
RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 4 and 5, 2012

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

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(Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <http://oba.od.nih.gov/oba/index.html>.)

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting¹**

December 4 and 5, 2012

The Recombinant DNA Advisory Committee (RAC) was convened for its 131st meeting at 2:00 p.m. on December 4, 2012, at the National Institutes of Health (NIH), Building 31-C, Room 10, in Bethesda, Maryland. Dr. Yuman Fong (RAC Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 2:00 p.m. until 6:10 p.m. on December 4 and from 8:30 a.m. until 2:35 p.m. on December 5. The following individuals were present for all or part of the December 2012 RAC meeting.

Committee Members

Andrew D. Badley, Mayo Clinic and Foundation
Tianxi Cai, Harvard University (*via teleconference*)
Paula M. Cannon, University of Southern California
Saswati Chatterjee, City of Hope National Medical Center
E. Antonio Chiocca, Dana-Farber Cancer Institute
Rebecca Dresser, Washington University School of Law
Yuman Fong, Memorial Sloan-Kettering Cancer Center (RAC Chair)
Norman Fost, University of Wisconsin, Madison (*via teleconference*)
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Hans-Peter Kiem, University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Walter J. Koch, Temple University School of Medicine
Donald B. Kohn, University of California, Los Angeles
Margaret Mallino, Missoula, Montana (*via teleconference*)
David A. Ornelles, Wake Forest University School of Medicine
Joseph Pilewski, University of Pittsburgh
Susan R. Ross, University of Pennsylvania
Marcella Sarzotti-Kelsoe, Duke University School of Medicine (*Day 1 only; via teleconference*)
Marshall Strome, St. Luke's–Roosevelt Hospital Center/New York Head & Neck Institute
Dawn P. Wooley, Wright State University
Laurie Zoloth, Northwestern University

Office of Biotechnology Activities (OBA)

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

Ad Hoc Presenter/Speaker

Carlos Ramos, Baylor College of Medicine (*via teleconference*)

Non-Voting Agency Representatives

Denise Gavin, U.S. Food and Drug Administration (FDA)
Samantha Smith, Office of Human Research Protection (OHRP), U.S. Department of Health and Human Services

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

NIH/OD/OBA Staff Members

Linda Gargiulo
Robert Jambou
Maureen Montgomery
Marina O'Reilly
Gene Rosenthal

Attendees

There were 76 attendees at this two-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, ad hoc reviewers and speakers, and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III is a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Fong, RAC Chair, called the meeting to order at 2:00 p.m. on December 4, 2012. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 24, 2012 (77 FR 67826). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of six gene transfer protocols, and discussion of one protocol previously reviewed by the RAC.

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

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

II. Minutes of the September 12, 2012, RAC Meeting

RAC Reviewers: Drs. Cai and Wooley

Dr. Wooley stated that the September 2012 meeting minutes document was well written and accurate, with a few minor changes needed.

A. Committee Motion 1

A motion was made by Dr. Wooley, but not seconded, to approve the September 2012 RAC minutes. The RAC members voted orally and unanimously to approve the September 2012 RAC meeting minutes.

III. Review and Discussion of Human Gene Transfer Protocol #1210-1189: A Phase II, Randomized, Open Label Study of Ad-RTS-hIL-12 Monotherapy or Combination with Palifosfamide-Tris in Subjects with Recurrent/Metastatic Breast Cancer and Accessible Lesions

Principal Investigator: John Nemunaitis, M.D., Mary Crowley Cancer Research Centers—
Medical City

Additional Presenters: John Barrett, Ph.D., ZIOPHARM Oncology, Inc.; Mary Matthew, ZIOPHARM Oncology, Inc.; Andrea Vergara-Silva, M.D., ZIOPHARM Oncology, Inc.; Hagop Youssoufian, M.D., ZIOPHARM Oncology, Inc.
Sponsor: ZIOPHARM Oncology, Inc.
RAC Reviewers: Drs. Ornelles, Pilewski, and Sarzotti-Kelsoe

Dr. Wooley was recused from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

This trial is a randomized, open label, Phase II study of Ad-RTS-hIL-12, an adenoviral vector expressing inducible interleukin-2 (IL-2) under the control of RheoSwitch® Therapeutic System (RTS), or Ad-RTS-hIL-12 in combination with palifosfamide-tris in research participants with recurrent and/or metastatic breast cancer with accessible lesions. Three dosing arms will be assessed for safety followed by random assignment to Ad-RTS-hIL-12 monotherapy or combination therapy. Each participant will be dosed for up to six 21-day cycles. Safety and tolerability will be assessed by the incidence and severity of adverse events as determined by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. A Safety Review Committee (SRC), composed of the Medical Monitor, Principal Investigators, and sponsor representatives, will be convened to review safety information and to decide upon further participant enrollment. The antitumor activity of the experimental treatment will be assessed according to total measurable tumor burden. Immunological and biological markers of response may include examinations of tumor biopsy samples, cytokine levels, and peripheral blood mononuclear cells.

The primary objectives are to assess the safety and tolerability of the activator drug plus Ad-RTS-hIL-12 as monotherapy and in combination with palifosfamide-tris. In addition, the investigators will assess the efficacy of repeated cycles of intratumoral injections of Ad-RTS-hIL-12 with oral activator ligand as monotherapy or in combination with palifosfamide-tris as measured by the 12-week progression-free survival (PFS) rate, defined as the proportion of participants who survive progression free at week 12.

The secondary objectives are to estimate PFS by immune-related Response Criteria (irRC) and RECIST v1.1, estimate overall survival, assess objective response rate by irRC and RECIST v1.1, assess response duration, and assess clinical benefit rate by irRC and RECIST v1.1. In addition, an exploratory objective is to evaluate pharmacodynamic tumor markers in tumor tissue samples that may correlate with objective tumor response and/or clinical outcome. Only individuals who are at least 18 years old will be offered enrollment in this trial.

The investigators' working hypothesis is that low-dose palifosfamide, through its inter- and intra-strand cross-linking of DNA, should induce antigen shedding as a consequence of cell death, resulting in the activation of the immune system. In addition, agents of this class have been shown to transiently decrease in myeloid-derived suppressor cells and regulatory T cells (Tregs), both of which are known to have potent immunomodulatory effects and to allow dendritic cells to mature, express costimulatory molecules, and become potent antigen-presenting cells.

B. Written Reviews by RAC Members

Thirteen RAC members voted for in-depth review and public discussion of this protocol. Key issues included that palifosfamide is not a licensed chemotherapy agent but is currently being evaluated in Phase III trials for the treatment of soft tissue sarcoma and non-small cell lung cancer. The risks and benefits of combining this experimental chemotherapy agent were considered to warrant further discussion, and the combination has not been tested in breast cancer with the gene transfer agent, which to date has only been tested in melanoma.

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

Dr. Ornelles expressed concern about the potent effect of IL-12 therapy, particularly when delivered directly to individuals. He also was concerned about the occurrence of several adverse events associated

with a related clinical trial, although those events might have been due to the advanced state of the participants' disease as much as to the study agent. Dr. Ornelles asked the investigators to clarify the statistical criteria used to define safety and to justify progression from the safety run-in portion to the randomized trial, including the statistical power underlying the decision to include additional participants if three or more participants out of 13 survive progression free at week 12. He asked for discussion of (1) the guidelines for the multiple trial sites that would promote uniform interpretation of the presentation of the lesions in each research participant, (2) whether the safeguards to prevent injection in participants with ongoing or recent infection at the injection site are attributable to the nature of the IL-12 transgene or to the nature of the patient population, (3) what the investigators expect to learn from the tumor and blood sampling and whether the response is anticipated to be sufficiently long lasting such that it can be captured with the necessarily limited rate of sampling, and (4) whether the virus distribution will be examined only in the tumor and draining lymph nodes, as it may be valuable to know if the IL-12 expressing viral vector was found in other locations in the event of a strong systemic IL-12 response. Dr. Ornelles noted that the informed consent document was thorough but contained overly complex language.

Dr. Pilewski asked the investigators to discuss the safety record of administering two relatively novel agents in combination, other than in a murine model, requesting discussion of the potential for additive or synergistic toxicity although no apparent theoretical drug interaction exists based on metabolism. He requested clarification of the decision regarding injection site and maintenance of treatment and more specific description of the pharmacodynamic evaluations, which currently are vague and leave much to the investigator's discretion. Dr. Pilewski suggested including in the SRC oncologists not involved in the study to provide unbiased safety analysis. Regarding the indication in the protocol that research participants who experience a substantial benefit from the experimental treatment and for whom the SRC recommends continuation on the study could be reexposed at a lower dose on a case-by-case basis, Dr. Pilewski asked whether such reexposure would be considered compassionate use or an extension study, and how such a study would proceed with respect to an institutional review board (IRB) and why the dose would be reduced. He requested that the investigators resolve inconsistencies and explicitly define the samples that will be assayed for transgene expression. He also asked why samples to assess shedding of adenoviral DNA are not being proposed for collection as part of this protocol.

Dr. Sarzotti-Kelsoe asked the investigators to explain the rationale for the experimental design, particularly that they plan not to continue the Arm B (palifosfamide) monotherapy in the randomization part of the trial and how the number of research participants being enrolled in each arm was determined. She requested discussion of preliminary information about what the immunological studies, mentioned in several places in the protocol as secondary endpoints, are expected to uncover. Dr. Sarzotti-Kelsoe asked about the existence of additional data showing nonclinical efficacy of the proposed combined therapy, in addition to the studies (reported on page 26 of the protocol) that show dramatically positive protective effects of the combination therapy in murine tumor models of breast, melanoma, colorectal, pancreatic, leukemia, and lung cancers with very low p-values, and she requested that Table 1 and Figure 3 provide information about the number of mice studied in each group to enhance assessment of these results. She requested discussion of the rationale for injecting the experimental drug into a draining lymph node (if the tumor lesions are not sufficient in number), as well as how investigators will know what type of cell(s) the injection would target and how the risk of undesirable local and/or systemic effects would be controlled. Dr. Sarzotti-Kelsoe suggested that the investigators add a statement to the informed consent document to address the issues of what would happen to the participant's tumor tissue and other samples, archived for future studies, should she/he withdraw consent. She asked the investigators what type of antibodies and what antibody specificity they plan to investigate in these research participants post-mortem, as well as which sites in addition to the Mary Crowley Medical Research Center would be involved in this multicenter study.

