RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 10, 2015

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

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[Note: The latest Human Gene Transfer Protocol List can be found on the Office of Biotechnology Activities website at http://osp.od.nih.gov/office-biotechnology-activities.]

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

March 10, 2015

The Recombinant DNA Advisory Committee (RAC) convened for its 141st meeting at 8:00 a.m. on March 10, 2015, at the National Institutes of Health (NIH), Building 35, Conference Room 620/630, Bethesda, Maryland. Dr. Donald B. Kohn (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 3:41 p.m. on March 10, 2015. The following individuals were present, either in person or by teleconference, for all or part of the March 2015 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Tianxi Cai, Harvard University *(via teleconference)*
Paula Cannon, University of California, Los Angeles
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School
Kevin Donahue, University of Massachusetts Medical School
Rebecca Dresser, Washington University School of Law
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Angelica Hardison, Georgia Regents University
Patrick Hearing, Stony Brook University
Howard Kaufman, Robert Wood Johnson Medical School/Rutgers, The State University of New Jersey
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Donald Kohn (RAC Chair), University of California, Los Angeles
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago
Richard Whitley, University of Alabama at Birmingham
Dawn Wooley, Wright State University
Laurie Zoloth, Northwestern University *(via teleconference)*

NIH Office of Biotechnology Activities (OBA)

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

Nonvoting Agency Representatives

Kristina Borror, Office for Human Research Protection, NIH
Denise Gavin, U.S. Food and Drug Administration (FDA)

NIH/OD/OSP/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Maureen Montgomery

[1] The Recombinant DNA Advisory Committee is advisory to the 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.

Chris Nice
Marina O'Reilly
Gene Rosenthal
Aparna Singh

Attendees

There were 57 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains a list of RAC members, nonvoting agency and liaison representatives, and attendees present for the bioethics discussions. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 8:00 a.m. on March 10, 2015. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* was published in the *Federal Register* on February 20, 2015 (80 FR 9277). 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 five gene transfer protocols, and RAC review and discussion of proposed updates to the NIH Informed Consent Guidance for Human Gene Transfer Trials.

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 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 RAC Meeting, December 11, 2014

RAC Reviewers: Professor Dresser and Dr. Hearing

Professor Dresser and Dr. Hearing found that the minutes accurately reflected the business and the discussions that were conducted during the December 2014 meeting. Dr. Ross pointed out that the research described on page 8 of the minutes was conducted by Dr. Rossi, not Dr. Ross. No other changes to the minutes were noted or suggested.

A. Committee Motion 1

Dr. Kohn asked the RAC to approve the minutes of the December 11, 2014, RAC meeting with the stated correction. The RAC voted unanimously by voice to do so.

III. Review and Discussion of Human Gene Transfer Protocol #1412-1364: Phase I Study of Intracranial Injection of T Cells Expressing Human Epidermal Growth Factor Receptor 2 (HER2)-Specific Chimeric Antigen Receptors (CAR) in Subjects with Glioblastoma (iCAR)

Principal Investigator (PI): Nabil Ahmed, M.D., M.P.H., Baylor College of Medicine

RAC Reviewers: Dr. Atkins, Dr. Curry, and Professor Dresser

A. Protocol Summary

Glioblastoma (GBM) is the most common primary brain malignancy of adults (about 30 percent of all brain malignancies in adults) and is usually rapidly fatal. Approximately 20,000 individuals are newly diagnosed with GBM in United States each year, and the annual prevalence of GBM has been showing an upward trend in this country. The current standard of care for newly diagnosed GBM is gross surgical resection, followed by adjuvant radiotherapy and chemotherapy. In the best circumstances, surgery may provide significant tumor debulking, but the majority of tumors cannot be completely resected due to the highly infiltrative nature of GBM. More than 50% of patients relapse in four to 12 months, and almost 85% relapse within two years. The extent of surgical resection is frequently limited by the location of the tumor and complexity of the neighboring brain areas. After surgery, radiation remains the most effective adjuvant therapy. A randomized controlled trial of radiotherapy alone versus radiotherapy with concomitant and adjuvant temozolomide (TMZ) for patients with GBM showed that survival was higher for patients assigned combination treatment, but only by 2.5 months (14.6 versus 12.1 months). For newly diagnosed patients, the median survival is generally approximately 1 year from the time of diagnosis, and most patients die within 2 years. Relapse is inevitable in almost all patients with some exceptions, mostly in younger and pediatric patients. There are significant toxicities with TMZ as well as surgery and radiotherapy. Thus the current standard treatment aims primarily at preserving the quality of life and is considered largely palliative. Given these disappointing results, it is imperative to develop new therapies that will improve survival. Targeted immunotherapy has this potential and warrants further study. The proposed research will use T cells that have been modified to recognize a protein on the surface of GBM cells called HER2 (human epidermal growth factor receptor 2). HER2 expression is seen during development and is mostly turned off after birth. It becomes reactivated during tumorigenesis. HER2 is a cancer-specific protein that is expressed in at least 2/3 of GBMs but not in normal body cells.

This Phase I dose-escalating clinical trial will evaluate the safety of intracranial administration of autologous T cells expressing transgenic CARs targeting the HER2 molecule in patients with progressive recurrent or refractory HER2-positive GBM after standard of care interventions. The modified cells will be administered into the tumor or tumor resection cavity at three dose levels: 1.0×10^7 cells (dose level 1), 3×10^7 cells (dose level 2), and 1×10^8 cells (dose level 3). These modified T cells will be given directly through a special catheter ending in the patient's tumor or the cavity created by removal of the tumor. Patients will be treated in the clinic and monitored closely for several hours after infusion. The team will assess the safety, persistence, and anti-tumor activity of the infused T cells. The goal of this research is to determine whether these modified T cells are effective at fighting GBM.

To date, genetically modified T cells have been infused into more than 500 subjects by using the same or a similar retroviral system. The investigators have infused (via intravenous administration) more than 40 subjects with HER2-positive tumors using the same or similar constructs introduced into CD19 CAR T cells. Of the 40, nineteen research subjects with HER2-positive sarcoma were administered the same CAR T cell product that is planned for use in this trial; the other 21 were research subjects with GBM, and were administered a similar, but CMV-specific HER2-CAR T cell construct. Side effects have been mild, consisting primarily of transient fever. Intracranial injection has been achieved in more than 170 subjects with doses at or higher than what has been used previously. Use of an indwelling catheter has a very low risk of complications; the most common complication is migration of the tip of the catheter, and infection, which has been reported in less than 0.5 percent of patients.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included that it will be the first trial to administer second generation HER2-specific CAR (HER2-CAR) T cells intracranially. Administration of second-generation CAR T cells intracranially may raise unique risks due to the potential for significant expansion of the cells and the inflammatory response that could result from that anti-tumor activity.

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

Dr. Atkins noted that patients must be at least 18 years old to be eligible for this study but that the protocol discusses cell processing modification for minor patients (i.e., those less than 18 years old). Professor Dresser similarly noted that some materials say that the trial will be conducted only with adult subjects while Appendix M says that children may be included. Phase I trials addressing conditions occurring primarily in adults usually involve adults first. If minors will be enrolled, the reasons for their inclusion need to be provided and should be compelling. Professor Dresser also questioned whether children, if eligible, will be old enough to have some understanding of the trial they are entering. These points need to be clarified and reconciled with respect to the proposed trial.

Additional detail about the in vitro testing of cytotoxicity against HER2 expressing cell lines is needed, including how this testing relates to killing of autologous tumor, whether this test is suitable for the proposed research, and how the 20 percent cytotoxicity threshold was chosen.

Dr. Atkins asked whether the surgery to resect the tumor and insert the infusion catheter will be done in the relapse setting and, if so, whether this is considered a standard of care.

The intervention will be done after debulking surgery, but it is not clear whether any measurable disease will be present at the time of treatment.

Additional information regarding administration of dexamethasone (up to 2 mg/day) should be specified, including how often relapsed GBM patients can be tapered down to this dose of dexamethasone and whether use of this agent is expected to cause problems for patients.

Drs. Atkins and Curry asked about proliferation of T cells within the tumor and associated risks of significant cytokine release and cytokine release syndrome (CRS) within the central nervous system (CNS). If CRS development in the CNS is anticipated, how will it be monitored and managed, and does the planned approach require systemic or intra-CNS treatment?

Dr. Atkins also asked about the expression of *Her-2* in normal brain or other cells in the brain following surgery, whether T cells will proliferate in vivo when they recognize antigen, and whether retreatment will require additional surgery or involve re-generation of the autologous HER2-CAR T cells or there will be “leftover” cryopreserved HER2-CAR T cells.

Drs. Atkins and Curry questioned whether 4 hours are sufficient for an initial post-procedure monitoring period, given the activity that has been seen with CAR T cells in other diseases and the direct intracranial intratumoral injection. Although it is difficult to predict the time course of cytolysis in this setting, which is unlike intravenous (IV) delivery and may allow for more immediate and concentrated exposure to targets, observation (overnight) of up to 16 or 24 hours should be considered. If not, the rationale for not having patients remain at the hospital overnight should be delineated.

Dr. Curry noted that monitoring of “cerebral function” is included in the description of “neurological assessment” in the protocol. Additional detail as to how cerebral function will be monitored should be given, and this evaluation should be done objectively. For instance, the mini–mental status exam is a quantifiable measure that would supplement the other listed components of the exam.

Dr. Curry requested clarification regarding the following issues involving study eligibility:

- Are patients eligible for treatment at the time of any recurrence (e.g., first, second, third)?
- A Karnofsky Performance Status (KPS) score of 50 percent is a low threshold for eligibility. A low KPS is associated with diminished survival; patients requiring this much assistance typically are not eligible for clinical trials in glioma, particularly those who have invasive components. The prospect of a patient at his or her third recurrence of GBM who has a KPS of 50 percent opens the possibility of enrolling some very frail subjects.
- Is there a minimum fractional requirement of HER2 expression in the tumors? The literature shows variability in this parameter. A tumor with very low percentage expression of the target may not be a representative test of toxicity.

- The protocol states that HER2-CAR T cells are to be infused via a single catheter into either a resection cavity or an unresected tumor. However, no distinction is made for the surface area of the lining of the cavity or the volume of the tumor or the cavity. The dispersion of the cells may differ significantly in different scenarios and the microenvironments may differ substantially based on tumor burden. Can this be made more uniform and designed to maximize interaction between CAR T lymphocytes and target cells?
- One criterion for eligibility is as follows: "Available autologous transduced T lymphocytes with (about) 15 percent expression of HER2 CARs determined by flow cytometry, and killing of HER2-positive targets (about) 20 percent in cytotoxicity assay." How were these thresholds chosen, and how do they compare to those used in previous studies, both in the investigators' experience and in the CAR T cell literature? If expression of HER2 in the tumor is variable and idealized cytotoxicity against known HER2-expressing targets is 20 percent, is there a risk of assessing the safety of a functionally inert therapeutic agent?

Professor Dresser focused her review on the informed consent document (ICD). In general, a larger font size and greater use of an active voice would make the document easier for prospective subjects to follow.

Professor Dresser commented that characterizing and referring to the study intervention as a "treatment" could result in patients believing that the intervention has a good chance of benefitting them, which is unlikely. She suggested using the term "modified cells," "study procedures," or other words that clarify the intervention's investigational status. In the ICD section on subject rights, the information on freedom to withdraw from the study should clarify that once the modified cells have been injected, they cannot be removed from the body.

Professor Dresser noted the concern among RAC members that the 15-year follow-up statement in the ICD could mislead subjects about their life expectancy. The investigators should consider softening this language with statements such as the following: "To see whether there are any long-term side effects of gene transfer, regulatory authorities require us to follow people receiving cells with modified genes. This follow-up could last as long as 15 years, although it is unlikely that the study infusion will lead to long-term survival."

In the section of the ICD that discusses leftover samples, it would be helpful to note that participants may remain in the study, whether or not they choose to allow sample storage and sharing.

Description of the use of TMZ is difficult to follow. Alternative language such as "You may receive TMZ up to 2 days before you receive the study infusion, but you will then have to wait 6 weeks before you resume TMZ treatment" was suggested. Similarly, some of the information regarding HER2-specific side effects is confusing. For example, it is not clear whether the statement that starts with "If low doses of HER2 CAR T cells worked" relates to the death noted in the prior statement or describes a second risk. It would be helpful to clarify this point and revise the information to be better understood by a layperson.

Use of the term "surgical removal" instead of "surgical resection" would be easier for laypersons to understand.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found their questions and comments to be largely addressed by the investigators.
- Dr. Atkins requested further clarification regarding the plan for patients who do not have measurable disease about 4 weeks after debulking surgery, which is when the investigational agent will be given. Dr. Curry asked whether a patient could be excluded if he or she had too large a tumor or too much residual disease.
- Dr. Curry asked whether all patients with recurrent disease will be required to have a craniotomy for resection or whether placement of a catheter into a presumed recurrent tumor will be

acceptable for this research. In addition, he asked about experiments in which T cells were injected into the cerebrospinal fluid (CSF).

- Dr. Curry considered the change in the KPS cutoff for eligibility to more than 60 percent (from more than 50 percent) to be an important safeguard that will minimize risk to subjects. The higher level will help screen out more frail patients, such as those with multiple GBM recurrences.
- Professor Dresser found the revised statements regarding the 15-year follow-up to be clearer and more relevant to the patient cohort and the changes to the potential benefits language to be appropriately circumspect.
- Dr. Whitley advised the investigators to consult the FDA to ensure that the protocol is approved for all planned uses of the modified cells, particularly reinfusions, to avoid potential problems that could arise as the study proceeds.
- Dr. Kohn noted that patients in CD-19 CAR trials occasionally develop atypical CNS symptoms that are not well understood. It is not clear whether these cases are cytokine-related (e.g., post-CRS). Dr. Kohn recommended collecting CSF for cytokine analysis and having a formalized plan to monitor cytokines should any similar or other clinical cases present themselves in the proposed study.
- Dr. Kiem asked about the extent to which the 2-mg doses of dexamethasone allowed under this study might affect T-cell survival.
- Dr. Zoloth commented that the proposed research is very promising and important for the development of potential treatments for GBM. She commended the investigators on the revised ICD.

D. Investigator Response

1. Written Responses to RAC Reviews

Regarding eligibility criteria, the investigators clarified that the protocol will enroll only adults (age 18 and older); references to enrollment of minors and individuals under age 18 are incorrect and will be removed. Research volunteers must have recurrent or refractory GBM, and its resection must be medically indicated to participate in this study. Since repeat resections of locally recurrent GBMs are commonly performed, the investigators do not want to exclude potential study participants with multiple recurrences as long as they meet the other eligibility criteria. A paper highlighting the utility of multiple resections was submitted with the response to the reviewers' comments. The team recently completed a clinical study (RAC/OBA #0903-970) in which GBM patients received IV administration of cytomegalovirus (CMV)–specific T cells that were genetically modified to express HER2-CARs. That trial had no problems accruing patients, which allowed for a dexamethasone dose of up to 2 mg/day for relapsed patients, as planned for the proposed trial. The investigators agree with the concerns regarding the KPS threshold and have revised the inclusion criteria to require subjects have a KPS of at least 60 percent. This is the same KPS cutoff as another protocol in which the safety and efficacy of locally injected IL13Rα2-CAR T cells are being evaluated. The minimum fractional requirement for HER2 expression in tumors has been clarified to have more than a grade 2 intensity score. The tissue grading and staging system was adapted from Gilbertson et al. (*Br J Cancer* 71[3]:463-467, 1995); details of the parameters for grading and staging were provided in the response to the reviewers' comments. Pathological reading will be performed by a dedicated neuropathologist and an investigator on the proposed trial (Suzanne Powell, M.D., chief of neuropathology at Houston Methodist Hospital).

The procedure for generation of autologous HER2-CAR T cells yields up to 1×10^9 cells in a period of 10 to 15 days. The cells are then tested and cryopreserved. The cryopreserved cells retain up to 90 percent of their vitality and functionality for several years. There will a sufficient number of leftover cells for more than one infusion.

The investigators acknowledged that the parameters for infusion of HER2-CAR T cells (e.g., surface area of the lining of the tumor cavity, volume of the tumor or the cavity) ideally would be defined at the time of study entry. They noted, however, that it is very difficult to implement such stratification at enrollment due to the heterogeneity in tumor configurations, especially at relapse. These criteria will be included in the analysis of the secondary aims, including the response to T-cell infusion. The ability of T cells to migrate

to tumor deposits should mitigate some of the concerns raised. Relevant publications from the research team and others were attached to the response. The surgery to resect the tumor and insert the infusion catheter will be done in the relapsed setting. Repeat resection (whenever possible) is a standard of care. The insertion of a catheter, while not uncommon, is not standard of care and is thus included as an experimental intervention in both the protocol and the ICD. Because HER2-CAR T cells are a genetically modified product, research subjects will be infused only when measurable disease is demonstrated on imaging. The investigators noted that HER2 is not expressed in detectable levels by normal postnatal human brain cells. In another clinical trial conducted by this team, HER2 expression was not detected in normal brain tissue in surgically excised samples from patients with recurrent GBMs ("CMV-Specific Cytotoxic T Lymphocytes Expressing CAR Targeting HER2 in Patients with GBM [HERT-GBM]").

The threshold of 15 percent CAR transduction and 20 percent cytotoxicity are the same release criteria as on the team's three prior RAC- and FDA-approved protocols by using the same HER2-CAR. Two of these studies are ongoing. The initial results of one study (RAC/OBA #0903-969) have been accepted for publication in the *Journal of Clinical Oncology 33:1688 (2015)*. Briefly, the data show that a safe dose of HER2-CAR T cells can be established for patients with advanced stage sarcomas (highest dose tested: $1 \times 10^8/m^2$) and that these cells can traffic to tumor sites and persist at low levels for more than 6 weeks in a dose-dependent manner without dose-limiting toxicities. Of 17 evaluable patients, four had stable disease for 12 weeks to 14 months. Three of these patients had their tumor removed, with one showing more than 90 percent necrosis. The median overall survival of all 19 infused patients was 10.3 months.

Regarding the T-cell product in RAC/OBA protocol #0903-969, a median of 65.2 percent (range: 36.2 to 88 percent) of T cells were positive for HER2-CAR expression as determined by fluorescence-activated cell sorting analysis. In addition, in a standard 4-hour ^{51}chromium-release cytotoxicity assay, HER2-CAR T cells had significant cytotoxic activity against HER2-positive target cells (median: 48.3 percent; range: 30.5 to 70.5 percent), whereas non-transduced T cells did not. The investigators plan to use the same HER2-CAR T-cell manufacturing procedure for the proposed protocol. The investigators noted that based on their experience, if HER2-CAR T cells can kill 20 percent of tumor cells within 4 hours, the cells should be able to completely control tumor growth in a 48-hour co-culture assay.

T cells are expected to proliferate within the tumor upon antigen encounter and will produce immunostimulatory cytokines. However, rapid CD19-CAR T-cell expansion, as seen in lymphodepleted patients with high disease burden, which can result in CRS, is not expected in this trial, because patients in the proposed trial will not undergo lymphodepletion, and the tumor burden will be small to minimal post-resection. In addition, several investigators have infused T cells into resection cavities of brain tumors, and none have reported such toxicity (as summarized in the protocol). Because the specific risk of CRS after intracranial infusion of HER2-CAR T cells is unknown, the protocol includes guidelines for the prompt diagnosis and management of CRS that will follow a recently published algorithm that reflects a consensus of such measures at five major cell therapy centers (Lee et al., *Blood,* 124[2]:188-195, 2014). The management plan will include intravenous injection of dexamethasone, which rapidly crosses the blood brain barrier and has been successfully used to control inflammatory processes and edema in the CNS. Another agent, tocilizumab, has also been used successfully in reversing CRS; tocilizumab is an antibody (anti-IL-6 receptor antibody) that depletes IL-6 and works very quickly, in some cases within 2 hours. The investigators noted that intracranial administration of medications to treat CRS is not anticipated.

As recommended by the reviewers, the protocol has been revised to extend the initial post-infusion monitoring period from 4 hours to overnight observation that includes a formal objective neurological assessment every 4 hours (and as needed) by an on-call practitioner per the team's institutional guidelines.

The investigators agree that use of the terms "therapy" and "treatment" to describe the study intervention can be misleading. The protocol and the ICD have been revised throughout to use words that clarify the investigational nature of the intervention.

The investigators explained that the “third-party” voice is mandated by the Baylor College of Medicine Institutional Review Board (IRB), as are the font type and size (12-point Arial), and that certain protocol specific-content is included in the consent template and cannot be changed. The investigators agreed to revise and update the ICD to reflect the other additional suggestions by the RAC reviewers, including simplifying or clarifying terms, descriptions of procedures, and minor edits.

In response to specific comments about the ICD, the language on long-term follow-up has been revised to read, “To see if there are any long-term side effects of gene transfer, regulatory authorities require us to follow people receiving cells with modified genes. This follow-up could be as long as 15 years or until death of the research volunteer.” The section of the ICD titled “Potential Benefits” has been revised as indicated by the underlined text: “The benefits of participating in this study may be: that your immune system may begin to kill the cancer cells. This could make the cancer grow more slowly, or get smaller, or go away for a while. This benefit is <u>at best only</u> possible, and <u>may not happen to you</u>. Your participation may help the investigators better understand how the immune system can fight this disease. However, you may receive <u>no benefit from participating</u>.”

