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**RECOMBINANT DNA ADVISORY COMMITTEE**

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**Minutes of Meeting**

**December 13 and 14, 2011**

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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<sup>1</sup>**

December 13–14, 2011

The Recombinant DNA Advisory Committee (RAC) was convened for its 127th meeting at 2:00 p.m. on December 13, 2011, at the Hilton Hotel and Conference Center in Rockville, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 2:00 p.m. until 5:10 p.m. on December 13 and from 8:15 a.m. until 4:35 p.m. on December 14. The following individuals were present for all or part of the December 2011 RAC meeting.

**Committee Members**

Andrew D. Badley, Mayo Clinic and Foundation  
Michael J. Buchmeier, University of California, Irvine  
Saswati Chatterjee, City of Hope National Medical Center  
E. Antonio Chiocca, Ohio State University Medical Center  
Rebecca Dresser, Washington University School of Law  
Yuman Fong, Memorial Sloan-Kettering Cancer Center (RAC Chair)  
Norman Fost, University of Wisconsin–Madison  
Marie-Louise Hammarskjöld, University of Virginia School of Medicine  
Joseph A. Kanabrocki, University of Chicago  
Hans-Peter Kiem, University of Washington School of Medicine  
Walter J. Koch, Thomas Jefferson University  
Donald B. Kohn, University of California, Los Angeles  
Margaret Mallino, Missoula, Montana  
Anna C. Mastroianni, University of Washington School of Law  
David A. Ornelles, Wake Forest University School of Medicine  
Bernard Roizman, The University of Chicago  
Susan R. Ross, University of Pennsylvania (*Day 2 only*)  
Marcella Sarzotti-Kelsoe, Duke University Medical Center  
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute  
James R. Yankaskas, University of North Carolina at Chapel Hill

**Office of Biotechnology Activities (OBA)**

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

***Ad Hoc* Presenters and Speakers**

Glenn Dranoff, Dana-Farber Cancer Institute  
Cynthia Dunbar, National Heart, Lung, and Blood Institute (NHLBI), NIH  
Carl June, University of Pennsylvania  
Amy Klion, National Institute of Allergy and Infectious Diseases (NIAID), NIH  
Larry Norton, Memorial Sloan-Kettering Cancer Center  
Steven A. Rosenberg, National Cancer Institute (NCI), NIH

**Non-Voting Agency Representatives**

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

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<sup>1</sup> 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.

Denise K. Gavin, U.S. Food and Drug Administration (FDA)

**NIH/OD/OBA Staff Members**

Linda Gargiulo  
Chezelle George  
Robert Jambou  
Erin Luetkemeier  
Maureen Montgomery  
Marina O'Reilly  
Gene Rosenthal  
Yun Xie

**Attendees**

There were 66 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. Fong, RAC Chair, called the meeting to order at 2:00 p.m. on December 13, 2011. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 18, 2011 (76 FR 71580). 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 four gene transfer protocols, updates and discussion of two clinical trials previously reviewed by the RAC, and discussion of institutional biosafety committee (IBC) review of low biosafety risk human gene transfer protocols.

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

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

**II. Minutes of the September 13–14, 2011, RAC Meeting**

RAC Reviewers: Drs. Chiocca and Ornelles

Dr. Ornelles suggested that the RAC approve the September 2011 RAC minutes as written. No changes to the document were offered.

**A. Committee Motion 1**

Dr. Ornelles moved and Dr. Yankaskas seconded that the RAC approve the minutes of the September 13–14, 2011, RAC meeting. The RAC voted 14 in favor, 0 opposed, and 1 abstention.

**III. Review and Discussion of Human Gene Transfer Protocol #1108-1122: Phase I Trial of the Safety and Immunogenicity of a DNA Plasmid Based Vaccine Encoding the Amino Acids 1–163 of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) in Patients with Advanced Ovarian Cancer**

Principal Investigator: Mary Disis, M.D., University of Washington, Seattle  
Additional Presenter: Lupe G. Salazar, M.D., University of Washington, Seattle  
RAC Reviewers: Ms. Dresser, Dr. Fong, and Dr. Strome

Drs. Chiocca and Kiem and Ms. Mastroianni were recused from discussion of this protocol due to conflicts of interest.

**A. Protocol Summary**

Ovarian cancer is immunogenic, and immunity may confer a better prognosis. If immunity could be generated in the majority of advanced-stage ovarian cancer patients during treatment-induced remissions, clinical outcomes might be improved. A vaccine targeting immunogenic, biologically relevant proteins in ovarian cancer could potentially produce such immunity. Insulin-like growth factor binding protein 2 (IGFBP-2) has been identified as an ovarian cancer tumor antigen that is overexpressed in the majority of ovarian cancers, and the level of overexpression is associated with invasive disease. Immunologic eradication of tumor cells expressing IGFBP-2 could be beneficial in preventing disease relapse or tumor spread throughout the peritoneum.

Vaccine strategies designed to elicit Type I inflammatory CD4+ T helper immunity (Th1) can generate T cells that traffic to the tumor, modulate the tumor microenvironment through production of inflammatory cytokines, and enhance the development of an immune response to multiple immunogenic proteins expressed in the tumor through epitope spreading. In addition, by providing a robust CD4+ Th1 T-cell response, tumor-specific CD8+ T cells can be elicited and can proliferate endogenously. Antigen-specific CD4+ T cells could provide the environment needed to enhance and sustain tumor-specific T-cell immune responses over time. Multiple Th1 epitopes derived from IGFBP-2 can be delivered in an extended-epitope, DNA-based vaccine. The purpose of this study is to evaluate the safety and immunologic efficacy of an IGFBP-2 plasmid-based polyepitope vaccine in research participants with advanced-stage ovarian cancer who have been treated to the point of complete response.

The investigators propose a Phase I clinical trial of active immunization with an IGFBP-2 Class II polyepitope plasmid DNA vaccine in research participants with advanced-stage ovarian cancer in the adjuvant setting. Participants will receive three vaccines one month apart.

**B. Written Reviews by RAC Members**

Four RAC members voted for in-depth review and public discussion of the protocol. Key issues included the proposal to administer a novel transgene encoding IGFBP-2 to research participants who are in complete clinical remission. Although patients with Stage III and Stage IV disease have a poorer prognosis than those with disease limited to the ovaries, the two-year and five-year survival rates are different for Stage III patients than for Stage IV patients. The risks and benefits of using an experimental vaccine in research participants with Stage III disease when they have recently entered remission were determined to deserve further discussion. In addition, this novel vaccine could have the potential to sensitize the immune system to noncancerous cells that express insulin growth factor-binding proteins.

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

Ms. Dresser asked the investigators to explain the prognosis for research participants with Stage III disease and wondered whether it would be feasible to include only participants with Stage IV disease as a way to improve the study's risk:benefit ratio. She suggested specific wording for the informed consent document for two purposes: providing participants with more information about the nature of this study, including that it is a first-in-human study, and including more explicit information about the chance that

participants would benefit personally from study participation. Ms. Dresser's other comments on the informed consent document included two suggested wording changes and a request for clarification of the information about costs of care in the event of adverse effects.

Because some potential participants already have immunity against IGFBP-2, Dr. Fong asked whether patients' specific T-cell and B-cell immunity against IGFBP should be a consideration for enrollment in this trial, and whether preimmunization IGFBP-2 immunity should be used for stratification and analysis of response or toxicity. He suggested that participant tumors could be stained for IGFBP-2 and expression of target could be used for inclusion criteria; alternatively, elevated serum IGFBP-2 levels could be used for inclusion. Noting that IGF has physiologic effects in humans, Dr. Fong queried the investigators about the anticipated off target effects if an over-vigorous response to vaccination is observed, whether there are specific off target tissues that should be assessed, and what tissues express IGFBP-2 in humans. He suggested including research participants with Stage III or Stage IV ovarian cancer not in remission, especially if they have exhausted standard therapy. Dr. Fong requested that the investigators list the contraindications to granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy.

Noting the survival data for Stage III ovarian cancer, Dr. Strome stated that including Stage III research participants in this clinical trial is problematic and suggested limiting participation to those with measurable disease following completion of all usual and customary therapeutic regimens. He asked whether over-expression of IGFBP-2 would be required for inclusion in this trial and requested more detailed information regarding the presented animal data. He questioned the addition of GM-CSF given the stated objectives, and he suggested that a trial with and without GM-CSF to assess efficacy should be considered after completion of Phase I. Dr. Strome asked the investigators to explain why a 60-minute outpatient observation period is being considered for a Phase I trial, noting that 24 hours would be more reasonable. Given that the survival of African-American women with ovarian cancer is significantly lower than the survival of Caucasian women, he questioned the limited enrollment of this group (1 in 22 participants). Dr. Strome suggested several clarifications to the informed consent document, including defining technical terms, stating what each test represents and why it is of specific interest, explaining why one cup of blood and leukapheresis is necessary, expanding on the risks of vaccination and on the adverse events for GM-CSF, and adding a sentence describing what participants should do if they are injured or if they develop an illness as a result of this experimental protocol.

### **C. RAC Discussion**

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

- Dr. Fong suggested that the protocol include a standard procedure that delineates the criteria determining how steroids will be given to participants.
- Dr. Fost suggested that the investigators discuss with their institutional review board (IRB) a rewording of phrases in the informed consent document that might lead participants to anticipate benefit. He noted the helpful guidance available on this issue.
- Dr. Hammarskjöld asked whether given expression of this protein in ocular tissues, do the investigators plan to test for ocular toxicities.

### **D. Investigator Response**

#### **1. Written Responses to RAC Reviews**

In response to concerns from all three reviewers, the investigators explained that their rationale for including Stage III or Stage IV research participants in remission after any therapy was based on three factors: (1) the high relapse rate, (2) the need to be able to follow research participants for toxicity during the evolving immune response that could take months, and (3) the extensive data in the literature and collected by the investigators that immunization against overexpressed self antigens is associated with low toxicity.

Despite complete response to standard therapy in Stage III and Stage IV ovarian cancer patients, the relapse rate is well above 50 percent, and patients eventually will succumb to their disease. Taking into account the frequency of each stage of disease and its projected relapse rate, the likelihood of relapse after initial therapy for women who present with Stage III or Stage IV disease is 60 percent to 85 percent. Although 5-year survival is 72 percent for Stage III patients and 27 percent for Stage IV patients, longterm relapse-free survival remains low at 30 percent in optimally debulked Stage III disease and only 5 percent in Stage IV disease. Therefore, only a small percentage of Stage III patients alive at 5 years will be disease free. Given these high relapse rates, which are associated with high morbidity and eventual mortality, clinical trials are needed to refine existing therapies and test the value of different consolidation approaches after adjuvant therapy for all ovarian cancer patients. The latest version of the NCI Ovarian Epithelial Cancer Treatment PDQ<sup>®</sup> states that “Patients with any stage of ovarian cancer are appropriate candidates for clinical trials.”

The rationale for not enrolling patients who have measurable disease is based on the nature of the intervention. The primary potential toxicity of the approach would be the development of immunity against tissues that express basal levels of IGFBP-2. Thus, a key feature of the study is to enroll research participants who are able to develop an immune response and who could be followed for months after vaccination as the immune response evolves. For instance, a Phase I study of adoptive T-cell therapy being conducted concurrently by the investigators enrolled relapsed patients with stable but measurable disease; the dropout rate to date has been 50 percent (8 out of 16) prior to study completion due to disease progression and the need for further treatment, thus limiting the understanding of the safety of the approach.

A substantial body of literature describes the toxicities of patients immunized against overexposed self-tumor antigens similar to IGFBP-2. Many of the antigens that have been identified in common cancers are nonmutated self proteins with an altered expression that may make them immunogenic. No clinical trial using these antigens as vaccines with standard adjuvants has demonstrated significant toxicity.

To answer the primary endpoint of safety and the secondary endpoints of immunogenicity, research participants who have achieved a complete remission or minimal residual disease state after standard therapies (including cytoreductive surgery and chemotherapy) will provide a sufficient window of disease stability for assessing a safe therapeutic immune response against the low microscopic tumor burden.

The investigators believe it is premature to choose research participants based on pre-existent immunity or to stratify participants in the context of this early clinical trial. Recent data indicate that pre-existent immunity may benefit immunologic interventions. The potential for pre-existent, tumor-specific immunity to be predictive of response is currently under intensive investigation, and the observation has not been validated. The investigators hope to elucidate this question in the context of the immunologic assessments proposed in this study.

The investigators do not believe that serum IGFBP-2 can be used as a test for inclusion. No data show correlation of serum IGFBP-2 levels with protein expression, and the investigators have demonstrated that some patients have antibodies directed against IGFBP-2. Those antibodies could form complexes with circulating IGFBP-2 and the protein would be cleared from the sera, thus limiting the use of serum IGFBP-2 as a marker.

IGFBP-2 is expressed in a wide variety of tissues at basal levels. The proposed monitoring program is designed to give broad organ coverage to assess toxicity. The investigators will evaluate blood counts and serum chemistries, including blood glucose and liver functions; a physical examination will be performed by one of the study physicians at each vaccine visit; and participants will be monitored for subclinical autoimmune toxicity by assessing common serologies.

All patients with invasive ovarian cancer, with the exception of clear cell histology, will be eligible for the study because they all have some level of overexpression of IGFBP-2 in their tumors (based on published literature). The rationale for inclusion is that nearly all patients upregulate IGFBP-2 to some extent in their

tumors. The largest study of protein expression was performed in 441 tissue samples from patients with a variety of ovarian cancer histologies using tissue microarray technology. All ovarian cancer specimens had some level of upregulated expression of IGFBP-2 ranging from 1+ to 3+ over normal tissue controls; only low malignant potential specimens had no overexpression compared to normal ovary. Studies of active immunization against HER2 have been conducted in research participants whose tumor expression of the protein ranges from 1+ to 3+, and the results indicate that individuals with lower levels of protein expression develop immunity and some clinical benefit.

Self-tumor antigens are weakly immunogenic, because tolerance is a major mechanism by which tumors evade the immune system. DNA vaccines are weakly immunogenic and require immunologic adjuvants to better stimulate immunity; every standard-of-care vaccine uses an immunologic adjuvant of some type to boost immunity. The investigators have extensive experience in using low doses of GM-CSF intradermally as a vaccine adjuvant, and they have shown that GM-CSF stimulates the egress of Langerhans cells (skin dendritic cells) to the site of vaccination. GM-CSF is associated with the development of immune responses to the self antigen HER2 using peptide, protein, and DNA vaccines. Moreover, GM-CSF is one of the few adjuvants used in cancer vaccines that is associated with the development of Type I immunity, a goal for this study. GM-CSF administered intradermally or subcutaneously is one of the most common adjuvants used with cancer vaccines today. This adjuvant is used in the only FDA-approved therapeutic vaccine for cancer, the prostate cancer vaccine Provenge, which targets prostatic acid phosphatase, an overexpressed self protein in prostate cancer.

The proposed 60-minute post-vaccination monitoring period is based on previous vaccine trials that have demonstrated, on rare occasions, acute hypersensitivity or anaphylactic reactions that would require emergent care, usually occurring within minutes of exposure to the allergen. Although other rare systemic allergic reactions (i.e., generalized skin rash) might occur within 24 hours, these are rarely associated with life-threatening adverse events that would require emergency treatment. To date, the investigators have conducted six Phase I clinical trials and have vaccinated more than 250 participants with intradermal injections; aside from expected and transient vaccine-related side effects of injection site redness, warmth, edema, and induration, and occasionally malaise, low-grade fever, and arthralgias, they have not observed any allergic-type reactions. In the recently completed Phase I DNA vaccine study of 66 participants who were immunized with the same plasmid construct proposed in this study, the investigators encountered no vaccine-related Grade 3 or Grade 4 adverse events within the first 24 hours. Most vaccine clinical trials monitor participants for 30 to 60 minutes post-vaccination for acute toxicity that may warrant immediate medical intervention. This time interval has been deemed appropriate and safe by the FDA in reviews of various vaccine studies, including this proposed trial.

Regarding the apparent limited enrollment of African-American women, the investigators noted that the Phase I clinical trials they have performed in ovarian cancer draw participants primarily from the greater Puget Sound, Washington, area. The enrollment estimates are based on the population statistics of Washington State from the recent 2010 U.S. Census data; the population pool from which participants will be drawn is 83.8 percent Caucasian, 7.0 percent Asian American, 3.9 percent African American, 1.8 percent American Indian or Alaska Native, and 0.5 percent Native Hawaiian or Other Pacific Islander. The investigators have established outreach programs to attempt to increase minority enrollment, so they hope to enroll more than one African-American woman.

The investigators clarified that the aims of this study, as listed in the protocol, are correct as stated.