Dr. Cai provided a written review of the statistical elements of this study, to which the investigators were not required to respond in writing. Noting that the primary endpoint of the study is the 12-week PFS rate, she explained that the Simon two-stage design was adopted in this trial to minimize the expected sample size if the regimen has low activity. However, Dr. Cai noted that the sample size determination does not account for the potential loss to followup prior to the 12-week evaluation time point. In addition, she was

uncertain why the investigators included Arm B (palifosfamide monotherapy), since the toxicity of palifosfamide alone may not shed light on the toxicity of the other two arms and palifosfamide alone is not one of the targets for efficacy evaluation.

C. RAC Discussion

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

- Dr. Fong stated that advanced breast cancer is never a local problem; it is a systemic problem. He assumed that the investigators' hypothesis is that by delivering the IL-12 virus locally a systemic response would be generated. He requested that the investigators discuss whether any functional parameters have demonstrated an immune response systemically against melanoma. He also asked whether they have seen a remote melanoma deposit shrink as a result of local direct injection.
- With regard to the death in the investigators' previous (melanoma) trial, Dr. Fong noted that the research participant who died had cellulitis in the lower extremity. Given that fact, he asked whether patients with inflammatory breast cancer would be considered as candidates for this trial. He explained his concern that it is difficult to discern whether inflammatory breast cancer is merely a lymphatic invasion in the subcutaneous tissue or whether there is a component of cellulitis.
- Dr. Fong suggested that, if the investigators see shrinkage at a remote site, they consider biopsying that remote site to prove that no vector is present.
- Dr. Strome noted that this trial proposes to include 32 sites, which could make participant selection difficult to control. Another death could signal the end of this study, so how the investigators choose sites will be critical.
- Dr. Hammar skjöld asked if the investigators had looked at the melanoma trial to see how long after one week this vector persists.

D. Investigator Response

1. Written Responses to RAC Reviews

For the criteria to evaluate safety, the investigators have applied the standard Phase I oncology 3+3 design, with the modification of evaluating six participants in total. For justifying progression from the safety run-in phase to the randomized trial, the investigators added an additional go/no-go decision point for Ad-RTS-hIL-12 monotherapy after the safety run-in phase: if at least one of six participants is progression free at week 16, then this experimental treatment will be included in Part 2. Based on a binomial probability calculation using the targeted 16-week progression-free rate of 35 percent, the probability of observing zero out of six participants surviving progression free at week 16 is 0.047. Therefore, a futility rule to stop further enrollment in this experimental treatment arm would be invoked at that point.

The power for determining sample size for each arm is 80 percent. The investigators applied a Simon two-stage design.

The sponsor allows some discretionary decisions regarding the selection of which lesions to inject at which cycle (assuming a participant presents with multiple injectable lesions). However, the sponsor provides thorough guidance regarding the administration of INXN-2001. Furthermore, the sponsor also has provided clear, objective guidelines for the decision to inject either multiple lesions during one cycle or a draining lymph node. The primary objective of this trial is exploratory in nature and, as such, necessitates innovative approaches to pragmatic issues.

The nature of the immune response that might differ among research participants is unknown. With this in mind, the sponsor has made a concerted effort in developing the schedule of assessments in order to ensure that biological sampling coordinates with imaging timepoints. This coordination provides the best opportunity to correlate biological responses with objective clinical outcomes.

The SRC has met regularly to discuss the emerging safety profile of this experimental therapy. To date there have been three safety cases in the ongoing related trial, one of which was an unrelated deep-vein thrombosis. Analysis of these adverse events shows that it is difficult to establish to what degree these events can be attributed to the patient population versus the mechanism of action of the study therapy.

The potential exists for the viral vector to leave the site of administration and transduce distant tissues and immune cells. However, the viral vector is nonreplicative and under the control of an oral activator ligand (INXN-1001). *In vitro* studies were performed to demonstrate inducible expression of murine and human IL-12 in murine and human cell lines following transfection with Ad-RTS-mIL-12 or INXN-2001 (Ad-RTS-hIL-12). The results of this study demonstrated that following subcutaneous injection, both Ad-RTS-hIL-12 and Ad-RTS-mIL-12 concomitant with the oral administration of the activator, INXN-1001, vector distribution was predominantly localized to the injection site, with low levels observed in the draining lymph nodes. There were no noteworthy differences in biodistribution between the two vectors. A second study will be conducted during 2013 to confirm the safety and tissue distribution of the viral vector in the presence of the oral activator through six cycles. The investigators are currently assessing the potential for viral shedding and neutralizing antibody formation in cynomolgus monkeys; they plan to use the results of this study to validate the assays for both viral shedding and neutralizing antibody in nonhuman primates as well as tissue distribution prior to performing studies in research participants.

The existing safety data for the proposed first-in-human combination therapy is from a murine model, and these agents have not yet been tested in humans for the targeted disease state. Acknowledging those facts, ZIOPHARM has designed a safety run-in for the study that includes testing the safety and tolerability, after one cycle of therapy, of each agent as a monotherapy in the proposed indication; an additional safety run-in for the combination therapy consisting of three participants with staggered enrollment to address the possibility of additive or synergistic toxicity; and a low-dose palifosfamide study based on the data from the murine study and in order to minimize the risk of overlapping toxicity.

When viewed overall, the profile of adverse events experienced by research participants administered palifosfamide-tris is consistent with the profile observed with other cytotoxic agents. The most commonly reported adverse events include nausea (62 percent), fatigue (55 percent), and alopecia (59 percent). The most commonly occurring Grade 3 or greater adverse events were neutropenia (22 percent of palifosfamide-treated individuals), followed by anemia (13 percent) and febrile neutropenia (13 percent). In addition, 14 percent of research participants receiving palifosfamide-tris as monotherapy reported at least Grade 3 fatigue. From the clinical studies conducted to date with Ad-RTS-IL-12 and INXN-1001, the investigators have identified two potential safety signals that are suspected to be attributable to these two study drugs. A number of flu-like symptoms (e.g., fever, chills, myalgia, fatigue) have been reported that are likely due to an increase in cytokine production. Gastrointestinal symptoms (e.g., nausea and vomiting) also were observed and are presumed to be related to the activator ligand (INXN-1001). ZIOPHARM will include language in the protocol and the informed consent document to more clearly acknowledge that this combination of therapeutic agents has not yet been studied in humans, particularly in this disease; therefore, the safety profile is not yet clearly defined. ZIOPHARM will make clear that safety is one of the primary objectives of this study.

Decisions regarding the selection of which lesions to inject at which cycle as well as decisions regarding maintenance of injection sites are intentionally left to the discretion of each research participant's primary treating physician. However, the sponsor provides thorough guidance regarding the administration of INXN-2001, as well as courses of action for scenarios such as if the number of injectable lesions is limited and if the lesion to be injected does not support the volume of injection.

The protocol is written intentionally to leave pharmacodynamic evaluations vague. The Schedule of Assessments in the protocol clearly defines which samples are to be taken on which days, and an additional laboratory manual will provide explicit details on sampling procedures for the trial sites. Section 7.4.4 of the protocol also provides a list of assays that the sponsor will conduct in order to assess immunologic activity and mechanism of action. The informed consent document addresses the reason why these samples will be taken.

While the sponsor agrees that including an additional oncologist in the SRC can easily be done, it is the sponsor's preference to keep the SRC limited to the medical monitor and the primary investigators participating in the study. Because the SRC is focusing its review on safety information and not efficacy signals, the treating physicians are the most appropriate to comprise this committee.

The sponsor agreed to clarify the language in the protocol regarding research participants who experience a substantial benefit from the experimental treatment and for whom the SRC recommends continuation on the study.

Assays for the quantification of IL-12 mRNA expression [quantitative (q) PCR] and protein expression [immunohistochemistry (IHC)] in the tumor were chosen specifically to align with the type of clinical sample collected from the study participant on a specific study day. If a punch biopsy is scheduled for collection, then IL-12 IHC for IL-12 protein expression will be performed with a portion of that sample; if a fine needle biopsy is performed, then qPCR will be used to determine IL-12 mRNA levels in the tumor. The sponsor attempted to minimize serial punch biopsies in this population.

Palifosfamide will not be continued in Part 2 of the study because palifosfamide monotherapy in breast cancer will be evaluated further as part of the palifosfamide clinical development plan and not within the current protocol.

The clinical protocol is intended to be used as an instructional document for the participating centers so that the sites are well informed as to how to conduct the study appropriately. In-depth scientific background information regarding the study therapy and the rationale for using IL-12 as an immunotherapy is provided in a separate Investigator's Brochure, which is a mandatory part of the initial submission package for every clinical site.

Results from mouse studies suggest that local immunotherapy combined with low-dose chemotherapy with palifosfamide offers a distinct advantage over either therapeutic modality alone. This supports the investigators' hypothesis that cytotoxic agents at low doses may prime the immune system to enhance immunotherapy, which could potentially be translated into a safe clinical regimen for treating metastatic breast cancer. Additional studies are ongoing to further test the hypothesis in other tumor types and anti-tumor agents.