2. Responses to RAC Discussion Questions

GBM is a microscopic disease, and GBM cells are mobile. To receive the investigational product, patients must have some measurable disease on imaging (gadolinium-enhanced magnetic resonance imaging (MRI)) following debulking surgery. The proposed study does not have a pre-specified “maximum” amount of residual disease that would exclude a patient. The design of this pilot-style dose escalation trial should help guide the investigators in refining the eligibility criteria and other aspects of the study (e.g., stratification by disease characteristics) to inform future protocols. As previously noted, factoring in the volume and surface area of the resection cavity is difficult. Resection cavities vary in size, and the potential effectiveness of the planned intervention relies in part on the mobility of T cells. For example, experiments in mice show that T cells injected into the left caudate nucleus traveled to the right caudate nucleus (about 8 mm) within 3 to 6 days. Experiments have also been done in which the modified T cells were injected into the CSF to target the ependymomas, another region that expresses HER2; tumors were eliminated in the majority treated mice. An older study involving medulloblastoma (a pediatric cerebellar tumor) showed an innate attraction of HER2-specific T cells to the tumor locality via chemokine signaling between tumor cells and endothelial cells. Results showed that T cells injected on the opposite side of the brain traveled through the superior peduncle into the tumor.
The proposed study does not include a dedicated surgery arm, and patients with recurrent disease will be eligible for the study only if they will, by conventional therapy, receive another craniotomy. Thus, patients will be a priori determined to be candidates for craniotomy (or subsequent craniotomy).

Regarding availability of cells for patients who undergo repeated infusion, the PI commented that production of the modified cells is very robust. About a billion cells are generated from a single blood draw of less than 90 mL, which is equivalent to six to eight doses for each research subject.

Dr. Ahmed noted that two changes to the KPS cutoff have been made: The KPS criterion was changed to more than 60 percent at the time of T-cell infusion (when patients are postoperative) and to 70 percent at procurement. Dr. Curry agreed that these values, including the 60 percent threshold, reflect reasonable levels of patient functionality and performance.

The investigators recognize the importance of monitoring CSF cytokines to manage and learn from patients who have clinical complications and to evaluate differences between patients who respond versus those who do not respond to the intervention. Dr. Ahmed noted that the protocol originally included a plan to regularly monitor CSF by having participants return to the clinic for CSF sampling. However, as neurosurgeons, the investigators had concerns about technical problems that could arise with the study procedure, including the location and any movement of the catheter tip, and potential risks to subjects with repeated sampling (e.g., infection). In the end, the team decided to remove this additional monitoring.

Dr. Ahmed commented that 2 mg dexamethasone could affect the T cells, but not substantially. The preference is not to use dexamethasone, which is a very strong medication, but use of the agent as

outlined in the protocol is expected to be safe. Other cytotoxic T lymphocyte (CTL) studies have shown a response in some patients on dexamethasone. In addition, the team has experience with patients who had an adoptive T-cell transfer and were on 2 mg or less of dexamethasone, and did have responses.

E. Public Comment

Dr. Borror noted that the ICD does not appear to describe dose escalation or its purpose and asked whether the investigators plan to add information about this aspect of the study to the ICD. Dr. Ahmed replied that the ICD has a brief description of the incremental doses that will be used in the study and that he will elaborate on the dose escalation plan and describe the three different doses.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- The HER2-specific CAR T cells will be infused into the tumor cavity of participants who are undergoing resection for relapsed disease. The inclusion criteria should be explicit that patients are only eligible for this study if they would be undergoing a craniotomy procedure as part of the standard of care; the craniotomy would not be done for the trial.

- It is stated in the protocol that additional doses of HER2-specific CAR T cells using intracranial administration may be an option for some research participants in this trial. To facilitate implementation of this plan, consider discussing this redosing plan with the regulatory bodies at the time of initial approval and add more information on redosing patients to the protocol and the informed consent document.

- In trials using anti-CD19 CAR T cells for hematologic malignancies there have been several cases of neurologic toxicities, a number of which were associated with other systemic symptoms of cytokine release syndrome (CRS), such as hypotension and fever, and others that appear to be primarily neurologic in nature (e.g., mental status changes, seizure, and encephalopathy). The etiology of these neurologic toxicities are not well understood. Since participants will have intracranial catheters, if participants experience neurologic toxicities you should consider measuring cytokines in the cerebral spinal fluid (CSF) if possible, to determine whether the neurologic toxicities are related to the same cytokines that are associated with CRS. This would likely inform this trial and the field.

Ethical/Legal/Social Issues

- Research participants in this trial first sign a procurement consent to collect blood for T cell product generation prior to signing a second consent for participation in T cell infusion. It should be clarified in the procurement consent that T cell infusion will only occur if the participant continues to meet the eligibility criteria for the study, including the presence of residual tumor after tumor resection.

- This is a dose escalation trial, but that is not clear in the informed consent document. You should consider adding additional information about this design (i.e., a more explicit discussion of the dose escalation).

G. Committee Motion 2

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 14 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IV. Review and Discussion of Human Gene Transfer Protocol #1501-1378: A Phase I/II Open Label Clinical Trial Evaluating the Safety and Efficacy of MAGE-A10c796T in Patients with Advanced Breast Cancer

PI:	Edith Perez, M.D., Mayo Clinic
Sponsors:	Marcia Gaido, Ph.D., and Bent Jakobsen, Ph.D., Adpatimmune, LLC
RAC Reviewers:	Drs. Cannon, Kiem, and Ross

A. Protocol Summary

Breast cancer is the most frequently diagnosed cancer in women and ranks second as a cause of cancer death in women (after lung cancer). An estimated 233,000 and 2,360 new cases of invasive breast cancer were expected to be diagnosed in women and men, respectively, and approximately 40,000 breast cancer deaths were expected in the United States in 2014. Overall, 61 percent of breast cancer cases are diagnosed at a localized stage, for which the 5-year survival rate is good (99 percent). However, 20 percent to 85 percent of women treated for localized disease will ultimately relapse with distant metastases. Second- and third-line treatment options for locally advanced non-resectable and metastatic tumors are limited. For patients with metastatic breast cancer, the prognosis is poor, with a median overall survival of approximately 2 years and a 5-year survival rate of 24 percent. Although outcomes for patients with small primary breast cancer can be reasonably good, therapeutic treatment options for patients with non-resectable refractory locally and metastatic tumors are limited. This limitation, in turn, is associated with poor prognosis and highlights the need for development of new biologically based therapies.

Melanoma antigen family A10 (MAGE-A10) is a cancer testis antigen that has restricted expression in normal tissue and is expressed across a range of solid tumors at varying frequencies, including melanoma, bladder cancer, non–small-cell lung cancer, liver cancer, and gastric cancer, and at a high frequency in breast cancer. In a subset of cancers, expression of MAGE-A10 has been shown to be associated with advanced disease and poorer outcomes. The study investigators hypothesize that incorporation of optimal higher-affinity T-cell receptors (TCRs) specific for cancer testis antigens, which have enhanced efficacy against antigen-expressing tumor cells, into autologous CD4 and CD8 T cells of patients with advanced breast cancer will allow immune recognition of the tumor antigens and consequently delay progression or promote regression of the tumor. One such affinity-enhanced clone is called MAGEA10^{c796}. MAGE-A10^{c796}T cells are autologous CD4 and CD8 T cells that have been genetically modified to express an affinity-enhanced TCR directed against a peptide of MAGE-A10 that is present at high frequency in breast cancers but has limited expression in normal tissues. The MAGE-A10 TCR has been affinity optimized to enable detection of the low levels of antigen present on immune-edited tumors. Spontaneous CTL responses to MAGE-A10 have been observed in melanoma against the peptide to which MAGE-A10^{c796} is directed.

Previous clinical trials using the same method of TCR affinity enhancement and T-cell manufacturing (selection, expansion, and transduction) as planned for the proposed study have been evaluated by the sponsor, Adaptimmune, including one directed against a different peptide in MAGE-10 (MAGE-A3ca3aT). The prior trial employing MAGE-A3ca3aT cells was closed after two serious adverse events (SAEs) resulted from off-target recognition of a peptide in the protein titin and ended in death related to the cardiac toxicity (presented at the June 19, 2012, RAC meeting, and reported in Linette et al., *Blood*, 2013). Based on these events, an enhanced screening program was discussed with the FDA and found satisfactory to allow the first-in-human testing of a new affinity-enhanced TCR specific for a different target.

The proposed research is an open-label dose escalation Phase I/IIa trial in adults with locally advanced or metastatic breast cancer whose tumor expresses the MAGE-A10 tumor antigen. Subjects who meet the eligibility criteria will receive autologous genetically modified T cells expressing enhanced TCRs that are

specific for MAGE-A10 (i.e., MAGE-A10^{c796}T). The trial will evaluate three doses of transduced cells administered with a standard dose of the cytoreductive chemotherapy by using an adaptive 3+3 dose escalation design. Participants in group 1 will receive 1×10^8 (plus or minus 20 percent) transduced cells, those in group 2 will be given 1×10^9 (plus or minus 20 percent) transduced cells, and those in group 3 will receive 5×10^9 (range: $1–6 \times 10^9$) transduced cells. Upon enrollment, subjects will undergo leukopheresis for T-cell collection, and their cells will be genetically engineered and expanded ex vivo. Before receiving T cells, subjects will undergo cytoreductive chemotherapy with cyclophosphamide. The cell product will be administered as an IV infusion (day 0). Once the maximum tolerated dose (MTD) is established, the target enrollment for this trial will be approximately six subjects treated and evaluated for safety at the MTD. To be considered evaluable, a subject must receive the target dose of engineered cells per cohort. Unevaluable subjects will be replaced. A second infusion of MAGE-A10^{c796}T cells may be given, but only to subjects who have documented progression of disease following response to the initial infusion and whose tumor continues to express the appropriate antigen target.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included that it will be the first-in-human clinical trial of an affinity-enhanced TCR specific for a MAGE target.

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

Dr. Cannon raised the following questions and issues regarding the protocol and study design:

- How was the target peptide in MAGE-A10 chosen?
- No animal studies have been performed to evaluate the safety of this TCR, but it is not clear why this has not been possible (e.g., there is no analog of human MAGE-A10 in rodents or primate models).
- Why was expression of MAGE-A10 on at least 50 percent of the patient's tumor cells chosen as an eligibility criterion? Is there a concern that treatment could simply select for the less-than-50 percent MAGE-A10–negative cells? Does loss of MAGE-A10 occur in breast cancer? Is it itself a marker of tumor aggressiveness?
- This will be the first-in-human study with MAGE-A10^{c796}T. A 3+3 dose escalation study design will be used, with 12 patients to be enrolled. The rationale for the three doses is provided. Were any adverse events (AE) seen at the lowest dose (1×10^8) in the prior Mage-A3ca3aT TCR trial, which is the same as the lowest dose for the proposed trial?
- A second infusion is described as an option for subjects initially responding to the therapy but then relapsing. The protocol does not specify how long this option would be available or how or whether an additional infusion would be done for relapsed subjects without any stored cells. Dr. Cannon also asked whether levels of expression of MAGE-A10 during progression be compared to the initial tumor, and if so, whether this value would be considered in the decision to move forward (e.g., if the positive percentage had decreased).

Dr. Cannon had the following comments about the ICD:

- Subjects are required to abstain or use birth control starting at the first dose of chemotherapy through four months (males) or 18 months (females) after the infusion of cells and until genetically modified cells are not found in the blood. Since this may be a subject population anxious about conceiving, information on how long the cells are expected to persist should be delineated. Issues related to an extended duration, if applicable (e.g., if cells are still present 5 years out), should be addressed. A reasonable expectation of this timeline should be included in the ICD.
- The protocol states that MAGE-A10^{c796}T is expected to be embryotoxic or abortifacient, but the basis for this statement is not clear. Is this potential toxicity of MAGE-A10^{c796}T due to expression of MAGE-A10 in the placenta? This information should be included in the ICD.
- The terms used in the diagram and the visit descriptions should match each other to avoid confusion. The diagram has some abbreviations that should be defined for subjects (e.g., F/U, I/E).

- The section titled "Heart Complications and Death" incorrectly states that T cells had MAGE-A3 when they actually had an anti–MAGE-A3 TCR. Similarly, in the section "Risks with Genetically Changed T Cells," it is stated that MAGE-A10 will be added to T cells when it is actually the anti–MAGE-A10 TCR.

Dr. Kiem found the protocol and Appendix M overall to be well written. He identified the following issues for clarification and discussion:

- The rationale for the dosing based on the previous MAGE study and the currently ongoing studies with NY-ESO-1c259 should be provided.
- The rationale for the dosing of cyclophosphamide in this setting needs to be delineated, especially in light of the potential cardiac toxicity of cyclophosphamide. The protocol does not specify whether an echocardiographic evaluation will be done before the initiation of the treatment (in addition to the ECG evaluation).
- As also requested by Dr. Cannon, limitations to the use of animal models for this approach and this target need to be clearly delineated.
- Additional information is needed regarding tissue expression of MAGE-A10^{c796}T and why the level targeted in tissue will be presumably sufficient for this study.
- The ICD should also inform patients that they could face significant costs if their insurance does not pay for side effects from the chemotherapy or the infusion of the T cells.
- The ICD should clearly inform participants that once the modified cells have been injected, they cannot be removed from the body.

Dr. Ross found the protocol, answers to Appendix M, and ICDs easy to read and honestly presenting potential risks. However, the section on privacy in Appendix M refers to the University of Texas MD Anderson Cancer Center with respect to Health Insurance Portability and Accountability Act (HIPAA) regulations and HIPAA certification of study investigators, but it does not refer to the Mayo Clinic or the PI, Dr. Perez. Dr. Ross agreed with Dr. Kiem that the ICD needs to explain that once the experimental treatment is completed, it cannot be undone. Participants can choose to withdraw from participating in the study, but the modified cells cannot be removed. The word "patient(s)" is used throughout the ICD, but technically it should read "patient-participant(s)". The section of the ICD that describes screening period 2 can be rewritten to exclude the word "patient," because the subject would be a research participant at this point (suggested revision: "The Study Doctor will let you know whether you can enter this study and, if you are not eligible for the study…"). Since the individual has not yet enrolled and is not eligible, there will be no physician-"patient" relationship.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Several clarifications and minor corrections, primarily to the ICD, were identified. Dr. Cannon noted that the revised ICD does not appear to include specific language regarding the upper limit of six months following confirmed progression for a second infusion. In addition, the ICD does not discuss the potential adverse impact of cyclophosphamide on oocytes and the placenta and the risk of miscarriage with the drug; participants should understand this as a possible risk and a reason that contraception or abstinence should be used. Dr. Zoloth asked whether banking of eggs and sperm would be offered due to potential toxicity of cyclophosphamide. Dr. Cannon asked whether the research intervention could be a fertility-ending treatment in part because of how long the modified T cells may persist. Given the detection limits of available assays, the investigators may wish to consider how to convey in the ICD that while subjects or their partners should not become pregnant while the modified cells can be detected, there is a possibility that the cells may be present at a level that is undetectable; because of this possibility, the risk to future pregnancies is unknown. Dr. Ross recommended that participants be advised to not plan to become pregnant over the course of the study and for 18 months post-treatment. The language in the ICD that discusses what happens if the subject's cancer "gets worse" could be softened so

that it is not so bleak. Drs. Cannon and Kiem noted that the information about payment for research-related injuries is contradictory and needs to be clarified and clearly stated. There is a least one reference to lung cancer instead of breast cancer.

- Dr. Cannon requested further clarification regarding the off-target peptides that could be recognized by the TCRs. The protocol identifies eight such peptides but also states that this motif was present on 41 or 100 different human proteins with a Basic Local Alignment Search Tool search. Furthermore, the literature cited appears to refer to eight proteins, not eight peptides.
- Dr. Cannon noted that clinical experience to date with enhanced TCRs is limited to two trials, one with good outcomes and one not. She asked the rationale for proceeding directly to an affinity-enhanced TCR for a new cancer antigen target as planned in the proposed study.
- Dr. Kiem considered the cyclophosphamide dose and the cardiac screening and criteria to be reasonable and appropriate. However, he questioned whether such a high dose of cyclophosphamide (45 mg/kg over two days) is necessary.
- Dr. Zoloth requested clarification on the exclusion criteria for clinically significant psychiatric illness and social situations that would limit compliance of the study requirements and how those criteria would be interpreted for the purposes of this research. A clearer definition of “socioeconomic situations (or standards)” and the process for how and who judges “patient readiness” need to be delineated. Dr. Zoloth expressed concern that vulnerable women who otherwise would be eligible for the study could be excluded for economic reasons (e.g., lack of family support to meet a rigorous study visit schedule). The exclusion criteria are well articulated but may be too broad.
- Dr. Kohn found the pipeline developed in response to the previous AEs to screen new TCRs going forward impressive. He asked whether an Epstein-Barr virus (EBV) peptide is predicted to be presented and recognized (for human leukocyte antigen [HLA]-B cell line 1501 with EBV transformation) or whether the EBV-transformed cells are recognized by the MAGE-A10 or the NY-ESO–enhanced TCR.

D. Investigator Response

1. Written Responses to RAC Reviews

The peptide MAGE-A10 (peptide sequence GLYDGMEHL) was selected for evaluation based on spontaneous CTL responses to MAGE-A10 specifically against this peptide, which have been detected in a number of HLA-A2 melanoma patients. The MAGE-A10 peptide GLY was identified by mass spectrometry twice in targeted searches on tumor cell lines at Adaptimmune.

The problems with animal testing for establishing the safety of a human TCR are related to cross-species incompatibilities inherent to adoptive T-cell therapy approaches. There currently is no animal model that can suitably evaluate TCR-specific toxicities, because the major histocompatibility complex (MHC) molecules in animals (from rodents to nonhuman primates) are different from those in humans. A comparison of MHC loci between humans and nonhuman primates has found no identical MHC class I alleles between African monkeys (chimps and gorillas) and humans and some conservation of the A and B loci for Old World and Asian monkeys. Thus, human cells will be rejected from immune competent animal models, thereby excluding the use of most spontaneous tumor models and requiring the use of complex immunodeficient humanized animal models. Even in the humanized mouse model, antigen, T-cell trafficking, and MHC expression across various tissues will be different and not representative of humans. In addition, human T cells in the humanized mouse model result in lethal xenograft versus host disease within eight weeks, which masks evaluation of potential toxicity. Engineering a corollary product of an animal TCR for use in animal model testing was considered, but this would not reflect the specificity and safety profile of the human TCR. Therefore, this would also be uninformative. Toxicity studies were considered for nonhuman primates; however, the MAGE-A10 peptide is not completely conserved between primates and humans, which would lead to considerable differences in epitope presentation. The search for a homologous peptide for Mage-A10 in primate sequences failed to identify potentially cross-reacting related peptides. Therefore, off-target screening would be unlikely to be predictive of the human response even if the MHC issue could be overcome. Furthermore, differences between the HLA genes in nonhuman primates and humans may lead to alloreactive or cross-reactive responses that would not be

observed in humans and would therefore be misleading. Given this information, the investigators have concluded that off-tumor interactions between the study drug (human T cells) and tissues in vivo cannot be performed in animals. The sponsor has presented this argument to the FDA, and the FDA reviewers have agreed that animal studies are not informative as part of the preclinical safety package for this product for the above reasons.

Regarding the inclusion criterion of at least a 50 percent level of expression of MAGE-A10 on a patient's tumor cells, the investigators noted that since this is a first-in-human Phase I study, it has not yet been determined how best to define a tumor as "positive" for MAGE-A10. This criterion was set following discussions with pathologists at the appointed central laboratory (QualTek) and involves two key factors: the intensity and the extent of the staining. The intensity of staining is rated on an ordinal scale. For the extent of staining, the pathologist scores only the tumor cells and requires more than 100 cells to evaluate. Since staining may be heterogeneous, the pathologist will review all supplied materials (5 to 10 slides) and give an estimate of the extent of the staining of tumor cells overall. The threshold for inclusion has been set at 2+ or 3+ expression in at least 50 percent of the patient's tumor cells, as this would indicate that MAGE-A10 is clearly being expressed in the majority of tumor cells. Experience with T-cell therapy to date suggests that the amount of antigen present may drive the expansion and activity of the modified T cells; hence, a better response may occur in tumor cells that express more antigens. However, because of the phenomenon of antigen spreading, the immune response initiated by the infused modified T cells may stimulate further anti-tumor response from the patient's natural immune system directed towards other, potentially MAGE-A10–negative antigen-expressing tumor cells. The balance between the activation of T cells by MAGE-A10 antigen and the subsequent effects of antigen spreading on MAGE-A10–negative cells is unknown at present.

Information on MAGE-A10 expression in breast cancer is limited. It is not consistently associated with clinical-pathological features or predictive of prognosis. The expression of cancer testis antigens is known to be heterogeneous and may change over time. Due to the tumor micro-environment, however, the available data do not show a trend toward a loss of MAGE-A10 expression with disease stage. In a subset of cancers, expression of Mage-A10 has been shown to be associated with advanced disease and poorer outcomes.

MAGE-A10^{796}T was evaluated against a panel of 36 HLA-A2 positive tumor cell lines originating from bladder, breast cervix, kidney, liver, lymphoid, ovary, prostate, and skin tissue. MAGE-A10^{796}T efficacy was assessed by interferon gamma (IFNγ) secretion by Enzyme-Linked ImmunoSpot (ELISPOT), and MAGE-A10 expression was confirmed by quantitative polymerase chain reaction (PCR) in both the positive and negative cell lines. The expression levels in many of the tumor samples are comparable to levels in cell lines where T-cell responses were seen in vitro. Mage-A10^{796}T cells secreted IFNγ and showed cytotoxic activity in response to GLYDGMEHL peptide-pulsed antigen-presenting cells (APC) and MAGE-A10–positive target cells, providing support for such responses in vivo against MAGE-A10–positive tumor targets.