The investigators altered, clarified, and reworded the informed consent document to incorporate suggestions by the three RAC reviewers. Clarifications included why 1 cup of blood must be collected, why leukapheresis is necessary, the risks of vaccination, and the procedure for participants who experience an injury or illness resulting from this trial; discussion was added about the adverse events for GM-CSF.

## **2. Responses to RAC Discussion Questions**

Ovarian cancer is a peritoneal disease that cannot be measured. Testing CA125 does not provide an accurate measure of tumor burden, and it is operative in only about 80 percent of patients; 20 percent of patients do not have elevated levels of CA125. Ovarian cancer is the most immunologically mediated disease in the sense that almost every mechanism of tumor immune suppression was first identified in ovarian cancer.

Regarding available animal models, Dr. Disis explained that three transgenic animal models of ovarian cancer exist, two of which were recently established. These models cannot be used to develop spontaneous tumors. The investigators recently acquired two of these cell lines and are hoping to develop a peritoneal implant model. Because these animals do not breed, it is not possible to conduct *in vivo* experiments with a pure ovarian cancer model without generating a cell line and doing peritoneal implant studies.

The investigators explained that if the research participants were immunized without an adjuvant, the trial likely would show no immune responses in these participants.

In response to concerns about recruitment diversity, Dr. Salazar stated that the investigators have been involved with two major advocacy groups in the Seattle area. CRS Sisters is an advocacy group for African-American breast, ovarian, and colon cancer survivors. The investigators have been working with CRS Sisters for the past 5 years on recruitment for breast cancer and ovarian cancer studies. To target the Latina population, the investigators are becoming more involved in the Sea Mar Community Health Centers, a Hispanic community clinic. The investigators pledged to continue to recruit aggressively throughout Washington State.

Regarding analysis of samples from research participants, Dr. Disis explained that the investigators are looking at whether levels of immunity pre-vaccine predicted the highest level of response. In the first 66 participants, there is no correlation between baseline immunity and response. However, the investigators will continue to gather data, from their own experience and from the experience of others, because their goal is to be able to pick the potential research participants who are most likely to respond.

In this patient population with a median recurrence rate at 18 months, the investigators are assuming that a majority of the participants would be able to provide at least two time points in the extended followup period, which is adequate for the purposes of this trial. The followup protocol is built on the median time to relapse in this patient population.

Dr. Disis emphasized that the relapse rate for patients with Stage III ovarian cancer is greater than 65 percent, and the Stage IV patient relapse rate is 85 percent to 90 percent. She emphasized that there is no cure available for ovarian cancer patients, and they will die of their disease.

With regard to testing ocular toxicities, Dr. Disis explained that seven such toxicities were tested; three of these had basal levels and four gave no signal. Ophthalmologic evaluations are conducted.

## **E. Public Comment**

Dr. Borrer stated that “treatment” and “therapy” language should be avoided in the informed consent document. She also noted that language about compensation was confusing, and she suggested that the investigators check with their IRB regarding standard language for that information.

## **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

### Clinical and Trial Design Issues

- The immune response of research participants to this vaccine may differ based upon several factors: (1) their baseline immune response to IGFBP-2 prior to vaccination, (2) expression of IGFBP-2 in their tumors, and (3) their circulating levels of IGFBP-2 protein. Although the planned

study size may not allow for stratification of these factors, analysis of the immune responses should examine these factors to the extent possible.

- Because IGFBP-2 is expressed on normal cells as well as tumor cells, autoimmunity may be an observed toxicity to this vaccine. Steroids can be used to try to eliminate such autoimmunity should it occur. However, in addition to safety, the secondary endpoints include measurement of an immunological response to the vaccine. Premature introduction of steroids may abrogate this immune response, and therefore the protocol should include specific criteria for starting steroids and the doses to be administered.
- Because all participants enrolled in this study will receive GM-CSF, the protocol should include information on potential contraindications to GM-CSF that would preclude enrollment on the trial.

#### Ethical, Social, and Legal Issues

- In a Phase I trial, it is important that potential participants understand that they are unlikely to benefit from the study. The NIH Guidance on Informed Consent for Gene Transfer Research (available at [http://oba.od.nih.gov/rdna/informed\\_consent\\_intro.html](http://oba.od.nih.gov/rdna/informed_consent_intro.html)) includes discussion of this issue. The current informed consent document states that “we do not know if this study will benefit patients with ovarian cancer.” Given that most participants will experience no benefit in Phase I trials, this statement may be overly optimistic or subject to misinterpretation. It may be better to include a statement such as: “It is unlikely that the intervention will change the natural course of your disease.” A direct statement such as this is less likely to lead to a misinterpretation of possible benefit.

#### **G. Committee Motion 2**

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Ornelles moved that these comments be approved by the RAC, and the motion was seconded. The RAC voted to approve these summarized recommendations by a vote of 14 in favor, 1 opposed (Dr. Strome), 0 abstentions, and 3 recusals.

#### **IV. Review and Discussion of Human Gene Transfer Protocol #1110-1133: A Phase I Clinical Trial of mTOR Inhibition with Sirolimus for Enhancing ALVAC(2)-NY-ESO-1(M)/TRICOM Vaccine Induced Anti-Tumor Immunity in Ovarian, Fallopian Tube, and Primary Peritoneal Cancer**

Principal Investigator: Kunle Odunsi, M.D., Ph.D., Roswell Park Cancer Institute (RPCI)  
Additional Presenter: Protul Shrikant, Ph.D., RPCI  
Sponsor: RPCI  
RAC Reviewers: Dr. Chatterjee, Ms. Mastroianni, and Dr. Sarzotti-Kelsoe

#### **A. Protocol Summary**

The purpose of this research is to study a vaccine treatment called recombinant canarypox-NY-ESO-1/TRICOM [also called ALVAC(2)-NY-ESO-1(M)/TRICOM]. The study is limited to patients with epithelial ovarian, fallopian tube, or primary peritoneal carcinoma whose tumors express certain proteins (antigens) called NY-ESO-1 or LAGE-1. The vaccine in this clinical research study will contain the antigen with recombinant canarypox virus. To make the vaccine, the investigators started with the canarypox virus and genes coding for molecules that stimulate the immune system to it. The virus reproduces in birds but not in humans.

Canarypox can be used to immunize humans and does not cause any known disease in humans. The virus is considered a low-rate-replicating virus in humans. The virus works to present the NY-ESO-1 protein to the immune system and thus elicit an immune response. Antigens such as NY-ESO-1 protein

are found on many cancer cells. The hope is that the vaccine will cause the immune system to produce immune cells and antibodies that will help locate the NY-ESO-1 or LAGE-1 antigens on cancer cells; once they are found, the immune system could work to control or eliminate the remaining cancer cells. Similar vaccines have been tested in various tumors. Although some patients make immune cells and antibodies to the vaccines, it is too early to know if the vaccines are helpful in preventing cancer from returning. In a study on patients with skin cancer in which NY-ESO-1 peptide (a portion of the protein) or the whole protein was given, some of the research participants who had an immune response to the vaccine experienced regression of their disease, and in others the disease stopped growing for a period of time. The most common side effect in the study was minor skin irritation at the injection site.

The purpose of this research is to study the safety of this vaccine approach when combined with sirolimus (also called rapamycin), which is a specific inhibitor of the mammalian target of sirolimus (mTOR). Sirolimus binds to an intracellular protein (FKBP-12), and the protein-drug complex binds to mTOR to inhibit its kinase activity. The research also will study whether this vaccine can stimulate the body to form an immune response.

This study involves placement into one of seven experimental treatment groups in which ALVAC(2)-NY-ESO-1(M)/TRICOM will be given to the participant by subcutaneous injection. All participants will receive this vaccine via a small needle inserted under the skin of the arm on Days 1, 29, 57, 85, and 141. Sargramostim (a recombinant GM-CSF that functions as an immunostimulator) will be given under the skin around the vaccination site for four consecutive days, starting on the day of vaccination. The seven groups will differ in the following ways:

- Cohort 1A low-dose sirolimus pills on Days 1–14.
- Cohort 1B low-dose sirolimus pills on Days 15–28.
- Cohort 1C low-dose sirolimus pills on Days 1–28.
- Cohort 2A higher-dose sirolimus pills on Days 1–14.
- Cohort 2B higher-dose sirolimus pills on Days 15–28.
- Cohort 2C higher-dose sirolimus pills on Days 1–28.
- Cohort 3 sirolimus at a low or higher dose on Days 1–14, Days 15–28, or Days 1–28, depending on the results from Cohorts 1 and 2.

A total of 18 to 42 research participants will be enrolled, and the duration of enrollment is expected to be four years. Toxicity and immunological assessments will be made at baseline and after vaccination.

## **B. Written Reviews by RAC Members**

Ten RAC members voted for public review and in-depth discussion of the protocol. Key issues included the novel combination of sirolimus with this vaccine, the limited preclinical data to support the hypothesis underlying this combination, and the doses of sirolimus proposed. In addition, the decision to test this potent immunosuppressant in cancer patients who are currently stable, including those in complete remission, was deemed to deserve further discussion.

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

Dr. Chatterjee stated that the choice of the modified canarypox virus is reasonable, given its success in other vaccine trials, and the choice of the tumor antigens NY-ESO-1 and its variant LAGE-1 also are reasonable, because they have been shown to be immunogenic and their ability to elicit humoral and cellular responses may be important in engendering effective antitumor responses. She asked whether the investigators have supporting data to suggest that a robust antitumor immune response would be elicited in the presence of sirolimus. She also asked about the basis for the choice of the doses and schedule of sirolimus, since the difference between immunosuppressive and immune-potentiating effects appears to be primarily dose related; accelerated relapse is possible if the chosen doses induce immune suppression. She noted that data on antiviral immune responses mounted by transplant patients while on maintenance doses of sirolimus may be informative with regard to dose in this trial, and she asked the

investigators to discuss data on possible “bystander” effects of sirolimus inhibition that could affect normal physiologic processes. Dr. Chatterjee requested that the investigators discuss why they propose to enroll participants in complete remission before a safe and effective dose and schedule for sirolimus administration have been established. She noted that inclusion of research participants in complete remission seems unwarranted at this time, particularly in light of the pleiotropic effects of sirolimus. Because the administration of a relatively high dose of sirolimus, similar to that used in renal transplants, is planned, it is possible that participants could become immunosuppressed, and if so, the tumor relapse rate in these individuals could increase significantly. In addition, because mTOR affects diverse pathways, mTOR inhibition could have multiple effects that could lead to serious adverse events.

With regard to the informed consent document, Professor Mastroianni noted that “treatment” and related words could lead participants to overestimate the potential for personal benefit from this Phase I safety trial; she suggested that the investigators revise the document appropriately. It should be made clear that this is a Phase I safety study, why the study is an important contribution to science, and what is known and not known about the safety and efficacy of the intervention and each of the proposed components. She noted seven pieces of missing information from various sections in Appendix M, including information about request for autopsy, availability of compensation in the case of research-related injury, and the procedures to avoid possible conflicts of interest if an investigator is also providing medical care to potential participants. Additional suggestions regarding the informed consent document included the following:

- Section 3 states that the investigators will “continue to keep track of you for the rest of your life.” More specific information should be provided about how this tracking will be conducted.
- Section 4 explains the study intervention but does not explain all of the responsibilities and tests that will be conducted throughout the trial. A one-page summary/timeline of the requirements of the study would be helpful.
- In Section 5, under “Treatment,” the IRB should pay particular attention to this statement regarding consent to indefinite storage and use of research participants’ biological samples in future unspecified research. A broad consent to unlimited, unspecified future research is of little value and therefore not recommended. The investigators should consider a separate checkbox so participants can make a conscious choice to opt in to future research.
- In Section 7, the investigators should indicate how specimens will be treated if a participant leaves the trial.
- Section 8 states that participants will not be able to receive any other cancer chemotherapy while they are on this study. All other prohibited treatments should be included here, as should information about at what point chemotherapy would be permitted if the disease recurs or progresses.
- The first two sentences in Section 10 should be revised to clarify the absence of personal benefits from this Phase I trial.

Dr. Sarzotti-Kelsoe noted that the components in this trial have been tested in previous clinical trials, but the combination of sirolimus with the other three components has not been tested in animals before being proposed for this human clinical trial. She stated that the effects of sirolimus are complex and currently under study: sirolimus is cytostatic, immunosuppressive, and immunomodulatory. Its proposed effect is to enhance memory T cells, but she expressed concern that it may have undesirable effects on the vaccine-induced immune response and in research participants in clinical remission. Given these possibilities, Dr. Sarzotti-Kelsoe suggested that this approach be tested in animal models before its application in human clinical trials. She suggested that the investigators clarify the possibly misleading statement that the canarypox virus is considered dead and that they explain and define sirolimus and sargramostim in simple terms. With regard to the informed consent document, Dr. Sarzotti-Kelsoe offered two corrections, asked whether TRICOM and GM-CSF would be provided free of charge, and suggested adding an explanation of what would happen to the participant’s specimens upon withdrawing consent.

## C. RAC Discussion

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

- Dr. Fong asked whether reactivation occurs when someone who has had a kidney or liver transplant and has hepatitis B or hepatitis C is given rapamycin. He asked for scientific rationale as to why patients who are hepatitis B or hepatitis C positive are not permitted to participate in this protocol.
- Dr. Fong asked for specifics about the two boards that comprise the safety assessment board for this proposed trial.
- In addition to using cytomegalovirus (CMV) antigenemia to monitor CMV, Dr. Kiem suggested that the investigators consider using polymerase chain reaction (PCR) assay. Agreeing with Dr. Kiem's comment, Dr. Badley stated that antigenemia is a bulky tool for assessing CMV and that most institutions would encourage using a PCR assay to monitor.
- Dr. Strome expressed concern about the investigators' plan to administer sirolimus for a short period of time, as longer-term administration may be associated with anti-tumor effect. In a series of experiments, Dr. Strome and colleagues looked at rapidly growing sarcomas in the transplant model in squamous cell cancer and used everolimus; as long as the therapeutic dose was administered the tumors did not grow, but when the dose was stopped, tumors grew.
- Dr. Badley noted accumulating data on the role of rapamycin in human immunodeficiency virus (HIV) and hepatitis C. It appears to have a protective effect and to block transcription indirectly, and it is used in the HIV transplant setting as well as for liver transplants for hepatitis C patients. There may be a lessened incidence of allograft reinfection with hepatitis C in the presence of rapamycin.

## D. Investigator Response

### 1. Written Responses to RAC Reviews

The investigators enumerated the key changes to the protocol in response to RAC review, which included the following:

- Elimination of Cohort 2C from the experimental design (4 mg on Days 1–28 of each cycle)
- Modification of the protocol to include pharmacokinetic (PK) assessment of sirolimus
- Based on concern about antiviral immune responses, incorporation of measurement of CMV antigenemia during the course of treatment in all research participants—to identify participants with CMV antigenemia who may require antiviral therapy and to document whether the proposed regimen compromises antiviral immune responses as measured by CMV antigenemia
- Based on concern for other “bystander” effects, inclusion of lipid profile measurements on all participants
- Changes to the informed consent document in response to reviewers' comments

The investigators provided a brief summary of the published preclinical study results supporting this clinical trial; these data have been reviewed and funded by the NCI. Some of the data were recently published and indicate that mTOR inhibition conditions antigen-specific CD8+ T cells for enhanced persistence, antigen recall, and tumor efficacy. These results indicate that inhibition of mTOR programs antigen-specific CD8+ T cells for durable responses that show greater tumor efficacy. The investigators also provided data from unpublished preclinical studies.

Regarding the rationale for testing the schedule of sirolimus in this clinical trial, the investigators explained that the preclinical data indicate that both the dose and schedule of sirolimus are critical in determining the fate of vaccine-induced T cells. Based on the preclinical data and the concerns of the reviewers, Cohort 2C has been eliminated from the experimental design; the investigators now propose to test short courses of low doses of rapamycin, short courses of a higher dose of rapamycin, and a

prolonged course of low-dose rapamycin. In renal transplant patients with low to moderate immunologic risk, the adult maintenance dose of oral sirolimus is 2 mg per day; in renal transplant patients with high immunologic risk, the adult maintenance dose is 4–6 mg/day. The investigators have chosen to provide doses that are known to be associated with immunologic effects, and the literature supports these dose levels of sirolimus and other rapamycin analogs. For sirolimus as an anticancer therapy, no regular dosing is known, although reports mention effective doses between 0.5 mg and 10 mg daily.