Investigators are instructed to inject lymph nodes only in cases in which no injectable lesions remain available or the lesions can no longer support the volume of injection. The rationale behind this instruction is exclusively to provide a way in which patients can remain on study therapy. The cells that comprise the lymph nodes are markedly different from other possible lesions, such as chest wall masses. In the case of the lymph node, cells of the immune system, such as lymphocytes, would be the targets of viral infection and cells in the injection tract might be targeted as well. Unintended autoimmune consequences of this and other immunotherapies are always taken into account in the risk/benefit balance of these therapies. A certain degree of systemic response is desirable in order to achieve an effect in lesions distal to those injected and that might imply some degree of autoimmune effects, whether the virus is injected into chest wall lesions or lymph nodes. However, in the case of Ad-RTS-IL-12, the therapy has the unique ability to turn the expression of IL-12 on and off at will by administration (or withdrawal) of the oral activator and, by association, has the ability to control the level of expression of IL-12.

Language that addresses the archival tumor tissue and other samples has been added to the informed consent document to make clear that, if a participant withdraws informed consent, any stored samples will not be tested further and will be destroyed.

The sponsor has realized during the course of the current melanoma study that implementation of post-mortem studies would be impractical. Additionally, post-mortem studies would not add useful information to the assays currently being conducted in samples obtained while the research participants are on study. Therefore, no post-mortem studies will be conducted.

This proposed trial will be conducted at multiple centers in the United States. Additional participating sites will not be listed in the protocol. Prospective additional research sites have been identified but no other research sites are currently activated. All additional research sites added to the study will be made known to all other participating research sites.

The informed consent document will be reviewed and refined as necessary to ensure that it remains thorough, is as non-technical as practical, and is understandable to all potential research participants (written at the 8th grade reading level or below).

2. Responses to RAC Discussion Questions

Dr. Vergara-Silva explained that the cause of death of a participant in the investigators' previous trial was septicemia and multi-organ failure. The cause of the septicemia was posited to be the colitis that this individual presented for previous treatment with ipilimumab. However, the investigators could not rule out injection into an infected site. Whatever the cause of this death, the investigators and the SRC agreed that the death from septicemia was not related to the study drug. It occurred 48 to 72 hours after injection of the vector and, for 48 hours before injection, the research participant had discontinued steroids such that this individual's colitis could have flared in that interim.

Dr. Vergara-Silva pledged that all research participants would be monitored closely to detect any signs of autoimmunity.

With regard to toxic systemic effects resulting from injection into the lymph nodes of animals, Dr. Vergara-Silva agreed that it is not possible to rule out that cells from the injection lymph node could circulate the viral vector into other organs in the periphery. However, no indication exists from the animal models that this is the case, and the investigators will address in the breast cancer study the possibility of vector appearing in other locations.

Regarding shrinkage of remote melanomas, Dr. Nemunaitis said that the investigators hope to see multiple lesions regress as this trial moves forward. He noted a significant number of animal studies for contralateral limbs in which an injection in one limb resulted in response in the other limb. For humans, however, only local-regional results have been observed; the investigators have injected eight melanoma patients but have not seen distant results such as injection in the skin causing a liver lesion to disappear. Dr. Nemunaitis explained that he does not believe that tumor shrinkage or disappearance is necessary to indicate success because progression-free survival at 16 weeks has been chosen as the measure of efficacy for this trial. He stated that the trial will attempt to discern whether palifosfamide contributes to a systemic effect and whether a Treg inhibitory effect occurs with the palifosfamide above and beyond the expected cytotoxic effect.

Dr. Nemunaitis agreed that the investigators will attempt, as diligently as possible, to sort out cellulitis from infection in the breast cancer patients enrolled in this study.

Dr. Vergara-Silva explained that the investigators have not addressed directly how long the viral vector persists; however, they have treated the melanoma patients for up to six 3-week cycles. The viral vector is injected, the oral ligand is provided for seven days and then interrupted for two weeks, and then another cycle of oral ligand is begun. The investigators do not know if those viruses have persisted from the previous cycle. Dr. Barrett added that several nonclinical studies were conducted in which the oral activator was continued to be administered for up to 28 consecutive days. Although no tumor escape from the B16 melanoma was observed, the investigators do not have information as to how much of the viral vector remained at the conclusion of the study.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- The protocol states that, in the absence of an available tumor lesion, the study agent can be injected into a lymph node. This will be clarified in the protocol to indicate that injection should be into a lymph node expected to be draining a tumor lesion, not an unaffected node. There are a number of different cells that the adenoviral vector could transduce in a lymph node, such as lymphocytes, which could traffic to other sites. For lymph node injections, the investigators should consider including further studies to understand the types of cells that are transduced by the Ad-RTS-hiL-12 vector, the biodistribution of those cells, and the persistence of the vector in those cells.
- In response to the death of a research participant who reportedly had a cellulitis at the site of administration of the same study agent in a previous trial for melanoma (OBA protocol #1007-1060), the protocol was amended to exclude participants with localized infection at the site of a lesion to be injected that requires anti-infectives within 2 weeks of planned dosing. It will be important to provide specific guidance and training to investigators to ensure consistent assessment of lesions. Certain lesions, such as those in research participants with inflammatory breast cancer, may be more difficult to assess with respect to presence of a superficial cellulitis.
- The goal of this trial is to inject a single lesion and generate an anti-tumor immune response that could result in regression of distant lesions. If such a response is observed, it will be important to consider performing a biopsy of the distant lesion to determine whether the vector has trafficked to that site directly, stimulating a local anti-tumor immune response, or whether the tumor regression resulted from an immune response generated by injection into the primary lesion.
- Understanding the nature of the immune response, especially correlating any systemic effects with immune system modulation, will be important to the design and interpretation of future studies. While maintaining some flexibility in the design of the immune studies may be desirable, consideration should be given to providing more specific information in the protocol regarding the types of assays that likely would be most appropriate. Undertaking this analysis in advance may reduce the likelihood of omitting an essential test. The protocol could be written in such a way that additional tests can be added without requiring a formal amendment to the protocol.

G. Committee Motion 2

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made but not seconded to approve these recommendations, and the RAC approved these summarized recommendations by a vote of 18 in favor, 0 opposed, 0 abstentions, and 1 recusal.

IV. Review and Discussion of Human Gene Transfer Protocol #1210-1188: Phase I Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1 Delivering the Survival Motor Neuron Gene by Self-Complementary AAV9

Principal Investigator: Jerry R. Mendell, M.D., The Research Institute at Nationwide Children's Hospital

Additional Presenters: Arthur Burghes, Ph.D., The Research Institute at Nationwide Children's Hospital; K. Reed Clark, Ph.D., The Research Institute at Nationwide Children's Hospital; Kevin Foust, Ph.D., The Research Institute at Nationwide Children's Hospital; Brian K. Kaspar, Ph.D., The Research Institute at Nationwide Children's Hospital; John T. Kissel, M.D., The Research Institute at Nationwide Children's Hospital

RAC Reviewers: Drs. Chatterjee, Chiocca, and Zoloth

A. Protocol Summary

The primary objective of this study is the assessment of the safety of intravenous administration of self-complementary adeno-associated virus, type 9 (scAAV9) carrying the cDNA of the human survival motor neuron (SMN) gene under control of the chicken β -actin hybrid promoter (CB) (scAAV9.CB.SMN). Spinal Muscular Atrophy (SMA) is the most common autosomal recessive disease of early childhood with an incidence of 1:10,000 live births. SMA is a single gene defect that most frequently results in low amounts of the SMN protein versus a total deficiency. SMN is a ubiquitously expressed protein that is essential in all tissues and is not associated with toxicity when over expressed. In addition, disease severity correlates with SMN protein levels emphasizing the potential therapeutic benefit for SMN as a treatment strategy. There is no treatment available to slow or halt disease progression, but recent preclinical studies utilizing gene delivery in newborn rodent models of SMA suggest gene transfer may hold promise, including encouraging results of gene transfer in preclinical testing in newborn rodent models of severe SMA and non-human primates. Intravenous delivery of scAAV9.CB.SMN in mice with severe SMA on post natal day 1 (P1) results in the expression of transgene in 70% of spinal cord motor neurons and complete rescue of motor function and strength, muscle physiology, and life span. Treated mice had life span up to a year as compared with approximately 16 days in the untreated animals. However this rescue effect by gene transfer is time sensitive so that the survival rate is significantly less effective with treatment on P5, and has virtually no effect on P10 as compared to P1 treatment. This is also the case with SMN inducible transgenic SMA mice, thus for any SMN induction therapy early induction is critical. Non-GLP toxicology studies in mouse and non-human primates showed no evidence of treatment-related toxicity found in hematology, clinical chemistry, and histopathology.

In the proposed human trial, scAAV9.CB.SMN will be delivered one-time through a venous catheter inserted into a peripheral limb vein in SMA type 1 patients with 2 copies of SMN2. The goal of this project is to increase the expression levels of the SMN protein in the motor neurons at the right time, and modify the SMA1 phenotype leading to a milder course and prolonged survival, similar to what we see in SMA2 and SMA3 patients. Dosing regimens have been chosen based on preclinical dose escalation studies and on-going toxicology studies showing no organ system pathology. Three cohorts will be enrolled: Cohort 1 (n=3) 6.7×10^{13} vg/kg; Cohort 2 (n=3) 2.2×10^{14} vg/kg; and Cohort 3 (n=3) 3.3×10^{14} vg/kg. Doses are based on the full range of efficacy (least to most) encountered in preclinical studies in SMN Δ 7 mouse.

The primary outcome for this clinical trial is safety. Research participants will be monitored closely, and motor function will be assessed. Blood and urine tests as well as physical examination will be conducted during the screening visits and on days 0, 1, 2, 7, 14, and monthly thereafter for a total of 2 years, to detect whether any side effects occur from the gene injections.

B. Written Reviews by RAC Members

Fifteen RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novel use of the SMN2 transgene and an AAV9 vector in a pediatric population.

