible in each participant. However, allowing injection of different lymph nodes would confer flexibility in the design that could help clarify, once a larger number of participants are enrolled in each group, whether or not injection of different lymph nodes results in differences in toxicities or response.

The investigators explained that the tests on the optional blood samples would be conducted in their laboratory (by Drs. Castro and Kipps) and the samples would be banked under HIPAA guidelines at the CLL Research Consortium Tissue Repository Bank. The samples will remain de-identified and will be identified only by protocol and participant number.

Regarding the possibility of conflict of interest, the investigators explained that the principal investigator of this study, Dr. Castro, does not have any financial interest or business relations with Memgen LLC. Dr. Kipps is the inventor of this technology, which has been issued a patent awarded to the University of California and licensed by Memgen LLC. While Dr. Kipps is on the scientific advisory board of Memgen LLC, he neither receives a salary from Memgen nor has any control over its business operations.

The investigators agreed to modify the informed consent document to include the suggested clarifications and language modifications, and then plan to send the revised draft to the IRB for final guidance and review.

2. Responses to RAC Discussion Questions

Dr. Castro stated that preliminary data (that the investigators were not able to present at this RAC meeting) indicate no antibody production against the recombinant ISF35 molecule they have produced. However, this is an active area of research in their laboratory and they hope to generate reportable data soon.

In this clinical trial, the investigators believe it would be challenging to increase the maximum dose to a level higher than 3.3×10^{11} vp. The primary reason is because research participants typically develop a myriad of flu-like symptoms (fevers are most common); higher doses would likely result in unacceptable adverse effects.

Regarding how many cells receive the vector and the nature of those cells, Dr. Castro averred that obtaining better assessment of the architecture and phenotypic changes in the lymph node would be of interest. However, he noted that some morbidity is associated with the procedure to collect repetitive lymph node samples. The investigators believe that the correlative data being generated from the peripheral blood will provide adequate information about the changes that might be happening as a result of this experimental treatment.

Dr. Castro explained that Memgen LLC no longer supports both of these trials financially, although the company will be supplying the adenovirus vector. The Orphan Drug Development Program at the FDA will support the CLL study and the Gateway Foundation is funding the NHL study.

With regard to the information that is proposed to remain with the samples, Dr. Castro stated that the samples would be de-identified, although not completely anonymized. Especially for research participants whom the investigators will follow longterm, being able to go back to the clinical data will be extremely valuable. Although the samples will be able to be re-identified, he noted that the samples would be identified only with a code number and research personnel would not have access to personal

identifying information; the only exception would be the need to identify individuals as a result of clinical implications.

The informed consent document requires two weeks of abstention from unprotected sex after the last administration of the vector. In response to Dr. Kahn's concern as to whether this was an adequate timeframe, Dr. Castro responded that the data on virus shedding, from his and others' studies, indicates that a recombinant incompetent virus is cleared very quickly, and two weeks of abstention is adequate.

Regarding whether the adenovirus vector replicates, Dr. Castro stated that the investigators already have in hand *in vivo* data in humans, so that assays using mouse models and cell lines would not be enlightening at this point. An assay looking for viral particles in different human tissues could provide information as to whether copy numbers increase or persist longer than in control patients. He noted the difficulty of creating models that would reflect truly the *in vivo* effect in humans. Dr. Kipps summarized that these replication-defective adenovirus vectors show no evidence of ongoing viral replication. Because the individuals who would enroll in this proposed clinical trial are immune suppressed, viral replication is a major concern. The molecular data to date has shown no evidence that complementation with the deleted E1 region would account for the observed clinical effects.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

It was noted that, contrary to the *NIH Guidelines*, the investigators initiated these studies and enrolled 11 research participants before the protocols had been submitted to the NIH for registration and RAC review. The investigators' institution discovered the oversight when the protocols were placed on hold because the Data Safety Monitoring Board identified major deficiencies in the conduct of these studies. The OBA has contacted the Institutional Biosafety Committee and the Vice Chancellor of Research regarding the implementation of a corrective action plan to address these violations. In addition to the belated review of this protocol, the RAC assessed the initial data submitted by the investigators regarding the 11 participants who were treated with the study agent during this period. According to data in the protocol, three participants had partial responses, five participants have stable disease, and three participants have experienced progression of their disease.