The investigators stated that they agreed with the reviewer comments about the complexity of the effects of mTOR inhibitors, noting that inhibition of the mTOR pathway has the potential to inhibit tumor progression at multiple levels. Given the concerns raised, the investigators modified the protocol to include PK assessment of sirolimus with a goal of keeping sirolimus levels at no greater than 4–12 µg/mL. For most indications, the target serum blood level of sirolimus is 10–20 µg/mL. Experimental animal and clinical data suggest that adverse events and their associated severity are correlated with blood concentrations.

Indirect data exist with regard to antiviral immune responses mounted by transplant patients while on maintenance doses of sirolimus. CMV is an opportunistic infection that causes substantial morbidity and mortality in solid organ transplant recipients and after allogeneic hematopoietic stem cell transplantation (HSCT). In studies using daily 5 mg or 10 mg of sirolimus, no increased incidence of opportunistic infection occurred. Randomized trials of sirolimus-based immunosuppressive regimens in solid organ transplantation have observed no increase in cumulative incidence of CMV disease in patients receiving sirolimus. Moreover, sirolimus-based graft-versus-host disease prophylaxis protects against CMV reactivation after allogeneic HSCT. These results indicate that the ability to mount effective antiviral immune responses is preserved in transplant recipients receiving prolonged maintenance doses of sirolimus. In response to a RAC reviewer's concern, the investigators have added measurement of CMV antigenemia during this trial for all participants. This measurement will serve two purposes: to readily identify patients with CMV antigenemia who may require antiviral therapy and to document whether the proposed regimen compromises antiviral immune responses as measured by CMV antigenemia.

With regard to why they plan to enroll research participants who are in remission, the investigators responded that more than 70 percent of ovarian cancer patients in "complete remission" will relapse following firstline therapy, and more than 90 percent in "complete remission" will relapse following second or additional lines of therapy. Thus, the majority of ovarian cancer patients will develop recurrent disease and, once disease recurs, there is no evidence for the curative potential of any secondline regimen. As a result, several investigators have proposed that ovarian cancer patients in "remission" in reality have minimal disease burden and are excellent candidates for consolidation strategies such as immunotherapy.

Several lines of evidence support the notion that the majority of ovarian cancer patients in "complete remission" actually harbor subclinical gross or microscopic residual disease. In second-look surgery, a comprehensive surgical reassessment procedure in a patient clinically free of disease, evidence indicates that approximately 50 percent of patients in "complete remission" beforehand will be found to have residual disease of no more than 2 cm in diameter. Even among those with a negative result (no disease identified at the time of second-look surgery), the risk of recurrence exceeds 50 percent. The recognition of the need to treat patients in "complete remission" has been explored in several Gynecologic Oncology Group (GOG) and non-GOG trials in an effort to improve the outcome of these patients. This proposed trial is congruent with the underlying rationale for these previous GOG studies targeting patients in "complete remission" to improve the outcome of ovarian cancer patients. In summary, the investigators stated that they have chosen a population of patients who are most likely to experience disease relapse and for whom no effective strategies currently exist to minimize the risk of relapse.

With regard to possible "bystander" effects, the investigators explained that sirolimus may have a number of adverse effects as detailed in the clinical protocol and informed consent. While on protocol, participants will be monitored for hematologic and non-hematologic toxicities using lipid profile measurements, PK measurements, and CMV antigenemia.

The word “treatment” has been replaced with the word “study” or modified with “investigational” appropriately throughout the informed consent document. Language has been added on pages three and four regarding the safety and efficacy of the study along with a description of all components used. Language has been added on page four that more specifically describes how tracking of participants will be conducted. Regarding treatment of specimens if a participant leaves the trial, the investigators have added language on page 16 giving the participant a choice either to allow samples to be used for future research or for samples to be discarded. Language has been added on page eight stating that if at any time during the trial a participant decides that she needs to be treated with chemotherapy or any other prohibited medication, she may decide to withdrawal from this study.

The investigators have indicated that cells may be kept indefinitely to perform additional analysis of immune cells if necessary, and a checkbox has been included that allows participants to make a conscious choice to opt in to that research.

The additional information requested for Appendix M was provided.

With regard to testing this approach first in animals, preclinical animal studies conducted by the investigators indicate that the quantity and quality of vaccine-induced CD8+ T-cell memory responses with differential tumor efficacy can be regulated by varying rapamycin treatment regimen (dose and schedule). Thus, it is important to validate this approach in a Phase I clinical trial.

The investigators explained that TRICOM and GM-CSF will be provided free of charge, and clarification has been provided on page 16 of the informed consent document as to what would happen to a participant’s specimens should she withdraw consent.

## **2. Responses to RAC Discussion Questions**

Dr. Odunsi explained that canarypox does not lead to productive infections in humans, so it is unlikely that any research participant would be infected with canarypox.

Patients with metastatic renal cell carcinoma receive up to 10 mg of rapamycin, and there is no evidence that they develop opportunistic infections. The 2 mg dose proposed for this trial is the lowest dose of rapamycin that has been shown to be associated with immunologic effects, and it will be used for a comparatively short period of time. The low dose of rapamycin proposed for this trial is much lower than what is generally used in patients with metastatic breast cancer, which is a maximum of 4 mg to 5 mg. The investigators showed data that patients with gliomas take up to 5 mg orally continuously for several months, and some take up to 10 mg. The literature is convincing that these patients are not at increased risk of opportunistic infections.

Regarding rapamycin’s maintaining immunosuppression in renal transplant patients, Dr. Odunsi pointed out that suppression is not entirely convincing with rapamycin alone; therefore, it is used in conjunction with cyclosporin or other calcineurin inhibitors and other immunosuppressive regimens, such as steroids.

Dr. Odunsi acknowledged that the investigators have not conducted chimeric experiments but have looked at human lymphocytes. In some experiments, they looked at NY-ESO-1–specific T-cell clones in the laboratory to determine what happens in the presence or absence of rapamycin, and the results have been confirmed. However, they have not looked at a chimeric mouse model because there is no mouse homolog of NY-ESO-1.

When the investigators have looked at human lymphocytes *in vitro* with rapamycin, they have observed the switch from effector to memory and have seen generation of more memory cells when they stimulate the cells in rapamycin conditions.

The investigators clarified that, overall, no evidence indicates that hepatitis B or hepatitis C patients develop fulminant hepatitis when given rapamycin; however, their rationale is to be cautious because of that potential. Because of the background of rapamycin as an immunosuppressant, the investigators

believe it is important to exclude those individuals from this trial. Transplant patients receiving rapamycin-based regimens generally do better in terms of their infectious complications and the rate of various infections, including hepatitis, than those receiving non-rapamycin-based regimens. Although there are no strong scientific data to support exclusion of hepatitis and HIV-positive patients from this trial, the investigators prefer to be cautious by not including them.

Dr. Odunsi explained the proposed safety assessment board, which comprises two separate committees. One is a Phase I committee that meets weekly to review all participants on Phase I trials, assesses adverse events, determines the significance of adverse events, and reacts in real time on an ongoing basis to what is going on in all Phase I clinical trials at RPCI. The Phase I committee is chaired by the senior vice president of clinical research and includes Phase I investigators across different departments but does not include external members. The data and safety monitoring board (DSMB) is a separate entity that meets quarterly as an institutional committee; its membership includes people from outside RPCI.

Regarding short-term versus long-term use of rapamycin, Dr. Odunsi stated that the investigators are initially restricting vigorous exuberant clonal expansion of the T cells so that those T cells can quietly transition into memory cells. Without rapamycin, cells are vigorously expanded. If the goal was to test rapamycin as an anticancer agent, then prolonged courses of rapamycin would be necessary. Dr. Shrikant added that it could be possible to kill the tumor cells directly with rapamycin at high doses for a long period of time, but the research participant might die as a result of other issues, such as renal failure and immune suppression. The goal is to allow the immune response to kill the tumor rather than to directly affect the tumor with rapamycin.

#### **E. Public Comment**

No public comments were offered.

#### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

##### Clinical and Trial Design Issues

- The sirolimus dose will be adjusted if serum levels rise above a certain level. This could lead to considerable variation in the doses that research participants receive. For the design of future trials, it will be important to do a rigorous correlation between sirolimus serum levels and biological readouts.
- CMV is an opportunistic infection that causes substantial morbidity and mortality in solid organ transplant patients. Although data in the literature show that sirolimus-based immunosuppressive regimens following solid organ transplant do not lead to an increase in the incidence of CMV disease, the investigators have nonetheless amended the protocol to monitor for CMV antigenemia during dosing. This will allow the investigators to readily identify participants who may require antiviral therapy and to document whether the sirolimus compromises antiviral immune responses as measured by CMV antigenemia. The investigators should consider monitoring for CMV infection by a PCR-based assay rather than by CMV antigenemia, because the PCR assay is potentially more predictive of disease.
- Immunocompromised research participants (including those with acute or chronic hepatitis B or hepatitis C virus infections, as well as those who are HIV positive) are excluded from this Phase I study because sirolimus has immunosuppressive properties. Currently, patients with these infections undergo solid organ transplant and receive sirolimus, and therefore data exist in the literature regarding whether the immunomodulatory properties of sirolimus lead to infectious complications in these patients. For completeness, this protocol would benefit from a discussion of the clinical experience regarding the use of sirolimus in these transplant patients.

## Ethical, Social, and Legal Issues

- The language used to describe sirolimus in the informed consent document is overly complex and should be simplified.

### G. Committee Motion 3

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. No official motion or second was offered that these comments be approved by the RAC, but a vote of the RAC members was taken. The RAC voted to approve these summarized recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

## V. Gene Transfer Safety Assessment Board Report

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

### A. GTSAB Report

Dr. Badley reported that the OBA had received 15 protocol submissions in the past three months, 11 of which were not selected for public review at this RAC meeting. Of the 11 protocols not selected for public review, seven were oncology protocols and the remaining four protocols were for hemophilia, peripheral artery disease, wound healing, and HIV-uninfected donors. Five of the 11 protocols proposed to use plasmid vectors and one each used adenovirus, plasmid-adenovirus combination, adeno-associated virus (AAV), vaccinia virus, retrovirus, and Venezuelan equine encephalitis replicon vectors.

Twenty-one serious adverse events (SAEs) from 12 protocols were reviewed by the GTSAB, including initial and followup reports. After analysis of these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

The OBA received notification from investigators that 15 protocols were newly open to enrollment. Eight of those 15 had previously been reviewed at a RAC public meeting.

The GTSAB highlighted several protocols that reported their responses to RAC concerns:

*OBA Protocol #707, reviewed by the RAC in June 2005: A dose-finding and safety study of an oncolytic polio rhinovirus recombinant against malignant glioma.* Preclinical, biodistribution, toxicity and neutralizing antibody studies in cynomolgus macaques were submitted. Concern had been expressed that neutralizing antibody against the polio virus might be present. In response, preclinical studies were conducted and showed that pre-existing immunity enhanced rather than decreased polio virus oncolysis.

- *OBA Protocols #1028 and #1029, reviewed by the RAC in June 2010: Phase II studies of repeat intranodal injections of adenovirus CD154 in patients with chronic lymphocytic leukemia (CLL) or lymphocytic lymphoma and in patients with non-Hodgkin's lymphoma.* The RAC expressed concern about which cells would be transduced by this adenoviral vector. In response, the investigators are looking into whether the leukemic cells or other cell types present in the lymph node are transduced by the vectors. The investigators also noted that, because the rate of transduction is low (possibly as low as 1 in 10,000), it is unlikely that the cells themselves are mediating all of the antitumor effect. Therefore, the investigators will evaluate a variety of other possible mechanisms, including cytokine profiles and alteration of a variety of genes, including pro-apoptotic and anti-apoptotic genes.
- *OBA Protocol #1034, reviewed by the RAC in June 2010: A study looking at Epstein-Barr virus (EBV) cytotoxic T cells (CTLs) expressed in CD34 chimeric antigen receptor (CAR) in patients*

with Hodgkin's disease or non-Hodgkin's lymphoma. The RAC expressed concern that the spacer domain could potentially bind to IgG Fc receptors on innate immune cells as has been seen in some preclinical data. The investigators stated that they have checked this possibility in eight individuals so far and toxicity was not associated with the administration, even after a twofold to tenfold expansion *in vivo*. The inclusion criteria have been modified to limit enrollment to participants with active disease who have failed standard therapy.

- *OBA Protocol #1091, reviewed by the RAC in June 2011: A protocol looking at EBV-specific CTLs that have been genetically retargeted for CD19 for therapy of acute lymphoblastic leukemia after transplantation.* Because this study is the first to enroll both EBV-positive and EBV-negative subjects in a protocol that uses EBV specific T cells, the investigators will examine the effect of prior EBV infection on the anti-T cell activity.

Dr. Badley highlighted notable publications and awards. A *New England Journal of Medicine* paper reported on a novel approach that was developed for a safety switch in transduced T cells. This approach was to transduce T cells with an inducible caspase 9 construct that contained an FK binding protein such that when exposed to a synthetic dimerizing drug, the inducible caspase 9 becomes activated and leads to rapid elimination of the transduced T cells. In addition, Jerry Mendell, M.D., Director of the Center for Gene Therapy in the Research Institute at Nationwide Children's Hospital, and his colleagues received the *Annals of Neurology* prize for outstanding contribution to clinical neuroscience for their publication titled "Sustained Alpha-Sarcoglycan Gene Expression After Gene Transfer in Limb-Girdle Muscular Dystrophy, Type 2D." The vector used in this protocol, OBA Protocol #815, was an AAV with a muscle-specific promoter and was administered into the extensor digitorum brevis muscle; administration resulted in improved function. Dr. Mendell has been awarded a new NIH grant to test regional vascular delivery to the lower extremities.

## **B. RAC Discussion**

No discussion occurred.

## **C. Public Comment**

No public comments were offered.

## **VI. Day 1 Adjournment**

Dr. Fong, RAC Chair, adjourned Day 1 of the December 2011 RAC meeting at 5:10 p.m. on December 13, 2011.

## **VII. Day 2 Call to Order and Opening Remarks**

Dr. Fong, RAC Chair, called to order Day 2 of the December 2011 RAC meeting at 8:15 a.m. on December 14, 2011.

## **VIII. Review and Discussion of Human Gene Transfer Protocol #1110-1130: An Adaptive Phase I/II Study of the Safety of CD4+ T Lymphocytes and CD34+ Hematopoietic Stem/Progenitor Cells Transduced with CAL-1, a Dual Anti-HIV Gene Transfer Construct, in Busulfan Conditioned HIV-Infected Adults Previously Exposed to ART**

Principal Investigator: Ronald Mitsuyasu, M.D., University of California, Los Angeles (UCLA)  
Additional Presenters: Louis Breton, Calimmune; Bryan Burke, Ph.D., Calimmune; Alison Knop, Calimmune; Jacob Lalezari, M.D., Calimmune; R. Jude Samulski, Ph.D., University of North Carolina; Geoff Symonds, Ph.D., Calimmune

Sponsor: Calimmune, Inc.  
RAC Reviewers: Drs. Badley, Hammarskjöld, and Ross

Drs. Kiem and Kohn were recused from discussion of this protocol due to conflicts of interest.

### A. Protocol Summary

It is estimated that 30 million individuals are currently infected with HIV. HIV/AIDS is a disease that impairs immune function, primarily by decreasing CD4+ T lymphocytes. Its progression can be contained by highly active antiretroviral therapy (HAART), but the side effects can be severe, and the development of resistance means that the physician must modify the HAART regimen from time to time.

No effective vaccines are currently available to prevent HIV infection. An alternative approach that could provide a path to a curative therapy is the use of cell-delivered gene transfer in which an anti-HIV gene(s) is introduced into white blood cells and bone marrow stem cells to produce a population of these cells that are protected from the effects of HIV. The proposed approach seeks to make target cells—CD4+ T lymphocytes and the progeny of bone marrow stem cells (primarily lymphocytes, monocytes, and macrophages)—resistant to HIV by reducing the expression of the primary HIV co-receptor, the CCR5 chemokine receptor. This will be accomplished by using a short hairpin (catalytic) RNA directed to CCR5 (sh5). Because resistance can develop and not all HIV strains use the CCR5 co-receptor, another anti-HIV factor, a fusion inhibitor known as C46, also is used within the lentiviral gene transfer vector.

Cal-1 (LVsh5/C46) is a self-inactivating lentiviral vector encoding the sh1005 short hairpin RNA (sh5) targeted against the HIV-1 co-receptor known as the human c-c motif chemokine receptor 5 (CCR5). Driven by the H1 promoter, short hairpin RNA (shRNA) is used to produce small interfering RNA (siRNA) within the transduced cells resulting in a reduction in the expression of CCR5 on the cell surface. An additional component is C46, a membrane-anchored C-peptide derived from the HIV-1 envelope glycoprotein gp41 and driven by the ubiquitin C promoter. The peptide is expressed on the surface of the transduced cells and acts as a fusion inhibitor, thereby blocking the entry of HIV into the cell.