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

Dr. Chatterjee asked why the investigators decided not to use the wildtype *SMN1* gene, which might be more efficient, and how many copies of the vector genome are present per cell. Noting that the data provided in the protocol was from macaques treated at the lowest dose to be used for the human studies, she asked the investigators whether more recent data exists in macaques injected with the highest dose (3.3×10^{14} vg/kg) because vector genome frequencies and potential immune responses could be relevant. Because the AAV9 genome most likely persists in an episomal state, Dr. Chatterjee asked the investigators to explain what happens to the vector genomes as the transduced cells divide as the individual grows. She requested that the investigators provide an estimate of the frequency of the vector genome copies per cell early after vector administration and at later time points, an estimate that could be important in predicting the stability of this experimental therapy. Regarding vector shedding, Dr.

Chatterjee asked the investigators to elucidate what samples they analyzed, at what time points the samples were analyzed, and what was the sensitivity of the assay. If AAV9 shedding were found, she inquired about the precautions to be taken to prevent inadvertent transduction to family members and health care workers.

Dr. Chiocca asked the investigators to discuss why patients who are taking drugs for myopathy or neuropathy or who are taking antidiabetics would be excluded from this clinical trial, and whether there would be a time period during which such patients could stop taking these drugs and still be enrolled in this trial. He noted that the last cohort is not expanded to six research participants as per usual dose-escalation trials, and wondered whether it would be possible to increase the dosing to the maximum achievable dosing, thereby including the entire dosing schema in the protocol upfront. Dr. Chiocca wondered why SMA type 1 patients with one copy of *SMN2* are excluded from this trial; these patients live less than 11 months, but this trial is a Phase I trial for which efficacy is not an objective. Noting that the starting dose in humans appears low, he requested that the investigators provide justification for that starting dose based on the safety data in mice and primates, and whether the dose was calculated on a muscle-weight or body-weight basis. Dr. Chiocca inquired about the preclinical data that excludes patients with antibodies to AAV9. He expressed concern that safety stopping criteria of one Grade 3 or two Grade 2 toxicities might be too harsh and could lead to premature closure of the trial at an ineffective dose; he suggested that investigators consider changing the stopping definition to the more-typical occurrence of two Grade 3 toxicities.

Dr. Zoloth noted that this trial attempts to develop a minimally invasive (via intravenous access) intervention using AAV viral vectors. One problem she pointed to is that the consent form is written as if directed to an adult, language that obviates the core ethical issue of whether parents with a newly diagnosed infant should consent to a first-use gene transfer trial that will involve unknown and untested risks, with no direct benefit to their child. Because parents have been told that their child has about two years to live, it is likely that the entire lifespan of the child will be that of a research subject; Dr. Zoloth stated that the informed consent document must explain this reality to the parents. She also noted that the discussion of uncompensated harms is replete with confusing language, and parents could be left wondering who will sort out the etiology of the harms and who will be responsible for compensation, should harm occur. Because the patient population is extremely vulnerable and the number of research participants is small, Dr. Zoloth stated her belief that the investigators or their institution should take responsibility for participants' health if harm occurs that is related to this trial.

C. RAC Discussion

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

- Dr. Hammarskjöld asked whether the investigators will study protein expression in individual cells, or DNA or RNA expression.
- Dr. Kohn asked about the level of transplacental transfer of antibody to AAV. He assumed that most mothers would have high levels of AAV antibodies and wondered how much would be transferred to their fetuses. He wondered, as a result, how many infants would be eligible for this study.
- Dr. Kohn suggested that the RAC recommend that this trial start at a therapeutic dose.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators clarified that they intend to use the wildtype, full-length cDNA for SMN.

When the investigators presented the toxicology data from nonhuman primates, the FDA commented that the data were sufficient with no need for further studies in nonhuman primates. GLP toxicology studies are ongoing in the mouse with the two clinical trial doses including the lowest (6.7×10^{13} vg/kg) and the

highest (3.3×10^{14} vg/kg). Final toxicology data are expected to be completed and reported to the FDA by early 2013. Preliminary evaluation of all tissues from these mice have not demonstrated any toxicity.

Based on current knowledge, the motor neurons are the critical target cells for SMA therapy, and they do not divide after birth in mice or humans. Therefore, SMN expression will be lost from other noncritical and dividing cells but not from the critical cells (motor neurons). The loss of vector genomes from neurons that are not dividing will be minimal.

The investigators expressed agreement about the importance of monitoring for shedding, particularly in light of the proposed doses. Published studies have observed vector shedding in the plasma, urine, saliva, and stool through the first week post injection of nonhuman primates intravenously injected with self-complementary AAV8 at 2×10^{12} vg/kg. These studies will serve as the basis for the post-administration monitoring in research participants. Designing preclinical shedding studies is challenging, particularly in mice, due to the youth and small size of the mice. In the investigators' previous studies, AAV9 injection in mice was performed during the first or second postnatal day. However, neonatal mice do not transition to solid food until approximately 2 weeks of age, and therefore do not defecate regularly when fed mother's milk alone. Repeated blood draws are not available during the first few weeks post injection due to the animals' size. Therefore, the investigators did not collect this data as part of their preclinical package.

The risk of exposure to AAV9 (via shedding) to health care workers and parents or caretakers is likely high, due to the feeding and diapering needs of the proposed patient population (younger than six months-old children). The investigators believe the dangers of exposure to scAAV9.SMN from viral shedding to be low due to the non-pathogenicity of the parent vector, the replication-deficient nature of the recombinant vector, and the excellent safety profile of AAV in clinical trials. Nevertheless, caretakers should use caution when interacting with excreta from dosed participants and, to minimize exposure, caretakers will follow a plan approved by the institutional biosafety committee that follows Biosafety Level 1 (BL1) containment as described in the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, including use of proper personal protective equipment and cleaning, storage, disposal, and transfer of BL1 agents.

Regarding exclusion criteria, the investigators explained that they will exclude anyone previously or currently on ISIS-SMNRx, sodium phenylbutyrate, and valproic acid because these agents are currently used as strategies to influence SMN gene expression and therefore might influence participant safety and study trial results. Diabetes will be an exclusion criterion; if a research participant has an immune response to virus that requires treatment with corticosteroids, such treatment would be contraindicated because of an effect on glycemic control. The investigators will exclude patients taking drugs for myopathy and neuropathy or who have evidence of these diseases, as these diseases may increase risk or have an effect on adverse study outcomes.

The investigators explained two reasons for the selected dosing: (1) FDA requests all initial dosing subjects be on a dose-escalation scale starting at the minimally effective dose, and (2) for the final dose escalation, the investigators believe they are currently at the limit of the maximal achievable dose. Exceeding the highest proposed clinical dose (3.3×10^{14} vg/kg) runs the risk of irreversible vector aggregation (precipitating out) during product manufacture. Promising rescue data exists in the preclinical studies in the SMA mouse model with the highest dose proposed.

Regarding justification for the starting dose in humans, the investigators explained that the FDA asked to start dosing at the lowest dose showing efficacy in mice (6.7×10^{13} vg/kg), a dose that was established based on body weight. At this dose, the SMN mouse survives to 35 days on average (predicted to die at 13 to 16 days undosed). The investigators' ongoing investigational new drug (IND)-enabling toxicology studies are exploring safety at the low and high clinical doses (6.7×10^{13} and 3.3×10^{14} vg/kg). The effect of doses on the survival of the SMA mouse model demonstrates the importance of dose range predicting therapeutic response.

Patients with one copy of *SMN2* are very rare and are known as “type 0 SMA,” with onset either prior to birth or at birth. Including such patients in the clinical trial would pose major challenges to distinguish whether a death within the first four to six weeks of life was due to natural history since death occurs frequently at one month of age, or a treatment-related serious adverse event (SAE).

Accumulating evidence in the investigators’ gene transfer studies as well as in other studies suggests that the cells of participants with preexisting immunity to AAV are poorly transduced upon gene transfer.

The investigators agreed to change the safety stopping criteria to the more-typical “occurrence of two Grade 3 toxicities,” and will present this approach in their IND submission.

The language relative to participant injury is the current IRB-approved language and follows the DHHS/OHRP/FDA guidance on exculpatory language. The language is intended to advise the parents that, if the subject is harmed as a result of the trial, neither the researcher nor the institution will be responsible for the costs of care associated with the research-related injury. The current language further recognizes that the parents are not waiving their legal rights to hold the investigators and/or the institution responsible for any research-related injury that is the result of error or wrongdoing. The investigators noted that the language in question also is consistent with the examples of acceptable exculpatory language provided by the DHHS/FDA/OHRP in their 2011 Draft Guidance on Exculpatory Language in Informed Consent. While the investigators are aware of a few institutions that provide some version of participant injury compensation, the position taken for this protocol is not uncommon to that taken by many peer institutions. A 2005 assessment of medical school IRB policies on informed consent published in the *American Journal of Medicine* indicated that coverage for medical bills was offered only 22 percent of the time (22 out of 102 institutions) when studies had no industry sponsor and, in those instances in which coverage for medical bills was extended, it was limited to emergency bills only (11 out of 22 institutions). The investigators reported that they presented RAC reviewer comments to members of their executive leadership committee for further discussion.

The investigators agreed to modify the informed consent document as suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Foust stated the investigators plan to move forward with an intrathecal gene delivery program as well as the delivery method proposed in this trial. If successful, the investigators will be able to evaluate a head-to-head comparison of the same vector, production style, and purification with two delivery paradigms. The ultimate result would be to establish the most clinically meaningful outcome for patients.

Dr. Mendell explained that, for the last three years, the investigators have studied re-dosing extensively in a nonhuman primate with AAV8 antibody. Repeated plasma pheresis in those monkeys has resulted in success. They achieved better success with plasma pheresis in a window of time for achieving a high transduction efficiency than they did with immunosuppression compared to prednisone and triple therapy. The investigators are about to publish that data.