The response to the reviewers' comments included a detailed explanation of how the doses for the proposed trial were chosen. In original studies evaluating the sponsor's affinity-enhanced MAGE-A3a3a TCR, the starting cell dose was 1×10^{10} total cells, of which at least 10 percent were transduced. The mean transduction typically was 30 percent to 60 percent, with the target dose translating to a dose of three billion to six billion TCR-engineered T cells. The MAGE-A3a3a study was initiated in 2011; at that time, there had been several TCR-engineered T-cell therapy studies using both native (high- and low-affinity) and engineered TCRs. These studies reported clinically manageable on-target toxicities (in the case of the native TCRs) and no unexpected toxicities, including no off-target toxicities with the affinity-enhanced TCR. In addition, no MTD was identified in any of the studies, despite administration of doses higher than 10 billion and up to 130 billion cells. In 2010, the RAC published considerations for dosing of engineered T cells, which recommended that a starting dose should be relatively safe but have potential biologic activity. Given this experience and guidance, the starting dose of 1×10^{10} cells was selected for the MAGE-A3a3a TCR studies because it was considered safe and above the minimum anticipated biological effect level based on allometric scaling from murine tumor models performed at the time (5×10^{6} cells/animal). Notably, the dose was administered in a split infusion (30 percent on the first day

and 70 percent on the second day) if no acute infusion reactions were observed. There currently is general consensus in the field that a split infusion is not necessary, because acute infusion reactions have not occurred with this technology.

Since 2011, SAEs associated with either off-target toxicity (as with the MAGE-A3a3a TCR) or on-target off-tumor toxicity (as with a murine-derived MAGE-A3 TCR that recognized MAGE-A12 expression in CNS tissue) have been reported. Subsequent to these and other SAEs in studies using chimeric antigen receptor technology, the consensus in the field has evolved regarding how to approach dosing for first-in-human studies toward a dose escalation design. The sponsor of the proposed study, Adaptimmune, has discussed the dosing scheme for this first-in-human study with the FDA, which has confirmed the recommendation to proceed initially with a dose escalation design.

Dose escalation with cell therapy is complex due to the living nature of the product. Once in vivo, T cells rapidly proliferate and have been reported to expand as much as 1,000-fold. Because of this possibility, it is unknown whether a lower starting cell dose in prior studies would have helped reduce toxicity or allowed more time for clinical management of toxicity. Close monitoring of targeted medical events informed by the preclinical safety package is essential to help mitigate emerging events in a timely manner. The investigators note; however, that this approach would not have altered the outcome of MAGE-A3a3a–related toxicity because cardiac toxicity had not been detected in the preclinical screen.

In determining the starting cell number for the dose escalation for the proposed trial with the MAGE-A10^{c796} TCR the following points are taken into consideration: the cleanliness of the target, the specificity of the TCR, and the generation of the receptor (e.g., if there are any additional co-stimulatory domains or other genes that increase cell potency). For the purposes of setting boundaries on the lower dose limit, dose escalation studies using second-generation CARs against high-risk targets (targets with known expression on normal tissue) typically start dose escalation at 1×10^7 cells when using lymphodepleting chemotherapy, which the investigators stated is an extremely low (murine-size) dose. The MAGE-A10 target is considered a very clean target, and the engineered TCR uses natural signaling machinery in the cell. The preclinical screen of this TCR has demonstrated specificity of the TCR. To balance the risk-benefit ratio for patients with advanced cancer, the team selected an initial dose of 1×10^8 transduced cells, which is 1/50 of the ultimate target dose of 5 billion transduced cells, based upon observations from the NY-ESO study, where doses at this level routinely result in robust expansion of the modified cells and objective clinical responses. This dose is likely to be high enough to allow detection of gene-modified cells in the blood (particularly after expansion in vivo), which will enable safety assessment and possible clinical response and will be consistent the RAC's recommendations regarding starting cell dose.

The protocol currently does not specify a time-window for a second infusion. Experience with one engineered TCR T-cell intervention (the NY-ESO^{c259}T cell program) suggests that the option of a second infusion is usually considered around the time of progression (before initiation of alternative treatment). Adaptimmune proposes to restrict the option for a second infusion to six months following confirmed progression post-initial infusion. This will be clarified in the protocol and ICD. A second infusion would first use any modified T-cell product left over from the original manufacture, although this is unusual. Cells are reserved from the initial apheresis for use in a second manufacturing process (modification and expansion). For patients without any stored cells, the protocol allows for second infusion using product from a second apheresis providing there is no persistence of gene-modified cells (from the first infusion) in peripheral blood detected prior to apheresis by PCR.

The levels of MAGE-A10 following progression will not be formally compared to the initial tumor results as a requirement for inclusion or exclusion of a subject to receive a second infusion. However, to move forward with a second infusion, the subject's tumor must continue to express the appropriate antigen target at the same levels specified in the inclusion criteria for study eligibility (at least 50 percent of cells that are 2+ or 3+). These criteria will be confirmed using tissue from another biopsy at or after progression for MAGE-A10 expression. Other factors will be taken into consideration when making a decision about a proceeding with a second infusion.

The investigators noted that the proposed protocol has been designed to incorporate birth control procedures and pregnancy monitoring to protect against any potential toxicity during pregnancy. Cancer testis antigens have restricted expression on normal tissue and have been found in testicular germ cells and placentae. Toxicity against testicular germ cells in men enrolled in this study is a theoretical risk potentially mitigated by the testes being an immune privileged site. The requirement for birth control for males is therefore set in accordance with the cyclophosphamide label, which advises the use of birth control for four months after treatment. The cyclophosphamide label also advises that women of childbearing potential who were exposed to cyclophosphamide during any maturation phase have a risk of failed pregnancy and fetal malformations for potentially more than 12 months after use of cyclophosphamide. MAGE-A10 has a high level of expression in normal placenta; MAGE-A10^{c796}T is therefore expected to be embryotoxic or abortifacient. This information will be included in the ICD. Women of childbearing potential will be required to use acceptable methods of birth control (or abstain) for 18 months and until the gene-modified cells are no longer detected in the blood. As this is the first-in-human use of MAGE-A10^{c796}T cells, however, there are no data on the persistence of these cells T cells. NY-ESO-1^{c259}T cells used in studies of sarcoma, melanoma, and ovarian cancer have been shown to persist consistently in subjects for up to six months after infusion and in many subjects after one year. Longer-term data are available from ten subjects with myeloma in whom at year two or later, two subjects had quantifiable persistence of gene-modified cells and seven patients had unquantifiable but detectable levels. Since the factors governing persistence are not yet fully elucidated and gene-modified cells may persist for a period of several years, the potential need for prolonged use of birth control will be clarified in the ICD.

The study involves risks to patients with preexisting cardiac conditions due to the use of cyclophosphamide and to the effect of cytokine release on cardiac function. The proposed trial will use a cyclophosphamide dose of 45 mg/kg for two days, which is below the doses reported to cause cardiotoxicity in patients. The planned doses are also consistent with doses required to promote efficacy of T-cell therapy. Doses as low as 120 mg/kg have been associated with increased risk of developing cardiotoxicity, and total doses of 180 to 200 mg/kg administered over two to four days have caused symptomatic cardiomyopathy. Several safeguards are in place to minimize these risks.

Patients will be screened by echocardiography or Multi-Gated Acquisition Scan before enrollment and must have a normal left ventricular ejection fraction greater than 55 percent, which is a high-threshold normal function (45 percent is considered acceptable). Patients age 55 or older will also be required to undergo a cardiac stress test per institutional standards. To monitor the effect of cyclophosphamide conditioning on cardiac function and to establish a differential to any infusion-related effects, EKGs will be performed and troponin levels will be monitored at baseline, within seven days of initiating chemotherapy, on the days of chemotherapy, on the day of T-cell infusion (day 0), and post-infusion (days three, four, seven, and 14 and month three). Analysis of EKG results and troponin or creatine phosphokinase (CPK) levels pre- and post-chemotherapy and post–T-cell infusion in subjects in the ongoing NY-ESO study shows that to date, there have been no clinically significant elevations in cardiac enzymes and no cardiac dysfunction.

The reference to the University of Texas MD Anderson Cancer Center was a cut-and-paste error and will be corrected. The study is being conducted at the Mayo Clinic.

The investigators agreed to revise and update the ICD to reflect suggestions by the RAC reviewers, including simplifying or clarifying terms, descriptions of procedures, and other minor edits. Regarding specific comments, the investigators noted that the term “patient(s)” has been changed to “study participant(s)” throughout both the Tissue Screening and Main Consents and that the two now state that once the T-cell treatment is received, it cannot be undone. The investigators clarified that Adaptimmune will pay for medical expenses associated with study-related AEs not covered by insurance. Standard language addressing this provision is included in the Clinical Trial Agreement.

2. Responses to RAC Discussion Questions

Dr. Jakobsen clarified that eight peptides on 41 off-target proteins may be recognized by the TCR that will be used in the proposed trial. He noted that the TCRs isolated for MAGE-A10 are naturally occurring and optimized to reduce risks that occurred with the MAGE-A3 agent. In addition, the protocol includes a comprehensive testing system to maximize safety of the MAGE-A10 study intervention and minimize off-target toxicities. While all risk cannot be eliminated, the design of the test article and safety assessments should significantly reduce the potential for cross-reactivity.

Dr. Perez and Dr. Tom Holdich, medical director at Adaptimmune, noted that the proposed 45-mg/kg dose of cyclophosphamide was chosen based on results of the NY-ESO study, which showed good responses with that dose. The 30-mg/kg dose did not appear to be as effective as the 45-mg/kg dose, while a 60-mg/kg dose was considered too high due to potential risks based on other findings.

Dr. Jakobsen confirmed that Adaptimmune will pay for medical expenses related to AEs associated with the clinical study that are not covered by a subject's insurance.

Dr. Perez explained that to be eligible for the proposed study, patients must understand the information in the ICD and the need for and importance of complying with follow-up visits. This assessment is left to the investigator's discretion and judgment in terms of discussions with the patient. The protocol will not require a psychiatric consultation or determination of whether the patient is competent from a psychiatric standpoint. Dr. Perez underscored the responsibility that physicians take when asking patients for consent to participate in a clinical trial. Part of that responsibility lies in understanding, to the extent possible, whether the patient is going to be compliant with the requirements of the study. The assessments and evaluation plan for the proposed study are consistent with what is done in the team's other clinical trials. The intention is not to be discriminatory in any way, but since the study requires multiple daily, weekly, and monthly visits over an extended period, patients must return to the clinic for ongoing follow-up. This aspect of participation is discussed with each potential participant and is part of the assessment of whether a patient will be able to comply. Dr. Perez acknowledged that this is a difficult issue and a societal problem that the team has tried to address through investigator-patient discussions, physician assessments, and the study criteria. Dr. Kohn pointed out that as a first-in-human safety study, the investigators need to ensure that the subjects can complete the planned safety visits in balance with the patient's right to participate in the research protocol.

Banking of sperm and eggs is not part of the proposed study. It is not known how long the modified T cells will persist. In some studies, the T cells have remained for an extended period. Data from the NY-ESO TCR trial show that for a few subjects, the cells are still detected after more than a year. For most patients, however, the cells cannot be detected at a year post-infusion. Dr. Jakobsen noted that while the TCR assay is sensitive, it is not possible to determine whether there is a central memory T cell somewhere that could suddenly or unexpectedly mount a response. The functional assay would be able to detect only a relatively large number or reasonable percentage of circulating cells. The consent language will be revised to clarify the unknown risk of long-term persistence of undetectable levels of T cells on pregnancy.

Dr. Jakobsen noted that the EBV (or other) peptide that the TCRs might recognize has not been identified yet. EBV might also be activating something else in the T cells. The NY-ESO TCR has not been through the predictive screening assay, but a TCR very similar to the NY-ESO TCR has successfully passed this screening. While the investigators do not want to restrict further testing of products that might have clinical value or potential, given prior experience, it is especially important to proceed with caution.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- The protocol indicates that subjects may have the option for a second infusion of the gene modified T cells if the research participant's disease progresses within six months of the first infusion. The informed consent document should include this information as well.

- The Pregnancy Risks and Birth Control section of the informed consent document would benefit from some additional information and clarification:
    - First, the section states that women should not become pregnant for 18 months after treatment or while the genetically engineered T cells are still in their blood. The 18 months was chosen in part because of the toxicity of cyclophosphamide on oocytes. The risks of cyclophosphamide on reproduction should be added.
    - Second, since women are being instructed to not attempt pregnancy until the genetically engineered T cells are no longer detected in their blood, it would be helpful to provide some information about how long genetically modified T cells have been reported to persist and how often women will be monitored for persistence of the cells.
    - Finally, consider rewording the statement that women may not become pregnant for 18 months after treatment to "women should not attempt to become pregnant."

- You stated that expenses related to research-related injuries will be covered, but the informed consent document currently states, "In the event you suffer a research-related injury, your medical expenses will be your responsibility or that of your third-party payer, although you are not precluded from seeking to collect compensation for injury related to negligence or intentional wrongdoing on the part of those involved in the research." This apparent contradiction should be clarified.

- As written, multiple statements in the informed consent document suggest that research participants will experience disease progression. For example,
    - "You will be in the active part of the study until your breast cancer gets worse with progression of disease."
    - "After your cancer worsens, you will have a CT or MRI scan and a biopsy."
    - "Some patients are able to have a 2nd T cell infusion, after their cancer has gotten worse." It may be more sensitive to address the possibility of progression of disease by replacing statements such as "after your cancer worsens" with a statement such as "if your cancer gets worse ...."

G. Committee Motion 3

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Review and Discussion of Human Gene Transfer Protocol #1411-1357: Phase I, Randomized, Open-Label, Active-Controlled Dose Escalation Study to Evaluate the Safety, Tolerability, and Immunogenicity of INO-1800 Alone or in Combination with INO-9112 Delivered Intramuscularly Followed by Electroporation in Select Nucleos(t)ide Analogue-Treated, Hepatitis B “e” Antigen (HBeAg)-Positive, Chronic Hepatitis B Patients

PI:	Douglas Dieterich, M.D., Mount Sinai
Sponsors:	Ami Shah Brown, Ph.D., M.P.H., and Mark Bagarazzi, M.D., Inovio Pharmaceuticals
RAC Reviewers:	Drs. Kohn, Ross, and Whitley
Ad Hoc Reviewer:	Dr. Marc Ghany

A. Protocol Summary

Hepatitis B virus (HBV) infection is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Despite the availability of effective vaccination in many countries, hepatitis B remains one of the most common infectious diseases in the world. Worldwide, there are more than 240 million individuals with chronic HBV infection (CHB) and an estimated 660,000 deaths due to CHB each year, with about half of these deaths caused by HCC. An important goal of therapy is to suppress HBV replication in a sustained manner that leads to biochemical remission and histological improvement. CHB goes through several stages, distinguished by the presence or absence of a protein called HBeAg in the blood of infected individuals. CHB infection is also defined by the continual presence of an additional protein called hepatitis B “s” antigen (HBsAg). The loss of HBsAg and concurrent appearance of antibodies against HBsAg is associated with a better prognosis, including lower rates of cirrhosis and slower disease progression to liver cancer. An immunotherapy which leads to a strong immune response against HBsAg as well as another protein, hepatitis B “c” antigen (HBcAg), may help to increase this loss.

The two main types of treatment, IFN-α and nucleos(t)ide therapy, have limitations that exist from both clinical and immunological perspectives. Although both types of treatment demonstrate virological (reduction in HBsAg) and biochemical (normalization of liver enzymes) responses, neither treatment offers a high rate of clinical cure. New treatments for CHB that are of finite duration, are well tolerated, and can produce greater and more durable HBsAg loss and seroconversion are therefore urgently required. These concerns highlight the demand for novel, safe, and effective therapeutic options that can generate immune responses that ultimately mediate biochemical remission and histological improvement in chronic HBV patients. Targeting multiple HBV antigens is proposed as an immunotherapy approach to drive viral clearance in chronic HBV infection. The planned trial will use multiple plasmids (in a single product, INO-800) that target HBsAg and HBcAg, either alone or in combination with another plasmid (INO-9112) expressing human IL-12. The addition of INO-9112 is expected to produce very small amounts of IL-12, which in turn are meant to boost the ability of the body to generate immune responses to HBV antigens.

This Phase I, randomized, open-label, active-controlled study will evaluate the safety and tolerability of escalating doses of INO-1800, with or without INO-9112, delivered intramuscularly followed by electroporation (EP) to enhance DNA uptake and expression. Individuals with chronic HBV infection who are positive for HBeAg and have been on treatment with entecavir (ETV) or tenofovir disoproxil fumarate (TDF) for at least 6 months before randomization will be eligible for this trial. Subjects will be randomized to one of three arms: (1) the plasmid expressing HBV antigens, (2) the plasmid expressing HBV antigens and IL-12, or (3) the control arm. Approximately 126 subjects will be enrolled into one of seven treatment groups under arms 1 and 2 or into the active control arm, for a total of 16 subjects per treatment arm and 14 subjects in the control arm. Dosing will start at 0.3 mg INO-1800 (0.1 mg of each plasmid) and progress to 2 mg and then to 9 mg. Each INO-1800 dose level will be tested with and without IL-12; the IL-12 dose will be 0.25 mg. The design includes a standard dosing regimen (five different groups) and an expanded dosing regimen (two different groups) to determine whether there is any improvement in immune response with more rapid dosing and additional doses. Active control subjects (receiving ETV or TDF) will be enrolled in the standard regimen arms. All subjects will remain on entecavir or tenofovir during the study.

B. Written Reviews by RAC Members

Seven RAC members recommended in-depth review and public discussion of the protocol. Key issues included the evaluation of a novel approach for treatment of hepatitis B infection and the unique combination of a multivalent vaccine composed of multiple HBV antigens with a second plasmid that expresses IL-12.

Three RAC members and one ad hoc reviewer provided written reviews of this proposed Phase I trial. Dr. Kohn found the proposed trial to be well designed, with an acceptable dose escalation schema. Appropriate precautions are used in subject selection, including documentation of viral suppression on

current regimen and the exclusion criteria. The ICD is thorough and does a good job of explaining the protocol and what it entails for subjects, the risks, and the unknown prospect for benefit.

The percentage of hepatocytes typically infected by HBV in patients of the type to be enrolled should be specified (e.g., 0.01 percent, 0.1 percent, 1 percent, 10 percent, etc., based on immunohistochemistry of in situ hybridization). Dr. Kohn noted that an effective immune response against infected cells could lead to cytolysis, and if a higher percentage of hepatocytes is at risk, then liver dysfunction could occur. Risks from liver biopsy (bleeding or other) should be added to the protocol if subjects may have this as part of screening tests.

SAEs have occurred from IL-12 administration, either as recombinant protein or from expression systems. Given this possibility, additional information was requested regarding how the amounts of IL-12 plasmid to be given compared to other studies that have expressed IL-12 as an adjuvant and how the levels of IL-12 produced from the plasmid in preclinical studies compared to those reported in patients receiving IL-12 by other methods.

Dr. Ross found the protocol and answers to appendix M easy to read. Her comments focused on issues related to human subject protections. The stopping rules are clear and appropriate. Dr. Ross noted that the list of questions for the participant to review is excellent, and will help potential participants know what they should understand, and gives them the opportunity to ask questions. The ICD is clear, including the discussion of risks and explanation of randomization, but written at a level beyond that of the average layperson. The description of the viral envelope and core in the ICD (under the purpose of the study) is accurate, but the relevance of this information to participants is not clear. Adding the word “inactive” to describe the virus when saying that the immune system will be exposed to parts of the virus may help subjects better understand the gene transfer process. Dr. Ross recognized the sponsors for agreeing to pay for expenses not covered by third parties, as stated clearly in the ICD.

Consistent with the other reviewers, Dr. Whitley considered the study to be well designed and the ICD to be appropriate for the study. In addition, he found the selection of antigens for inclusion to be carefully thought out. Dr. Whitley posed the following issues and questions to the investigators:

- The protocol is dated October 2014. Have any changes been made to the study since then?
- All patients will receive either ETV or TDF as noted, given the potential concern for reactivation of virus. Is there a rationale for having a group of patients who receive no antiviral therapy after the completion of this trial?
- Who will make the decision to advance to the next dosing level? The protocol indicates that this decision will be made internally by Inovio personnel (i.e., by an internal monitory committee), but is there an independent Data Safety and Monitoring Committee that will make this decision? Dr. Whitley also asked whether this decision should be made later than currently proposed; if not, would a later time impede enrollment?
- Under inclusion criteria, the protocol allows for either biopsy or FibroScan®, but should this not be standardized to one or the other?
- Is there a benefit to repeating either the biopsy or FibroScan® at the completion of the study, as an exploratory endpoint?
- The ICD states that the study will be performed at approximately 30 sites around the world. It might be helpful to simply describe this aspect of the study as a multicenter clinical trial.
- Any concerns about the amount of IL-12 produced by the plasmid and enhanced by electroporation should be clearly described.
- Dr. Dieterich should attach a more recent curriculum vitae (CV) than one dated 2013.

Dr. Ghany commented that the protocol is well written and that the primary investigator is well trained and experienced to conduct the research. He noted, however, that a DNA vaccine strategy that requires multiple injections, and the use of painful electroporation is unlikely to find widespread clinical application. With that in mind, he raised some concerns about the ability of the study to recruit subjects who are otherwise doing well on nucleos(t)ide therapy, including the following:

- Will the study be providing the nucleos(t)ide analogue during and after completion of the study?

- If the purpose of the adjuvant is to permit lower doses of the DNA vaccine to be used, why is the low dose regimen not being tested with IL-12?
- With respect to study eligibility and the inclusion and exclusion criteria, what cutoffs of FibroScan® will be used to exclude bridging fibrosis, and how was the DNA cutoff for entry selected? The choice of less than 90 international units (IU)/mL seems somewhat arbitrary; the limit of quantification of the proposed assay (less than 20 IU/mL) should be considered.
- Will patients with delta hepatitis be excluded?
- Is monitoring CPK levels useful for monitoring muscle injury after electroporation? If so, should levels be monitored more frequently?
- Urine pregnancy tests should be performed monthly if there are any concerns about harm to the fetus.
- HBV genotyping is unlikely to be achievable in subjects with HBV DNA levels of less than 90 IU/mL.
- Despite HBV DNA suppression with ongoing nucleos(t)ide analogue therapy, there is a theoretical risk for an HBV-specific T-cell mediated flare, because many patients with complete viral suppression continue to demonstrate HBsAg and, to a lesser extent, HBcAg on liver biopsy. Given this possibility, the protocol should include a plan to manage such a flare.
- The treatment progression scheme provides a comprehensive plan, but details of the specific stopping rules to be used in the planned interim analyses are lacking. This study is relatively large for a Phase I trial, and given the large number of subjects to be exposed, the investigators should consider including an independent data and safety monitoring board (DSMB) to review the safety data.
- The primary and secondary endpoints should be more clearly delineated with respect to what would be considered a positive or negative result.
- It is very unlikely that antiviral resistance will occur; however, because subjects will be monitored for development of viral resistance, a management plan should be outlined.
- The patient ICD should include the results of another HBV vaccine or IL-12 in combination with nucleos(t)ide analogue studies to provide a balanced perspective to the ICD. The benefits of staying on the nucleos(t)ide analogue alone also should be discussed in the ICD.
- The protocol should specify who will be performing the immunological studies (the primary investigator or the sponsor).