This trial is the first-in-human clinical study of Cal-1. The CD4+ T lymphocytes and the mobilized and harvested CD34+ hematopoietic stem cells (HSPCs) will be cultured *ex vivo* and transduced with Cal-1 to yield sufficient numbers of viable, sterile cell populations for intravenous (IV) infusion back to the same person. Once transduced with Cal-1, CD4+ T lymphocytes and the progeny of CD34+ HSPCs may be protected from HIV infection and its pathogenic sequelae. This protective effect may act to lower HIV viral load and elevate CD4+ T lymphocyte counts.

The study aims to assess the safety, feasibility, and tolerability of Cal-1 in HIV-infected individuals who have previously been on HAART but are not currently taking any antiretroviral agent. In addition to routine clinical and laboratory assessments to monitor general health and HIV infection, the study will monitor the presence of Cal-1 in various cell types in the blood and lymphoid tissue. Other analyses will be employed to monitor the safety of Cal-1.

### B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novelty of the vector, which expresses an shRNA and the C-peptide derived from gp-41, and because an integrating vector is being used in CD34+ stem cells. In addition, although single-dose busulfan has been used as a nonmyeloablative conditioning agent prior to infusion of genetically modified autologous hematopoietic stem cells in some gene transfer trials, with the most experience in patients with ADA-SCID, it is novel for HIV gene transfer trials.

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

Noting that he had only minor concerns with this protocol, which he characterized as an important study that is likely to generate meaningful results, Dr. Badley suggested making longterm followup mandatory

rather than optional, allowing participants to drop out if desired. Doing so would encourage longterm safety studies of an integrating retroviral vector from all participants. He noted that it is important to understand more fully whether transduced cells remain functional. Therefore, it would be helpful to conduct *in vitro* studies of the ability of Cal-1 transduced CD4<sup>+</sup> T cells to respond to chemotactic signals and antigenic and mitogenic responses, to monitor these outcomes in participants who received the transduced cells, and to monitor proinflammatory cytokines in participants who receive transduced cells. Dr. Badley suggested that the investigators consider monitoring antibody responses to the C46 peptide, as well as CD4 and CD8 T-cell responses, to determine whether cognate immune responses occur to the likely antigenic C46 peptide and whether these responses are altered over time. He asked the investigators to explain the relationship between the DSMB and the medical review committee and to comment on the stopping rules for this proposed trial. Dr. Badley suggested that the investigators state explicitly in the protocol and in the informed consent document who would be responsible for the cost of treating a malignancy that might result from insertional mutagenesis.

Dr. Hammarskjöld noted that the protocol is clearly written and is supported by a large number of preclinical *in vitro* experiments. Although Cal-1 is a new vector, there is an FDA-approved drug targeting the CCR5 co-receptor and another approved drug based on a different peptide derived from gp41 that inhibits fusion. Although both of these drugs seem to be generally well tolerated, she noted that clinical resistance has been observed in patients due to the emergence of resistant HIV variants. With regard to preclinical studies, Dr. Hammarskjöld requested an explanation of why a vector expressing only the shRNA against CCR5 inhibits replication of CXCR4 viruses to some extent, as presented in the peripheral blood mononuclear cell (PBMC) HIV challenge assays. She asked the investigators to share their results from *in vivo* efficacy experiments with Cal-1 in a humanized bone marrow/liver/thymus (BLT) mouse model, and she asked them to discuss attempts made to select for HIV variants *in vitro* that show resistance to the shRNA targeting CCR5. Noting that preinfusion PBMCs will be screened for the delta 32 mutation, Dr. Hammarskjöld asked whether a finding of this mutation would lead to exclusion from the study. With regard to study endpoints, she noted that the outcome measures would include assessment of potential resistance by a tropism shift from R5 to dual/mixed X4; therefore, she asked whether the potential selection of resistant viruses would be analyzed in any other way and whether viruses would be analyzed for changes in gp120/gp41 that may signify resistance to C46. Dr. Hammarskjöld indicated that the statement about insertional mutagenesis in the informed consent document is potentially misleading and should be reworded to reflect that a lentivirus is a retrovirus, that few trials have been conducted with lentiviruses compared to other retroviruses, and that there is a lack of data to indicate that lentiviruses are likely to pose a lesser threat than other retroviruses.

Dr. Ross stated that the protocol is clear and the investigators have conducted extensive preclinical work with immortalized and primary cells showing that the vector is expressed and can limit HIV infection. With regard to preclinical safety studies, she noted that the investigators provide no data demonstrating whether the shRNA encoded in the vector could have off target effects and asked whether they plan to test this possibility in cultured cells or mice. Noting that all the pilot studies tested HIV infection of immortalized or primary cells already transduced with Cal-1, with the vector showing efficacy at inhibiting subsequent infection, Dr. Ross asked whether the investigators have conducted studies to examine virus spread in mixed populations of transduced and untransduced cells, similar to what will occur *in vivo*. She requested discussion of how the different endpoints examined in the mice (e.g., lack of engraftment and poor hematopoiesis) would be used to determine whether to proceed with the clinical trial. Dr. Ross suggested that integration site analysis for transduced HSPCs should be performed in addition to the planned analysis of the transduced T cells. She asked the investigators to show data from the mice indicating that integration and expression of the vector in non-HIV target cells would have no consequences. Dr. Ross suggested that insertional oncogenesis should be presented as a potential risk in the informed consent document. In addition, she asked the investigators to discuss the following:

- The sensitivity of detection in a mixture of transduced and untransduced cells (e.g., the level of detection of engraftment in the trial)
- What would happen if the investigators were unable to achieve the level of transduction proposed ( $\geq 30\%$  transduction), including whether more than one apheresis would be attempted

- Whether data exist on busulfan or Neupogen treatment in HIV-infected individuals with measurable virus levels/active infections, and whether this preconditioning would be expected to affect virus loads
- The treatment options available to participants who withdraw, especially those who could not tolerate or were noncompliant with antiretroviral therapy
- What would be the negative clinical endpoint of the integration site analysis to be performed on PBMCs
- Whether integration-mediated oncogenic events should be included in the longer-term followup study
- Clarification of the criteria for enrollment in the study
- Why individuals with CXCR4-tropic virus will be excluded from this study, and whether individuals with dual-tropic virus would also be excluded
- Whether delta 32 heterozygotes would be allowed to participate in this trial even though their inclusion could complicate interpretation of the results from this small cohort size

### C. RAC Discussion

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

- Dr. Badley noted that the investigators should not be concerned about immunizing against any particular viruses or bacteria but that close followup and adequate access to medical care will be important. There exists an increased risk of worse outcome from a variety of illnesses, notably malaria and tuberculosis.
- Dr. Fong asked about the mechanisms of resistance to the drugs that are used for treatment directed at CCR5. He further asked whether the investigators are planning to do repeated administration or to gather data directed at that resistance and what endpoints they might add to this protocol.
- Ms. Dresser asked the investigators to add to the section in the informed consent document about possible benefits a statement making clear that it is unusual for research participants to benefit from an experimental agent.
- Dr. Hammarskjöld counseled the investigators to conduct as many resistance studies as possible, stating that those studies would inform the investigators about the specificity of this experimental therapy and what they and the rest of the field could expect from it.

### D. Investigator Response

#### 1. Written Responses to RAC Reviews

The investigators have demonstrated that there is no modification in the proliferation of PBMC transduced with Cal-1, Cal-1 does not induce apoptosis in transduced PBMC, and there is no induction of interferon  $\gamma$ , TNF  $\alpha$ , or IL-6 in PBMCs transduced with Cal-1. They are currently conducting additional experiments to monitor the level of activation and type of CD4 cells (naïve/memory with and without transduction). Due to limitations on the amount of blood that can be drawn and the variety of causes for proinflammatory cytokines in humans, the investigators plan to remain focused on the endpoints proposed for this trial but will consider monitoring for proinflammatory cytokines in future trials.

There is no evidence that the C46 peptide is antigenic, and there is no evidence that cells expressing this peptide could be eliminated by antibody-dependent immune effector mechanisms or that CD8 responses to C46 could be detected in preclinical testing in nonhuman primates or in a clinical trial. However, the investigators will conduct C46 antibody screening, as indicated in the protocol. The test will be performed using a portion of the archived samples that are collected at regular visits, pre- and post-infusion.

The Medical Review Committee (MRC) evaluates realtime participant data, including adverse events, SAEs, serious unexpected associated adverse events, and laboratory data, whereas the DSMB conducts periodic safety evaluations. A member of the MRC will be present at the DSMB meetings. The

investigators updated the appropriate section of the protocol to reference the relationship of the MRC to the DSMB.

The investigators amended the protocol to specifically reference provisions in the event of a study-related injury or health problem.

Regarding the preclinical data that showed an apparent sh5 inhibition of CXCR4 virus, this is not the usual result seen, and it could be due to low HIV infection in this experiment compared to the other preclinical studies shown. Potential off target effects of sh5 have been addressed extensively in tissue culture, in nonhuman primates, and in BLT mice with no toxicity or offtarget effects encountered.

A key aspect of this proposed approach is the use of a bifunctional vector to enable target cells to be protected from both types of HIV and to mitigate potential resistance to one of the elements. The aim is to affect the CCR5 coreceptor and viral fusion to the target cell. Such a dual approach is important because HIV has been shown to overcome monotherapy. The dual therapy provides protection against different steps of viral entry, thus effectively inhibiting HIV. In addition, preliminary data indicate that Cal-1 inhibits C46-resistant HIV-1 strain BaL.

The experiments in a huBLT mouse model of a vector expressing sh5 with Cal-1 are currently in progress. Results will be available in 1 to 2 months.

Currently, the study design does not exclude participants who are heterozygotes for the delta 32 mutation in CCR5. The investigators explained that they are screening for the mutation as part of the exploratory analysis, but including it as an eligibility criterion would further complicate the screening process and the study timeline. Any delta 32 heterozygotes will be enrolled and will provide useful, exploratory information.

Samples will be collected and banked, as described in the protocol, and will be available for further analysis if clinical data suggest resistance independent of a tropism switch from R5 to X4.

The investigators agreed to alter the wording in the informed consent document to more accurately reflect the nature of a lentivirus, the experimental experience with lentiviruses, and its potential for safety in relation to other retroviruses.

The sh5 has been extensively studied in previous publications. No cytotoxic effects have been seen when the shRNA is expressed from the H1 promoter with no alteration in the growth kinetics of cells expressing the shRNA. In addition, the shRNA has been modified with a single nucleotide mismatch to ensure 100 percent homology to the rhesus macaque CCR5 sequence, and no toxicity has been seen in a nonhuman primate model. In addition, no toxicity/off target effects were seen in BLT mice.

All PBMC challenge experiments were performed on populations of between 20 percent and 50 percent transduction, so the investigators were able to address virus spread in mixed populations. In addition, experiments are currently in progress with Molt4/CCR5 cells in which Cal-1 transduced cells are mixed with untransduced cells at varying percentages and subsequently challenged with various HIV strains. Preliminary results indicate a transduced cell dose-dependent effect on HIV replication.

Humanized NOD-scid-gamma (NSG) mice are currently being used in the GLP safety study to determine *in vivo* safety of Cal-1. Data generated during this study will be used to support the proposed clinical protocol. This safety study addresses engraftment, acute or subacute toxicity, hematopoietic lineage reconstitution, and predominant integration site analysis in human CD34+ hematopoietic stem cells transduced with Cal-1 versus control. All of these measures are important precursors to the clinical trial. In addition, the investigators have conducted the *in vitro* immortalization assay, and preliminary evidence indicates that Cal-1 showed no genotoxicity.

The protocol states that the investigators will perform integration site analysis for the transduced T cells, and it has been amended to state that they also will perform integration site analysis for the transduced HSPCs. Both are warranted due to safety considerations.

The investigators accepted the need to be flexible on the number of transduced cells infused, and they will lower the acceptance criteria to no more than 10 percent transduction. This level is consistent with previous clinical studies. Given this change, only one apheresis will be proposed for each cell type.

With respect to busulfan conditioning, the AIDS Malignancy Consortium trial dosed 20 individuals (who had recurrent and extensively chemotherapy-pretreated HIV-associated non-Hodgkin's lymphoma or Hodgkin's lymphoma) with a dose-reduced busulfan and cyclophosphamide preparative regimen with autologous stem cell transplantation. Regardless of baseline viral load, the conditioning regimen did not appear to modify either CD4 count or HIV viral load. With respect to Neupogen conditioning, results from a Phase II study in which 74 individuals were mobilized with high-dose growth colony stimulating factor (GCSF) while on HAART showed no increase in viral load. An earlier study reporting the results of the Adult AIDS Clinical Trials Group 285 Study Team demonstrated that GCSF at a dose of 10 mcg/kg/day for 7 days caused a transient increase in plasma HIV-1 RNA level that resolved by days 27 to 30 after initiation of GCSF.

The treatment options available to participants if they withdraw from this trial will not be prescribed in the protocol but will be left to the investigators' best judgment. Those who withdraw can (1) go on their last tolerable regimen (which may not be fully suppressive, but will keep viral fitness down), (2) go on the most effective regimen as determined by prior treatment history and prior available genotyping or phenotyping, or (3) join a clinical trial of a new antiretrovirus, if available. The investigators revised the protocol to clarify these options.

Three sections of the protocol have been modified to describe clearly what the investigators would consider a negative clinical endpoint of integration site analysis performed on PBMCs.

The sponsor intends to perform integration analysis as part of the scheduled followup. This topic will be addressed when the separate longterm followup protocol is written.

The clinical investigators will assess the most suitable candidates for this trial. The acceptance criteria are general and do not specifically exclude individuals who decide on their own to discontinue therapy to participate in this study. These participants may go back on their last reported suppressive regimen at the time of withdrawal from or completion of the study.

The investigators reported that, in the pre-IND meeting, the FDA requested that the investigators ensure that only individuals who do not have CXCR4-tropic HIV are enrolled in this trial. The reasons for this request were to simplify analysis and to allow both mechanisms to be effective against the virus. With an inclusion criterion that requires potential participants to have a CD4 count greater than 500 off therapy, it is unlikely that these individuals will have anything other than CCR5-tropic virus.

The investigators reported modification of wording in the informed consent document to include the potential risk of insertional oncogenesis. They also agreed to alter wording in the document to indicate that the planned longterm followup is a mandatory part of this clinical trial.

## **2. Responses to RAC Discussion Questions**

Dr. Symonds explained that viral resistance to maraviroc is both viral and cellular—either a switch from R5 to X4 or the R5 virus mutates in such a way that it can bind to the CCR5 with maraviroc (a FDA licensed anti-viral medicine that is designed to block CCR5) already bound. The investigators would be looking for any potential resistance to the switch from R5 to X4 and any resistance to the CCR5 binding. Repeated administration might be needed, but resistance would be monitored by the level of marking and the increase in stability of marking over time. Dr. Mitsuyasu added that the investigators will monitor CD4 count and viral load. An increase in viral load or a decrease in CD4 count would suggest that the effect

may be wearing off or that there may be resistance, in which case they would look for both. At that point, the investigators would decide whether or not additional dosing is needed.

The investigators agreed to add a statement making clear that it is unusual for research participants to benefit from an experimental agent to the section in the informed consent document about possible benefits.

#### **E. Public Comment**

No public comments were offered.

#### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

##### Preclinical Issues

- Maraviroc (Selzentry) is an FDA-licensed drug that acts as a CCR5 receptor antagonist. Resistance to maraviroc has been detected in some strains of HIV. Studies should be conducted to determine whether the sh5RNA vector can inhibit infection by maraviroc-resistant HIV strains. This will shed light on whether the mechanism that confers resistance to maraviroc also will confer resistance to the sh5RNA.
- To confirm that the inhibition of HIV replication is due specifically to the shRNA action on CCR5 receptor expression, the investigators should consider making a cell line expressing a CCR5 protein that is resistant to the CCR5 shRNA but still permissive for HIV infection. These cells should be transduced with the shRNA vector, and HIV replication should be compared in transduced and untransduced cells. A finding that HIV replication is the same in both cell lines would further confirm the specificity of the shRNA effect.