Dr. Burghes explained that the critical issue is not how much SMA is produced from a vector, but how many vectors are put in each cell. Therefore, enhancing the biodistribution of the vector is a significant improvement. The intrathecal delivery that the investigators have conducted in pigs has resulted in an 80 percent transduction in the lumbar cord and slightly less in the cervical cord.

Dr. Foust explained that AAV2 is the most predominant AAV serotype, and the numbers are quite high for the adults who have high neutralizing antibodies against AAV2. For AAV9, approximately 15 percent of adults have neutralizing antibodies. The investigators have discussed the chance of placental transfer of neutralizing antibodies, and they plan to check the babies and the mothers for those antibodies. Dr. Clark added that the investigators have screened their primates for anti-AAV8 and anti-AAV9 antibodies and have discovered, uniformly, no seropositivity in the newborn animals. Transplacental transmission is believed not to accrue efficiently, although the reason is unknown.

E. Public Comment

Note: These public comments are presented essentially verbatim.

Vincent (and Catherine) Gaynor. My name is Vincent Gaynor and I have traveled here with my lovely wife, Catherine. It is really a hard meeting for us to attend because we have never left our child before. This is the first time in her life but we thought it was an important meeting, so we had to. This is my daughter, Sophia Gaynor. She has SMA Type 1. We've watched this disease ravage her body. It has stolen everything from her. Every movement, even her smile.

Too many children are lost every year to this disease. With a heavy heart I came this morning. I got a phone call that a very close friend of Sophia's passed away.

This is a patient population that is always avoided. We need something for the Type 1s. This is some of the greatest preclinical data ever seen in this disease and some of the safest data as well. We've gone in hundreds of animals. There are many questions about SMA that are left unanswered but there are some answers that we do have. We know what happens if we do nothing: these children will die. I don't know how many more funerals I can go to where the coffins are 2 feet long.

Please take that into consideration and thank you for your time.

Jennifer Sloan. My name is Jennifer Sloan. I am from Orange County, California.

Because of SMA, my son has almost as many therapy appointments in a week as he does school days. but he doesn't have SMA. His sister does. He was 3 years old when she was diagnosed, and for the longest time he thought that she was incapacitated because she was a baby. Now he understands differently. He understands more. He feels wracked with guilt when he comes home with a cold from kindergarten and it goes through the house. He fears the death of everyone in the house. He asked me in September when he passed strep throat to me, he begged me, "Mommy, please don't die." We're considering anxiety meds because he is under the care of a psychologist and a psychiatrist. This is what SMA has done to my healthy child.

If there had been an opportunity for a clinical trial like this when my daughter was diagnosed. we would have jumped at it. We would have hoped to have prevented the loss that happened within weeks of her diagnosis. Within 6 weeks she was starting to lose her swallow. By 8 weeks she needed bipap. By 10 weeks she had her first pneumonia.

We want more for our daughter and our son. Not a week ago my son said to me, "Mommy, what if Kennedy could grow up to be an adult and she could walk and talk and eat like me?" And I said, "Jacob, we don't know if that's going to happen." He said, "But, Mommy, what if it did?" Indeed!

Matt (and Deb) Chambers. My name is Matt Chambers. My wife, Deb, and I are here from Indianapolis. We're the proud parents of Emerson Grace, an energetic 3 year old with Type 2. I can tell you that the day Emerson was born was the happiest day of our life. From early on she was happy, a good eater, and had the energy and spark of both of us combined.

She made all of her early milestones that most doctors look for. She sat up by the age of 6 months. She was rolling. She could even get herself up into the crawling position ready to explore the space around her.

The day we found out she had SMA was the worst day of our lives. We watched the slow decline in her abilities. To see her struggle doing the things that all of her peers do on a day-to-day basis ravaged our lives. Even more so when she learned how to talk and was able to ask, "Why can't I walk? Why can't I climb the stairs?" Things such as her going up the stairs in her wheelchair, wanting to drive up the stairs because she thinks she's walking. Those are the things we deal with.

Nothing can prepare a parent for having to deal with this and having to make the decisions that we have had to make. Whether they are popular or not, we make these because we love our daughter and we want the best for her.

We realize that this trial is targeting the younger Type 1s and we support that because we believe that is the way we can move this forward so that our children might have a chance at even maintaining what they have now, if not for some type of reversal.

So we appreciate your time today and we thank everybody here for listening to us.

Sarah Turnbull. My name is Sarah. This is my daughter, Marcella. She was a healthy 9 pound, 12 ounce baby girl and she did everything early. She was smiling. She was pulling at her toy bar. Everything so early to indicate that she was quite advanced. And at 1 month of age SMA just ravaged her. Hers was a very rapid decline. I went to give her a bath and all of a sudden her arms flailed. She couldn't hold her head up and it was a very sudden thing for my Stella.

We went to Mayo Clinic and we were given the same thing that everybody is told: take her home and love her. There is absolutely nothing you can do. Because she was the weakest case they had seen to date, five and a half years ago they said, "There's just simply nothing."

Had this been an opportunity for us we would have absolutely latched on to it. We have very few options when we are dealing with absolute devastation. What more do you turn to?

What I can tell you is that we were willing to fight. We didn't know that at 2 months she would quit breathing in her car seat. We didn't know that we'd have episode after episode throughout her life but she has been so worth it. She is such a blessing. At 5½ years old she goes to school. She has a trach. She has a vent. She has a feeding tube. She has a catheter. She has an IPV machine. It's around-the-clock care.

I will also tell you that she thanks you. This morning before I hopped a plane from Iowa I went in to turn her like I do every night. She was wide awake looking with her big brown eyes. I said, "Honey, I am going to go fight for your friends and for all the thousands of children that we represent here today that weren't as lucky as our children." With the very little movement that she has, she raised her head and she blinked for yes.

So I just want you to know that our children thank you. We thank you so much for having the hope for our children that we have for them.

Cameron Gephardt. My name is Cameron Gephardt. This is my son, Charleston Gephardt. We live in Northern California currently.

I'd like to, first of all, in plain language address the issue of informed consent. I think that the honor of being a parent is something that I hold especially sacred. In that honor, every decision I make for my son—what we feed him, what television programs he watches, everything we do for him—directly affects his overall outcome as a potential adult someday. Every decision I make as a parent is with his best interest in mind.

Just like the other parents, if I had the opportunity to say you wouldn't have to have your son on a ventilator, you wouldn't have to have the emotional scars that every SMA family lives with, if I had the opportunity I would have jumped on that bandwagon wholeheartedly. Ethically I would not have allowed myself to have any sort of regret for doing the best thing that I could for my son, the best thing that was available. We all know if you do nothing what happens. It's confirmed. It's widely known.

I'd like to thank the team and their efforts and all the nights of sleep that they lose for giving a family like mine hope and potential long-term life of our child. The clinical data is promising obviously. With your continued support as the guardians of the medicine we receive and the opportunities for hope we receive, I hope you acknowledge each and every one of us. These kids are cognitively gifted. Their bodies fail them.

Each one of us sitting here has the benefit of being able to adjust ourselves when we're not comfortable or being able to swallow if we have too much saliva in our mouths. My son doesn't. Some day he may have that opportunity.

Thank you for your time.

Rosemary Hilson. I'm Rosemary Hilson and I'm from Cincinnati, Ohio. This is our grandson. As you can see, he is a big boy. He is 14 years old and in the eighth grade. He has Type 2. You saw in the graph that Type 2 doesn't go downhill so fast, but they still go down. He never crawled. He never walked. He never sat without support. So he has been very disabled and uses much of the equipment that Type 1 children do. He is in puberty now and he likes school and wants to have a girlfriend, loves cars, wants to learn to drive and get a Camaro, and lots of aspirations for a boy who cannot feed himself.

Alex was in the VPA trial that Dr. Kissel talked about, and within 14 years we have seen so much come and go. This is promising. This is what we need for our future generations.

Matt Hiley. My name is Matt. I'm from Rochester, Minnesota. This is my daughter, Briana. She was diagnosed as Type 1. Five years later we got told the same thing. These children, even being the Type 1,

a lot of the misconception is that they cannot make choices, but they can, just like Mr. Chambers' and the other child. They are fully cognitive. My daughter is already running a computer faster than we can. They are fully cognitive and they are a blessing to us all.

I appreciate your time. Thank you.

Kristen Bausch. My name is Kristen Bausch and I'm representing my family with Kendra, my daughter. We are from Lancaster, Wisconsin, where we have two families in our community and two additional families within half an hour that have children with SMA.

The birth of your child—everyone imagines what great joy it brings, but until you have that child and get to experience it, the immense joy is indescribable.

We have been able to see her develop milestones early on and later lose those milestones. Something that no parent should have to go through is the loss of those milestones. We bargain for their life. We ask God just one day to be able to take a breath.

Kendra uses her pulse ox. We have all of the correct respiratory equipment and we take all of the protocols to stay proactive in her care. Last week coming home from a Thanksgiving family event, when we tried communicating with her she wasn't responding and it was dark in our vehicle. When we turned the lights on we saw that she was blue. Her pulse ox was not going off. She was not breathing. She was not responding to us. The immense joy that you feel on your child's birth is the immense devastation I felt when I thought that we were losing her.

This whole research has brought a lot of hope to our lives and we hope that it will go further so that we don't have to experience that fear of what if the day comes that we have to say goodbye.

Jennifer Swann. My name is Jennifer Swann. I am from Las Vegas, Nevada. This is my daughter, Fallon. She is now 2 years old. She is a Type 1. Every night she falls asleep on the couch. I pick her up and put her over my shoulder, and I'm grateful for being strong enough to tolerate that. From our walk from the family room to our bedroom to lay her down I have a few moments every night where I'm a normal mom and she is sleeping and I'm putting her to bed. But the second I lay her down it is back to reality. I hook up the feeding tube and pulse ox, put on her biopac and suction. At 6 weeks old, my daughter could hold her head; at 2 years old she can't even move her head. I do it for her.