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

<u>Preclinical Issues</u>

- The rationale for the protocol's approach is that injection of the study agent into a lymph node will result in transduction of CLL cells in the lymph nodes. However, since CLL cells do not express the coxsackie/adenovirus receptor, it is not clear which cells are being transduced in the lymph node. Further preclinical studies in an appropriate model with a smaller tumor burden should be performed to better understand which cells are being transduced.

- The adenoviral vector (inactivated E1 and E3 genes) is replication defective, i.e., its capacity to replicate in a cell-type dependent manner is reduced. However, because inadequate preclinical data were provided (the data were from studies conducted in a mouse model that does not support adenovirus replication), studies should be performed to determine whether the vector replicates in CLL cells or other cells that could be transduced in the injected lymph nodes. If the vector is able to replicate, the risk of vector dissemination is increased. These studies may also shed light on the vector's apparent antitumor effects in the individuals dosed to date. Such studies can be conducted concurrently with the clinical trial.

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

- Given that a very small number of cells in the lymph node are modified by the vector (as little as 1:10,000), the significant change in the expression of cell signals on the surface of the unmodified CLL cells is unlikely to be due to cell-to-cell interactions as proposed. Additional studies, including analysis of cytokines and gene expression by PCR, should be conducted to understand the cytokine or other mechanisms underlying these changes. Broader analysis (e.g., microarrays) of participant specimens, the collection and banking of which is already part of the protocol, should also be considered.

- The murine sequences in ISF35 could lead to an antibody response to human CD154. Although such a response was not seen in any of the 11 enrolled participants, there is uncertainty about the specific sensitivity (nanograms/ml) of the screening assay that was used, and it is important that the assay be validated before conclusions are drawn about this risk.

Ethical/Social/Legal Issues

- The informed consent document does not provide participants with sufficient information about how their biological samples will be used in the protocol and whether those samples will be used for other research purposes in the future. While it may not be possible to describe all potential future uses, additional information should be provided regarding the general purpose(s) for which the optional blood samples will be collected, who will have access to the samples, whether the samples will remain identifiable, and where they will be stored (e.g., at UCSD or as part of a larger biobank). From an ethical standpoint, the consent should also enable the participants to decline to permit the future use of their specimens, e.g., through an opt-out provision.

- The University of California, San Diego, holds the patent for Ad-ISF135, which is being licensed to Memgen LLC (the sponsor of the related trial involving intravenous infusion). However, Memgen is only providing clinical grade vector and is not otherwise involved in the conduct of these studies. Funding for these studies is from the FDA and a private funding source. The informed consent document should inform the participants of the financial interests to make it clear that potential conflicts of interests have been mitigated.

- Until definitive data have excluded the possibility of vector replication in the cells that could be transduced in the lymph nodes, barrier contraception methods should be recommended for a time period similar to that used in gene transfer trials involving replication-competent oncolytic vectors.

G. Committee Motion 10

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. It was moved by Dr. Flint that the RAC approve these summarized recommendations. The vote was 12 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XVI. Closing Remarks and Adjournment/Dr. Federoff

Dr. Federoff thanked the RAC members and the OBA staff, and adjourned Day 2 of the June 2010 RAC meeting at 2:40 p.m. on June 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.]

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Jacqueline Corrigan-Curay, J.D., M.D.