##### Clinical and Trial Design Issues

- Mutations that knock down CCR5 expression can affect an individual's ability to respond to certain infections, such as malaria and tuberculosis. To determine whether Cal-1 has any effect on the function of the transduced T cells, participants' immune responses should be monitored pre- and post-infusion by measuring T-cell proliferation response to recall antigens, including HIV antigens.
- Because the C46 peptide may be antigenic, it is important to monitor whether cognate immune responses develop against this peptide and whether these immune responses change over time. The investigators should consider monitoring antibody responses to the C46 peptide as well as CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses; this could be accomplished with ELISpot measurements.
- The protocol provides specific rules regarding withdrawal of individual participants based on CD4<sup>+</sup> lymphocyte counts. However, for other adverse events, clinical judgment will be used to determine whether the protocol should be stopped. Although this is reasonable for certain Common Terminology Criteria for Adverse Event Grade 3 toxicities, the investigators should consider defining additional rules for certain Grade 4 toxicities.
- Research participants who are heterozygous for the delta 32 mutation in CCR5 have partial protection against HIV infection. Inclusion of these participants may confound analysis of the biological activity of the investigational agent. To maximize the information obtained from this small Phase I trial, the investigators should consider limiting enrollment to individuals who do not carry the CCR5 delta 32 mutation or, if such an individual is enrolled, the investigators should expand the cohort size to include additional participants who do not carry the mutation.

- Because this protocol uses an integrating vector in hematopoietic stem cells, longterm followup is warranted to monitor for potential genotoxicity. The investigators propose to conduct a separate protocol for that longterm followup and have included information on longterm followup in the informed consent document. For completeness, information on longterm followup also should be included in this protocol.

#### Ethical, Social, and Legal Issues

- The section in the informed consent document regarding who will bear the costs of care for adverse events was amended in response to the RAC written review to state, “If you have an injury that is directly due to taking part in this study, you will receive tests and treatment at no cost. This includes care that you need right away and longer term care.” Longterm followup will be done to monitor for any genotoxicity that may occur years after the study is complete. This section on coverage for study-related injury should make it clear that it will cover an injury that is related to taking part in the study even if it occurs during longterm followup, which is not currently part of this protocol.
- Because this is a Phase I trial, it is important that participants understand that the likelihood that they will benefit from the trial is extremely low. The investigators could state this more explicitly by adding the following language to the informed consent document: “It is unusual for participants in an early trial like this to benefit from the intervention.”

#### **G. Committee Motion 4**

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. No official motion or second was offered that these comments be approved by the RAC, but a vote of the RAC members was taken. The RAC voted to approve these summarized recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 2 recusals.

#### **IX. Update Discussion of Recent Results on Protocol 793 titled: Pilot Study of Redirected Autologous T Cells Engineered to Contain Anti-CD19 Attached to TCR $\zeta$ and 4-1 BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma**

Presenter: Carl June, M.D., University of Pennsylvania

#### **A. Presentation**

Dr. June reviewed the status of his research, discussed lessons learned, and presented information about the field of CAR therapy. The two major approaches to overcome immune tolerance to tumor cells, 1) the use of the T-cell receptor (TCR) heterodimer approach and the CAR or T-body approach. The use of CARs has engendered issues such as what to select as a target and what to use as a signaling domain.

CARs were developed in 1989 *in vitro* by the laboratory of Eshhar and colleagues at the Weizmann Institute, but the first CAR trial was not reported until 2000—an HIV trial that retargeted cells to CD4zeta to attack the high-affinity receptor for HIV. Much safety information was gleaned from the first CAR trials, in which more than 40 research participants were dosed, and recently submitted results show that 37 out of 40 individuals continue to have engrafted cells at stable levels ten years after a single infusion. These results indicate that a first-generation CAR can establish memory in humans without genotoxicity or other SAEs.

Despite strong preclinical rationale, technical difficulties have prevented clinical translation for use in cancer patients until recently, when improved T-cell culture systems and efficient gene transfer systems

were developed. The first CAR trial was reported from the NCI in 2006, for a target folate receptor 1a for ovarian cancer; subsequently in 2008, another first-generation CAR trial was reported. Both trials showed safety but neither evidenced substantial antitumor effect. The possibility that these trials failed because of poor T-cell engraftment has been hypothesized but not proven. A number of trials have tested CD19 or CD20 CARs for B-cell malignancies; most of these trials have been reviewed by the RAC.

Safety principles applicable to clinical trials using second- and third-generation CAR T cells are taking the field forward as more investigators become interested in CARs. Caveats suggest that dosing should be based on the transfer of unselected CAR T cells, which have the potential for proliferation and long term engraftment; survival and engraftment likely are enhanced by preconditioning. Cytokine support of transferred cells may enhance initial cell proliferation and survival and should be investigated in more depth. Initial toxicity might be avoided by reducing cell dose and/or by using a split-dose infusion strategy to administer infusions over several days. Based on data from trials to date, a moderate initial cell dose might be appropriate for targets already tested in other trials; a lower dose should be considered for CAR T cells directed at novel targets. Second- and third-generation CAR T cells proliferate, so the dosing and scheduling may be different than for first-generation CARs. One of Dr. June's protocols, reviewed by the RAC in 2007, featured a split-dose infusing strategy in which the research participants received the specified doses over 3 days—10 percent, 30 percent, and 60 percent; this strategy mitigated the potential of cytokine-release syndrome.

Other strategic safety considerations include whether a target has been tested before and whether to do preconditioning. For hematological malignancies, the most beneficial approach appears to be using conditioning therapy, which allows the induced lymphopenia to enhance engraftment and the effects of the CAR T cells. Whether to give cytokine support is an open issue, and a number of protocols have tested IL2 following CAR administration and now propose to test IL7 and other cytokines such as R15.

CD19 was considered the first ideal CAR target, and B-cell malignancies were determined to be the best tumors to test these cells because of the known distribution of CD19. CD19 is known not to be expressed on hematopoietic stem cells so that aplastic anemia would not be a potential on-target, off-tumor effect. CD19 expression is restricted to B cells and possibly follicular dendritic cells, is not expressed on pluripotent bone marrow stem cells, and is expressed on the surface of most B-cell malignancies. Antibodies against CD19 inhibit growth of tumor cells.

A protocol conducted by Dr. June and colleagues was the first to use CD137, which is a 4-1BB member of the tumor necrosis factor (TNF) family. A lentiviral vector was used, and a high level of T-cell expression resulted. The conclusion from this protocol was that the 4-1BB signaling domain promotes CAR T-cell proliferation.

In OBA protocol #0607-793, "Pilot Study of Redirected Autologous T Cells Engineered to Contain Anti-CD19 Attached to TCR $\zeta$  and 4-1BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma," Dr. June and colleagues proposed three new approaches that had not been tested in a clinical trial: the use of a lentiviral vector to deliver the CAR construct, the use of a dual-signaling domain of 4-1BB and CD3zeta, and the use of an anti-CD3 and anti-CD28 monoclonal-antibody-coated bead stimulation. The protocol was a single-infusion testing using split dosing of the CAR construct in research participants who had relapsed and refractory B-cell malignancies. The primary objectives were (1) to determine the safety and feasibility of the CAR T cells transduced with the anti-CD19 lentiviral vector and (2) to determine the duration of *in vivo* survival of the CAR T cells; secondary endpoints included immune assessment of the research participants. Enrolled participants had refractory and relapsed CLL and were heavily pretreated with standard treatments. Surprisingly, all three participants in this pilot study showed clinical responses: one individual had a longterm partial response and the other two had complete hematologic malignancy remissions. All three participants entered into this protocol with high tumor burdens, between three and seven pounds of tumor each. In the first research participant, each infused cell killed on average 2,200 tumor cells, supporting the hypothesis that T cells are attractive for tumor immunotherapy because they can kill multiple times and have proliferative capacity to continue to do so.

The third participant in this clinical trial developed delayed-onset tumor lysis syndrome three weeks after infusion, when the number of CAR cells had peaked. He developed transient renal failure, high levels of uric acid, and elevated lactate dehydrogenase, all of which resolved to normal within one to two weeks, at which time tumor became undetectable in his bone marrow.

Secondary endpoints in the study were to determine the function and persistence of these cells. The three research participants were monitored intensively, and all had CAR T cells persisting to at least 180 days after dosing. This result—persistent levels of memory CAR T cells—was not seen in previous CAR trials, so this trial represented the first documentation of substantial *in vivo* proliferation. The investigators were able to measure and track expression of the CAR T cells using an anti-single-chain variable fragment (scFv).

Persistence-flow cytometry showed that CAR T CD19 cells could be detected in periphery and marrow at 12 months post-infusion for two of the three participants. Long term persisting CD19 cells were shown to retain anti-CD19 functionality directly *ex vivo*, with the CAR antibody receptor functioning for at least six months after infusion. Suppressor T cells expressing the CAR are not present, but the three participants did have effector cells, so the long term persistence allowed the investigators to track the function of these cells. Followup data from the participants' one-year visits shows that participants 1 and 3 were expressing CARs in their periphery and in the bone marrow. The 30-fold variation between these two participants has yet to be explained, although this variation also occurs one year after vaccinating individuals against, for example, varicella.

Other markers that showed biologic effect were cytokines in the serum. Cytokines increased in participants 1 and 3, correlating with the peak of CARs in the blood. The cytokine signature is the same in both participants, with interferon gamma increasing 100-fold followed in extent by interferon-gamma-related cytokines. The investigators saw no TNF-alpha or IL2 in the serum of the participants, no indication of cytokine storm, and no cytokine release in the first five days, which they posited was a result of having used the 4-1BB signaling domain. Cytokine secretion was seen at the time of on-target effects with tumor reduction, thus the investigators posit that cytokine release will occur when a target is hit. Although they are not sure why this secretion is delayed, the investigators noted that at follow-up all the cytokines returned to baseline.

In summary, the synthetic biology of engineered T cells shows promise in treating B-cell malignancies. These cells can perform in humans similarly to what was seen in mouse models, and their performance is enhanced compared with the function of normal T cells. Longterm persistence has been shown to be possible, and CD19 CAR T cells can be used for longterm immuno-surveillance with an integrating viral vector and in the setting of heavily immunosuppressed CLL patients. It is as yet unknown how well these CD19 cells will work in other hematologic malignancies and whether CARs will be effective against solid tumors.

## **B. RAC Discussion**

Dr. Fong asked about Dr. June's next trial. He responded that he and his colleagues are testing ten participants to fully define the safety effects. After that, they want to determine the required dose, because an important lesson from trials to date is that the response is dose dependent. They plan to conduct a dose de-escalation trial to find an optimal biologic dose.

Noting that many promising results have occurred in this field, Dr. Fong asked whether the strategy will be to conduct Phase III trials and what forms those might take, or whether investigators are more likely to concentrate on Phase II trials in patients who are completely resistant to current therapy. Dr. June explained that the most difficult aspect of working with CLL patients is that they were treated previously with very toxic immunotherapy. Before getting FDA approval, it will be necessary to know what setting, where to treat, and the role and necessity of having memory cells or repeat infusions. This trial represents the first therapy that has gotten this far solely in an academic setting; pharmaceutical companies have not been interested in these types of trials. It will be a challenge to get these trials approved by the FDA because they are first-in-class therapies.

Dr. Fong asked about the obstacles for development of this therapy, and how the government, public, or other sectors will need to push this therapy to the forefront. Dr. June opined that gene transfer might be more cost effective in the same way that vaccines are more effective for infectious diseases—prevention is less expensive than treatment. In many ways, cell therapies and gene therapies are going to be much less expensive than current treatments. No effort to develop this technology has been expended other than a cottage industry; antibodies used to be expensive to manufacture but now the cost of goods is low. These therapies can be developed using cell and vector manufacturing approaches, but such approaches are not well done in a university setting.

In response to regulatory concerns, Dr. June explained that regulation is similar to standard transfusion medicine that is based on stem-cell regulation. Cells are stored, programs look at the product's stability, and barcoding and product identity are being used. T cells and stem cells are amenable to long-term storage.

Noting that until now every cancer product has been organ based, Dr. Fong asked whether future therapies should be approved based on target approaches instead. Dr. June responded that some chemotherapy combinations already go across different organ types and cancers. It is likely that future therapies will be developed as a class, but likely for specific indications.

Dr. Chiocca stated that three main advances made this research possible and effective—preconditioning, the intracellular signaling molecule, and changing dose schedules to eliminate toxicity. He wondered whether these three areas would need to be tweaked for every tumor. Dr. June noted that one lesson learned is that vector design, small differences, and how the cells are manufactured do matter. The major variable in CAR trials is swapping out scFvs and redirecting their specificity; the rest of the process has been well tested. The dose is less important because the cells are self-replicating and have an integrated transgene. This situation is quite different from standard drug development.

Dr. Yankaskas asked why the delayed tumor lysis syndrome happened when it did. Dr. June answered that it takes some time for infused genetically modified cells to traffic to tumor sites, and those cells can be trapped in vessels and pulmonary vasculature. The delay could be related to a rate-limiting step or the dose to the effector or the amount of targets, which could be residual normal B cells as well as leukemic cells. Another variable is how much of tumor cell killing is accomplished directly by the CAR T cells or whether bystander killing is occurring. Much pharmacology is yet to be worked out. CD19 negative escapes have not been observed in humans or in the preclinical studies.

Dr. Fong asked whether Dr. June and his colleagues are considering including a suicide gene in future trials, especially trials that would treat individuals with minimal residual disease. Dr. June responded that he has not taken that approach in his laboratory, and that he is relying on other colleagues in the field to do that. He predicted a variety of approaches would be tested in future protocols.

Dr. Sarzotti-Kelsoe inquired as to whether this therapy is tailored to each patient. Dr. June explained that, at this point, it is an autologous, closed manufacturing system. It remains a personalized therapy from the research participant's own cells. Investigators are working on making a universal donor CAR cell; such an advance could be the next generation of this therapy. A major challenge is to have longterm CAR therapy with a cell that is not one's own. He predicted the development of engineered cells for knockdown therapy and induction of remission that then disappear as well as cells for long-term immunotherapy.

Dr. Hammarskjöld asked whether lentivirus vectors provide an advantage for transduction with less insertional mutagenesis. Dr. June explained that it depends on which cell type is being targeted. For hematopoietic stem cells, lentiviral vectors have a number of advantages with regard to biosafety, expression and ability. With T cells it is an open question as to what will be useful. For a self-selecting system such as this, transduction efficiency does not matter because the cells will expand. It is very different than in stem-cell-directed therapies, in which transduction efficiency has been the rate-limiting step.

### C. Public Comment

Lloyd Klickstein, M.D., Ph.D., Novartis Institutes for Biomedical Research, asked which steroids are used with these research participants, how much is used, what was the effect on the CAR T CD19 cells. Dr. June summarized the case of participant two, who experienced the delayed cytokine release. He was treated with dexamethasone that was tapered rapidly. Corticosteroids have also been found to be effective and could be used as a safety switch, and are being listed as the first line of toxicity management in Dr. June's protocols. It could affect long-term engraftment, so retreatment might be necessary.

### X. Review and Discussion of Human Gene Transfer Protocol #1110-1127: Pilot Clinical Trial of Autologous Met Redirected T Cells Administered Intratumorally and Intravenously in Patients with Operable Triple Negative Breast Cancer

Principal Investigator: Julia C. Tchou, M.D., Ph.D., University of Pennsylvania  
Sponsor: Carl June, M.D., University of Pennsylvania  
RAC Reviewers: Drs. Fost, Kiem, and Yankaskas  
*Ad Hoc* Reviewer: Larry Norton, M.D., Memorial Sloan-Kettering Cancer Center (*via teleconference*)

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

### A. Protocol Summary

Triple negative breast cancer (TNBC) lacks expression of the conventional prognostic markers, i.e. estrogen and progesterone receptors, and Her2-neu expression. Therefore, patients with TNBC derive no benefit from molecularly targeted treatments such as endocrine therapy or trastuzumab, because they lack the appropriate targets for these drugs. Treatment of this more aggressive breast cancer is challenging especially in the recurrent and metastatic setting when conventional chemotherapy combinations have been exhausted.

c-Met is a receptor tyrosine kinase encoded by the *c-met* proto-oncogene and has been widely implicated in tumor progression and invasion. The ligand for c-Met is the hepatocyte growth factor (HGF), and this pathway has been linked to the cancer progression by driving proliferation, motility, invasion, and angiogenesis. Both c-Met and the ligand HGF are overexpressed in most human malignancies including TNBC.