All of us want that chance for our children and we want the new generation to have a better shot than we did. Thank you.

Dr. Fong thanked the families and caretakers for their comments, noting that RAC members share their desire to achieve improvements in treatment of this disease and are working through a process to perfect the protocol in order to do that.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- To further the understanding of transduction efficiency and expression, the investigators should compare the AAV vector genome copy number per cell in the nonhuman primates who received the vector by intravenous infusion, the SMA mouse model, and the pig model with intrathecal delivery. In the mouse model, studies should also be conducted to examine the decrease in genome copy number over time after AAV vector administration to help predict the potential durability of efficacy. Episomal copies of the AAV vector may be lost in dividing cells as the research participants grow.

Clinical and Trial Design Issues

- The primary objective of this study is to establish the safety of this approach. Unacceptable toxicity is defined as the occurrence of one Grade 3 (as determined by the CTCAE) or higher related toxicity, or two or more Grade 2 treatment-related toxicities. While safety is paramount in any pediatric trial, in this very ill population, development of two Grade 2 related toxicities may not necessarily be determinative as to whether the risk/benefit of this agent favors further development. The investigators should consider whether the primary objective should be

amended to allow for more flexibility in assessing which types of Grade 2 toxicities should lead to failure to meet the primary endpoint and possible termination of the trial.

Ethical, Legal, Social Issues

- Children enrolled in this study will receive a first-in-human gene transfer agent, which carries more than minimal risk. This risk should be balanced by the prospect of direct benefit. Based on the animal models, the current starting dose is very unlikely to provide clinical benefit compared to the higher doses. If higher doses do prove efficacious but research participants in the lower starting dose cohort do not experience clinical improvement, these children are not likely to have the option of receiving a second dose because the first dose is likely to generate an immune response to the AAV vector. Therefore, the investigators should consider whether a higher initial starting dose would better balance the risks compared to the benefits.
- This is an extremely vulnerable population lacking a curative therapy for a fatal disease, and the parents who will be asked to consent will likely have a strong desire for any possible therapeutic option, even if the likelihood of benefit is remote. Therefore, the consent process must be meticulous in providing the required information on risks while avoiding the creation of the therapeutic misconception. Along these lines, it is important to highlight that there are many unknown risks with this gene transfer agent. The current informed consent document states, "Studies on animals that received larger doses via infusion showed no side effects that were directly related to the vector. Our group has conducted three previous gene therapy studies using different genes and similar viruses without significant side effects." More emphasis should be placed on informing the parents that there may be other serious risks that are not known at this time despite the experience in animals and other gene transfer trials with different diseases and agents.
- The preclinical animal data, on which this trial is based, indicated that dosing at a younger age could lead to better clinical outcomes than dosing at a later stage of the disease. While there are many limitations to extrapolating mouse data to humans, the decision to intervene with this agent in this young population is based on that data. While parents should not be pressured into a rapid decision regarding enrollment and should have ample time to review the consent form, ask questions, and consider their decision, the data regarding greater efficacy in younger animals should be shared, along with information on the limits of extrapolating animal data to humans. This full disclosure will assist parents in making an informed decision. The informed consent document should also include information about the alternative of home ventilatory programs.
- The protocol and informed consent document state that in the event of injury resulting from participation on this trial, the institution "makes no commitment to pay for the medical care provided for you. No funds have been set aside to compensate you in the event of injury. If no one else pays for your care, you may have to pay for the cost of this care." Given the small number of research participants, the severity and burdens of their disease, and that by enrolling in a trial, these children may be research participants for much of their lives, the investigators should consider whether a mechanism could be developed to provide compensation for care for research-related adverse events.

G. Committee Motion 3

Dr. Fong summarized the RAC recommendations to be included in the letter, expressing the comments and concerns of the RAC to the investigators. A motion was made but not seconded to approve these recommendations, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Gene Transfer Safety Assessment Board Report

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

A. GTSAB Report

Dr. Fong presented the GTSAB report for the fourth quarter of 2012. The OBA received 15 protocol submissions in the past three months, nine of which were not selected for public review at this RAC meeting. Of the nine protocols not selected for public review, seven were oncology protocols, one was for sickle-cell disease, and one was for HIV vaccine. In these nine protocols, two each used retrovirus, lentivirus, and plasmid vectors; and one each used adenovirus, herpes simplex virus (HSV), and RNA transfer vectors. Dr. Fong noted that information about these trials would be available on the OBA Web site after this RAC meeting.

For all of 2012, 59 protocols were submitted for review at RAC meetings. Of those submissions, 73 percent were for cancer, seven percent each were for single gene disorders and cardiovascular disease, five percent were for infectious diseases, and eight percent were for other diseases. The 2012 gene transfer trials featured a variety of delivery systems: adenovirus (24 percent), retrovirus (19 percent), lentivirus (15 percent), plasmid DNA (15 percent), AAV (eight percent), poxviruses (three percent), HSV (two percent), and other delivery systems (14 percent).

Ten SAEs from nine protocols were reviewed by the GTSAB, including initial and follow-up reports. After analyzing these events, the GTSAB concluded that none warranted public discussion at this RAC meeting.

The OBA received notification from investigators that 12 protocols were newly open to enrollment. Two of those 12 had been reviewed previously at a RAC public meeting.

Dr. Fong reported on two noteworthy protocol changes that represented responses to RAC review:

- *OBA Protocol #880, reviewed in December 2007: A Phase I/IIa Study of Safety and Efficacy of Modified Stromal Cells (SB623) in Patients with Stable Ischemic Stroke.* The investigators have examined SB623 cells, adult bone marrow-derived cells that have been transiently transfected with the plasmid construct encoding the intracellular domain of human Notch-1. They have conducted a 1-year study in immune compromised rats to examine the tumorigenicity of the Notch-1 intracellular domain. Neither tumor formation nor cell proliferation was observed, and the revised clinical study no longer employs cyclosporin A or any other form of immunosuppression.
- *OBA Protocol #1087, reviewed in March 2011: A Randomized Phase I/II Trial Using a GM-CSF-Producing and CD40L-Expressing Bystander Cell Line (GM.CD40L) Vaccine in Combination with CCL21 for Patients with Stage IV Adenocarcinoma of the Lung.* Based on data from a previous trial using the GM-CSF and CD40L-expressing bystander cell line, it is expected there will be a time interval between dosing and development of an antitumor immune response. One of the RAC's recommendations was to modify the protocol because during that period the research participants would not be treated. The inclusion and exclusion criteria have been updated to define more precisely patients whose disease is not likely to progress significantly during the course of the trial. Participants have been provided a study card containing summary information about the trial and the investigator's contact information.

B. RAC Discussion

No discussion occurred.

C. Public Comment

No public comments were offered.

VI. Update Discussion on Protocol #0604-776 titled: A Phase I Study of CD19 Chimeric Receptor Expressing T Lymphocytes in B-Cell Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia

Principal Investigator: Carlos Ramos, M.D., Baylor College of Medicine

A. Presentation by Dr. Ramos

Dr. Ramos discussed a proposed amendment to this protocol, which was submitted to the OBA in April 2006; at that time, public discussion and review at a RAC meeting was deemed not warranted. Chimeric antigen receptor (CAR) is the molecule that combines the recognizing portion of monoclonal antibodies with the signaling portion of the T-cell receptor. When inserting these molecules into a T cell, the T cell is freed from MHC restriction; therefore, any cell that has this antigen on the surface should be recognizable by these T cells and then able to deploy the signaling T cells. Although this process is known to work well *in vitro*, the first study conducted *in vivo* showed limited success; therefore, the goal of subsequent research was to improve both the T-cell activation and the proliferation.

The original trial proposed by Dr. Ramos and colleagues sought to answer the question of whether these CARs containing the CD28 co-stimulatory domain were superior to CARs that did not contain this domain. The target group of diseases was CD19 positive non-Hodgkin's lymphomas. The investigators proposed to prepare for each research participant two autologous products containing activated T cells that expressed a high rate of first-generation or second-generation receptor. Both T-cell populations were prepared by retroviral transduction, and after a period of expansion, both were infused and tracked. *In vitro* the characteristics of these cells were similar in terms of the degree of transduction with both the first generation and the second generation CARs. The phenotype of these cells was similar as was the killing ability; no major differences in laboratory assays were discovered.

Dr. Ramos provided data regarding the first six research participants who completed dose escalation; most of these individuals had aggressive lymphomas and had been treated extensively for severe refractory disease. These first six participants showed that the adoptive transfer of these CAR-transduced cells was safe and that the second-generation T cells expanded better and persisted longer *in vivo*. Because of the limited clinical response attributed to limited expansion and persistence *in vivo*, Dr. Ramos and colleagues modified the trial to include other mechanisms that might further improve persistence and clinical activity, attempting three approaches: activation of homeostatic expansion mechanisms including lymphodepletion prior to adoptive infusion, provision of growth factor IL-2, and blocking negative feedback pathways using CTLA-4 antibody.

The proposed amendment focuses on increasing the expansion of CAR cell persistence by using a single dose of CTLA-4 antibody. To reduce the risk of toxicity, the proposed dose of ipilimumab will be between 0.75 mg (25 percent of the middle dose in a new dose-ranging Phase II trial in which three doses were studied), and the lowest dose used in that trial (0.3 mg). Ipilimumab is a monoclonal antibody that blocks CTLA-4 and recently has been approved to treat metastatic melanoma. The investigators plan to administer the drugs to coincide with the follow-up clinic visit at two weeks, the point at which the T-cell expansion begins to decline.