C. RAC Discussion

The reviewers found the responses to their questions and comments to be comprehensive and well addressed.

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Dr. Ross noted that the information at the start of the ICD about the virus is still very technical but that her questions, which focused on the ICD, had been answered.
- Dr. Whitley revisited his question regarding the plan to allow for liver biopsy or FibroScan® at screening and whether it would be preferable to do only FibroScans®. Dr. Ghany noted that FibroScan® has been evaluated most widely in patients with hepatitis C and those with micronodular cirrhosis, which is present in persons with hepatitis B. The FibroScan® works by passing a low ultrasound wave through the liver and then measuring the speed of the wave through the liver. In the presence of larger nodules, this test can underestimate the degree of scarring in the liver, which could be an issue when assessing patients for the proposed trial. There is a concern for flares in patients who still have large amounts of either core or surface antigen in their liver. These patients, in turn, are at increased risk of developing decompensation. To address this issue, Dr. Ghany suggested that a platelet count be used in addition to the FibroScan® to decide whether to proceed with a liver biopsy. Those with a platelet count below a predetermined value (e.g., fewer than 150,000) might undergo biopsy, which would provide more specific information about core and surface staining.
- Dr. Kohn asked whether the low platelets in this patient population are due to sequestration or deficient liver production.

- Dr. Ghany was concerned about having an internal committee be responsible for data and safety monitoring of this study, even with the modifications made with the revised version of the protocol. For such a complicated trial and the potential risks, additional consideration should be given to having an outside DSMB. It might be helpful to consult the FDA further regarding this question.
- Dr. Atkins noted that the small percent of patients that are under antiviral therapy have elevated antigens. He asked whether these patients have a drug-resistant virus. If so, how would this be determined, and would these patients be eligible for the proposed trial?

D. Investigator Response

1. Written Responses to RAC Reviews

The prior version of the protocol (version 1.1, dated October 21, 2014) was modified based on comments by the FDA during the 30-day review for the associated investigational new drug (IND) application. The current version of the protocol (version 1.2, dated December 16, 2014) includes a listing of significant changes to the study in the Summary of Changes section of the updated protocol. These changes were also provided in their entirety in the investigators' response to the reviewers' comments.

The literature regarding hepatocyte infection and expression of HBV proteins by infected hepatocytes indicates variable results. Examination for the presence of hepatic HBV protein during chronic infection reveals that even in the absence of suppressive therapy, as low as 2 percent to 3 percent of hepatocytes may be found to actively produce HBcAg. Low-frequency expression of HBcAg was also noted in a study of 39 chronically infected individuals on nucleos(t)ide analogue suppressive therapy for 48 weeks, in which 89 percent of patients showed HBcAg expression in less than 6 percent of hepatocytes; however, HBcAg was expressed in as many as 50 percent of the hepatocytes in the other 11 percent of patients. Expression of HBsAg in chronically infected patients on active suppressive therapy for at least 5 years is similarly low but also variable. One study reported a range of 0 percent to 1 percent of hepatocytes staining positively for antigen, while another study identified two patients on lamivudine for an undefined period exhibiting HBsAg-positive hepatocytes at frequencies of 28 percent and 77 percent. In addition to a lytic response, infected hepatocytes would be subject to the non-lytic arm of the cellular immune response that has previously been established in chimpanzee models to play a role in elimination of HBV covalently closed circular DNA. Production of IFNγ by liver-infiltrating CD8-positive T cells has previously been identified as an important element of an effective cellular immune response in clearing hepatitis B in relevant models of infection and provides a mechanism of viral clearance that does not contribute to liver disease based on destruction of hepatocytes. In contrast with these findings, several other studies of immune therapy in CHB patients have not reported hepatotoxicitiy, including three studies of DNA-based therapy, highlighting the importance of evaluating and comparing the safety of immunotherapy in the setting of effective antiviral suppression.

The investigators postulated that if liver dysfunction were to occur, it would be a result of a tissue-infiltrating immune response in the presence of a high frequency of HBV-infected hepatocytes producing and expressing antigen that would make them targets of HBsAg- and HBcAg-specific CTLs. The proposed trial includes individual subject safety monitoring of liver function, HBV DNA and serology, and the development of any AE throughout the study. All patients will continue their antiviral therapy (ETV or TDF) to minimize the likelihood of acute HBV "flares." The protocol includes stopping rules, which dictate criteria for pausing of further enrollment and administration of the study intervention on a protocol-wide basis. The liver function panel will be reviewed prior to each subsequent dose in all subjects with pre-specified cutoffs for alanine aminotransferase, total bilirubin, and international normalized ratio for blood clotting time to discontinue dosing of an individual participant if any limits are exceeded. In the event that an HBV-specific T-cell–mediated flare is suspected, watchful waiting would likely be the first line of management, because patients are known to flare as they clear surface antigen. If the flare appeared to be leading toward hepatic decompensation, then the study intervention would be discontinued and the treating investigator would be supported in determining the optimal course of clinical management (e.g., immunosuppression in the form of corticosteroids). The sponsor or its designee will perform all immunology assessments.

The protocol has been revised to present a clearer definition of CHB; for eligibility, an individual must be HBsAg positive for at least 6 months before randomization. Screening assessments to determine eligibility include serology testing for the presence of hepatitis D total antibody. Per inclusion criterion c, subjects must have negative hepatitis D serology to qualify for enrollment into the study. Since some degree of variability exists in the criteria for fibrosis, for this Phase I study, investigators will use their clinical judgment and experience in assessing that the FibroScan® result is “without evidence of bridging fibrosis or cirrhosis” to meet the inclusion criteria per the protocol. The investigators noted that while the HBV DNA cutoff value of less than 90 IU/mL may initially seem arbitrary, this DNA level was intentionally set higher than the limit of quantification of the cobas® assay (cobas® is a fully automated PCR-based device) to accommodate for minor fluctuations in HBV DNA level that would not be considered significant virological breakthrough. The cutoff of less than 90 IU/mL represents a value that is roughly midrange among suggested values signifying well-controlled viremia. Since subjects participating in the study must have HBV DNA levels of less than 90 IU/mL at screening and will likely maintain this level of HBV DNA throughout the course of the study if viremia is well controlled, genotyping will be performed indirectly through serology rather than as a sequence-based assessment. HBV genotype will be determined based on serological results obtained by the IMMUNIS® Enzyme Immunoassay, which detects genotype-specific HBsAg epitope combinations. HBV genotyping will be performed as part of the exploratory assessments.

Subjects with well-controlled viral DNA levels are not likely to develop resistance to their antivirals. The proposed trial includes frequent HBV DNA testing, and virological breakthrough is indicated if the increase in HBV DNA is greater than 10-fold above the nadir (defined as the HBV DNA load at screening). In the event that virological breakthrough occurs, a confirmatory sample will be drawn for HBV DNA determination and ETV or TDF resistance monitoring. Subjects who develop confirmed resistance to ETV or TDF will immediately stop receiving study treatments and will be closely monitored during the post-treatment follow-up period for safety and the persistence of ETV or TDF resistant mutation(s). The investigators commented that a general management plan in the case of viral resistance will be difficult to provide because patient care will vary depending on the standard of care in the country in which the subject is being treated. The treating investigator will determine the most appropriate course of action using the country’s recommended treatment guidelines.

A ClinicalTrials.gov website search for completed and active clinical studies using plasmid-based IL-12 as an adjuvant (regardless of target tissue or use of EP) found that plasmid-based IL-12 doses evaluated or proposed for evaluation range from 0.1 to 5.8 mg. The 0.25-mg dose of plasmid IL-12 planned for the proposed clinical trial is well within this range. As described in the protocol, the DNA sequence of INO-9112 was optimized to achieve the same improvement in antigen-specific immune response to the protein encoded by the co-administered plasmid (INO-1800) with a lower dose of IL-12 DNA by increasing expression levels of IL-12 (to that of the bioactive form) at lower doses of plasmid. Specifically, in vitro expression levels of the bioactive form of IL-12 (p70) were approximately three to five times higher with the “optimized” IL-12 DNA (INO-9112) sequence than with the “non-optimized” IL-12 DNA. The intramuscular (IM) dose of optimized IL-12 DNA selected for the proposed study was accordingly reduced to 25 percent of the doses of non-optimized IL-12 DNA administered in the prior studies (1 and 1.5 mg, respectively). The 1-mg dose of non-optimized IL-12 DNA showed an acceptable safety profile and some immunological benefit and was subsequently used as the starting point to select the dose of optimized IL-12 DNA (INO-9112) in the proposed trial.

The investigators noted that past attempts to detect systemic levels of IL-12 produced by plasmid delivery in clinical trials have shown the levels to be below the limits of detection (about 5 pg/mL). Intratumoral delivery of up to 5.8 mg of IL-12 DNA with or without electroporation resulted in local expression of IL-12 and anti-tumor effect in some patients, but systemic IL-12 was not detected and no safety signals were observed. In contrast with human studies, systemic levels of IL-12 have been detected in primates (rhesus monkeys and macaques) with IM administrations of 0.1 and 0.4 mg of IL-12 plasmid DNA followed by in vivo EP without evidence of systemic toxicity. Preclinical and clinical studies using adenoviral vector for IL-12 delivery have reported systemic levels in the nanogram range, as defined by

IFNγ production (serum IL-12 levels of about 500 pg/mL) and serum IFNγ levels (about 1000 pg/mL). SAEs attributable to adenoviral-based delivery of IL-12 have not been reported. However, as noted by Dr. Kohn, SAEs have been reported following administration of recombinant IL-12 protein at doses of 500 to 1,000 ng/kg. For example, severe toxicity was observed with daily IL-12 recombinant protein administration for 5 days; in these studies, mean systemic IL-12 levels in subjects from the 500-ng/kg dose cohort reached about 10,000 pg/mL immediately after infusion and were about 2,000 pg/mL 24 hours later.

Data from completed clinical studies indicate general safety and tolerability of plasmid-based IL-12, both with and without EP, by using plasmids encoding an IL-12 protein identical in amino acid sequence to the agent planned for the proposed study. In addition, INO-9112 shows an acceptable safety profile with none of the previously reported toxicity with administration of recombinant IL-12 protein. The investigators commented that based on the available data, the starting dose of 0.25 mg IL-12 plasmid in the current protocol is not expected to approach levels associated with systemic toxicity as seen after administration of IL-12 recombinant protein.

The primary reason for using IL-12 as an adjuvant is to improve immune responses overall to a level not achievable through delivery of the other three plasmids without IL-12, as shown in primate studies. In these studies, co-administration of IL-12 DNA enhanced the magnitude of both functional cellular and humoral immune responses. Thus, IL-12 would not be added to the combination product only to lower the dose of the other three plasmids, given that manufacturing a fourth plasmid would likely exceed the value of lowering the dose for the other three plasmids and may theoretically pose unknown safety concerns.

The selection of treatment arms was based on multiple factors. In a dose-ranging study involving treatment of human papilloma virus–related disease in the absence of any adjuvant, a trend toward greater magnitudes and more frequent T-cell responses with no difference in safety profile was observed when higher DNA doses were administered. To avoid having a separate arm for each of the 12 possible combinations of INO-1800 dose, IL-12 use, and regimen under the current protocol, the decision was made to study IL-12 DNA with the high dose of INO-1800 in an attempt to maximize the immune response based on results of prior dose-ranging studies.

The investigators noted that noninvasive methods are more commonly used than liver biopsy to assess the evidence of fibrosis or cirrhosis. In current practice, liver biopsy and FibroScan® are both acceptable methods of demonstrating liver disease consistent with chronic HBV infection without evidence of bridging fibrosis or cirrhosis. Due to the global nature of this clinical trial, both these methods will be used to evaluate eligibility to offer maximum flexibility for clinical sites and potential subjects. While repeating either the liver biopsy or FibroScan® at the completion of the study would be interesting as an exploratory endpoint, evidence of disease progression should be detected from the results of other repeated testing during the study (quantitative HBsAg, qualitative HBsAg and HBeAg, hepatitis B s-antibody [α-HBs], hepatitis B e-antibody [α-HBe], HBsAg/α-HBs, HBV DNA, and HBV RNA) before results are forthcoming from a liver biopsy or FibroScan®.

Regarding the question about having a group of patients who receive no antiviral therapy after the completion of this trial, the investigators noted that special consideration has been given to the design of this Phase I trial in accord with current treatment guidelines for management of CHB in the United States and Asian-Pacific countries. For HBeAg-positive patients, the American Association for the Study of Liver Diseases guidelines recommend nucleos(t)ide analogue therapy may be discontinued only if the following three conditions are met: (1) The patient achieves an undetectable HBV DNA level, (2) HBeAg seroconversion occurs, and (3) the patient completes at least 6 months of therapy after α-HBe has appeared. Per the Asian Pacific Association of the Study of the Liver, nucleos(t)ide analogue therapy is recommended for at least 12 months after HBe seroconversion occurs. Long-term (less than 1 year) viral suppression with antiviral therapy is associated with partial restoration of immune response, but the limitation is that HBsAg seroconversion is rare. The specific patient cohort for this study (CHB patients whose HBV DNA is effectively suppressed with ETV or TDF) was chosen with the aim of increasing the probability of HBsAg clearance and seroconversion and ultimately leading to finite (as opposed to lifelong) duration of antiviral treatment. This patient population represents individuals most likely to benefit

from an enhancement of T-cell responses by building on a process that may already be under way as a result of the virological suppression. Study participants will be monitored for changes in HBeAg and α-HBe, but this first-in-human trial will be focused primarily on safety and immunogenicity with an initial evaluation of antiviral dynamics. Subsequent studies may assess stopping nucleos(t)ide treatment when HBsAg loss has been attained.

Safety assessments to determine escalation to the next dosing arm will be conducted by an internal monitoring committee consisting of sponsor representatives not directly involved in the conduct of this study or in direct communication with clinical sites participating in this study. The internal monitoring committee will include representatives of the sponsor's collaborator, none of whom will have direct contact with clinical sites or subjects participating in this study. Clarification regarding membership of the internal monitoring committee was made upon recommendation by the FDA following review of the prior version of the protocol. The investigators explained that an internal monitoring committee was favored over an external committee to avoid impeding study enrollment given the complexity of the study and the amount of safety data to be reviewed. Criteria for pausing or stopping the study and rules for discontinuation of an individual subject (as delineated in the protocol and the response to the reviewers' comments) would apply at the time of any interim safety assessment. However, specific rules and criteria are not defined for these assessments, because each and every possible scenario cannot be adequately predicted in preparation for these reviews. The investigators noted that there are no specific established levels of safety for HBV-specific immunotherapy in this patient population that can be applied in this Phase I study. Similarly, while the primary objective of this Phase I study is evaluation of safety and tolerability of the study intervention, no formal criteria pertaining to positive and negative results are defined. The primary endpoints are a collection of safety data represented as incidence of AEs, including frequencies of injection site reactions and changes in safety laboratory parameters. Primary safety and secondary immunogenicity and antiviral data will be summarized by descriptive statistics, including means, percentages, and confidence intervals, stratified by treatment arm.

Regarding the question as to whether CPK levels should be checked more frequently to monitor muscle injury after electroporation, the investigators provided data on experience with the EP device that will be used in the proposed trial. They noted that as of August 5, 2014, more than 560 human subjects have been treated with EP using the Inovio CELLECTRA® device in 14 different studies. More than 1,300 injections (IM or intradermal) of DNA followed by EP and approximately 186 injections of placebo followed by EP have been administered. More than 2,000 CPK assessments have been performed across 10 studies of Inovio's DNA vaccines delivered by IM EP. Of those 2,000 assessments, nine events involving elevated CPK levels considered as at least possibly related to study treatment were reported (seven mild, one moderate, and one severe). The moderate CPK elevation was reported in a subject taking Midol® and naproxen for menstrual cramps with no other history available. The severe CPK elevation was reported in a subject who denied recent exercise or injuries but reported mild malaise or fatigue and moderate myalgia 30 to 90 minutes post-dose that resolved within 1 month; this subject was later diagnosed with ovarian cysts and gastroenteritis due to food poisoning. The mildly elevated levels were reported in subjects following physical exertion (e.g., sports participation, packing and moving boxes) or after use of non-steroidal anti-inflammatory drugs. All mild and moderate AEs of elevated CPK resolved without sequelae. These findings indicate that evaluation of CPK for muscle damage has been relatively unremarkable. Unexplained mild elevations in CPK have been reported after less than 1 percent of EP procedures. Based on the data available, the investigators have chosen to assess CPK at screening, day 0, and week 6. More frequent CPK monitoring is not planned, but any reports of clinical symptoms consistent with muscle damage would be evaluated by testing for CPK and further evaluation for evidence of rhabdomyolysis as indicated by clinical symptoms.

The investigators noted that there is an absence of data with regard to potential harm to a fetus, but there are no known increased concerns regarding harm to the fetus. As an added safeguard, all women of childbearing potential in this study will have a urine pregnancy test before receiving the study intervention; a negative result must be confirmed prior to each dosing. The investigators state that monthly pregnancy testing would not reduce the subject's exposure to the research intervention because additional doses are administered only upon confirmation that the subject is not pregnant. If a participant becomes pregnant at any point during the study, she will not receive any additional doses of the investigational product but will

be asked to continue safety monitoring during all follow-up assessments. If the partner of a male subject becomes pregnant at any point during the study, the pregnant partner will be asked to consent to obtain follow-up on the pregnancy. Procedures that are contraindicated during pregnancy will not be performed. Investigators will use clinical judgment regarding subsequent study-related blood collection based on the presence or absence of anemia in each subject, as well as continuation of ETV or TDF usage. Subjects who do not withdraw from the study will continue to be followed for safety assessments until completion, per protocol. Both male and female participants must agree to remain abstinent or practice effective contraceptive methods. Subjects who are pregnant during screening or expect to become pregnant during the course of the study will be excluded from participation. These requirements are discussed in both the ICD and protocol and apply during the entire study treatment period and through 12 weeks after the last dose.

The investigators understand Dr. Ghany's concern regarding multiple injections delivered by EP in individuals whose viremia is well-controlled by nucleos(t)ide therapy. They noted that the DNA delivery platform to be used for this research has been evaluated in previous Inovio-sponsored clinical trials for various indications and has been found to be associated with a low study drop-out rate due to EP. For this particular study, extensive feasibility efforts were expended prior to finalization of the protocol. This process involved outreach to investigators in 18 countries to gauge the level of interest and feasibility of the specific study design and inclusion or exclusion criteria. A total of 33 investigators responded to the feasibility questionnaire to guide decision making in the design of the protocol. From a patient management perspective, Dr. Ghany's reservations on the applicability of widespread clinical use of this immunotherapy and delivery platform is reasonable, considering potential differences in acceptance of injectable therapeutics between U.S. and non-U.S. patients. The investigators pointed out, however, that nucleos(t)ide therapy is of indefinite duration and does not offer a high rate of clinical cure, as it is associated with only modest levels of HBsAg loss and α-HBs seroconversion. Given these deficits, a finite series of HBV-targeted injections designed to enhance the immune response (and perhaps achieve greater rates of HBsAg loss) delivered by EP may be attractive to a subset of patients interested in increasing the probability of discontinuing antiviral therapy.

All subjects are required to be receiving ETV or TDF at randomization and for at least 6 months before randomization; one of the two antivirals is considered standard maintenance therapy for their disease. The sponsor will not provide nucleos(t)ide analogue therapy to subjects during or after completion of the study. However, reimbursement will be provided over the course of the study (including the follow-up phase) if the cost of the antiviral medications is not covered by the subject's insurance or other government program. Neither supply nor reimbursement will be provided to subjects upon completion of the study (after the last follow-up visit).

The investigators appreciated the feedback and suggestions regarding the ICD. They explained that the ICD has been designed and written per regulations for the protection of human subjects (45 CFR 46) to provide a comprehensive and thorough description of all aspects of the study, including the potential risks and benefits of participation, and a balanced perspective of the options available to patients that would be eligible for this study with and enough information for a truly voluntary and informed consent. The investigators pointed out that the ICD is designed not as a standalone document but to supplement an informed consent process between potential participants and the study investigators to facilitate understanding of the research and its risks. They agreed to revise and update the ICD to reflect suggestions by the RAC reviewers, including simplifying or clarifying terms, descriptions of procedures and investigational products, discussion of risks, and minor edits and corrections.

Regarding specific points raised by the reviewers, the potential risks of liver biopsy and a description of the proposed immunotherapy as an "inactive"/noninfectious DNA plasmid will be added to the ICD. In addition, to make the technical concepts more understandable to the patient population, specific instructions will be provided to study investigators for additional emphasis and explanation of the concepts in the "Purpose of the Study" section of the ICD. The investigators considered discussing results of similar trials or one or more other ongoing trials with similar objectives to be out of scope for the ICD for the proposed trial. Available data do not identify any particular risks that might represent a "class effect" applicable to all immunotherapy approaches other than the theoretical risk of an immune-mediated flare

which was included. A comparison of this study to previously conducted HBV vaccine trials may not be as informative as intended given the different antigens, delivery platforms, and vaccine technologies used across protocols. While the ICD does not include a discussion of the results of other HBV vaccine trials, potential side effects of INO-1800 and INO-9112 are detailed. The option of continuing nucleos(t)ide analogue therapy is briefly discussed within the "Purpose of the Study" section of the ICD. This section highlights the importance of adhering to antiviral medication while participating in the study and explains that the greatest benefit from participation is expected if subjects continue receiving antiviral medication.