The protocol proposes an adoptive immunotherapy approach with autologous T cells modified to express a chimeric antigen receptor (CAR) specific for c-Met using the sequences from the 5D5 mAb as a potential therapy for breast cancer. The c-Met directed CAR is engineered to express a single chain antibody variable fragment (scFv) linked to the intracellular CD3 $\zeta$  T cell receptor domain and the 4-1BB costimulatory domain (cMet.BBZ). Preclinical studies indicate that human T cells electroporated to express the cMet.BBZ CAR specifically respond *in vitro* to target cells expressing cMet by cytokine secretion and CD107 degranulation. Furthermore, the *in vivo* efficacy study using the NSG immunodeficient mouse model transplanted with an ovarian cancer cell expressing cMet indicates that cMet.BBZ CART cells are able to halt tumor development compared to control groups. The proposed study is a first in human study targeting cMet with redirected T cells. To maximize safety, the approach is to conduct the first trial using T-cells electroporated with the c-Met CAR mRNA which will allow for only a limited time of expression. If side effects are noted, toxicity will rapidly abate because expression of the mRNA CAR is limited to a few days and T cells dilute the CAR expression following proliferation triggered by interaction with the target antigen, thus making side effects transient and manageable. T cells will be given first by intratumoral (IT) injection, as a further safety feature, and then only after the clearance of cMet CAR T cells given by IT injection, a single injection of Met CAR T cells will be given by IV injection. This is a 3x3 designed dose-escalation protocol. Cohort 1 (n=3 patients) will receive IT injection of 3x10<sup>7</sup>

autologous RNA Met CAR T cells followed by  $3 \times 10^8$  cells/m<sup>2</sup> by IV infusion. Cohort 2 (n=3 patients) will receive IT injection of  $1 \times 10^8$  autologous RNA Met CAR T cells followed by  $3 \times 10^9$  cells/m<sup>2</sup> by IV infusion. Cohort 3 (n=3 patients) will receive IT injection of  $3 \times 10^8$  autologous RNA Met CAR T cells followed by  $3 \times 10^9$  cells/m<sup>2</sup> by IV infusion. Toxicities related to the therapy will be managed by dosing interruption and administration of corticosteroids to induce lysis of cMet-redirectioned T cells. These measures in addition to temporary expression of cMet receptor allow for efficient monitoring of safety. If safety is established in this protocol with cMet RNA CARs, then the investigators plan to submit a new protocol using T cells that permanently express the Met CAR, and to further evaluate safety of cMet CAR T cells.

## 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 CAR and the target population. Although TNBC generally has a poorer prognosis than other breast cancers, some of the enrolled participants may be cured by standard therapy; the risks and benefits of enrolling these individuals in a Phase I trial were deemed to deserve further discussion.

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

Dr. Fost noted that the informed consent document was unusually well done. Absent concerns about scientific issues, he stated that his review did not uncover any ethical problems associated with this proposed trial.

Dr. Kiem noted that the study and protocol documents were written clearly and the research team possesses significant expertise in T-cell therapies. Although the investigators anticipate that their proposed study should minimize fatal risks because of the use of electroporation and thus transient expression of the CAR construct, Dr. Kiem expressed concern about the outcome of another study in which the adverse event occurred shortly after infusion of the T cells. With regard to nonspecific targeting (nontumor tissue) based on naturally expressed Met, he asked the investigators whether data exist that show T-cell binding to nontumor tissue. Dr. Kiem requested that the investigators clarify at what point and how often the protocol-specific Monitoring Committee would review adverse event data. Although the investigators mention that a potential immune response against CAR T cells would limit maintenance of c-Met-expressing T cells, he wondered whether a potential immune response to the intratumoral injection could strengthen an immune response reaction to the IV infusion and potentially cause more adverse events; he asked whether such a possibility could or would be tested. Dr. Kiem suggested that the informed consent document list the fatal SAEs from the previous two studies discussed in the protocol.

Dr. Yankaskas asked the investigators to include in the protocol information about the survival of the infused cells, and he asked if it is feasible to measure the persistence of the infused Met CAR T cells and the fraction of those cells that express the scFv antibody. If adverse side effects are observed in this trial, he wondered how the investigators would distinguish toxicity due to T cells from toxicity due to scFv. Dr. Yankaskas requested discussion of the option of starting the IV doses at a lower level than proposed, considering the participant death after infusion of  $1 \times 10^{10}$  third-generation CAR cells as reported by the an NIH trial in 2010. In that trial, the participant received  $1 \times 10^{10}$  retrovirally transduced cells via IV infusion and developed respiratory distress within 15 minutes, and the investigators reported marked increases in IFN- $\gamma$  and other cytokines (Morgan, R. *et al*, *Molecular Therapy* (2010) 18 (4), 843–851). In addition, he suggested two minor wording changes in the informed consent document.

*Ad hoc* reviewer Dr. Norton noted that if the results in humans mimic the effects seen in animals, this proposed approach would represent a major advance. He focused his review on the breast cancer patients because most of those patients could be cured by standard therapy. With regard to experimental design, he suggested that the investigators might want to focus eligibility on c-Met-positive tumors regardless of cell type defined by other characteristics or ancestry. Based on the data provided by the investigators, Dr. Norton wondered whether the proposed dose for this trial would reflect the multiple dosing likely required for therapeutic efficacy. His primary concern was safety, and he requested that the investigators discuss why they have elected to start with primary breast cancer participants to ask an acute safety question that might be better investigated by enrolling participants with advanced, incurable

disease who are c-Met positive regardless of cell type or ancestry. Dr. Norton asked whether the investigators plan to conduct a Phase I trial to determine safety in participants with advanced incurable disease with multiple exposures. He requested clarification as to whether the primary goal of this study is to determine the occurrence of an anticancer response, downregulation of c-Met, or some other effect.

### C. RAC Discussion

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

- Noting that breast cancers are generally rock-hard tumors, Dr. Fong asked the investigators what approximate volume of injectate they plan to use and whether it would be a peritumoral injection or an intratumoral injection. He added that, after the first few research participants, the investigators should examine the margin positivity rate and ensure that no participants' excisions are misguided by either the injection volume or other issues.
- Dr. Fong asked the investigators whether waiting three days after injection is enough time to see a biologic effect from a T-cell therapy directly administered to tumor.
- Dr. Fong asked about the correlation between the percentage of CAR cells identified in the periphery and a biologic response in humans.
- Regarding the initial surgical site, Dr. Strome noted that bleeding will occur and additional tumor will need to be removed. He suggested trying the intratumoral injection in advanced-stage breast cancer patients, which could result in obtaining preliminary data quickly.

### D. Investigator Response

#### 1. Written Responses to RAC Reviews

Regarding the feasibility of measuring the persistence of the infused Met CAR T cells and the fraction of those cells that express scFv, the investigators explained that one of the secondary endpoints of this protocol is to assess the pharmacokinetics of c-Met T-cell clearance after IV infusion. This will be assessed by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) for the fraction of electroporated T cells and flow cytometry for the fraction of electroporated T cells expressing the scFv CAR at days one and seven post-infusion. The investigators currently are conducting similar pharmacokinetic studies on a different protocol testing the safety of mesothelin-specific RNA CAR T cells in mesothelioma patients (OBA protocol #1010-1072), and the preliminary results indicate that it is possible to detect RNA CAR T cells in blood after IV infusion at the dose proposed in this c-Met protocol. Analysis of these results has revealed that mesoCAR T cells can be detected immediately and up to 3 days after each infusion but could not be detected at later time points; persistence of these cells is transient, as predicted by the investigators' preclinical studies, and the expression is dose dependent.

This protocol is designed to test the safety of c-Met CAR-expressing T cells. The protocol features designed to increase participants' safety are the use of RNA CAR for transient expression and self-limited toxicities, the use of intratumoral injection as the first route of administration, and the use of a starting IV dose that is 100-fold lower than that used in the Morgan study. Toxicities that may be induced by c-Met CAR are unknown but would be expected to be similar to those elicited with MetMab 5D5, the anti-Met antibody that is in late-stage development for lung cancer. c-Met is expressed at lower levels in the epithelial cells of many organs; however, it is not found in the intravascular sites that may provoke cytokine release syndrome.

The investigators have substantial experience in the field of adoptive therapy using autologous *ex vivo* expanded T cells that are manufactured in the same way but have not been engineered to express scFv. Several hundred research participants have been enrolled on Phase I–II translational trials, testing a variety of biologic and targeted cell therapies, including more than 350 T-cell infusions. Experience to date indicates no safety concerns related to infusion of CD3/28-stimulated and *ex vivo* expanded autologous T cells. Several of the studies sponsored by Dr. June used non-gene modified *ex vivo* expanded T cells in individuals with HIV and myeloma at doses of up to  $6.5 \times 10^{10}$  cells. All T-cell infusions were well tolerated with no SAEs and no significant clinical or laboratory toxicities. Expected infusion-

related adverse effects have included fever, chills, nausea, back pain, joint pain, headache, myalgia, and itchy/sore throat. These grade one and grade two symptoms typically resolve within two days.

Toxicity due to scFv would be the consequence of c-Met CAR T cell interaction with c-Met antigen on tumor cells or normal tissues. “On-target on-tumor” inflammatory toxicity would indicate the efficacy of c-Met CAR T cells in lysing tumor target cells and can be managed as in previous studies. Due to the generally low tumor burden of the research participants on this protocol, the investigators do not expect significant systemic toxicity from CAR-induced inflammation and cytokine release at the tumor. “On-target off-tumor” toxicity would indicate c-Met CAR T-cell interaction with c-Met antigen expressed at physiological levels on normal cells. Such toxicities have been seen in other CAR studies; they were specific to each CAR scFv specificity, and their intensity varied. Any clinical or laboratory toxicities occurring after c-Met CAR infusion would be considered possibly related to the study agent; stopping rules and dose-limiting toxicity are defined in the protocol.

In this proposed trial, Cohort 1 is flat-dosed at  $3 \times 10^7$  cells intratumorally and then, if safe,  $1 \times 10^8/m^2$  cells IV. The first dose is below the minimum anticipated biological effect level (MABEL) as well as being 100-fold lower than the  $1 \times 10^{10}$  cell dose used in the NIH study, and the second is at the anticipated MABEL dose. The investigators are proposing to use the 4-1BBzeta domain that they have tested in leukemia and in one individual with mesothelioma. Total CAR cell doses used in other trials suggest that large numbers of CAR T cells can be administered to cancer patients with the exception of ERBB2, which is a target antigen known to be expressed in the pulmonary capillary endothelium.

In addition, the investigators have incorporated two other safety features into this proposed study: (1) In the trials using permanently modified CAR T cells, the infused cells are likely to expand, making the effective dose higher. In contrast, with the RNA electroporated CAR studies, the effective dose may be lower because, as the cells expand, the RNA CAR is diluted and cells’ responsiveness to c-Met is reduced. Thus, c-Met CAR T cells encountering antigen would be triggered to proliferate, which reduces their functional potency, resulting in self-limited toxicity. (2) Two forms of “on-target” toxicity are attributable to CAR T cells—one that results from synchronized activation of CAR T cells occurring during first-pass biodistribution and another that is dependent on biodistribution of CAR cells trafficking to sites of antigen, a process that does not occur for at least 24 hours after infusion. The fatal case in the NIH study was attributed to synchronous activation of  $1 \times 10^{10}$  CAR T cells encountering specific antigen upon passing through the pulmonary capillary bed, which resulted in rapid and profound systemic cytokine release following infusion.

The second type of toxicity reported with CAR T cells is consequent to cells trafficking after initial biodistribution and encountering cognate antigen on normal tissues; it develops with delayed kinetics and in a nonsynchronous fashion. Given the known c-Met distribution (i.e., not expressed within the vascular system), the investigators do not anticipate immediate toxicity but rather the potential for delayed toxicity that may become evident several days post-infusion. However, by this time, the CAR expression is already waning, further reducing the intensity of any potential toxicity that is expected to be self limited.

The investigators are not aware of any publicly available information about non-tumor tissue binding, so they have designed a Phase I protocol to evaluate whether there are any significant effects from short-term binding of CAR T cells to non-tumor tissue. Anti-mesothelin RNA CAR T cells, which are quite similar to Met RNA CAR T cells, have an *in vivo* expression that is limited to approximately three days, so any resultant toxicity should be self limited.

The protocol-specific safety monitoring committee will review the safety data after dosing the first three research participants, after completion of each cohort, and at least every six months. The members of the safety monitoring committee are listed on the protocol.

The participants will be evaluated for the development of anti-CAR immune responses by humoral and cellular assays against natural autologous T cells and against c-Met CAR T cells. Michael Kalos, Ph.D., of the University of Pennsylvania, has experience in evaluating such responses, and the procedures are established in the laboratory. To date, no clinically evident adverse effects have been associated with

CAR immunogenicity, and the effects have been limited to rejection of CAR T cells and loss of engraftment. The c-Met CAR in this trial is derived entirely from human sequences, so the development of an immune response against the engineered T cells is unlikely. Immune responses to CAR constructs containing mouse sequences were detected in other studies and were associated with a loss of CAR T-cell engraftment. In this proposed protocol, the investigators believe that an anti-CAR response is unlikely following the single intratumoral infusion, and serious side effects are not expected as a consequence of redirected T-cell rejection or as a result of the self-limited expression of the RNA CAR T cells.

c-Met-positive breast cancer is expected in all subtypes of breast cancer in the selected patient population. The investigators selected TNBC because of the lack of targeted therapy for it.

Regarding the patient population from which participants in this trial would be recruited, the investigators explained that a novel immunotherapy is being tested, and it is known that immunotherapy works better on early-stage patients. Because the investigators are proposing to use a small dose injected directly into the tumor, they do not anticipate any effects on late-stage breast cancer patients. This “Phase 0” approach will test whether this immune-based intervention can kill a c-Met-positive tumor, and, in the long run, it is hoped that an immunogenic event will trigger a vaccine response. The investigators want to gather data at this proposed low dose to indicate whether this intervention will trigger inflammation and reduce c-Met expression; it is a test of drug potency.

This proposed trial is a first-in-human trial but not a first-in-class test. Numerous other CARs have been given to humans; so much is known and understood about the behavior of CARs. The investigators for this trial propose using several orders of magnitude below what is considered a therapeutic dose. In an advanced cancer patient,  $1 \times 10^{11}$  cells would be needed to elicit a therapeutic effect, according to the modeling experiments in mice. Although the investigators would like to test that population of patients, they first want to ascertain whether this CAR kills the c-Met-expressing tumor cell *in vivo* in patients in whom there have been no trafficking issues. Immune-based therapies are more appropriately assessed for toxicity in earlier-stage patients than in late-stage patients who have had cytotoxic therapies and are immunosuppressed. The goal is to look at an early-stage cohort of patients and first find out if the c-Met CAR works and can hit a target, and then later progress to a standard advanced cancer patient population and do dose-dense testing to identify a potential efficacy signal compared to the safety signals.

The investigators reported that the informed consent document was modified to add a summary of the fatal SAE reported on an NIH trial in 2010.

## 2. Responses to RAC Discussion Questions

The investigators are considering conducting this experiment in the metastatic patient population for their Phase I trial.

Dr. Tchou explained that the anticipated injectate volume is between 1 ml and 3 ml and an intratumoral injection, guided by ultrasound as needed, is planned. Most breast cancer biopsies are done by core needle biopsy that penetrates the tumor, so the investigators expect to be able to inject into the tumor from a deep to superficial fashion. The tumor is removed along with healthy tissue all around it; wide excisions are done so as not to compromise the tumor margins for assessment.

With regard to the adequacy of waiting only three days to see a biologic effect, Dr. June explained that the investigators have direct demonstration in a patient with pancreatic cancer who had ascites and malignant peritoneal ascites. The investigators were able to obtain explants of those tumor cells *in vitro* and then add mesothelin CAR T cells; within 48 hours, all the tumor cells were dead.

The research participants will have the surgery usually two to four weeks after signing on to the protocol with a target of three weeks after enrollment. The investigators stated that they are able to produce the T cells in approximately ten days.

In their experience to date, the investigators determined that between 10 percent and 90 percent of CAR cells in the periphery of circulating cells were needed to produce a biologic response, depending on whether the individual had prior chemotherapy. The absolute number of T cells that have the transgene likely determines the mechanism of action and will define the ultimate dose required. In animal models of leukemia, the investigators have determined the need to administer about 50-fold more cells than are required with lentiviral vectors.

Because their goal is to conduct a Phase 0 trial, the investigators hope to be able to look at effects of a small dose in primary operable breast cancer patients. Although participants with cutaneous metastases would be ideal for this trial, the investigators encounter hardly any such patients each year in their clinic.

#### **E. Public Comment**

Dr. Borrer suggested replacing the discussion of participants' blood cells on page 2 of the informed consent document with language that will better convey that participants are unlikely to benefit from this clinical trial. She also suggested defining some of the more technical terms, such as the discussion on page 4 about T-cell injections into a vein.