B. RAC Questions and Discussion

Dr. Fong asked about the Phase I data combining CAR and CTLA-4 antibodies, which Dr. Ramos acknowledged existed only for both agents independently but not the combination. Dr. Ramos further explained that, because he and his colleagues have established the safety of their CAR system, it would be easier to use a dose that is thought to be safe and that is lower than any doses previously used for trials involving ipilimumab. Their goal is to discover whether ipilimumab helps with T-cell expansion, so the endpoint of this amended study will be more biological than clinical.

Dr. Kiem asked why the cyclophosphamide did not work. Dr. Ramos responded that one possible explanation is that all of the research participants had received several lymphodepleting regimens to treat

their lymphoma. Although the biological actions of cyclophosphamide are dose dependent, the investigators did not want to increase the dose because most of these individuals at this point have limited marrow reserve.

C. Public Comment

No public comments were offered.

VII. Day 1 Adjournment

Dr. Fong, RAC Chair, adjourned Day 1 of the December 2012 RAC meeting at 6:10 p.m. on December 4, 2012.

VIII. Day 2 Opening

Dr. Fong, RAC Chair, called to order Day 2 of the December 2012 RAC meeting at 8:30 a.m. on December 5, 2012.

IX. Review and Discussion of Human Gene Transfer Protocol #1208-1180: Local Modulation of Immune Receptor Function To Enhance Immune Responses to Dendritic Cell Vaccination in Subjects with Triple Negative Breast Cancer

Principal Investigator: Scott K. Pruitt, M.D., Ph.D., Duke University Medical Center
Additional Presenters: Kimberly Blackwell, M.D., Duke University Medical Center; Bruce Burnett, Ph.D., Duke Translational Medicine Institute; Shelley Hwang, M.D., Duke Cancer Institute; Paul Kelly Marcom, Duke Cancer Institute
RAC Reviewers: Ms. Dresser, Dr. Hammarskjöld, and Dr. Kiem

Drs. Fost, Ross, and Sarzotti-Kelsoe were recused from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

More than 230,000 new cases of breast cancer will be diagnosed in the United States this year. Of these cases, 15 percent to 20 percent will be triple-negative breast cancer (TNBC) in which the cancer cells do not express estrogen or progesterone receptors or Her2/neu. As such, TNBC does not respond to targeted therapies such as tamoxifen, aromatase inhibitors, or Herceptin, and has a poorer prognosis compared to other breast cancers. Treatment of TNBC includes surgery, radiation, and chemotherapy, but currently no targeted therapies are available to improve outcomes in this more aggressive form of breast cancer. Therefore, a pressing medical need exists to develop novel therapies that are safe and effective to combat TNBC. In this Phase I clinical trial, the investigators will test a novel approach that will employ the immune system to fight TNBC.

This cancer vaccine approach involves culturing dendritic cells (DCs) from the research participant, loading them with proteins unique to TNBC, and injecting them back into the participant as a cancer vaccine. The investigators have pioneered a method of having DCs express tumor proteins by loading them with RNA. Once inside the DC, the RNA serves as a template from which the tumor protein is made. These tumor proteins are then chopped into pieces that are displayed on the surface of the DC, and these pieces are then recognized by the killer cells of the immune system, cytotoxic T lymphocytes (CTLs). Each CTL is specific to the protein sequence it recognizes, and this stimulation and expansion of tumor protein-specific CTL is critical for tumor elimination. In early cancer immunotherapy trials, vaccination with RNA-loaded DCs expressing tumor proteins has been shown to stimulate anti-cancer

immune responses that could be measured in the laboratory; however, only a few research participants had clinical cancer responses such as reduction of tumor size.

One reason for the lack of clinical responses to cancer vaccination in patients with breast cancer, as well as other cancers, is the normal immune mechanisms that serve to control immune reactions. Normally these control mechanisms are thought to inhibit the body's immune system from attacking a person's own cells, preventing autoimmunity. However in the setting of cancer, this down-modulation of the immune system may blunt responses to tumor vaccines. Studies in mice and in patients with cancer suggest that anti-cancer immune responses are improved by blocking these control mechanisms using antibodies that specifically bind to immune control receptors expressed on T cells. Two blocking antibodies that have been studied extensively are specific for the receptors GITR and CTLA-4. Cancer patients given an injection of antibody against CTLA-4, even in the absence of a cancer vaccine, showed clinical benefit, but there were serious and sometimes fatal side effects because the antibody was given at a high dose and apparently stimulated autoimmunity such that killer immune cells attacked normal cells in the body.

In this study, the investigators propose to vaccinate TNBC patients with DCs that have been loaded with RNAs that produce the TNBC associated antigens, MAGE-3, MUC1, and EGFR, in combination with DCs that contain RNAs that encode anti-CTLA-4, anti-GITR, or both. These DCs will produce a small amount of immune-modulating antibody compared to the much greater doses that have been injected in other studies; however, this immune-modulating antibody will be secreted locally in immune tissues at the site where DCs loaded with TNBC proteins are stimulating tumor-specific killer immune cells. This approach eliminates the need for large amounts of antibody, reducing the cost of immunotherapy and minimizing the side effects seen when large doses of immune-modulating antibodies are injected into the blood and non-specifically activate killer immune cells. These locally secreted immune modulators will enhance the induction of anti-TNBC immunity in response to vaccination. TNBC patients participating in this trial will be monitored for vaccine-related side effects as well as immunological and clinical responses.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of this protocol. Key issues included the fact that it will utilize a novel construct for breast cancer. In addition, the investigators propose to enroll research participants who, despite having a less favorable prognosis compared to those with hormone receptor positive breast cancer, can be cured by standard therapies. The risks and benefits of enrolling these individuals in a Phase I trial deserve further discussion.

Three RAC members provided written reviews of this proposed trial.

Ms. Dresser asked whether it would be reasonable to exclude women with node-negative disease, since this group has significantly better survival rates than do women with node-positive disease. With regard to the informed consent document, she noted that prospective participants would benefit from having more information about the investigational intervention and the potential for direct benefit. Ms. Dresser suggested that the investigators consider adding language resembling the following statements, which are based on samples from the NIH Guidance on Informed Consent for Gene Transfer Research:

- Page 2, "Why is this study being done?" — Add: "This is the first time that this vaccine will be tested in humans."
- Page 10, "Are there benefits to taking part in this study?" — Add: "Treating your disease is not a purpose of this study. It is very unlikely that getting the study vaccine will improve your health."

Dr. Hammarskjöld noted that patients with no known lymph node involvement (N0) as well as patients with T2 tumors are believed to have an improved prognosis; therefore, she asked for discussion of the rationale for including N0 and T2 patients in this trial, noting the importance of selecting only individuals with the poorest prognosis. She asked whether the participants and/or their tumors would be tested for markers that have been shown in some studies to affect prognosis (e.g., p53 status or BRCA1/2 deletions) and how this information would be used to determine potential inclusion in this study. Dr. Hammarskjöld queried as to the estimated necessary minimal level of gene transfer and/or expression for this gene transfer protocol to be successful in humans, and how that level was determined. She asked

whether data is currently available from the previously approved DC-based trial (RAC Protocol #0708-874). Dr. Hammarskjöld asked the investigators to discuss what they will do to ensure that one of the key endpoints of this study—testing the functional activity of the induced T cells to kill autologous tumor associated antigen (TAA) expressing DC or HLA-matched breast cancer cell lines—can be done. She inquired as to the expression of the TAAs in normal tissues and whether the investigators plan for tests to determine potential auto-immune reactions to such cells *in vivo* and/or *in vitro*. In addition, Dr. Hammarskjöld suggested several wording changes to the informed consent document to improve clarity and accuracy.

Noting that this study is well written, Dr. Kiem's primary comment was with regard to the inclusion of N0 patients: Given that this is a first-in-human study, he requested justification for enrolling N0 patients and suggested initiating this study with more advanced patients. He asked how the investigators plan to assess the electroporation efficiencies within one arm, given that they will be comparing different co-transfection strategies. Dr. Kiem asked the investigators to discuss whether the gene is expressed in cells other than the target cells, what other tissues are expected to express the targeted TAAs, and what responses to other tissues have been observed in previous studies targeting these TAAs. He suggested clarification of the follow-up after the last leukapheresis, including how often research participants will be tested and evaluated for autoimmune abnormalities especially given that a significant percentage of these individuals will survive long term.

C. RAC Discussion

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

- Dr. Fong asked whether the 14 percent of male breast cancer patients who are triple negative would be eligible for this trial.
- Dr. Strome requested that the investigators share data regarding the time course of recurrences in triple negative versus other breast cancer patients.
- Dr. Zoloth expressed concern about three ethics-related issues from this protocol and others under review at this RAC meeting: (1) therapeutic anticipation on the part of the researcher, (2) whether participants really can withdraw from a gene transfer trial, and (3) how adverse incidents are treated and, if care is needed, who pays for that care.
- Dr. Zoloth emphasized the need for this trial to be conducted with the subject population that is most affected by this disease. Accrual should occur keeping in mind that African Americans are twice as likely to have TNBC.
- Dr. Kiem asked when the investigators anticipate that autoimmunity might be seen as a side effect.