The "Clinical Protocol Synopsis" section of the protocol refers to the global nature of the proposed trial, which will conducted at approximately 15 to 30 study sites in North America, Europe, and Asian-Pacific regions of the world.

A current version of Dr. Dieterich's CV (dated July 21, 2014) was provided (Attachment 2).

2. Responses to RAC Discussion Questions

Dr. Dieterich acknowledged the concerns regarding use of FibroScans® and added that large nodules can also be a problem with liver biopsies. In addition, liver biopsy is not the standard of care for hepatitis B. He noted, however, that there are sufficient data and experience with FibroScan® and hepatitis B to be confident with the results. He will consider adding platelet counts but found the suggested threshold of 150,000 to be somewhat high. Another option would be to use an aspartate transaminase-to-platelet ratio index or fibrosis index score. Patients with previously diagnosed cirrhosis will not need a liver biopsy, and determining their eligibility based on platelet count or a related score would be reasonable. Dr. Dieterich explained that a low platelet count in patients with hepatitis B is due largely to portal hypertension and to pancytopenia from the cirrhosis to a lesser degree. Dr. Ghany added that the thrombocytopenia may also be due in part to reduced thrombopoietin levels in the cirrhotic liver.

To be eligible for the proposed trial, patients must have undetectable HBV DNA, which is defined for this study as less than 90 IU/mL. Patients who have detectable virus per the protocol even while on medication would therefore be excluded. Drug resistance testing can be done to determine if a patient has drug-resistant virus.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

- FibroScan® is part of the screening procedures in order to demonstrate that there is no evidence of bridging fibrosis or cirrhosis. A FibroScan®, however, may underestimate the amount of scarring in individuals with Hepatitis B, since this procedure has been evaluated more extensively in individuals with Hepatitis C and the liver pathophysiology is different in Hepatitis C. Therefore, in some patients with Hepatitis B, a FibroScan® might underestimate the amount of liver damage. To avoid enrolling a patient with cirrhosis, who would be at high risk of an adverse event, consider using a biochemical measure (for example reduced platelet count) to decide whether a liver biopsy rather than a FibroScan® is indicated to exclude research participants that have, or are at high risk of, cirrhosis based on serum markers.

- The proposed safety monitoring committee will be comprised of members from Inovio Pharmaceuticals. An internal committee was chosen over an independent committee because the former would likely be able to more rapidly respond to the review of safety data and allow enrollment to proceed without delay. However, there may be advantages to have independent monitors and you should consider whether it is feasible to include one or more outside experts on the committee without impeding efficiency.

Ethical/Legal/Social Issues

- The initial paragraphs of the informed consent document contain a very detailed description of the Hepatitis B virus. You state that some participants may want this level of detail. For ease of reading, it may be preferable to place this description later in the document or as supplementary material.

G. Committee Motion 4

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Donahue, Kaufman, Kiem, Kohn, Pilewski, Sadelain, and Whitley

A. GTSAB Report

Dr. Kiem presented the GTSAB report for the first quarter of 2015. Within the past 3 months, the OBA received a total of 27 protocol submissions, 22 of which were not selected for public review at this RAC meeting. Of the 22 protocols not selected for public review, 14 were oncology protocols and three were monogenic disease protocols; in addition, there was one protocol for each of the following conditions: heart failure, stroke, peripheral artery disease, tendon injury, and macular degeneration. Among these 22 protocols, five used adeno-associated viruses, four used plasmids, four used retroviruses, four used attenuated *Listeria monocytogenes*, three used lentiviruses, and two used pox viruses.

For the first quarter of 2015, the GTSAB reviewed initial and follow-up reports on 24 SAEs from 18 protocols. (Information about these trials will be made available on the OBA website after this RAC meeting and in the future, will be available in the NIH Genetic Modification Clinical Research Information System.)

Over the past year, the GTSAB has reviewed several events in vaccine trials in which subjects with adrenal insufficiency, who were on maintenance steroid doses, have had serious reactions following the vaccine that included fever, hypotension, and mental status changes. All subjects recovered, but their adrenal insufficiency was considered to have contributed to these reactions. While such subjects may be appropriate for the trial, closer monitoring or follow-up instructions may be warranted, as well as confirmation that the subject has been compliant with their steroid therapy.

Dr. Kiem noted awards to two pioneers in the field of gene therapy. In the overall field of Medical Science and Medicinal Science, 2015 Japan Prize Laureates include Dr. Theodore Friedmann of the United States and Professor Alain Fischer of France, who were selected for the “proposal of the concept of gene therapy and its clinical applications.” During the 1970s, Dr. Friedmann became the first to propose the concept of gene therapy, and Professor Fischer was the world’s first to clinically prove the dramatic effectiveness of gene therapy. The third recipient is Dr. Yutaka Takahasi of Japan, who is being recognized for his work in the Resources, Energy, and Infrastructure field. To honor the three distinguished scientists, an award ceremony will be held in Tokyo on April 23, 2015.

A joint meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee and the Oncologic Drugs Advisory Committee, scheduled for April 29, 2015, will focus on the Biologic License Application for Talimogene laherparepvec, an attenuated Herpes virus with the transgene for granulocyte-macrophage colony-stimulating factor (Amgen, Inc.) for the treatment of patients with metastatic melanoma (injectable

regionally or distantly). The meeting will be streamed live; the link for the webcast is available at https://collaboration.fda.gov/ctgtacodac/.

In the first quarter of 2015, OBA received notification that 12 new protocols opened, four of which were publicly reviewed. Dr. Kiem reviewed the list of the 12 new protocols and reported on the following noteworthy changes that represented responses to RAC public review:

- *OBA Protocol #1288 reviewed in March 2014: Phase I Study to Determine the Effects of Mesenchymal Stem Cells Secreting Interferon Beta in Patients with Advanced Ovarian Cancer.* The use of autologous cells instead of allogeneic cells is not feasible as most research participants are unwilling to undergo the necessary bone marrow harvest. Transfected mesenchymal stem cells (MSCs) that fail to express interferon beta (IFNβ) or that stop producing IFNβ may have the potential to differentiate into tumor-supporting fibroblasts. Tumor biopsy samples will now be evaluated for proliferation and evidence of tumor-supporting fibroblasts. Since data are limited regarding repeat administration of allogeneic MSCs to immunocompromised research participants, peritoneal fluid will be removed before each of the weekly doses and stored for possible future analysis of inflammatory markers and MSC-specific immune cells.
- *OBA Protocol #1320 reviewed September 2014*: *Phase I Study of T Cells Expressing an Anti-CD22 Chimeric Receptor in Children and Young Adults with B Cell Malignancies.* The Informed Consent Document has been revised to indicate that the modified cells are an experimental intervention and that while these cells are able to attack cancer cells in animals, it is not known whether the cells will have the same effect in humans. To facilitate assent, age-appropriate information sheets have been added for ages 5 to 7 years, 8 to 12 years, and 13 to 17 years.

B. RAC Discussion

RAC members had no comments or questions.

C. Public Comment

No public comments were offered.

VII. Follow-Up: Proposed Updates to the NIH Informed Consent Guidance for Human Gene Transfer Trials Subject to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*

Presenter/Moderator: Dr. Corrigan-Curay, OD, NIH

A. Proposed Updates

Dr. Corrigan-Curay provided a recap of the key points raised during the discussions on bioethics and informed consent guidance at the December 2014 RAC meeting. A formal summary on the various opinions and suggested language on how to discuss risk, including language for trials that enroll pediatric patients, is being prepared.

Three topics addressed at the end of the discussion at the December meeting involved withdraw from a study. One topic focused on the potential long-term effects of gene transfer agents and whether patients understand that the agent itself could not be withdrawn. Another topic involved how long-term follow-up might confuse patients and foster therapeutic misconceptions, especially with respect to a 15-year follow-up period. The third issue involved on contraception and pregnancy, with a focus on patients in end-stage cancer trials.

Dr. Corrigan-Curay reviewed recommended guidance and consent language suggested by RAC members. With respect to withdrawal, the ICD should clarify that withdrawal from the study refers to withdrawal only from study follow-up and procedures and that the product itself cannot be withdrawn

because it has the ability to persist. The language on withdrawal should indicate that once a subject receives the modified cells, the can persist indefinitely; withdrawal from the study therefore means only withdrawal from monitoring for potential long-term adverse effects of the experimental intervention. Withdrawal from study monitoring, in turn, can carry its own risk because long-term events might not be detected.

The current considerations for subject withdrawal, as delineated in the NIH Informed Consent Guidance for Human Gene Transfer Trials, already include the concept that the intervention cannot be undone (per the first bullet):

- **"Irreversible course of action:** Whenever applicable, potential participants should be informed that, in the event that they withdraw after receiving the experimental intervention, the intervention cannot be undone. If early withdrawal could pose any special risks of harm to participants or others, they should be described.
- **"Request for follow-up:** If follow-up is necessary or highly desirable for health and safety purposes, participants should be encouraged (but not required) to return for needed follow-up in the event that they withdraw from the study.
- **"Risks to close contacts:** If early withdrawal could pose risks to third parties, for example, transmission of vector to close contacts if the participant withdraws during a period of viral shedding, participants should be strongly urged to comply with any necessary precautions to minimize the risks."

The proposed changes (in italics) to the sample informed consent language to clarify subject withdrawal per the RAC's recommendations are as follows:

- **Sample 1:** "You do not have to be in this study. You can say no. If you join the study, you can leave at any time; *however, if you received the gene transfer agent, the effects may not be reversible, and withdrawing from the study means you will not be followed for long-term effects under this study.* If you choose not to participate, there will be no penalties, and no bad effects on any benefits or medical care that you are entitled to get from this hospital or from your health care providers. If you leave the study, please tell the investigator or research coordinator. We will ask you to come back to the clinic for a final assessment and discussion of future treatment options."
- **Sample 2:** "Being in this study is completely voluntary. You may withdraw at any time without penalty, without loss of benefits to which you are entitled, and without affecting the medical care you get at our hospital. However, once you receive the gene transfer product, there may be some effects that cannot be reversed. *If you withdraw from the study, you will be withdrawing from monitoring for long-term effects. You may be monitored by your own doctor.*"

Regarding long-term follow-up, the RAC found the consent language regarding the requirement for long-term follow-up of at least 15 years to be potentially misleading for research participants with a disease with limited survival. Given this concern, the RAC noted that it might be helpful to clarify with the regulatory authorities whether 15-year follow-up is actually required, since the most recent FDA guidance indicates that not all gene transfer products require follow-up lasting 15 years. Any statement about long-term follow-up should be clarified to explain why this language is included and that it is not indicative of the investigators' expectations regarding the potential efficacy of this approach.

Dr. Corrigan-Curay clarified that the 15-year follow-up is a regulatory requirement to ensure that participants are followed for a sufficient length of time to capture side effects and AEs that may be related to the research intervention and may affect their care. This follow-up should not be confused with clinical benefit, however. The existing guidance stresses the importance of long-term follow-up also recognizes that the language and plan for follow-up should be tailored to the individual research study being conducted.

The informed consent guidance on long-term follow-up includes the following, with proposed modifications in italics:

- "Because gene transfer is innovative and its long-term risks are not well understood, it is important to try to obtain long-term toxicity data on participants and to provide to participants any new significant clinical information that might affect their future care.

- "Investigators need to inform prospective participants that they will be expected to participate in long-term follow-up that extends beyond the active phase of the study. Investigators should explain the rationale for long-term follow-up and describe the specific follow-up activities planned, including the desire to collect information about new cancers, blood disorders, autoimmune diseases, and neurologic disorders. Long-term follow-up may be viewed by participants as a benefit, a burden, or both. Potential participants need to understand what long-term follow-up activities will occur; how long follow-up will continue; and what, if any, procedures they will be asked to undergo.
- "Both the design and extent of long-term follow-up and its discussion in the ICD and process should always be tailored to the specific study design and vector used. *Fifteen-year follow-up may not be required for all vectors (per FDA guidance).*
- *"It is important that the discussion of long-term follow-up be sensitive to the clinical prognosis of research participants and that the requirements for monitoring for long-term safety be presented in a manner that will not lead to therapeutic misconception regarding long-term clinical benefit."*

Dr. Corrigan-Curay noted that the last bullet point was added to underscore that these issues not only need to be included in the ICD, but they need to be discussed with patients.

Sample informed consent language in accord with this guidance includes:

- **Sample 1:** "Long-term follow-up in gene transfer research allows for the collection of important information on the long-term safety and effects of the gene transfer intervention used in this study. The long-term follow-up planned for this study will occur [*frequency*] for [*length of time*]. It includes [*study-specific information, as available; e.g., drawing a small amount of blood once a year; completing a health history questionnaire every year; having a biopsy of the injection site every 5 years*]. The investigators will try to make it easier for you to participate in long-term follow-up by [*study-specific information as available; e.g., using mail and telephone to collect some information, arranging with your local doctor to collect blood or biopsy specimens and having them sent to investigators*].
- **Sample 2:** "At the end of the experimental phase of the study you will be asked to participate in the *long-term follow-up phase for the rest of your life*. Once a year you will be asked to have your blood drawn (~[amount]) and answer questions about your general health and medical condition. The investigators may ask you to report any recent hospitalizations, new medications, or the development of conditions or illness that were not present when you enrolled in the study and may request that physical exams and/or laboratory tests be performed if necessary. We will also ask you to participate in the long-term follow-up phase if you leave the study early."

The RAC expressed concern that statements in the ICD and discussions regarding protecting against pregnancy seem unnecessary and possibly upsetting to potential research participants in specific trials (e.g., those in patients with end-stage or terminal cancer). The RAC understands that a local IRB may require certain standard language, but investigators are used to discussing this issue within the context of the specific disease of the patient cohort. At the December RAC meeting, there was general consensus that language about pregnancy prevention needs to be in the ICD due to the reproductive risks involved with gene therapies. However, the ICD also needs to reflect the nature of the patient population, include abstinence as an acceptable form of birth control, and to be tailored carefully to the protocol. The current guidance on pregnancy and contraception speak to those concepts but was modified as follows (in italics):

- "Some vectors used in gene transfer experiments have the capacity to integrate and alter the germ line. When data are inadequate to rule out the possibility of inadvertent germline alteration, non-sterile participants should be informed that the biological consequences of this procedure are not known, and therefore unborn children, children who are breastfeeding, and mothers could be harmed.
- "Discussion of the risk of reproductive harm should always be study specific. Study-specific factors include but are not limited to frequency of pregnancy testing and the possibility of inadvertent germline effects, which could be teratogenic.

- "Reproductive considerations may be unique to one gender or may need to be discussed differently for men and women. It may be worthwhile to have separate sections in the ICD for issues especially pertinent to men or to women.
- *"Discussions of pregnancy risk may need to be tailored to the clinical condition, for example certain diseases or their treatment may preclude pregnancy, and discussions of pregnancy in such situations should be sensitive to these issues*."

Sample ICD language for reproductive risks was revised as indicated (in italics) below:

- "You should not become pregnant or father a child while taking part in this study. Women who are pregnant or breast-feeding may not be in this study. If you are a female who can have children, you will take a pregnancy test and the results will be given to you. You must confirm that you do not plan to become pregnant while on this study. If you are capable of giving birth or fathering a child, you must use an acceptable form of birth control (*which may include abstinence*). For women, contraception should go on for [time period] after the last dose of the [gene transfer agent] to ensure that it has completely cleared from your body. For men, contraception should go on for [time period] after the last dose of the [gene transfer agent] to make sure that all sperm in the body during the trial have been replaced. If you or your partner becomes pregnant or you suspect that you or your partner is pregnant while in this study, notify the investigator at once."

The goal of the current presentation and discussion was to review the proposed changes to the existing guidance and language per the RAC's most recent recommendations. The added concepts and language can be further refined and modified per agreement by the Committee.

Dr. Corrigan-Curay noted that the OBA has volunteered to go through the current guidance and update links and references in consultation with a bioethicist. In addition, staff plan to make the informed consent guidance more prominent on the OBA website to increase awareness of the guidance. The American Society of Gene & Cell Therapy already has a link to the guidance to increase outreach.

B. RAC Discussion

Dr. Zoloth found the term "long-term effects" to be too vague and recommended that the ICD explicitly state what it means not to be followed if a subject decides to no longer participate in the study. Since "withdrawal" is from ongoing monitoring, potential harms from the experimental intervention might go undetected. The language could be strengthened by including a statement that explains why it is important to remain in the study for ongoing follow-up. Dr. Ross noted that the main concerns are the long-term risks for the individual past participant and the possibility of obtaining information that might be relevant to the research but that would not be detected without the lengthy follow-up. Dr. Zoloth agreed that investigators cannot anticipate the full range of effects that might occur in the future, which is a main reason to follow participants carefully over several years. Subjects have the right to not be followed, but they also need to understand that withdrawing from ongoing monitoring is not necessarily a benign choice. Drs. Ross and Zoloth agreed, however, that because there can be risks of being followed, continuing with long-term monitoring also may not be a benign choice. Being watched carefully does not mean that something can be done for all AEs that might arise over the course of follow-up; it is therefore important not to promise or imply that if something is found, it can be fixed. Given the tension between these perspectives, it might be helpful to balance the language to support the choice of withdrawing while still being clear about the potential drawbacks of not being followed and the realistic expectations with long-term monitoring. Upon further discussion, the preference was to use neutral language where possible. Dr. Kohn suggested the term "long-term side effects," which most people understand and which is relatively neutral. Dr. Corrigan-Curay will modify the proposed language to include this term.

Professor Dresser inquired about criteria used by the FDA to determine when or whether a 15-year follow-up period is needed. Drs. Corrigan-Curay and Gavin provided additional detail regarding these criteria, noting that a follow-up phase as short as 1 to 2 years may be acceptable depending in part on the vectors used and the length of the study. For example, protocols evaluating T-cell immunotherapy and using retroviruses tend to have the 15-year follow-up, while studies using plasmid-based investigational agents may have a shorter follow-up phase. Investigators are encouraged to discuss the issue and any

questions regarding the follow-up with the FDA. Dr. Corrigan-Curay will forward the link to the FDA guidance on long-term follow-up for gene therapy trials to RAC members.

C. Public Comment

No public comments were offered.

VIII. Review and Discussion of Human Gene Transfer Protocol #1411-1360: CAR Mucin 1 (MUC1) T-Cell Immunotherapy for Metastatic Breast Cancer

PI: Stewart Sharp, M.D., Harrington Cancer Center, Amarillo, TX (*via teleconference*)

Co-PIs: Sanjay Srivastava, Ph.D., and Stephen Wright, M.D., Harrington Cancer Center (*via teleconference*)

RAC Reviewers: Drs. Kiem, Pilewski, and Zoloth

A. Protocol Summary

Breast cancer is the second most common cancer in women and the second leading cause of cancer death among all ethnicities. An estimated 233,000 new cases of invasive breast cancer will be diagnosed among U.S. women this year, with approximately 40,000 people dying from the disease annually. While many patients recover, there currently are more than 2.5 million breast cancer survivors in the United States, and those whose disease metastasizes have an average survival rate of 3 years, despite regimes of chemotherapy and radiation. New treatments, even aggressive treatments such as bone marrow hematopoietic stem cell transplantation and drug-induced immunotherapy, do not help some 70 percent of patients, many of whom often suffer painful and debilitating side effects in their last few years of life.

Per these statistics, patients with metastatic disease are not considerable curable by current modalities of therapy. A predictor of clinical relapse in patients with localized (stages I–III) breast cancer is occult cytokeratin-positive (CK+) cancer cells in the bone marrow. Data indicate that one-half of these patients relapse. One study reported a statistically significant increase in cancer deaths of patients with CK+ cells (25 percent) vs. 17 percent for those without CK+ cells in bone marrow at 5 years. For patients with stages I and II breast cancer, CK+ cells in peripheral blood were associated with statistically significant reduced disease-free interval and overall survival. CK+ cells in peripheral blood have also a demonstrated predictive value in therapeutic response. Approaches used for patients with metastatic disease include high-dose therapy and autologous stem cell transplantation, allogeneic stem cell transplantation, and passive immunity with trastuzumab. Despite these advances, the long-term outlook for patients with metastatic breast cancer remains poor. Alternative treatments are needed that may be effective alone or combined with standard therapy. Adoptive T cell immunotherapy for viral infections and malignancies has been successful in a number of instances.

Mucin is a transmembrane protein expressed in a number of secretory and other epithelia, including breast, gastrointestinal (GI) tract, lung, liver, pancreas, ovary, skin, cornea, and prostate. Studies over the last few decades have demonstrated that one mucin, MUC1, is important in both transformation and metastatic progression in a number of cancers. High levels of MUC1 expression in some malignancies have been correlated with advanced disease and poor survival. MUC1 from normal tissue is, for example, immunologically different from MUC1 from breast cancer tissue. MUC1 has therefore been a logical target for a number of investigational therapies, including peptides to block MUC1 signaling, antibodies to the extensive ectodomain, and plasmid vaccines to enhance immune responses to MUC1.

A functioning immune system has a role in malignant cell elimination. The ability of the immune system to eliminate the malignant population appears to be dependent on both chemotherapy administration and specific reactive lymphocytes. Breast cancer-specific MUC1 CAR T cells have been designed to find and destroy breast cancer cells. Data indicate that that activity of the immune system produces clinical

responses and that breast cancer-specific MUC1 CAR T-cell infusions and immune modulation may have therapeutic benefit. Clinical data have accumulated to support earlier observations from animal studies.