#### **F. Synopsis of RAC Discussion and RAC Observations and Recommendations**

##### Clinical and Trial Design Issues

- The safety of this approach is predicated on the hypothesis that transient expression of the CAR will limit the risk of toxicity and that intratumoral injection of these T cells will not hinder the ability to completely resect the tumor. Because some of these research participants will be cured by standard therapy, the excised tumors in the first few participants should be carefully analyzed to make sure that the injection does not alter the surgical margins or tumor volume, which would change the relative risk of the protocol.
- Breast cancer tumors express c-MET at different levels and, appropriately, only potential participants whose tumors express c-MET will be enrolled. The level of c-MET expression required for enrollment has not yet been determined but should be set at the highest level feasible, given the need to recruit a sufficient number of participants to complete the trial in a timely manner.
- The safety of this approach is predicated on the limited expression of the CAR. The protocol should include collection of pharmacokinetic data documenting transient expression of the CAR, as is being done in the trial that is testing mesothelin-specific RNA CAR T cells.

##### Ethical, Social, and Legal Issues

- It is important in this early trial to make clear to potential research participants that they are unlikely to obtain any clinical benefit by participating in this study. This is particularly critical in this trial because it will most likely not have direct benefit to patients with potentially curable tumors. Examples of possible wording are available in the NIH Guidance on Informed Consent for Gene Transfer Trials at [http://oba.od.nih.gov/rdna/informed\\_consent\\_intro.html](http://oba.od.nih.gov/rdna/informed_consent_intro.html).
- The current informed consent document includes language regarding the possibility of these modified cells to have an "improved ability to attack breast cancer cells." Such statements can be misconstrued to imply potential therapeutic benefit and should be changed.

#### **G. Committee Motion 5**

Dr. Fong summarized the RAC recommendations be included in the letter to the investigators, expressing the comments and concerns of the RAC. It was moved and seconded that these comments be approved

by the RAC. The RAC voted to approve these summarized recommendations by a vote of 19 in favor, 0 opposed, 0 abstentions, and 1 recusal.

## **XI. Update on Discussions Regarding IBC Review of Low-Risk Protocols**

Presenter: Dr. Corrigan-Curay

### **A. Presentation**

Dr. Corrigan-Curay discussed the role of IBCs in review of human gene transfer trials, feedback from some investigators, a potential proposal for exemption of certain gene transfer trials from IBC review, the next steps for the IBC exemption proposal, and the OBA proposal regarding selection of protocols for in-depth public review.

The role of IBC review in human gene transfer trials is to identify and manage biosafety issues raised by gene transfer agents, including horizontal or vertical transmission risk, safe handling and administration, ensuring that the informed consent document incorporates information regarding risks that arise from the biological nature of the agent, examining the preclinical animal data that support the safety of the vector, identifying new biosafety issues through analysis of adverse event reports, and, for protocols that undergo in-depth public review by the RAC, ensuring that the RAC recommendations are considered.

Feedback from some investigators regarding IBC review of multisite trials has noted that a number of gene transfer clinical trials are conducted using vectors for which there is considerable clinical experience and the associated biosafety risks are well characterized. Multiple individual IBC reviews of low-risk trials may add little benefit to protect public health and can be costly. The conclusion was that a mechanism to streamline the review of low-biosafety-risk trials is needed to facilitate research, especially for multisite trials.

As a result of this feedback, the OBA is considering exempting multisite Phase II or Phase III low-risk trials from IBC review. IBC review would not be required if the vector is a plasmid or a specified non-integrating vector derived from a Risk Group 2 virus and if a previous safety study in humans tested the proposed dose for the Phase II or Phase III study. In addition, the prior safety study should have resulted in no unexpected toxicities related to the investigational agent using the same delivery method at the dose proposed, the concomitant interventions must be comparable to the previous Phase I safety study or the previous Phase II study, and the study populations must be comparable.

Gene transfer trials grouped by delivery system indicate that retroviruses make up 27 percent and adenoviruses make up 24 percent of the delivery systems used with naked DNA at 19 percent and DNA complex, poxviruses, AAV, herpes simplex virus, and other delivery systems comprising the remaining 30 percent of delivery systems used. These figures indicate that almost 50 percent of human gene transfer protocols could benefit from this change if they are in Phase II or Phase III and if they are using the same dose, population, and delivery as used in prior-phase studies.

Further specification of the following criteria would be developed by a RAC Working Group:

- Which non-integrating Risk Group 2 viral vectors will be considered low biosafety risk
- Whether “unexpected” toxicities will be based primarily on the absence of a dose-limited toxicity with the propose dose(s) to be tested in the Phase II or Phase III study What would be considered comparable concomitant interventions
- The definition of comparable study populations, including immune status, age, and geographic/infectious disease background

According to the proposal, a trial that meets all the criteria could be exempt from IBC review under the *NIH Guidelines*, although an IBC retains the discretion to review the trial in accordance with institutional policy. Though exempt, the protocol would still be required to register with the OBA in accordance with

the requirements of Appendix M, and the principal investigator would remain responsible for all reporting requirements under Appendix M. Reporting to the IBC would not be required under the *NIH Guidelines*, but institutions could establish their own reporting requirements in accordance with institutional policy.

The RAC Working Group will continue to refine this proposal and will present a final proposal to the RAC at its meeting in March 2012. The RAC recommendations will be considered by the NIH, and if they are accepted, a proposal will be published in the *Federal Register* for public comment.

To streamline the RAC review process, the OBA has been considering the selection process for trials reviewed publicly by the RAC and proposed a change to the initial review process. Currently, 15 percent to 20 percent of protocols are selected for indepth public review. Protocols are selected by the OBA if at least three members of the RAC recommend public review because of novel scientific, clinical, or ethical issues. The OBA proposes to change that threshold such that it will only accept a recommendation for public review if the recommendation is made by at least 20 percent of RAC members, a change that could be implemented immediately if the RAC concurs. This change could reduce the number of protocols selected for public reviewed.

## **B. RAC Discussion**

Dr. Fong summarized the task of the RAC discussion as determining whether it is reasonable to shorten the process of getting to a Phase II or Phase III trial by exempting a vector that is fairly well known, has gone through Phase I or Phase II human trials, has been reviewed by an IBC at one or more major institutions, and has shown no unexpected toxicities. He summarized the requested result of the discussion as whether the RAC should authorize a subcommittee to author a document that would formalize this proposal.

Dr. Kanabrocki suggested that registration, rather than exemption from local review, be pursued. Two rationales were that some institutions do not have access to an IBC and that not enough is known about some of the viral vectors currently in use. Dr. Chatterjee agreed, noting that AAV is categorized as Risk Group 1 but that new emerging AAV serotypes are untested. Dr. Kanabrocki further stated that a central IBC with an expedited registration process—with additional IBC involvement in quick reporting of adverse events regionally and locally—might be a satisfactory resolution.

Dr. Fost suggested using the IRB model, in which each institution does not need its own IBC but can defer to another institution's IBC. Dr. Corrigan-Curay pointed out that the rules of what constitutes an IBC state that each IBC must include two nonaffiliated local members, but it might be possible to state that certain protocols would be allowed to use a central IBC without nonaffiliated local members. Dr. Fost noted that such a change would be consistent with the evolving changes in the Common Rule. Dr. Kohn agreed with the idea in which a well-informed central IBC conducts a review and institutions can accept that review or choose to constitute their own IBCs.

Dr. Fong reiterated that this discussion of exemption centers around the bottom line that the concomitant interventions must be comparable to previous Phase I safety studies or Phase II trials and that the study population that is proposed for exemption must be comparable to the population that has been studied before.

Dr. Roizman stated that he is in favor of review by a national IBC with the local institutions then able to decide whether they want to conduct their own reviews.

Dr. Fong acknowledged that the RAC is having difficulty with the issue of the effect on other areas of use for vectors and genes used for vaccination that are granted blanket exemptions.

## **C. Public Comment**

Nancy Jones, Ph.D., NIAID, identified herself as part of a group that submitted a proposal to consider whether certain recombinant agents have safety characteristics for which no additional concerns exist.

She requested that the RAC continue to consider whether there might be certain classes or stages of investigation at which an agent is so well characterized that the local IBC review does not add anything substantively different from what would already be covered by other regulatory authorities, such as the FDA and the local IRB review.

Mary Enama, Vaccine Research Center, NIAID, opined that the exemption decision should be based on whether the findings are related to the fact that an agent is a recombinant DNA vaccine, as opposed to safety concerns in general. Whether a particular agent would need to continue to undergo IBC review should be based on whether those types of findings are related to the nature of recombinant DNA.

## **XII. Update on T-Cell Immunotherapy Targeting Human Cancer Antigens**

Presenter: Steven Rosenberg, M.D., Ph.D., NCI

### **A. Presentation**

Dr. Rosenberg provided an update on the NCI program and followup of research participants who have been enrolled on several trials. He provided a brief background on cell transfer therapies for cancer, which have many advantages compared to other forms of therapy. Once cells with antitumor activity are identified, it is possible to grow them *in vitro* to very large numbers. As many as  $6 \times 10^{11}$  cells have been given to cancer research participants. It is possible to select cells that have a high affinity for tumor recognition prior to administering the cells, and removing the immune cells from the body offers an opportunity to provide a favorable microenvironment prior to administering the cells. That manipulation makes it possible to eliminate T regulatory cells, myeloid-derived suppressors, and other cells that might inhibit antitumor immune reactivity.

Prior research conducted by Dr. Rosenberg and colleagues dealt with tumor-infiltrating lymphocytes (TILs) identified with antitumor activity. These trials, with a median of more than five years of follow-up, have shown that when administering a cyclophosphamide and fludarabine lymphodepleting regimen prior to administering TILs, it is possible to mediate substantial regressions of tumors with about a 50 percent response rate and, in some cases, complete regression in participants with advanced disease. Of the 93 research participants in one trial, all but five had previously had at least one systemic treatment for their metastatic cancer. No relationship was found between the bulk of disease and likelihood of having a complete regression. When 200 Centigray of whole-body irradiation was added, the complete remission rate went up to 20 percent, five out of 25 patients in one trial, and none of those participants has experienced a recurrence as of over five years of follow-up. In the last pilot trial of 25 participants in which research participants were given 1,200 Centigray of whole-body irradiation and then stem cells, the complete regression rate in participants with metastatic melanoma was 40 percent; all but one remain ongoing beyond four years and are likely cured. The conclusion is that, with lymphodepletion and the administration of antitumor T cells, it is possible to cure patients with large burdens of metastatic disease. These studies in melanoma showed that T-cell-based immunotherapy can be used to mediate long-term durable regression of large vascularized tumors regardless of prior therapy. The challenge was to improve the treatment for patients with melanoma and extend this treatment to additional cancer types.

Studies in a transgenic melanoma model using B16 melanoma-specific T cells showed that putting the single-chain IL-12 gene into transgenic T cells that recognize a melanoma antigen could substantially increase the antitumor effects of these transferred T cells and eliminate the need for IL-2; the B16 melanoma was eliminated completely. To take advantage of this striking finding in the mouse, Dr. Rosenberg and colleagues developed a clinical trial that began in 2011 in participants with metastatic melanoma who received TILs that were transduced with a single-chain IL-12 using an inducible promoter. This method was developed to have IL-12 produced only when the T cell was activated through reaction with its T-cell receptor.

The trial involved participants for whom it was possible to generate TILs with antitumor activity. They were stimulated with Orthoklone OKT3, transduced, and expanded. Subjects were given a conventional

cyclophosphamide and fludarabine preparative regimen, which causes about an eight-day depletion of T cells and myeloid cells before the subject's counts recover. Before the counts recover subjects are given a single infusion of TILs. One of the problems with this trial is the difficulty of predicting which TILs will have antitumor activity; only about half of the TILs have been demonstrated to have antitumor activity by virtue of their ability to mediate tumor regression under these conditions. Under this slow dose escalation, the investigators have not seen any unusual toxicities. The current cohort is receiving  $1 \times 10^8$  TILs, and the next level is  $3 \times 10^8$ .

One participant dosed on this trial had multiple lung metastases predosing. He had received a conventional TIL treatment ( $3 \times 10^{10}$  of his normal TILs with seven doses of IL-2 following nonmyeloablative chemotherapy); all the tumors expanded. After the same cells were transduced with the gene for IL-12, virtually all of the tumors disappeared. This individual received 1,000-fold fewer cells ( $1 \times 10^8$ ) and no IL-2, but he has undergone a complete regression of his lung metastases, which continues to the present time. No off-target toxicity has been seen with these TILs.

One potential target for extending this result to tumors other than melanoma is the cancer testis antigen NY-ESO-1. The more than 100 cancer testis antigens are expressed during fetal development but are not expressed in normal adult cells, with the exception of the male testes. They are upregulated in 10 percent to 80 percent of common epithelial cancers. Dr. Rosenberg and colleagues began their studies of these cancer testis antigens by studying the NY-ESO-1 family of antigens, and they recently began a trial with a MAGE family of antigens, starting with melanoma. Seventeen melanoma subjects were treated by lymphocytes expressing the gene for a NY-ESO-1 T-cell receptor. The objective response rate has been 47 percent. Three of the 17 research participants have had complete regressions, all of which are ongoing, and five had partial regressions, two of which are ongoing, demonstrating that it is possible to treat solid cancers with this approach.

The next step was to treat patients with synovial cell sarcomas. Ten subjects, all of whom had at least three prior regimens of high-dose chemotherapy or radiation therapy before being enrolled in this trial have been treated to date. Eight of the ten participants have had objective regressions of their metastatic synovial cell sarcoma. This 80 percent response rate was the first result in a solid tumor other than melanoma that showed regression using this genetic modification of normal lymphocytes. Disappointingly, all of these regressions were partial and, with the exception of one ongoing partial regression at 15 months, the participants went on to experience tumor recurrence.

Dr. Rosenberg and colleagues decided to improve upon this prior result by taking advantage of the observation that IL-12 substantially reduces the number of cells needed and does not require giving IL-2 systemically to sustain the cells. This trial, OBA protocol 1103-1097, titled "Phase I/II Study of Metastatic Cancer that Expresses NY-ESO-1 Using Lymphodepleting Conditioning Followed by Infusion of Gene Engineered Lymphocytes Cotransduced with Genes Encoding IL-12 and Anti-NY ESO-1 TCR," was reviewed by the RAC in March 2011 (but not selected for public review). The first research participant has been dosed, but there are no results yet.

Another trial was begun using targeting of MAGE/A3, which is a commonly expressed cancer testis antigen. Because Dr. Rosenberg and colleagues were not able to generate T cells that recognize MAGE/A3 from humans, they immunized an A2 transgenic mouse with a MAGE/A3 peptide, cloned the mouse cells, identified high-affinity T-cell receptors, cloned the mouse T-cell receptor into a retroviral vector, and then used that mouse T-cell receptor to treat humans. This trial was a dose escalation study to target an antigen expressed on many common epithelial tumors. Because no off-target toxicity had been encountered with NY-ESO-1, the investigators started at  $1 \times 10^{10}$  cells and then escalated to  $3 \times 10^{10}$  cells, using cells that were highly specific for small-cell lung cancers, melanoma, and non-small-cell lung cancers with high degrees of specificity. A dose of  $3 \times 10^{10}$  cells given to participants with melanoma resulted in an ongoing complete response of lung metastases and no toxicities. However, when the dose was escalated to  $7.9 \times 10^{10}$ , one research participant who had an ongoing partial response developed white matter changes and became comatose for unknown reasons. Two other participants experienced neurologic problems but have recovered completely. Five of the nine research participants have had objective responses, including three that are ongoing.

The investigators put this trial on hold to analyze what happened. It is possible that the T-cell receptor, which is derived from a mouse, recognizes something that looks like the MAGE/A3 peptide but is not the MAGE/A3 peptide. MAGE/A3 is a potentially exciting target that is highly expressed in patients with esophageal cancer and many other common epithelial cancers, so finding a full answer to this unfortunate result is important.

Dr. Rosenberg reported on the results of seven research participants in a trial reported in December 2010 for treatment of B-cell lymphomas and CLL using an anti-CD19 CAR. The first participant was dosed twice (due to recurrence at seven months after the first infusion) and has an ongoing partial regression of his follicular lymphoma. The second participant died of H1N1 influenza four days after dosing. Of the remaining seven research participants, six have had dramatic regressions of their B-cell lymphomas with ongoing partial responses at the 10-month time point. This experimental treatment results in elimination of normal B cells, although the T cells and NK cells rebound. Given that B-cell loss has been well tolerated following the use of rituximab and immunoglobulin G, Dr. Rosenberg and colleagues are continuing to use this regimen to treat participants with B-cell lymphomas.

A recently approved trial will seek to dose participants with glioblastomas, about 30 percent to 50 percent of which have an epidermal growth factor receptor variant-III activating mutation that is unique to the tumor and is not present on any normal tissue; this mutation is likely essential for the malignant phenotype of the tumor. The investigators have not dosed any research participants but are looking for individuals with recurrent glioblastoma following resection and radiation therapy; such glioblastoma patients experience 100 percent mortality at that stage of their disease.