RAC Executive Secretary

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

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

_____
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Recombinant DNA Advisory Committee

Attachment I: RAC Roster
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Attachment II
Public Attendees

Emmett Barkley, Proven Practices
Jack Bennich, NIH
Lilia Bi, Reviewer
M. Bishop, NCI/NIH
Adriana Byrnes, SAIC
Mark Cantwell, Memgen LLC
Dhana Chinnasany, NCI/NIH
Bob Coates, Memgen LLC
Romu Corbau, Pfizer
Steven Fleischer, FDA/CBER
Stephen J. Forman, City of Hope
Bernard Fox, Earle A. Chiles Research Institute
Elena Gribina, CBER/FDA
D. Gavin, FDA
Jennifer Goodrich, BNBI
S. Gordine, FDA
Jaydee Hanson, International Center for Technology Assessment
Mike Harvest, CBER/FDA
R. Hawkins, University of Manchester
Anthony Helstosky, Pfizer
Eric Hoffman, Friends of the Earth
Ying Huay, FDA/CBER
Syed Hussein, FDA
Michael Jensen
Carolyn Lawencot, NCI/NIH
Xiaodbin Lu, FDA
Elaine Meaker, Quark Pharmaceuticals
Andrea Miller, BGC
Rick Morgan, NCI/NIH
Brian Niland, Reviewer
Rachel Nosowsky, University of California
Rimas Orentas, NCI/NIH
Pam Pavco, RXI Pharmeceuticals
Janet Peterson, University of Maryland
Roxanne Phillips, UCSD
Vastan Ramjay, FDA
Graeme Rice, FDA/CBER
Mercedes Sebastian, CBER/FDA
Alfred Slaretz, Genesis, Inc.
Ken Smith, UCSD
David Sully, Tacere Therapeutics
Zenobia Taraporewala
James Thompson, Quark Pharmaceuticals
Akshay K. Vaishanaw, Alnyam Pharmaceuticals
Chenchong Wei, Reviewer
L.J. Wes, Harvard University
Keith Wonnacott, FDA
Yao Yaozhu, FDA
Ling Zhang

Attachment III
Abbreviations and Acronyms

ABSA	American Biological Safety Association
Ad-ISF35	Adenovirus-CD154
AE	adverse event
BL	biosafety level
BWG	Biosafety Working Group, RAC
CAR	coxsackievirus and adenovirus receptor or chimeric antibody receptor
CD40L	CD40 ligand
CDC	Centers for Disease Control and Prevention
cfu	colony forming units
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSAT	Division of Select Agents and Toxins, CDC
EBV	Epstein-Barr virus
EBV-CTLs	EBV-specific cytotoxic T lymphocytes
FDA	Food and Drug Administration, DHHS
FCR	fludarabine, cyclophosphamide, and rituximab
GTSAB	Gene Transfer Safety Assessment Board
HL	Hodgkin's lymphoma
IBC	institutional biosafety committee
IgG	immunoglobulin G
IL-2	interleukin-2
IL-12	interleukin-12
IRB	institutional review board
ISATTAC	Intra-Governmental Select Agents and Toxins Technical Advisory Committee
IV	intravenous or intravenously
$lcr^{(-)}$	low calcium response
LcrV	low-calcium response virulence
LTR	long terminal repeat
mRNA	messenger ribonucleic acid
miRNA	micro-RNA
MuLVs	murine leukemia viruses
NCI	National Cancer Institute, NIH
NFAT	nuclear factor of activated T cells
NHL	non-Hodgkin's lymphoma
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NK	natural killer
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
OSWG	Oligonucleotide Safety Working Group
PBMCs	peripheral blood mononuclear cells
PCR	polymerase chain reaction
$pgm^{(-)}$	pigmentation deficient
RAC	Recombinant DNA Advisory Committee
RG	Risk Group
RG-HR16	Type-16 Human Rhinovirus
RNA	ribonucleic acid
SA	Select Agent
SAE	serious adverse event

SDF	stromal cell derived factor
siRNA	short-interfering RNA
TBI	total body irradiation
TILs	tumor-infiltrating lymphocytes
UCSD	University of California San Diego
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptors
vp	viral particles
XMRV	xenotropic murine leukemia virus-related virus
Y. pestis	*Yersinia pestis*