The proposed Phase I/II study will test the safety and efficacy of a novel combination of adoptive immunotherapy by using MUC1 CAR T-cell infusions and immune modulation for the treatment of subjects with metastatic breast cancer. While there are several cancer vaccine trials targeting the tumor antigen MUC1, this is the first trial to do so by using a T cell that expresses a MUC1-specific CAR. In addition, the T cells are engineered to secrete IL-12 in order to enhance the activity of the MUC1 CAR T cells. The plasmid that expresses the CAR will also contain the caspase 9 suicide gene as an added safety feature. The study population will consist of a total of 232 patients with metastatic breast cancer, following planned standard hormonal therapy, chemotherapy, or radiation therapy; participants may currently be receiving hormonal therapy. Upon completion of the dose escalations in Phase I, both with and without lymphodepletion chemotherapy, the proposed plan is to randomize 160 subjects into the Phase II arm of the trial to assess the safety and efficacy of MUC1 CAR T cells versus untreated controls.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because of the novel approach of using a third-generation CAR targeting MUC1 with IL-12 for treatment of metastatic breast cancer

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

Dr. Kiem noted the investigators' preclinical research involving MUC1-loaded dendritic cells (DC), which were injected with CTLs into mice with MCF-7 breast cancer; results of these experiments showed that DC and CTL protected 50 percent of the mice. The investigators are now proposing to use MUC1 CAR T cells with IL-12 that are generated under the influence of interleukin 21 (IL-21) to keep the T cells in a "young" and undifferentiated state. They hypothesize that this should manipulate the microenvironment to prevent the development of suppressor T cells that would reduce the effectiveness of activated T cells. Dr. Kiem commented that it would be helpful to have preliminary data supporting the investigators' rationale and hypothesis for the planned research. He pointed out that other than the DC studies published in 2013, there seems to be no additional preliminary data with the CAR construct proposed.

Data supporting the statement that the transposon and transposase system is about 40 percent efficient, data supporting this should be provided along with a description of the method used. The numbers given for the gammaretroviral vector with 5 percent do not appear to be correct; with the appropriate pseudotype, efficiency is typically much better.

Dr. Kiem requested that the investigators provide the following information:

- A schematic of the constructs being used in conjunction with the structure of the cloned DNA.
- A detailed description of and preliminary data for the generation of the CAR T cells, in addition to the efficiency rate, should be provided.
- The method for assaying for the presence of transposase.
- Data using the K562 artificial APC (aAPC) cells clone D2 and expression of IL-21, which was introduced with the Sleeping Beauty (SB) system. All data cited are from Dr. Cooper's group, but the protocol does not specify whether Dr. Cooper's group (or another team) will generate the CAR T cells.
- Data for the third- versus second-generation CAR. The investigators propose the inclusion of iCaspase, but it is not clear when the suicide gene mechanism would be triggered.
- The rationale for including both cyclophosphamide and fludarabine for lymphodepletion after the initial phase and the investigators' experience with this strategy and regimen.

The ICD needs a much more extensive description of the side effects of infusion of CAR T cells, including the possibility that patients can die. The side effects and risks of chemotherapy, including immunosuppression and potential consequences, need to be clearly described in the ICD. Participants

need to understand that once the modified cells are infused, the cells cannot be removed and that there can still be consequences even if the patient withdraws from the protocol.

Dr. Pilewski inquired about preclinical safety data to provide a level of assurance that the CAR T cells to MUC1 will not target MUC1 on normal or regenerative tissues, where the expression or glycosylation of MUC1 may be abnormal or under-glycosylated. For example, Appendix M does not include a description of animal models to assess safety of the transduced T cells. Models are reported as similar to the proposed human treatment, but the referenced animal data are limited to a murine model with a human breast cancer cell line such that only the cancer cells express the target antigen. In the absence of preclinical safety studies in MUC1 transgenic mice or primates where the tandem repeat sequence is highly homologous, the risk of off-target effects is unknown and potentially significant.

Safety measures will include symptom assessment for evidence of GI toxicity and chemistry panels every 2 months for 2 years. The rationale for giving sodium bicarbonate and omega-3 fatty acids for prevention of colitis is not clear and should be provided. In addition, given high-level expression of MUC1 in lung and pancreas, the protocol should include the assessments that will be performed to ensure that there are no subclinical effects in these organs.

MUC1 is heavily shed from the cell surface and can be detected in serum. Dr. Pilewski asked whether this increases the risk of CAR T-cell activation in the circulation and potential cytokine storm and, if so, whether extrapolation of dosing from prior CAR T-cell studies is therefore less reliable than presumed. Given that MUC1 is shed in the circulation and may engage the MUC1 CAR T cells, the investigators should consider monitoring serum IL-12 and other cytokine levels in the first day after administration to assess for T-cell activation and detectable IL-12 in the circulation. Dr. Pilewski noted that such monitoring may prove valuable if there are significant reactions early after infusion.

Dr. Pilewski did not find the proposed design—combining a Phase 1 and 2 study in a single protocol—to be well justified, given the lack of preclinical safety data in a relevant model. He suggested that the design be revised as a Phase 1A dose escalation trial for MUC1 CAR T cells, followed by a Phase 1B study for MUC1 CAR T cells after lymphodepletion. Additional details regarding the design are needed, including how long each dosing cohort will be followed before proceeding to the next cohort. The timing of safety reviews and the composition and independence of a data safety review committee or DSMB also need to be addressed. Further, in the proposed Phase 2 portion, a plan is needed for the evaluation of the control group if other salvage therapies are pursued. Given that MUC1 expression is in part controlled by estrogen, the investigators need to specify how use of hormonal therapies will be stratified in the randomization, or explain why the study will not control for use of hormones.

Dr. Pilewski had the following additional comments and suggested modifications regarding the ICD:

- The word “treatment” appears repeatedly in the ICD and implies therapeutic benefit. Treatment should not be used aside from reference to therapies that are proven beneficial and approved for use in humans. “Treatment” should be replaced with “research study” or similar wording that makes the experimental and unproven benefit unambiguous to a lay person.
- The purpose of the study is stated as if this is an efficacy (late phase) study, not the safety study that it actually is.
- The number of people in the study should be listed as maximum with the provision that the intervention is safe in the first 36 patients.
- The ICD should acknowledge that this is the first human study of this specific intervention to induce an immune response to tumor cells.
- Leukopheresis should be listed or identified as a procedure rather than a test (e.g., in the table on page 3).
- Scientific jargon that should be edited for simplicity to the eighth-grade reading level.
- The section on possible risks and discomforts needs to include a description of autoimmune disease and potential for death from autoimmune disease.

- The statement “There is a possibility that you will not benefit at all from participating in this treatment” should be deleted and replaced with a statement that acknowledges that the benefit is completely unknown in humans and, at most, is possible but not expected.
- The document should acknowledge that the intervention may be permanent insofar as the half-life of the CAR T cells is unpredictable and that, unlike drug interventions with defined pharmacokinetics, withdrawal from the study in no way ensures that any adverse effects of the intervention will resolve.

Dr. Zoloth questioned the basis for proceeding with the proposed first-in-human Phase I/II trial, given the lack of nonhuman primate data. In addition, a clear understanding of the interactions between the intensive therapies used by this patient population should be provided, including whether the mice that were administered the breast cancer-specific MUC1 CAR T cells were also dosed with chemotherapy and radiation or hormonal therapy.

Dr. Zoloth commented that the ICD has many serious problems and needs to be rewritten. She raised the following key issues:

- The premise (as stated in the ICD) that the protocol will be limited to participants who can pay or provide payment for a costly “treatment” represents a serious departure from research, and it is particularly inappropriate when the research is being done on seriously ill patients. It appears that all potential participants, including those who will be in the control group, will be screened for the ability to pay. Dr. Zoloth noted that per the statements in the ICD, not only are the researchers refusing to take responsibility for harms that may ensue as a result of their experiment; they are suggesting that the insurance company would pay tens of thousands of dollars for the cost of the proposed intervention, which is another source of confusion about therapy and research. She added that the investigators must know that insurance companies, with few exceptions, do not pay for experimental interventions, much less for research-related harms to subjects. Dr. Zoloth found telling desperate and, in all likelihood, dying patients that they have to pay to participate to be deeply disturbing. Given the status of the planned patient cohort, the researchers are obligated to an even higher duty of care.
- The ICD confuses the idea of research and treatment form the first sentence, mixing the terms “routine care” and “best-known treatment.” It is also inappropriate to describe the proposed intervention as a sort of “treatment,” which could be misleading. The investigators should use phrasing such as “modified cells and other study procedures” or other words that allude to the intervention’s investigational status.
- The statements “We cannot promise that this research will help you” and “Just like regular treatment, the research can have side effect that can be serious or minor” need to be deleted. Alternative language such as the following should be used instead: “This is not regular treatment. It is not treatment because the goal is to test an idea and see if it works and if it is a safe idea. We can promise that you will help us in our research to help understand cancer. The benefit of the study to you is that you will have an opportunity to act as a courageous volunteer. The risk is that the experiment may be harmful or even fatal.”
- The statement “We believe treatment with specific immune therapy can effectively kill all the cancer cells” should not be in a research ICD. Dr. Zoloth found this phrase especially objectionable when the results described in the murine model show only 40 percent to 50 percent effectiveness. She noted that the subjects need to be presented with some reasons to agree to test a hypothesis, not an aspirational goal. The investigators should use language such as “We have tested this idea in mice and it appeared to work well with 50 percent of the mice. Other scientists have tried a similar idea (genetically boosting white blood cells) out on patients with other sorts of cancer and early results are promising. This is why we want to test our idea [or hypothesis] that if we can make your immune system stronger, it would kill some, many, or perhaps all of the cancer cells circulating in your body. We cannot know the answer unless we try.”
- The ICD should clearly state that three changes will be made in the DNA of the patient’s white blood cells and then briefly describe each change and its purpose. Example: “One change will make your white blood cells able to recognize cancer cells. One change will help kill cancer cells

better. One change will allow the researchers to 'turn off' (kill) the altered white blood cells. This is because we do not know whether our idea is completely safe, and we wanted to have a way to destroy the cells."

- The amount of time for this study, 6 months, is one-sixth of the time that most of these patients are estimated to have to live. The investigators should consider mentioning this in the ICD. The only mention of temporal duration is a reference to 15-year follow-up, which seems unduly optimistic without some framing of the patients' actual situation.
- The ICD does not mention that the side effects could be fatal.
- The final benefits description offers the hope of therapeutic effects when it should say that a benefit is the grace of altruism. Saying, "There is a possibility that you will not benefit at all," is misleading, because it sounds as though there is a possibility of benefit as well.
- The ICD states that the patient may withdraw at any time, which is not completely accurate. The patient cannot withdraw from the intervention once the cells are infused. There is no indication that the cells can be deactivated (or even that such a process is available and works) if the patient wants to withdraw. The ICD should clarify this issue and state that withdrawal is possible only from follow-up.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers and other RAC members asked whether the investigators have any preliminary data to support the rationale and hypothesis for the proposed research. Dr. Kiem inquired whether the investigators were aware that the transposases can integrate in the CAR Tcells and how they will assess that. In addition, he asked about any data for the transposon and transposase system, which reportedly has about a 40 percent efficiency rate. Dr. Kiem noted that it would be helpful to have the structure of the clone DNA and also a schematic of the entire construct being used for these studies. He asked about the preliminary data for the generation of CAR T cells and whether they have any experience with CAR T cells. He further inquired about the data using K562A artificial antigen presenting cells (aAPC) expressing IL-21 and their experience with this particular K562aAPC line. Dr. Kiem further inquired about the rationale for using a third-generation versus a second-generation construct and whether the group has any experience with CAR T cells and the K562 APC line. There was another question regarding the rationale for using lumphodepletion with cyclophosphamide and fludarabine after the initial phase. Dr. Pilewski commented that while MUC1 is a very attractive target for immunotherapy, as evident by the work toward developing vaccines against MUC1, safety remains a major concern, particularly with respect to off-target effects. The target region is the extracellular domain, where there is an abundant number of tandem repeats; the tandem repeat sequence, in turn, is not homologous between humans and mice. There essentially are no data to support safety in a model or even in vitro, where human MUC1 is expressed by normal cells. The investigators are relying on under- or de-glycosylated tandem repeat regions to be the target for the CAR T-11 cells, which make sense. However, very little is known about the glycosylation status in many normal tissues, and MUC1 is expressed in a large number of secretory organs (e.g., liver, lung, pancreas, intestine), where off-target side effects could be significant. Dr. Pilewski acknowledged that there are no good animal models, in part because of issues with a primate model, but it also appears that no in vitro experiments have been done to assess efficacy and tissue toxicity. The investigators noted that MUC1 expressed in normal tissues is shielded in some ways by glycosylation, which would prevent antibody binding. They also cited experiments where tumor growth from human breast cancer cells was slowed, delayed, or eradicated in mice following injection with MUC1 CAR T cells, but activity in normal cells and tissue is still largely unstudied. The investigators were asked to elaborate further on their plans for other in vitro or animal studies to assess safety and efficacy as raised by the reviewers rather than extrapolating from the experience with CTLs, which may not be appropriate for the proposed construct that includes IL-12.
- To monitor for potential off-target effects in the lung and pancreas, the investigators added a biochemical marker (lipase) of pancreatic inflammation and oxygen saturation monitoring to assess lung function. Dr. Pilewski commented that oxygen saturation is a very crude estimate

and suggested doing pulmonary function testing because MUC1 in the lung is expressed in airway cells and airway is likely to be a target, so pulmonary function testing would be a muchmore sensitive measure of whether you're having any off-target effects there. Several RAC members considered the plan to proceed with a Phase I/II study design in a single protocol to be premature. The investigators should consider conducting a Phase I trial first to assess the safety of the investigational product and determine the MTD and whether lymphodepletion is needed and then conducting a separate Phase II study that builds on results from the initial trial. Dr. Pilewski requested further clarification regarding the rationale for combining these different types of studies. Dr. Zoloth shared Dr. Pilewski's and others' continued concerns regarding the proposed combined study design. This first-in-human trial will enroll very ill patients, who will be given an investigational product with the potential to cause severe side effects. Dr. Zoloth noted that there is a place for Phase I/II trials, but in this specific case, there is very little preliminary data that would support the robustness of the hypothesis and inform the safety profile of the study intervention. Given the absence of such data, the investigators need to proceed very carefully. She strongly advised that they conduct this initial research as Phase I safety study.

- To control for participants receiving other salvage therapies or hormonal treatments, the Phase II arm of the protocol has been revised to include stratification of these groups. Dr. Pilewski commented, however, that the implications of these stratifications for the power analysis were not fully addressed, making it difficult to assess whether the sample will be sufficient to justify moving forward to a larger Phase III trial.
- Dr. Pilewski also asked about the rationale for having a 1-month follow-up period before proceeding to the next cohort. He suggested that the investigators take into consideration off-target effects in defining the timeframe for safety monitoring within the dose escalation plan. In addition, the protocol should include a more detailed plan for data safety reviews to assure careful oversight and monitoring, given the significant risks of this study.
- The revised consent addresses many of the reviewers' questions and concerns. Dr. Zoloth commented, however, that any language that suggests a treatment transaction (i.e., that the patient has to give money to the researchers) implies that the intervention is a treatment. The current language therefore needs to be as clear as possible with respect to this issue and to be reviewed with a critical eye. She questioned statements suggesting that current law requires third-party payers to pay for FDA-approved research and pointed out that in many other protocols, the researchers take responsibility for any untoward side effects and research-related injuries directly. Dr. Kohn noted that the Affordable Care Act includes language regarding third-party coverage of the cost of care provided through clinical trials but that such coverage by insurers is not an FDA rule.
- Dr. Zoloth appreciated the investigators' hesitancy to address the issues associated with the short life expectancy of this patient population more directly, but the duration of the study and the long-term follow-up may need further discussion in the consent in balance with the real-life situation facing the study participants.
- The issue of "withdrawal" within the context of ongoing follow-up may need further clarification. As previously discussed, because the cells persist in the body, participants can withdraw from ongoing monitoring but not from the intervention. Follow-up may continue for years because the long-term effects of the study agent are not known. Monitoring for 15 years presumably would not be recommended if it were known that the cells have no delayed effects. Thus, without an extended follow-up period, potentially serious research-related side effects might not be captured.
- Dr. Zoloth requested clarification regarding provisions to turn off or eliminate the modified cells (e.g., with a suicide gene) to reduce the risk of side effects. If such an approach has been demonstrated to be successful, it should be discussed in the consent. Dr. Donahue asked whether the team has tested use of cyclosporine or the caspase to reverse cytokine release effects with this cell product, or if the investigators are depending on published results in situations that may not exactly replicate the setting in the proposed trial. Ideally, the team has tested (or will test) these mechanisms to validate the results for the proposed research. Dr. Kohn asked why the investigators do not expect to encounter significant toxicity from the IL-12 as seen in other settings. He agreed with the suggestions to test the proposed stopping mechanisms to data showing their effectiveness in the planned setting.

- Dr. Donahue noted that the study will exclude individuals with significant co-morbid conditions, including serious medical illness and psychiatric illness. These criteria are vague, however, and should be more clearly defined within the context of potential adverse effects such as CRS. He encouraged the investigators to take into consideration all of the major organ systems with potential illnesses that could interfere with or be worsened by the investigational therapy or the side effects of the therapy. These exclusionary conditions should be clearly identified and defined so that eligibility is based on consistent, pre-specified parameters. Similarly, the inclusion criteria should be precisely defined to avoid ambiguity in understanding or interpretation.
- Dr. Zoloth advised including an individual who is independent of the study and research institution in the DSMB.

D. Investigator Response

1. Written Responses to RAC Reviews

In response to requests for additional data to support the rationale for the proposed study, the investigators cited a study that showed MUC1 CAR T cells eliminated human adenocarcinomas in immunodeficient mice. Since the tumors recurred, the team has applied for additional funding to add IL-12. The mice in these studies were not given chemotherapy or radiation therapy before MUC1 CAR T cell therapy. The investigators noted that there is no nonhuman primate model that could provide additional preliminary data for the proposed research.

Regarding questions about off-target toxicity, the investigators noted that MUC1 is expressed at low levels by several normal tissues, but it has a predominance of core 2 glycans preventing the MUC1 tumor-specific antibody (derived from de-glycosylated MUC1) from binding. Thus, the investigators state, toxicity has not proven problematic in antibody-based therapeutic protocols targeted to tumor-specific MUC1. Furthermore, despite low-level expression of MUC1, killing of a non-tumorigenic mammary cell line by MUC1 CAR T cells has not been observed. As discussed, a preclinical safety study in human MUC1 transgenic mice showed no toxicity of MUC1-specific CTL, which eradicated MUC1 tumor cells.

The investigators revised the classification of the proposed study from a Phase I/II protocol to a Phase IA/IB/II study, with the Phase IA component involving dose escalation for MUC1 CAR T cells, followed by the Phase 1B component for infusion of MUC1 CAR T cells after lymphodepletion. Each dosing cohort will be followed for 1 month before proceeding to the next cohort. The control group will be stratified with or without salvage therapies. Similarly, use of hormonal therapies will be stratified in the randomization. All problems or symptoms present at the time of registration will be monitored for progression or regression at each subsequent visit. At each visit during the trial, all new problems, progression of problems not present at the time of registration, and increased severity of problems reported at the time of registration will be recorded. Any new problem developing or progression since registration will be reported as an AE and treated according to good clinical practice.

Information on the composition and independence of the DSMB has been added to the protocol.

A detailed description of the different elements of the cloned DNA was provided.

The investigators noted that they have not performed any assays to determine efficiency of the transposon and transposase system, which the protocol states is about 40 percent. The efficiency of the gammaretroviral vector was revised from 5 percent, which was considered very low and likely incorrect, to 35 percent per a different reference.

A detailed description of the multi-step process for how the CAR T cells will be generated was provided. Autologous T lymphocytes will be electroporated with pT2/SVNeo IR H0X iCaspase-9 NFAT.hIL12.PA2 IR pT2/SVNeo and SB transposase plasmid 20207. The description includes the process for analysis of CAR expression by flow cytometry, preparation of aAPCs, aAPC-mediated stimulation of CAR-positive T cells, and harvesting and cyropreserving T cells for release testing and infusion. The investigators noted that they have not yet performed efficiency testing for generation of

these CAR T cells. In addition, because there is no integration of SB11 transposase in the electroporated and propagated T cells, the investigators stated that there is no need to assay for transposase. Furthermore, they do not have data using the K562 aAPC clone D2 and expression of IL-21, which was introduced with the SB system, because they have not performed this aspect of the T-cell generation process. The investigators clarified that Dr. Cooper's group will provide the reagents and procedures for generation of the CAR T but that the research team, not Dr. Cooper's group, will generate the cells.

A third-generation CAR was selected based on data showing benefits of the combination of CD28 and OX40 costimulatory molecules. Specifically, CD28/OX40-positive T cells produced significantly greater amounts of IFNγ than T cells that expressed CD28 or CD28/4-1BB.

The rationale for not using lymphodepletion initially is to determine whether it is necessary. If so, the protocol includes provisions for lymphodepletion with cyclophosphamide and fludarabine after the initial phase of the research. The investigators state that they have no experience with CAR T cells.

Sodium bicarbonate will be given because it reverses the acidic component of diarrhea and has been shown to be effective against diarrhea induced by irinotecan. A component of omega-3 fatty acids (available only in Japan) prevented systemic inflammatory response syndrome (SIRS) due to graft-versus-host disease after allogeneic stem cell transplantation, a condition due to activated lymphocytes. SIRS has been added as part of the rationale for using omega-3 fatty acids for prevention of colitis.

Given high-level expression of MUC1 in lung and pancreas, measurement of lipase and oxygen saturation will be done three times a week for the first 2 weeks post-infusion then monthly for 6 months and every 2 months for 2 years to monitor for any subclinical effects in these organs.

MUC1 is heavily shed from the cell surface and can be detected in serum. The investigators noted that soluble MUC1 may sustain proliferation of MUC1 CAR T cells based on data indicating that free immunoglobulins sustained proliferation of IgkCAR-CD28 transgenic T cells. Serum IL-12 and other cytokine levels will be monitored in the first day after administration of the test article to assess for T cell activation and detectable IL-12 in the circulation.