Dr. Rosenberg and colleagues also have begun research using gene modification to attack the tumor stroma rather than the tumor itself, because the stroma is critically important to tumor growth. In preclinical models, the investigators developed a gene transfer approach to target vascular endothelial growth factor receptor 2 (VEGFR2). Antibodies to VEGF can interfere with tumor angiogenesis and, when combined with chemotherapy, can extend survival in patients with metastatic colon cancer by approximately four months. The investigators hypothesized that if the cells that over-express VEGFR2 could be destroyed, then the tumor vasculature might be destroyed more effectively. Published findings reported that in mouse splenocytes transduced with an anti-VEGFR2 vector (which encodes a CAR that recognizes the VEGFR2 molecule), five different BALB/C mouse models showed inhibition—in melanoma, colon cancer, a sarcoma, a gastrointestinal cancer, and a renal cancer. This finding was surprising because it is unusual to find anything that affects multiple tumor types.

In additional research using a vector expressing the anti-VEGFR2 CAR administered with IL-12, complete tumor elimination in five different tumors in two different mouse strains, B6 and BALB, was observed with some long-term survival of 120 days to 150 days. These mice are cured, so the investigators would like to extend this approach to humans. Meanwhile, they have started a dose escalation study of the VEGFR2 CAR alone in participants with widely metastatic cancers that are refractory to standard treatments. With a slow dose escalation and testing in a variety of cancers, they have not seen any antitumor responses yet, only one research participant experienced toxicity but recovered completely, and no off-target toxicity has been attributable to this CAR.

## **B. RAC Discussion**

Dr. Fong asked how subjects are recruited to enter single-subject escalation trials. Dr. Rosenberg remarked on the tension when dealing with patients with metastatic cancer, because they come to these trials as a last resort, having been through multiple treatments that have failed. The tension is between wanting to offer something that is safe and wanting to offer something that has a chance of being at least somewhat effective. He and his colleagues try to minimize the number of research participants who receive ineffective doses. Their usual plan is to enroll one participant at each dose and keep escalating. Once any toxicity higher than grade 2 is encountered, six research participants are enrolled at that cohort and any subsequent cohorts have a minimum of three participants.

In response to Dr. Fong's query about the minimal mouse dataset that is acceptable to move forward for testing in humans, Dr. Rosenberg noted that many biochemical cytotoxic agents have similar but not identical effects in mice as in humans. For example, IL-2 alone does not work in the mouse but it can cure five percent to ten percent of patients with metastatic melanoma. Many of the target antigens are major histocompatibility complex restricted, so the mice do not have those restriction elements; even when the restriction element is added, it is variably expressed in other tissues. Therefore, a certain minimum needs to be done in animal models to show that the agents are not directly toxic, but careful dose escalations in humans are the most efficient way to move forward. These patients with widely metastatic cancer have limited life expectancies. It is unclear how predictive the mouse antibody will be compared to the human anti-VEGFR2 antibody.

Dr. Rosenberg agreed with Dr. Fong that a different paradigm of clinical trial approval should be considered—approval should be based on target rather than organ, especially at the Phase III level. The antigen expression is more important than the anatomic site of origin of the tumor.

Responding to Dr. Fong's question about how these data will be operationalized, Dr. Rosenberg explained that the process of creating a new drug for each patient—taking the patient's own cells, converting them, and giving them back—does not fit into the usual business model of pharmaceutical companies. He expressed frustration by the fact that immunotherapy, which was shown in the recent clinical trial to have cured 40 percent of research participants, has not been embraced commercially. He posited that blood banks and hospitals might have to embrace this therapy by receiving the apheresis product, transducing the cells, and returning the transduced cells back to the patient for reinfusion.

### C. Public Comment

No public comments were offered.

### **XIII. Discussion of a Serious Adverse Event on Protocol #937: Vaccination with Lethally Irradiated Autologous Myeloblast Admixed with Granulocyte Macrophage-Colony Stimulating Factor Secreting K562 Cells (GM-K562) in Patients with Advanced MDS or AML After Allogeneic Hematopoietic Stem Cell Transplantation**

Sponsor: Glenn Dranoff, M.D., Dana-Farber Cancer Institute  
Ad Hoc Presenters: Cynthia Dunbar, M.D., NHLBI, and Amy Klion, M.D., NIAID

#### **A. Presentation**

At least 23 trials have administered irradiated K-562 cells transduced with the gene for GM-CSF to approximately 400 research participants, many of whom have received more than one vaccination. In addition, at least 48 studies have administered a tumor vaccine consisting of irradiated tumor cells transduced with the gene for GM-CSF to approximately 1,400 participants. No comparable SAE has been reported on these trials. Prior to the SAE on OBA Protocol #937, 32 participants with either acute myelogenous leukemia or myelodysplastic syndrome had received vaccinations as part of this protocol.

One participant developed a significant and persistent skin reaction to the first vaccination. A similar vaccine reaction was not seen with the next three vaccinations. A significant rise in the peripheral white blood cell count occurred over several weeks with a predominance of eosinophils, and the elevation in the white blood cell count did not decrease significantly in response to steroids or hydroxyurea. After a biopsy of the vaccination site found persistent K-562 cells, nilotinib was started and the eosinophil count dropped significantly. However, the participant developed a number of medical complications, including pulmonary infiltrates progressing to acute respiratory distress syndrome, positive blood cultures for enterococcus and coagulase negative staphylococcus, positive PCR for respiratory syncytial virus from the upper respiratory tract, and renal failure and cardiac complications as evidenced by EKG abnormalities, elevations in serum troponin T, and hypotension. Unfortunately, despite intensive medical care, this research participant died.

The autopsy revealed that the cause of death was an acute, extensive myocardial infarction caused by intramyocardial small vessel thrombi containing eosinophilic debris. Extensive cardiac mural thrombi with numerous eosinophils and Charcot-Leyden crystals also were present. Although this individual had positive blood cultures, the autopsy did not show evidence that infection was causative in the death. K-562 cells were found only at the site of the first vaccination on autopsy. Although 80 percent to 90 percent of the cells were necrotic, viable cells were found.

Dr. Dranoff noted that there was no evidence that the irradiation protocol used for the K-562 cells was not followed, and the cells recovered did show sensitivity to radiation. He also reviewed the serum GM-CSF levels during the event, which became extremely elevated, peaking at approximately 60 ng/ml before nilotinib was administered. Data were also presented regarding several unique factors of this individual's immune system that may have led to impaired immune-mediated killing of K-562 cells, including impaired natural killer cell function.

The RAC heard from two expert consultants from the NIH, Dr. Dunbar and Dr. Klion. The discussion addressed a number of questions, including why only the cells from the first injection remained and were viable, whether the donor cells had unique characteristics that led to a hyper-responsiveness to the GM-CSF (this individual had 100 percent chimerism), and how the unique immune reconstitution of this individual may have contributed to this event. Dr. Klion raised a question regarding whether this event could be used to refine future protocols by identifying research participants who might have impaired immune systems that would put them at high risk for an event such as this. Uncovering immune criteria that could be used to identify an individual at risk of this rare reaction was determined to be difficult based on this single data point. However, Dr. Dranoff noted that rapamycin, which was used to prevent graft-versus-host disease in this research participant, can also impair natural killer cell function, and whether to use rapamycin in these protocols may need to be considered. This case also highlighted the importance of careful monitoring of lymphocyte counts, in particular absolute eosinophil counts. Dr. Klion noted it may be prudent to collect whole blood and fix it so that, if such an event were to reoccur, it would be possible to look at eosinophil activation by flow cytometry and to determine the source of GM-CSF production if serum levels are elevated. Another question raised by this case is, with a rising eosinophil count, when nilotinib should be considered. It was discussed that steroids are an appropriate initial therapy but nilotinib might be used; however, it was also noted that nilotinib can have cardiac toxicity. Extrapolating from this one case regarding optimum management was determined to be difficult. In addition, the persistence of viable K-562 cells after radiation was surprising, and there was considerable discussion regarding revisiting the radiation protocols to determine whether any changes are warranted.

This case highlights the benefit of close monitoring of eosinophil counts in research participants on similar trials and, given the small number of participants on any one trial, it may be beneficial to share such data among researchers conducting these protocols. Given that this is a single case and that many research participants have received GM-CSF transduced K-562 cells without such a reaction, the discussion did not result in any specific recommendations for protocol design.

After discussions with the IRB and the FDA, Dr. Dranoff stated that one option would be to continue this clinical trial, especially if the Hematologic Malignancies Group was comfortable moving forward.

## **B. RAC Discussion**

Dr. Kohn noted that his original concern about this adverse event was that it may have been caused by a radiation failure, but the documentation indicates that that was not the case. Because radiation kills cells logarithmically, there will be events in which not all cells are killed. Impressed by reading the documentation of the radiation and the standard operation procedures and monitoring of the site, he asked whether this commendable level of documentation was common at other institutions that test vaccines and whether the FDA reviews procedures at institutions that apply to conduct a vaccine radiation trial. Dr. Gavin responded that institutions conduct validation studies showing that the process to be used is sufficient to kill the cells. As part of the IND and as part of the validation of the irradiation, the FDA does look at the submitted materials.

In response to Dr. Chiocca's query, Dr. Dranoff reiterated that the primary finding of all the data is that something was different about this donor-host combination compared to the rest of the donor-hosts, but that "something" has not yet been discerned.

Dr. Fong asked what intervention might be done differently to stop the thrombotic event that killed this individual if it were to occur again in another research participant. Dr. Klion stated that she believed the investigators did everything that could have been done. The first intervention would be to give steroids to lower the eosinophil count acutely; the investigators did that and it did not work. Hydroxyurea is effective at lowering eosinophil counts, although it takes several weeks; the investigators tried that but it was not effective. It has been documented that the thrombosis cannot be prevented – heparin, Coumadin, and aspirin do not work; there are too many eosinophils and clotting occurs. Apheresis does not work— eosinophils in the blood represent only 10 percent of what is in the body, so the removed eosinophils are immediately replaced. Dr. Klion suggested that radiating the arm might be effective, since radiosensitivity was seen *in vitro*.

Dr. Dranoff noted that this participant was by far the most immunosuppressed participant in this trial. He acknowledged that the investigators considered radiating the arm. In retrospect, he thought the investigators would start erlotinib much earlier; every other usual response was tried, to no positive effect. Dr. Dranoff pointed out that the riskiest time from the immunosuppression is directly after transplant. A total of 84 other people who received this transplant have not shown any negative effect, so it is not possible to conclude anything definitive with one event.

If this event were ever to occur again, Dr. Klion suggested fixing whole blood and then looking at the lymphocytes, eosinophils, and neutrophils all at once. Dr. Dranoff acknowledged this helpful suggestion.

With regard to Dr. Fong's query about irradiation, Dr. Dranoff acknowledged the bottom-line thought that has troubled the investigators about this case: Either something went wrong with the irradiation that has not been picked up or what is known about irradiation is incomplete.

Dr. Chatterjee wondered if the individual who died was the only participant in the group who had two transplants. Dr. Dranoff responded that three participants had two autologous transplants each, and the other two have not experienced this problem. This participant also is not the only participant to be given rapamycin. He reiterated that a single clinical variable does not account for the SAE that occurred.

Ms. Dresser asked the investigators what they plan to tell future participants in this trial, assuming it goes forward. Dr. Dranoff explained that the trial's current informed consent document explains that there is no guarantee that the tumor cells that are irradiated will be 100 percent destroyed. The investigators would tell the potential participants exactly what happened in the proper lay language, because this toxicity is one that people must know about before they consider entering into a clinical trial. Every one of the research participants enrolled in this trial knowing there is a ten percent chance of death from the transplant, but they agreed to participate because this trial offered their only chance at any long-term survival. Patients treated with other major clinical advances also face the possibility of death associated with their experimental treatment. Dr. Dranoff emphasized that investigators try to minimize the risk based upon what is known and try to make sure that research participants are informed; it is then up to them to decide whether they want to assume the risk of a clinical trial.

Regarding Dr. Yankaskas' question about the best time after steroids to add erlotinib, Drs. Klion and Dranoff answered that erlotinib should be administered as soon as a problem is noticed. For example, a white cell count of 20,000 after a local reaction with some eosinophils present would warrant administering erlotinib. Dr. Klion further suggested choosing an eosinophil count cutoff number, using the median and confidence intervals for eosinophil counts.

While acknowledging this one possible risk, Dr. Dunbar suggested that it would be acceptable for the trial to go forward but with incredibly careful monitoring, gathering some of the data discussed at this meeting, and making sure that the human leukocyte antigen types are disparate enough that T-cell rejection can occur. These research participants are going through an experimental and dangerous therapy anyway,

even without this new potential risk, and their risk of dying from graft-versus-host disease is significantly greater than the risk of dying from K-562 cells.

Dr. Klion agreed that this trial could go forward, adding that it would be important to collect samples to be able to answer some of the questions about the origins of the GM-CSF with the expectation that eosinophilia will occur in some people. Why this person had such a dramatic outcome may be related to starting erlotinib too late, possibly because something else activated his eosinophils. The investigators should determine what is occurring in response to the dosing by using eosinophil flow to look at whether the eosinophils are activated. Whole blood should be collected and fixed for flow later on if this event recurs. PBMCs should also be collected.

Dr. Kiem noted that this research participant was a high-risk patient, and the risk of dying from something else was much higher than the risk of dying from K-562 GM-CSF.

Dr. Dranoff noted that if the investigators encountered another case in which eosinophil cells behaved like this, this trial would not be worth continuing.

### **C. Public Comment**

No public comments were offered.

### **XIV. Closing Remarks and Adjournment**

Dr. Fong thanked the RAC members and the OBA staff and adjourned the December 2011 RAC meeting at 4:35 p.m. on December 14, 2011.

*[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they 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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Yuman Fong, M.D.  
Chair  
Recombinant DNA Advisory Committee

**Attachment I: RAC Roster**  
**Recombinant DNA Advisory Committee**

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### **LIAISON REPRESENTATIVE**

**FAYL**, Gilbert, Ph.D.  
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## Attachment II Public Attendees

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

Gwen Binder-Scholl, Adaptimmune  
P.J. Brooks, National Institute on Alcohol Abuse and Alcoholism  
Karen Byers, Dana-Farber Cancer Institute  
Mark Charette, NIH  
Boro Dropulic, Lentigen  
Heather Embree, Lentigen  
Mary Enama, NIAID  
Hildegund C.J. Ertl, The Wistar Institute  
Nancy Jones, NIAID  
Lloyd Klickstein, Novartis  
Laura Mamounas, National Institute of Neurological Disorders and Stroke (NINDS)  
David Maybee, FDA  
Claudia Mickelson, Massachusetts Institute of Technology  
Ronald Mitsuyasu, UCLA  
Richard Morgan, NCI  
Robert Riddle, NINDS  
Lupe Salazar, University of Washington  
Jude Samulski, University of North Carolina  
Rita Sarkar, NHLBI  
Chun-Pyn Shen, Adaptimmune  
Danilo Tagle, NINDS  
Ramjay Vatsan, FDA  
Steven Winitsky, FDA  
Ran Zhang, NINDS  
Yeji Zhang, Rush University Medical Center

*(plus 4 other individuals whose names were not readable on the sign-in sheets)*

### Attachment III Abbreviations and Acronyms

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AAV	adeno-associated virus
CAR	chimeric antigen receptor
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
CTL	cytotoxic T cell
DSMB	data and safety monitoring board
EBV	Epstein-Barr virus
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
GCSF	growth colony stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GOG	Gynecologic Oncology Group
GTSAB	Gene Transfer Safety Assessment Board
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem cells
IBC	institutional biosafety committee
IGFBP-2	insulin-like growth factor binding protein-2
IRB	institutional review board
IV	intravenous
MABEL	minimum anticipated biological effect level
MRC	Medical Review Committee
mTOR	mammalian target of sirolimus (or rapamycin)
NCI	National Cancer Institute
NHLBI	National Heart, Lung, and Blood Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
NINDS	National Institute of Neurological Disorders and Stroke, NIH
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PK	pharmacokinetic
qRT-PCR	quantitative reverse-transcriptase polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
scFv	single-chain variable fragment
sh5	short hairpin (catalytic) RNA directed to CCR5
TCR	T-cell receptor
Th1	T helper immunity
TIL	tumor-infiltrating lymphocyte
TNBC	triple negative breast cancer
TNF	tumor necrosis factor
UCLA	University of California, Los Angeles
VEGFR2	vascular endothelial growth factor receptor 2