Regarding specific comments and suggestions to the ICD, the word "treatment" has been replaced with "research study" throughout the ICD to avoid wording that might imply that the intervention has proven therapeutic benefit. The language regarding anticipated benefit has been changed to state that the potential for benefit is completely unknown in humans and, at most, is possible but not expected. The investigators clarified that participants will not be required to pay for the intervention and that current law requires third-party payers to pay for FDA-approved research. To better distinguish between routine clinical care and the research aspects and aims of this study, the following statements have been added to the ICD: "We need to experiment on people with your disease to see if the research study is safe when used on people" and "This is a research study. It is not treatment because the goal is to test an idea and see if it works and if it is a safe idea. We can promise that you will help us in our research to help understand cancer. The benefit of the study to you is that you will have an opportunity to act as a courageous volunteer. The risk is that the experiment may be harmful or even fatal." The purpose of the study has been revised to include the reason why the research is being conducted based on the study hypothesis. Modifications to the patient's white blood cells and the reasons for those changes are now more clearly described in the consent. The consent has been revised to clarify that subjects cannot withdraw from the intervention once the cells are infused, only from ongoing monitoring. The PI did not consider it appropriate to add any discussion of lifespan or prolongation of life and pointed out that the 15-year "follow-up" is required by the FDA.

The investigators agreed with the other recommendations regarding the ICD and have modified the proposed consent document accordingly.

2. Responses to RAC Discussion Questions

Dr. Wright noted that the team has no preliminary data, including in vitro data, for the planned construct in support of the proposed study. The original construct was made by Dr. John Maher, whose study showed that MUC1-expressing tumor cells in mice were initially eliminated but subsequently recurred. No other studies or experiments with this construct have been conducted. The investigators agreed that there is a need for additional testing in a mouse model, such as the human transgenic MUC1 mouse model from Sandra Gendler's lab at the Mayo Clinic in Scottsdale, Arizona. Experiments using that model (by Dr. Gendler's lab and the study team) did not find any toxicity from MUC1-stimulated CTLs. Dr. Wright relayed the experience of one patient at their center who had post-infusion pain in the abdominal cavity at the location of the tumor; the patient's pain subsided after a brief time. In addition, he noted that the team's in vitro experiments with CTLs have always had a normal control, which showed no toxicity. These experiments were done by using a first-generation CAR T-cell construct, however, and similar experiments would need to be conducted with third-generation agents to show that there is no toxicity in vitro against normal cells that express MUC1 and also in the mouse model that is transgenic for human MUC1. Lymphodepletion would be done if initial studies fail to show clearance of the tumor from mice. Dr. Pilewski and others commented that such data are critical to providing some insurance against widespread T-cell infiltration and off-target effects. Plans to proceed with in vitro and animal studies with the proposed construct need to be completed before proceeding to Phase I and II human studies.

The investigators have not studied the transposon and transposase system described in the protocol, and they do not have experience with CAR T cells or the K562 APC line. Dr. Wright noted that he was not aware that transposases can integrate into the CAR T cells until reviewing material forwarded by Dr. Corrigan-Curay. Per that information, Appendix 5 of the protocol was revised to look for integration of transposases in the CAR T cells via PCR. Dr. Wright noted that the team does not have experience with CAR T cells or the K562 APC line. The decision to proceed with a third-generation construct was based on results from Dr. Malcolm Brenner's lab, which published data showing enhanced efficacy when using third-generation versus second-generation constructs. In these experiments, higher levels of IFNγ were produced with the third-generation construct.

Dr. Wright recognized the concerns raised regarding the combined Phase I/II study design but did not find this approach to be unusual. He noted that answers about the safety and potential efficacy of the investigational product could be obtained sooner with this approach versus separating the research into two trials. Dr. Wright explained that the 1-month timeframe for follow-up between dosing cohorts is based on results from the NCI Surgery Branch protocol, in which adverse events occurred between 10 and 20 days post-intervention. A detailed data safety monitoring timeline will be provided, as suggested.

Regarding the persistence of cells and ongoing monitoring for effects of the investigational product, Dr. Wright explained that the cells can be eliminated if needed by using the suicide gene icaspase-9 that is included in the CAR construct. The suicide gene mechanism would be triggered in the event of a life-threatening event thought to be secondary to the CAR T cells. The mechanism has been studied in humans and has been shown to eliminate lymphocytes causing graft-versus-host disease (GVHD). The team has not yet tested this mechanism or cyclosporine to reverse or cut off toxicities associated with the modified cells that will be used in the proposed trial. Several steps are in place in the planned protocol to reduce the risk of significant IL-12-related toxicities, particularly in the liver. Serum IL-12 levels will be monitored, with the goal of keeping IL-12 levels low. Participants with elevated serum IL-12 levels will be given cyclosporine, which blocks the NFAT and, in turn, the production of IL-12. If this approach does not lower IL-12 levels, a monoclonal antibody against IL-12 will be used. Dr. Kohn noted that while caspase has been shown to very quickly stop GVHD in patients (Brenner paper), CRS is a different entity, and it is not clear that the outcome with GVHD would directly extrapolate to CRS. Preclinical data to show that the icaspase gene in the construct for the proposed trial functions in the same way with CRS and/or IL-12 release should be pursued.

Dr. Wright agreed with the recommendation to include an independent external reviewer on the DSMB.

The investigators will address the additional recommendations for the ICD.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Cancer immunotherapy using engineered CAR T cells expressing tumor antigens is a very promising therapeutic strategy, but this experimental intervention has also been associated with considerable toxicity in certain settings. The lack of experience of this team with CAR immunotherapy and the complex pathway to bring them to the clinic, coupled with the lack of any in vitro or in vivo preclinical data with this construct is concerning. The RAC strongly suggests that the group consult FDA before proceeding further. In addition to consulting with FDA before further work is done on this approach, the following specific recommendations were made.

Preclinical Issues

- Preclinical studies to establish the efficacy of the MUC1 CAR T cells should be performed prior to moving into the clinic rather than relying on the results of another investigator's single mouse study in which the tumor regressed but then recurred (*Journal of Immunology*, 2008:180(7):4901-9). As this is a complex vector construct with which your group does not have experience, additional data are needed to establish that the vector will function as proposed in this protocol. Finally, further preclinical studies should be performed to establish that the MUC1 CAR T cells do not have any significant on-target off-tissue interactions on normal or regenerative human tissues.

Clinical and Trial Design Issues

- This study proposed to use a third generation CAR, i.e. one that has two additional signaling domains (CD28 and 0X40 (CD134)) in addition to the CD3 zeta chain. Although efficacy has been observed with both second generation and third generation CAR constructs in clinical trials, both constructs are associated with toxicities. Because more studies have been done with second generation CAR constructs, a larger body of data is available on the potential toxicities of the second generation CAR constructs. The protocol does not provide a clear explanation as to why a third generation CAR is preferred for this initial safety study.

- IL-12 is being included in this construct to modulate the immune response in the microenvironment of the tumor and thereby enhance the anti-tumor activity of the MUC1 CAR T cells. Your reference in support of using IL-12 is a publication from the Surgery Branch at the National Cancer Institute (NCI) using T cells that expressed a normal T cell receptor (TCR), not a CAR, in a murine model. However, this same group closed two clinical studies that used IL-12 under the control of a NFAT promoter in T cells expressing a tumor antigen-specific-TCR due to severe liver toxicity. The protocol does not explain why your construct would not be expected to lead to the same toxicity. Your plan is to prevent this potential toxicity by administering cyclosporine A (CsA), which targets NFAT, if the IL-12 levels are too high (>2 fold above normal levels). However, there is no data that you could abort this toxicity through the use of CsA. Furthermore, the protocol lacks data on how IL-12 levels will be monitored and how quickly the IL-12 levels can be obtained to guide clinical management.

- You plan to use ustekinumab, the anti-IL-12/23 p40 monoclonal antibody, to block IL-12, or the dimerizing agent API903 to activate the inducible caspase 9 suicide gene to eliminate the MUC1 CAR T cells, if severe life-threatening CRS develops and it is not reversed by corticosteroids, tocilizumab, or CsA. Additional data are needed to establish that the proposed abortive mechanisms would be effective in halting these toxicities in vivo. Of note, the inducible caspase 9 suicide system has been shown to be effective in controlling GVHD but not in the setting of CRS.

- The exclusion criteria states that individuals with serious medical illness will be excluded. Given the potential risks of CRS after T cell immunotherapy, additional criteria should be developed to

specifically exclude research participants with higher risks of adverse outcomes with CRS (e.g., those with history of cardiac dysfunction, liver dysfunction, significant COPD, etc.).

- Because MUC1 is expressed in the lung and the pancreas, there is a risk of on-target-off-tissue targeting by the modified T cells. Consider whether the 1-month proposed waiting period between dose escalations is sufficient to detect such toxicities. In addition, assessment of potential pulmonary toxicity should be done using the more sensitive pulmonary function test (PFT) rather than oxygen saturation levels.

- Upon completion of the dose escalations in Phase I, both with and without lymphodepletion chemotherapy, the proposed plan is to randomize 160 subjects into the Phase II part of the trial to determine the efficacy of MUC1 CAR T cells versus untreated controls. This study is designed as a combined Phase I/II trial to avoid resubmission of a new protocol at the end of the Phase I portion of the study. Given the novelty of this construct and the intention to enroll a large number of research participants in the Phase II portion of the trial, consider conducting a Phase I study initially. Subsequently, plan and proceed to a Phase II trial after the results of the Phase I study demonstrate the safety of your construct and inform the optimal design of the next phase of the study.

- Given the complexities of this study and the potential for toxicity due to known expression of MUC1 on non-tumor tissues and the use of IL12, this study would benefit from a dedicated external data safety monitoring board (DSMB). The protocol should articulate the time points at which the safety data will be assessed (e.g., after completion of each dose cohort or after dosing of a given number of subjects) and how that data will be used to make decisions on whether or not to proceed to the next cohort.

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

- The discussion of long-term follow-up should be balanced by some acknowledgement that prognosis for survival with the target disease is significantly shorter than the proposed 15 years of follow-up.

- The language regarding the ability of research participants to withdraw from the protocol should clarify that it is not possible to undo the infusion of the CAR T cells, despite the inclusion of a suicide system, which can potentially eliminate the majority of the CAR T cells. Some of the engineered T cells may persist and have future effects on the research participants, which can only be monitored in subjects who do not withdraw from the study.

- The informed consent states that "The processing of your WBC to make the (CAR) T cells ($30,000 (Carl June, personal communication)) will be charged to you or your insurance company." Charging for the gene-modified T cells implies that this is an established treatment. Furthermore, the assertion that the participants' insurance will pay tens of thousands of dollars to obtain the gene-modified T cells should be confirmed as it is not clear that this would be required under the current laws, since it is an experimental agent not a clinical standard of care.

G. Committee Motion 5

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IX. Review and Discussion of Human Gene Transfer Protocol #1501-1372: A Phase I Clinical Trial to Evaluate the Safety and Immunogenicity of a Personalized Polyepitope DNA Vaccine Strategy in Breast Cancer Patients with Persistent Triple-Negative Disease Following Neoadjuvant Chemotherapy

PI: William Gillanders, M.D., Washington University School of Medicine

RAC Reviewers: Dr. Hammarskjöld, Ms. Hardison, and Dr. Kaufman

Professor Dresser recused herself due to a conflict of interest.

A. Protocol Summary

Tumor-specific antigens that are uniquely expressed in individual cancers are typically the result of point mutations or other genetic changes that are present only in the tumor. As such, tumor-specific antigens represent the only antigens that are truly unique to the tumor and not expressed in normal tissues. Cancer vaccine strategies targeting mutant tumor-specific antigens are potentially safer and more effective than strategies targeting shared tumor antigens. Because mutant tumor-specific antigens are expressed only in the tumor, the risk of autoimmunity is decreased. One of the hallmarks of cancer is genome instability, and one clear weakness of cancer vaccines that target a single shared tumor antigen is antigen loss. Targeting multiple mutant tumor-specific antigens may preclude antigen loss and is likely to be universally applicable in solid tumors. Solid tumors appear to have a remarkable number of nonsynonymous mutations present, suggesting that a personalized vaccine approach could be used in most patients with solid tumors, regardless of intrinsic subtype or HLA type.

The personalized polyepitope strategy is based on a DNA vaccine platform. Advantages of the DNA vaccine platform include the safety profile of DNA vaccines; the relative ease of manufacture relative to proteins and other biologics; and the molecular flexibility of the DNA vaccine platform, which provides the ability to genetically manipulate encoded antigens and/or incorporate other genes to amplify the immune response. The molecular flexibility of the DNA vaccine platform also allows the investigators to target multiple mutant tumor-specific antigens using a single polyepitope DNA vaccine. Use of personalized polyepitope DNA vaccine as planned in the proposed trial may be a more effective way to generate an immune response to breast cancer cells.

This Phase I clinical trial will evaluate the safety and immunogenicity of personalized vaccines that express tumor-specific antigens identified by sequencing tumors from individuals with triple-negative breast cancer. The personalized polyepitope DNA vaccines are composed of plasmids that are designed to express mutant tumor-specific antigens identified by DNA and RNA sequencing. These plasmids will be formulated without the addition of lipids (i.e., as naked DNA) and will be administered intramuscularly by electroporation. The hypothesis is that personalized polyepitope DNA vaccines will be safe and capable of generating measurable CD8 T cell responses to mutant tumor-specific antigens. Fifteen evaluable breast cancer patients will be enrolled. Potential participants must provide consent for genome sequencing and must provide germline and tumor DNA samples for sequencing. Subjects meeting eligibility criteria (including having recently completed pre-surgery chemotherapy for their breast cancer) will be treated with 4 mg of a personalized polyepitope DNA vaccine at 3 time points. Peripheral blood specimens will be obtained at independent time points before and after vaccination to evaluate the immune response. Each subject will be followed for 12 months following the last vaccination. Additional follow-up visits or telephone contact will be scheduled annually thereafter if the patient is available for follow-up. Due to the generation of potentially unique products for each research participant, traditional toxicology and biodistribution studies are not planned.

B. Written Reviews by RAC Members

Six RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it involves a novel vaccine strategy whereby individualized vaccines targeting mutant tumor-specific antigens will be identified, in part, by computer modeling.

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

Dr. Hammarskjöld noted that one of the most serious adverse effects of the proposed intervention is the potential reactivity of the generated immune responses against non-tumor tissues. This is of special concern because each patient will be treated with a “different” plasmid. Thus, there will be limited informative value from each individual patient enrolled in this trial. Given that the antigens will be different in each case, the investigators need to expand on why they do not think that autoimmune responses will be common and a potential serious concern with their proposed strategy.

The investigators explained in the protocol why they do not think that further preclinical safety/toxicity studies would be meaningful. The rationale for this is largely based on the fact that each patient will be subjected to a unique plasmid. In addition, some preclinical studies with other antigens seem to validate that the prediction algorithm led to immune responses that were primarily directed to the mutated proteins. Regarding this issue, Dr. Hammarskjöld asked the following:

- How confident are the investigators that the selected “epitopes” will not generate immune responses to “normal” proteins as well as the mutated proteins?
- Are the investigators planning to assess this for each individual patient?
- Will expression and immunogenicity of expressed epitopes from the final plasmid constructs be determined in any way (beyond verifying if the mRNA is expressed after transfection)?
- How will the investigators verify that the mutated proteins are only present in tumor cells?
- How will potential cross-reactivity against normal tissues be assessed?
- How will patients be monitored for potential specific adverse effects of this nature?

The protocol notes the possibility of integration of the DNA plasmid vector into genomic DNA of transfected myocytes and that plasmid integration at a sufficiently high frequency carries the possibility of inducing deleterious mutations. Potential side effects of plasmid integration could include an increased risk of malignancy arising from the cells harboring the mutation. The protocol does not appear to address this potential safety issue, however. The reason for not pursuing this further is not clear.

After DNA exome sequencing, the investigators will perform a cDNA capture (mRNA analysis) on tumor tissue to verify that the proposed antigens are represented in transcribed mRNA. However, detection of mRNA does not necessarily mean that protein is expressed (resulting in sufficient epitope expression to make these cells efficient targets for immune responses). Dr. Hammarskjöld asked if the investigators have considered ways in which this potential concern could be addressed.

From the description of tumor DNA and RNA exome sequencing and tumor cDNA capture sequencing, it appears that the percentage of the tumor cells that express any of the mutated “antigens” could be determined as well as whether the antigens are co-expressed in these cells. It is not clear if the investigators plan to address these questions.

The investigators refer to the material that will be inserted into the plasmid as a “polyepitope”. Additional information was requested regarding the size of the individual antigen epitopes and of the total polyepitope insert.

Per the protocol, any significant autoimmune disease or phenomena presumed to be related to protocol therapy will lead to a participant being removed from the study intervention. Dr. Hammarskjöld requested additional information about how patients will be monitored for potential auto-immune reactions, how these patients will be further assessed, how follow-up and potential treatments will be paid for, and how this will affect enrollment of additional patients.

Dr. Hammarskjöld requested clarification as to who will determine if any of the stopping criteria have been met and if the PI is the only person who will review safety data before these data are reported to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC). Further, safety data will be reported to the QASMC every 6 months, but the protocol does not specify potential events that will trigger more frequent reporting.

The personalized nature of this research makes determination of the sample size more complicated than described in the protocol. As presented, the power calculations for safety will likely be applicable to more generalized effects of the proposed research (e.g., effect due to the plasmid backbone, injection method, etc.). The investigative team was asked to address this concern.

Dr. Hammarskjöld questioned the policy regarding research-related injuries, specifically (as stated in the consent), that Washington University School of Medicine will not provide long-term medical care or financial compensation for research-related injuries. This policy should be clearly explained to patients so they understand that they may have to pay for injuries sustained as a result of participating in this study. Any provisions in place to cover these kinds of medical costs, which often are not covered by individual insurance, should be specified.

The requirement that each participant must consent to genome sequencing and database of Genotypes and Phenotypes (dbGaP)- -based data sharing and provide germline and tumor DNA samples for sequencing is not clearly described in the ICD. In addition, the consent form does not mention dbGAP data sharing.

The consent needs to explain that because this is a “gene therapy” protocol, withdrawal from the study in the true sense of the word may not be possible and that withdrawal reflects withdrawal from ongoing monitoring.

It is not clear why immune reactions are described in the consent document as “less likely” risks of the study intervention. In addition, more serious consequences of autoimmune reactions should be discussed in the consent.

Ms. Hardison had the following comments and suggested modifications regarding the ICD:

- “Routine blood tests” should be described so that the subject is well informed regarding what the research is considering in determining his/her eligibility to participate in this trial. The specific blood tests that will be performed should be listed.
- The use of the term “research blood tests” and “routine blood tests” are too vague. The document should clarify that some blood tests will be considered part of standard care and other tests will be done for solely research purposes. There should be a way for subjects to distinguish between these tests.
- The description of the potential effects of the intramuscular delivery of DNA indicates that there is a risk of muscle damage, peripheral nerve damage, and/or injection site infection. Although the consent indicates that these risks are unlikely, the protocol states that the electroporation device may increase these risks. Subjects should be informed of this risk (perhaps under the description of risks associated with the electroporation).
- In describing the time frames for potential side effects, the term “short time” should be changed to “several days” to be consistent with the protocol.
- The investigators should consider adding a table that lists “routine” and “research” evaluations and when/how frequently each evaluation will be done to more clearly present what is done under this trial. This type of chart might also assist the subjects in adequately assessing their potential financial risks.
- The investigators should consider deleting the statement that subjects give up property rights when they agree to allow their samples and data to be used for this research. Subjects enrolled in this study do provide consent for their genetic material to be utilized and shared; however, they also have the right to revoke this consent for continued use. The document should explain that data and samples collected up to the point of revocation will continue to be part of the research data. It should not be conveyed in any way that subjects do not have rights regarding their personal health information.
- Regarding the autopsy request, Ms. Hardison recommended removing the sentence, “We do not expect that an autopsy will be necessary in all cases; we expect that it will be extremely rare for us to conduct an autopsy related to your involvement in this study.” She found this statement to

be unnecessary and misleading. The investigators should specify what would be considered clinically relevant information discovered from the autopsy that could affect family members.

- The information regarding privacy and confidentiality might fit better under the “Risks” section of the ICD instead under “Other Considerations.” Subjects should include this information as a risk when making a determination of the risk-to-benefit ratio for participating in this research trial.
- The ICD document does not outline what interventions would be considered routine if a subject opted for “routine clinical care of their condition.” In addition, there is no means for the subject to determine what their potential financial risks might be. Ms. Hardison recommended that the investigators explicitly state which blood tests will be monitored to measure the effectiveness of the vaccine and which tests will be done to assess safety.

The protocol and consent need to specify whether a sample will be excised for the purpose of this research in cases where a sample of the patient’s tumor is not already available.

Dr. Kaufman noted the complexity of the proposed trial and that the investigators have arranged a large number of processes to accomplish the goals of this research. The protocol has appropriate eligibility criteria, toxicity monitoring plans, lot release criteria, and stopping rules.

A major issue in this study is that the investigators have not provided a sense of the time it may take to develop the vaccine for each patient, many or most of whom may have an average of 3 years before recurrence or metastatic disease occurs. Using this patient population makes sense, but patients may ultimately receive the vaccine at variable stages of their disease. This factor may not be important for assessing safety or immunogenicity, but it could be an impediment to further development of this approach. Because the timing of vaccination may influence therapeutic activity, more information on how the vaccine may be standardized for preparation would be important when considering future studies designed to evaluate clinical responses.

Dr. Kaufman noted that to date, DNA vaccines have not proven to be clinically effective. However, given the complexity of the epitope selection process, it is reasonable to use this approach as a “proof-of-concept” strategy. In addition, the plasmid (pING) vector has been given to human subjects in other clinical trials, most notably in melanoma Phase I studies, in which no unexpected adverse events were reported. Dr. Kaufman identified the following additional issues for the investigators to consider or address:

- Information on how long it takes to establish the final vaccine product should be provided. The protocol should specify how subjects who may develop recurrent or metastatic disease in the interval between tumor collection and vaccine availability will be managed. A plan to either replace these subjects or remove them from study should be clearly provided.
- The use of intra-muscular injection is understandable, but this may not be an optimal strategy of inducing effector CD8+ T cell responses. The rationale for this approach as opposed to an intradermal or subcutaneous approach (which may result in more potent Langerhans’ cells as targets for antigen presentation) should be provided.
- The informed consent allows subjects to decline genomic data analysis for further research. However, one of the eligibility criteria is allowing germ-line genome sequencing. This information should be clarified and made consistent.
- The selection of three to five epitopes based on HLA binding algorithms and in vitro studies raises several questions, including how the epitopes will be selected. This process may be challenging, and more information would be helpful. For example, what is the basis for the number of epitopes? Because very high-affinity binding may result in T cell deletion, it may be preferable to select slightly lower affinity epitopes. This study will also require significant resources to generate reagents for in vitro binding studies and immune monitoring, including tetramers and ELISPOT assays. Given the numerous other complexities in this study, it is not clear why the subjects will not be HLA-restricted to one or perhaps a few different HLA alleles where reagents are more readily available.
- Although the underlying hypothesis of this study is that the mutated epitopes are unique to each individual tumor, it is hypothetically possible that cross-reactivity to native or other mutated

epitopes may result in off-target recognition. The investigators should specify how expression of selected gene/protein targets in normal tissues will be detected/determined. If the normal tissue distribution of the selected genes is known, the potential for autoimmune toxicity might be better anticipated. Because no formal good laboratory practice (GLP) safety studies are planned, it may be helpful to consider evaluating a series of normal tissue for gene expression of the selected antigens.

- The electroporation device has been associated with toxicity in patients with sinus bradycardia. The presence of sinus bradycardia is an exclusion criterion for subjects, but an EKG is only planned for the pre-study visit. An EKG should be considered prior to the three vaccinations to screen for sinus bradycardia and potentially other dysrhythmias, and if identified, patients should be excluded from the research intervention.
- A plan to measure anti-DNA antibody titers (if not already being done) might be helpful in evaluating potential immune reactions against the vector.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Dr. Hammarskjöld expressed concern that the protocol and consent do not fully convey the potentially serious nature of the immune response to the vaccine product, and specifically, to the mutant tumor-specific antigens. Not only is cross-reactivity to the “normal” protein represented by the specific epitope possible, but cross-reactivity to other T-cell epitopes may also occur. These responses are especially hard to predict, adding to the unknown risk of the investigational product. Dr. Hammarskjöld requested additional information on how the prediction algorithms will be able to identify the mutant tumor-specific antigens that will be less likely to be cross-reactive in normal tissue to minimize these risks. Dr. Kaufman suggested that the investigators develop all the reagents needed for the immune monitoring studies.
- The information in the consent about the databases and repositories is very general and only refers to sharing of “research data,” which will probably be interpreted as lab data but not specific genetic information. So that participants have a clear understanding of the scope of sharing that is planned, the document should needs to explicitly mention sharing of genomic data and the special protections in place for such sharing (e.g., removal of personally identifiable information). There was a question as to the impact of results of genomic analysis on the patient and potentially on the patient’s family as well. Dr. Ross noted that in forensic science, for example, individuals have been located based on testing of family members. Dr. Zoloth pointed out that genetic data cannot be completely de-identified or anonymous. Provisions can be put in place, however, to reduce the risk of disclosure. She suggested using language to explain the balance between risk and disclosure, such as the following: while sharing of genomic data on [dbGaP] poses some degree of risk for the patient and the patient's family members, the investigators will go to all possible lengths to prevent any disclosure of that information.
- In following up to responses to prior questions, Dr. Hammarskjöld asked whether the investigators consider verification of mRNA to be adequate or if other methods to confirm epitope expression should or could be added to the protocol. In addition, she asked whether use of filters to meet a minimum variant allele frequency can provide an estimate of the percent of mutant tumor-specific antigens within a tumor.
- Patients who develop significant autoimmune disease will be removed from the study. However, it is not clear how participants will be monitored specifically for autoimmune reactions. In addition, the investigators were asked how (or if) patients will be further assessed if they are withdrawn from the protocol, what provisions are in place to cover the cost of follow-up, and how withdrawal of participants due to a severe response will impact enrollment of additional patients. Dr. Kohn suggested adding a measure (e.g., ANA panel) at baseline and specific time points over the course of the study as part of ongoing monitoring.
- Ms. Hardison was not certain how a subject can do a risk-benefit analysis, especially with respect to the financial aspects of participating in this research, if they do not know what tests could be their responsibility.

- Ms. Hardison and Dr. Kaufman expressed concerns about the use of IM injections to deliver the investigational product. Ms. Hardison noted that risks of intramuscular delivery of DNA, including muscle damage, peripheral nerve damage, and injection site infection are increased with EP. The investigators plan to revise the consent to inform participants of these risks following consultation with the sponsor. Dr. Kaufman conveyed his concern that an immune response may not be detected with IM injections but acknowledged the investigators' experience with and preference for this route of administration
- Ms. Hardison agreed with the comments and suggestions about sharing of genome data, and noted that her queries to the investigators had been addressed for the most part.
- Dr. Kaufman commented that the consent should inform participants that they may be withdrawn from the study and not receive the vaccine if they develop advanced disease.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators anticipate that it will take several months to develop a personalized vaccine product. They took this timeframe into consideration when designing the study, which is one reason why patients in the adjuvant setting (patients with no evidence of disease, but who are at high risk of disease recurrence) will be targeted. The plan is to identify patients who are eligible for the study at the time of their initial diagnosis. While the patient is undergoing standard-of-care therapy (typically neoadjuvant chemotherapy, surgery, and then radiation therapy), the tumor will be sequenced, and the mutant tumor-specific antigens will be identified and prioritized for inclusion in the personalized vaccine. The vaccine will then be manufactured to be available when the patient has completed standard therapy, which is expected to take approximately 6 months (4 months for neoadjuvant chemotherapy, 1 month for surgery, approximately 1 month for radiation therapy). The investigators do not expect a significant dropout rate due to progression of disease during this time frame. However, if a patient's disease does progress, the patient will be given other standard-of-care therapies and will not be eligible for vaccine treatment; such patients will be replaced.

The route of and process for administration of the vaccine were carefully considered in the study design. The investigators have partnered with a medical device company, Ichor Medical Systems, to use an electroporation device for delivery of the DNA vaccine. They noted that intramuscular delivery with EP has proven to be very successful in nonhuman primate studies and in preliminary studies of healthy volunteers being treated with candidate HIV vaccines. The investigators commented that using an electroporation delivery device represents the state of the art for DNA vaccine delivery. The EP device has been associated with toxicity in patients with sinus bradycardia, however; as an added safeguard, the protocol will include an EKG or rhythm strip prior to the three vaccinations in addition to the pre-study visit. The ICD will be revised to include a discussion of the risks of IM administration of the vaccine with EP. The consent form (under risks of EP) already specifies that the potential side effects of EP typically last between 24 and 72 hours but may take up to 5 days to resolve.

The investigators acknowledged that selection of three to five epitopes for inclusion in the personalized vaccine is somewhat arbitrary. However, preliminary analyses by the team suggest that this is the most reasonable and pragmatic approach given the number of candidate epitopes that have been identified in triple-negative breast cancer patients to date. Significant resources are required for these studies, and the team will consider restricting study participation to specific HLA types as appropriate. (Extensive details about the epitope prediction algorithms developed and used by the team are provided in a recent *Nature* paper (Gubin et al., 2014; attached to the response to the reviewers' comments).) The goal of the sequencing and epitope prediction algorithms is not to determine what percentage of tumor cells expresses the mutant antigens. One of the filters used for prioritizing selection of mutant tumor-specific antigens involves meeting a minimum variant allele frequency. As such, the expectation is that only mutant tumor-specific antigens that are expressed in a significant number of cells will be targeted. The investigators acknowledged that cross-reactivity with the native protein may result in off-target recognition and potential autoimmunity. The epitope prediction algorithms are designed to minimize the potential for off-target cross-reactivity but cannot completely exclude this possibility. The FDA has recommended

additional steps in the algorithms to minimize the risk of cross-reactivity. A BLAST search will be performed on the final personalized vaccine construct to exclude any unanticipated cross-reactivities, and if any are detected, the construct will be revised.

Clinical assessment and laboratory evaluation will be done to determine if there is any cross-reactivity against normal tissues. If cross-reactivity is observed, additional studies will be performed to try to identify the etiology of the autoimmune reaction. Decisions about how these treatments will be paid for will be made on an individual basis, in collaboration with Washington University School of Medicine. SAEs will be reported (per the protocol) and may result in termination or amendment of the protocol. Significant adverse events that result in protocol/consent amendments will be reported to subjects considering enrollment.

Determination of the immunogenicity of the expressed epitopes is not planned for this Phase I protocol; verification that the mRNA is expressed after transfection will be done, however. To date, there have not been any reported cases of anti-DNA antibody responses to the pING parental vector. This issue was discussed as part of the pre-IND meeting; the FDA found that additional studies to assess integration of the DNA plasmid (prior to initiation of this study) are not needed. The investigators therefore do not plan to measure anti-DNA antibody titers routinely. If immune reactions against the vector are suspected, antibody titers can be measured.

The sample size calculations are designed to test the safety of the personalized vaccine strategy. Because the sequencing, epitope prediction protocols, and manufacturing are standardized, the personalized vaccine strategy is considered a single drug product. Each personalized vaccine will be distinct from the others, and this factor will be carefully considered when evaluating and reporting the results of the trial.

The PI will determine if any of the protocol stopping criteria have been met, in consultation with study coordinators and Dr. Lockhart (who runs the Developmental Therapeutics Group at Washington University School of Medicine). The QASMC may also determine that the protocol stopping criteria have been met following evaluation by the PI, study coordinators, and Dr. Lockhart. The reporting requirements and data and safety monitoring plan, including reporting of events to the QASMC (every 6 months, or more frequently as needed), the local IRB, and other regulatory agencies.

Exome sequencing of the tumor and normal tissues and RNA sequencing of the tumor is required for study participation but participants may decline to consent to future genetic research. Sequencing will be done to identify mutant tumor-specific antigens; the mutant proteins are not expected to be present in normal tissue if the mutant DNA is not present in normal tissues. The majority of patients are expected to have germline and tumor DNA samples of adequate quality for this purpose.

Information about sequencing and related requirements for participation will be stated more clearly in the ICD. The investigators agree that it is important for subjects to be well informed about study procedures and interventions, and they understand the concern that the consent does not list or provide additional detail on routine versus research blood tests and interventions. The language in the proposed informed consent document was developed in collaboration with the local IRB. This includes language that discusses provisions for saving subjects' samples and research data for use in future studies and references to sharing with the database of Genotypes and Phenotypes (dbGaP). In the past, the local IRB has asked the investigators to remove detailed lists of what tests and interventions are done for research versus clinical purposes to avoid confusing participants. The investigators believe that the current language in the consent form is consistent with expectations from the local IRB. The additional statements to clarify some language (e.g., that participants have the right to revoke this consent for the continued use, information about required tests) will be added to the consent as requested. The investigators pointed out, however, because the consent includes the preferred language of the local IRB, it is likely that they will be asked to remove any changes.

The description of what it means to withdraw (i.e., from ongoing monitoring versus from the intervention) has been clarified in the consent document. The investigators noted that the decision to cover medical

costs associated with research-related injuries is made on a case-by-case basis, and this issue is explained in detail to potential subjects. A chart to help subjects better understand the study evaluations and assess their potential financial risks will be added to the consent. The investigators agreed with the other recommendations regarding the ICD, including those related to information about autopsies, and have modified the proposed consent document accordingly.

2. Responses to RAC Discussion Questions

Dr. Gillanders acknowledged that the risk of cross-reactivity cannot be entirely eliminated. However, the epitope prediction algorithms have built-in safeguards to target the antigens that are least likely to be associated with cross-reactivity. The antigens will be prioritized with respect to the degree of difference in the predicted binding affinity between the wild-type and the mutant antigen. The highest priority is usually given to antigens with a mutation that significantly affects binding. Results of the investigators' research to date indicate that the mutation in these antigens is often in a known binding pocket residue. The biology of the peptide interaction with HLA Class I alleles suggests that using a strategy with those prioritization filters would reduce the likelihood of cross-reactivity. As previously noted, a BLAST search will be performed on the final personalized vaccine construct to exclude any unanticipated cross-reactivities, and if any are detected, the construct will be revised. Dr. Gillanders will review and, if needed, revise the language in the consent to assure that it is not misleading regarding the potential for and known as well as unknown risks of immune response to the vaccine construct.

Dr. Gillanders considered the suggested change to the consent language regarding sharing of genome data to be reasonable. The local IRB will likely find the revised language acceptable. Dr. Gillanders will confirm that provisions are in place to protect patients' privacy and that the consent describes those protections. It is not clear, however, if the consent needs to include language about the risks to family members with sharing of the patient's genomic information. Dr. Gillanders noted Dr. Zoloth's suggested language, and he requested NIH guidance and language for dbGaP data, if available, for further consideration.

The team's efforts to confirm expression of mutant tumor-specific antigens in preclinical studies required pushing the proteomics analysis to its limits, even when a large number of cells was used for the analyses. Give this experience, the investigators concluded that the technology is not robust enough at this time to be used in the context of a clinical trial. Technologies for detecting mutant protein expression may be used in the future, however.

The investigators use a set of filters in both exome and cDNA capture sequencing to minimize risk of false-positive cells. Mutant allele frequency is one of those filters. Exome sequencing is done in both breast cancer and normal DNA; cDNA capture sequencing is done to confirm expression of somatic mutations in the tumor. The mutant allele frequency filter prevents targeting mutations that are present in only a very small subset of the tumor. Results will vary somewhat given differences in tumor cell populations and mutational profiles. Thus, while the goal of the protocol is not to evaluate heterogeneity, including filters for a minimal variant allele frequency ensures that sequencing will target tumor-specific antigens that are present in a significant percentage of the tumors. The investigators are targeting a variant allele frequency of 40 percent (20 percent for each mutated allele). Setting the filter at 40 percent means that at least 40 percent of the tumor cells being sequenced expressed that allele.

Patients will be monitored for potential autoimmune reactions with clinical assessments and laboratory evaluations. Analysis of specific tissues for cross-reactivity is not planned. Further assessment of patients who are withdrawn from the proposed trial due to significant autoimmune disease would depend on the patient's symptoms and the results of the lab tests. Follow-up would therefore be specified to each individual. Dr. Gillanders expressed confidence that the investigators will be able to appropriately assess and follow patients based on the extensive clinical experience managing patients in investigational protocols. For trials conducted at the Washington University School of Medicine, the cost of treatment of all study-related injuries is covered by either the University or the sponsor. A subcommittee of the local IRB worked on the current consent language regarding who pays for these injuries. Specific stopping

criteria are built into the protocol. Thus, if there is a serious adverse event, it will be reviewed and may result in stopping the study.

All research tests and procedures done under the protocol will be paid for by the research study. Most of the tests done during the vaccination period will be research blood tests. The trial will also include some standard-of-care blood tests that also be covered under the study.

Dr. Gillanders noted the reviewers' concerns about the planned route of administration. The optimal site for delivery of DNA vaccines is not clear, but based on available data, intramuscular injection with EP seems to be the best option at this time.

The investigators will address the additional recommendations for the ICD.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- By design, each vaccine will likely be different and the peptides selected for the vaccine will be based in part on human leukocyte antigen (HLA) binding assays. The individualization of vaccines will raise some challenges in that many different reagents will need to be developed for in vitro binding studies and immune monitoring. One potential way to simplify this complexity, at least for this initial study, is to consider restricting enrollment to certain HLA-alleles where reagents are more readily available.

- The main concern in the reviews by the members of the RAC was that T cells generated in response to the vaccine might also recognize self-antigens on normal cells, leading to autoimmune disease. You have stated that it is possible that cross-reactivity to native proteins may result in off-target recognition and potential autoimmunity but that the epitope prediction algorithms that you have developed are designed to minimize the potential for off-target cross-reactivity. This possibility, however, cannot be completely excluded as demonstrated by the very unexpected off-target effects observed in a T cell protocol (Linette et al., *Blood*. 2013; 122[6]:863-871). To address this potential risk, you propose to clinically monitor participants for signs or symptoms of autoimmunity. In addition to a clinical assessment, diagnosis of autoimmune disease often involves serum markers and with any test some individuals may have results that are not "normal" despite not having clinical disease. You may want to consult appropriate specialists (e.g., a rheumatologist) regarding whether it would be helpful to draw certain baseline serum markers so that if an autoimmune response is suspected baseline labs will be available for comparison.

Ethical, Legal, and Social Issues

- As stated above, one risk of this approach may be autoimmunity and while this is mentioned in the informed consent as a less likely risk, consider adding additional information regarding why this could occur and what the clinical implications would be for the participant.

- The informed consent document states that "We may also share your research data with large data repositories (a repository is a database of information) for broad sharing with the research community" as a reference to the sharing of genomic data through the database of Genotypes and Phenotypes (dbGaP). While we understand that you and your Institutional Review Board do not want to use the term dbGAP in the informed consent, as that term is not particularly

informative, participants should be informed that it is genomic data that will be shared as they may have specific concerns about genomic data sharing. It should also be specified that such data will remain anonymous, but that de-identification of data cannot eliminate a risk of disclosure, which could affect the participant and even potentially the participant's family members.

- The informed consent document should explain to participants that if their disease reoccurs during the time that it takes to prepare the vaccine they may no longer be eligible for the trial.

G. Committee Motion 6

Dr. Kohn summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kohn requested a vote, and the RAC approved these summarized recommendations by a vote of 13 in favor, 0 opposed, 0 abstentions, and 1 recusal (Professor Dresser).

X. Closing Remarks and Adjournment

Dr. Corrigan-Curay thanked RAC members and guests for their participation. She also thanked staff for their hard work in putting together the current meeting. She announced that this was her last RAC meeting and that she will be moving to the National Heart, Lung, and Blood Institute's Office of the Director, where she will be working on a number of different issues related to clinical trials and strategic planning. Dr. Corrigan-Curay noted how much she has enjoyed working with RAC members and how much she has learned during her tenure with the RAC. Dr. Kohn thanked Dr. Corrigan-Curay on behalf of the RAC for her years of support in and commitment to the field of gene therapy and her unparalleled medical and legal knowledge, which contributed to the success of the committee. He wished Dr. Corrigan-Curay the best in her new position.

Dr. Corrigan-Curay introduced the new OBA Acting Director, Dr. Lyric Jorgenson, Dr. Carrie Wolinetz, the new Associate Director for Science Policy in the Office of the NIH Director, was introduced earlier.

Dr. Kohn thanked the RAC members and the OBA staff and adjourned the March 2015 RAC meeting at 3:41 p.m. on March 10, 2015.

(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.)

_____
Lyric Jorgenson, Ph.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: _____

_____
Donald B. Kohn, M.D.
Chair, Recombinant DNA Advisory Committee

Attachment I:
Recombinant DNA Advisory Committee Roster

Chair

KOHN, Donald B., M.D.
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Attachment II:
Public Attendees

(This list includes only individuals who are not identified elsewhere in this document. It does not include two individuals whose names are illegible on the sign-in sheets.)

Gwen Binder-Scholl, Adaptimmune
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Elizabeth Calleja, Inovio Pharmaceuticals
Gina Cravey, Harrington Cancer Center (*via teleconference*)
Marissa Diehl, Inovio Pharmaceuticals
Douglas Dieterich, Icahn School of Medicine, Mount Sinai
Marcia Gaido, Adaptimmune
Andrew Gerry, Adaptimmune
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Tom Holdich, Adaptimmune
Tom Kim, Inovio Pharmaceuticals
Stewart Sharp, Harrington Cancer Center (*via teleconference*)
Sanjay Srivastana, Harrington Cancer Center (*via teleconference*)
Maryann Toto, Shire
David Weiner, University of Pennsylvania
Joy Weir, Shire
Scott White, Inovio Pharmaceuticals

Attachment III:
Abbreviations and Acronyms

aAPC	artificial APC
APC	antigen-presenting cells
CAR	chimeric antigen receptor
CHB	chronic HBV infection
CK+	cytokeratin-positive
CMV	cytomegalovirus
COPD	chronic obstructive pulmonary disease
CPK	creatine phosphokinase
CRS	cytokine release syndrome
CsA	cyclosporine A
CTL	cytotoxic T lymphocyte
CT	computed tomography
CV	curriculum vitae
dbGaP	database of Genotypes and Phenotypes
DC	dendritic cell
DSMB	data and safety monitoring board
EBV	Epstein-Barr virus
ELISPOT	Enzyme-Linked ImmunoSpot
EP	electroporation
ETV	entecaviror
FDA	U.S. Food and Drug Administration
GI	gastrointestinal
GLP	good laboratory practice
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HBcAg	hepatitis B “c” antigen
HBeAg	hepatitis B “e” antigen
HBsAg	hepatitis B “s” antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HIPAA	Health Insurance Portability and Accountability Act
HLA	human leukocyte antigen
iCAR	intracranial chimeric antigen receptor
ICD	informed consent document
ID	intradermal
IFN	interferon
IFNβ	interferon beta
IFNγ	interferon gamma
IL-6	interleukin 6
IL-12	interleukin 12
IL-21	interleukin 21
IM	intramuscular
IND	investigational new drug
IRB	institutional review board
IU	international unit
IV	intravenous
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell
MTD	maximum tolerated dose
MUC	mucin 1
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction

PFT	pulmonary function test
PI	principal investigator
QASMC	Quality Assurance and Safety Monitoring Committee
RAC	Recombinant DNA Advisory Committee
SAE	serious AE
SB	Sleeping Beauty
SIRS	systemic inflammatory response syndrome
TCR	T-cell receptor
TDF	tenofovir disoproxil fumarate
TMZ	temozolomide
WBC	white blood cell(s)