D. Investigator Response

1. Written Responses to RAC Reviews

With regard to safety issues, multiple DC-based clinical immunotherapy trials have been conducted in research participants with a variety of cancers and no significant toxicity has been observed. In addition, Dr. Pruitt noted that he had recently completed a Phase I clinical immunotherapy trial using DCs transfected with RNAs encoding melanoma TAAs in which no toxicity was observed. Therefore, the investigators anticipate that the RNA transfected DC vaccine to be used in this proposed trial will be safe. Secondly, the investigators are initially evaluating the safety of vaccination with DCs transfected with TNBC TAA-encoding RNAs without any RNAs encoding immune modulators in Study Arm A; only if this approach proves to be safe will they proceed with co-transfection. Third, vaccinating with DCs co-transfected with RNAs encoding soluble immune modulators is designed specifically to deliver these immune modulators locally and to avoid the induction of autoimmunity seen when such immune modulators are administered systemically. As shown in mice in the investigators' recently published report, vaccination with DCs transfected with RNAs encoding immune modulators augmented anti-tumor immune responses to the same extent as did systemic immune modulator administration, while eliminating autoimmunity induced by the systemically administered immune modulator; this effect was

statistically significant. For all of these reasons, the investigators anticipate that DC vaccination in this study will be well tolerated and will not lead to significant toxicity in any of the study participants.

With respect to participant selection, the investigators have chosen to enroll any TNBC subject with T2 or greater disease, regardless of nodal status, for several reasons: (1) the risk of toxicity is low; (2) patients with T2 TNBC will be treated with chemotherapy, regardless of nodal status, consistent with the high likelihood that this subset of TNBC patients is at high risk for metastatic disease despite being node negative, and they are likely to benefit the most if effective adjuvant immunotherapy can be developed for TNBC; and (3) the 5-year survival for patients with node-negative TNBC is approximately 73 percent and the 5-year survival for TNBC patients with involvement of one, two, or three nodes is 62 percent, suggesting that a large number of node-negative patients have distant disease at the time of surgery and these patients are also more likely to benefit from an effective immunotherapeutic approach. Because patients with T2/N0 have an outcome similar to those with T1 tumors with nodal (N1) involvement, the investigators have decided to include T1 subjects. In addition, the correlation between lymph node involvement and survival is not as strong in TNBC as it is in other types of breast cancer.

Given that this Phase I study will not assess the effect of immunotherapy on survival, the investigators do not plan to test participants for BRCA1/2 mutations or to evaluate their tumors for expression of additional tumor markers. Such marker studies could be performed retrospectively during the analysis of the study results, but because of the small number of participants to be enrolled in this Phase I study, it would not be possible to determine a significant correlation between survival and the expression of such markers.

Expression of each of the proteins encoded by the transfected RNAs will be assessed by intracellular flow cytometry of the final DC vaccine product using monoclonal antibodies specific for each of these proteins. In the investigators' recently completed study in melanoma subjects vaccinated with melanoma TAA RNA-transfected DCs (RAC Protocol #0708-874), they assessed the intracellular expression of these TAAs in the DCs using a similar approach. In discussions with the FDA, it was agreed that the investigators would collect the intracellular expression data for each DC vaccine preparation and compare this expression with induction of anti-TAA immune responses retrospectively. The FDA further agreed that the investigators were not required to establish an arbitrary TAA expression-level cutoff for vaccine release. Recent analysis of the data from that trial did not detect any correlation between DC vaccine expression levels of any individual TAA with the immune responses generated against the given TAA. Anti-TAA immune responses were stimulated in all trial participants, regardless of the levels of TAA expression in the DCs administered.

Regarding followup after last leukapheresis, participants will be examined weekly during the vaccination period with a careful review of systems and physical examination. As part of this examination, skin condition will be assessed. Following vaccination, each participant will be followed with physical examination every two to four weeks in the immediate post-vaccination period. Two weeks following the final of six vaccinations, blood will be drawn from each participant for autoimmune testing, which will include measurements of anti-nuclear antibodies, rheumatoid factor, thyroid-stimulating antibodies, and anti-thyroglobulin antibodies. The time course of blood testing and physical examination of study participants is detailed in the Schedule of Activities. Thereafter, participants will be followed long term by their medical oncologists.

In all immune assays to be performed in this study, reactivity against targets not expressing the TAA of interest will be used as a negative control and will serve as a surrogate for "autoimmunity," at least when TAA RNA-transfected DCs are used as targets.

With respect to the discussion of "alternatives" in the informed consent document, no approved therapies exist for patients with TNBC after they complete surgery, radiation, and standard-of-care adjuvant chemotherapy. As such, these individuals can participate in this proposed trial, participate in another trial, or proceed with standard of care, which currently means continued clinical followup alone, with additional chemotherapy when recurrences develop.

Dr. Pruitt respectfully disagreed with the suggestion to add a statement that “it is very unlikely that getting the study vaccine will improve your health.” He explained that he would not conduct this or any other study of a vaccine if he thought there was no potential for benefit. To support this belief, he stated that, in his recently completed RNA-transfected, DC-based, first-in-human immunotherapy trial, a participant with metastatic disease experienced a complete clinical response.

With respect to enrolling patients with more advanced cancer, such as those with metastatic disease, the investigators explained that numerous studies suggest that such patients are immunosuppressed and fail to respond to vaccination. Conducting this proposed study in such patients would not provide meaningful information about induced antitumor immune responses or the potential safety and toxicity of the proposed immunotherapeutic approach.

Study participants will have undergone chemotherapy prior to study enrollment. Therefore, a comprehensive discussion of prognosis based on the individual patient’s tumor stage will already have taken place between the patient and her medical oncologist. Having undergone this previous counseling, the potential study participants should be better able to understand prognostic information included in the informed consent document.

Because the investigators plan to electroporate DCs with RNAs *ex vivo*, the labile RNAs will not be able to enter any other cells; therefore, none of the TNBC target antigens and none of the immune modulators will be expressed in cells other than the intended target DCs. In addition, because this study will use RNAs instead of DNAs, no incorporation into the genome of any transfected cells or any cells of the vaccine recipients can occur.

The investigators agreed to modify the informed consent document as suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Pruitt clarified that the investigators will not be enrolling male breast cancer patients. In his 15 years at Duke and in his breast cancer practice, he has never encountered a male who was triple negative.

Explaining the time course of recurrences of TNBC compared with other breast cancer patients, Dr. Pruitt stated that most TNBC recurrences occur in the first three years rather than in the first five years as with other, non-TNBCs. After 3 years, the TNBC recurrence rate flattens out more quickly than it does for non-TNBC. Dr. Blackwell added that the number most supported in the literature is that less than 5 percent of recurrences of TNBC will occur after the fifth year, with 60 percent of cases recurring within the first two years and 80 percent recurring within the first three years. This data makes the proposed study population ideal for studying short-term immune strategies.

The investigators pledged to do their best to accrue research participants who reflect the TNBC population, with nearly half of all TNBC patients being African American, although the number of patients to be accrued for this study might be too small to make any statistical representation.

Regarding the followup schedule and the potential for autoimmunity, Dr. Blackwell offered to build in a 3-month follow up for the first year to allay concerns about this possible adverse effect.

Dr. Pruitt explained that individuals who withdraw from this study during the vaccination course will not have accumulated any residual RNA or gene products.

E. Public Comment

Dr. Smith thanked the RAC reviewers for their germane comments about the informed consent document. She noted that the representation in the informed consent documents of protocols regarding withdrawal and the residual risk to participants in these trials has not fully been fleshed out. Gene transfer cannot be rescinded, which is a risk that participants need to be made aware of because they are unlikely to think about that aspect of risk on their own.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- One of the main questions raised during the discussion of this protocol was whether participants with early disease should be enrolled in this Phase I safety study. Although individuals with TNBC have a poorer prognosis than those with hormone-positive forms of breast cancer, the prognosis for those with TNBC and no known lymph node involvement is more favorable than for those with lymph node involvement. At least one study indicates that there is an overall survival of approximately 80 percent for those patients without node involvement (*J Clin Oncol* 29: 2628, July 2011). In addition, patients with small tumors (T1 and T2) may have a better prognosis. While the need to test new therapies is clear, even for node-negative patients, the enrollment criteria should be modified to at least exclude those who are node negative with tumors that are smaller than 3 cm.
- The presence of certain genetic markers, for example BRCA1, BRCA2, and p53, are predictive of disease prognosis. Although this is a Phase I safety and toxicity study (and not an efficacy study), it would be useful to examine whether clinical responses are correlated with these genetic markers.
- Because almost 50 percent of women with TNBC are African American, it may be beneficial, to the extent possible, to have the study population reflect this population. While this is an initial trial and will not enroll a large number of participants, nonetheless the investigators should consider recruitment strategies that might increase the diversity of participants enrolled.
- Systemic administration of an anti-CTLA-4 monoclonal antibody, in clinical trials for melanoma, has led to the development of severe autoimmunity. As CTLA-4 is one of the components of this dendritic cell vaccine, autoimmunity is a potential risk. Currently, the induction of autoimmunity will be monitored at only one time point after the last vaccination (2 weeks). In order to monitor for the development of autoimmunity at later stages, the investigators should consider checking for an autoimmune response 4 to 6 months after vaccination.

Ethical, Legal, Social Issues

- While every Phase I trial is conducted with the intent of developing a new therapeutic, the reality is that the majority of agents tested in Phase I trials will fail to become a licensed therapeutic. Therefore, it is important not to promise therapeutic benefit in this early Phase I trial. The statement regarding benefit in the informed consent document, "... there may not be direct medical benefit to you," implies that there may be a clinical benefit and likely reflects the study team's optimism. A more realistic expression of anticipated benefit would be, "Phase I studies such as this, which are first-in-human studies, usually do not provide any medical benefit to participants."
- In gene transfer trials, the agents have the potential to persist longer than traditional chemical drugs. The investigators should clarify in the informed consent document that withdrawal from the trial may be withdrawal from further testing but that the effects of the study agent, including any potential harms such as autoimmunity, have the potential to persist.

G. Committee Motion 4

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made but not seconded to approve these recommendations, and the RAC approved these recommendations by a vote of 15 in favor, 0 opposed, 1 abstention (disagreement with enrolling any participant without node involvement), and 3 recusals.

X. Review and Discussion of Human Gene Transfer Protocol #1210-1192: A Study to Infuse ROR1-Specific Autologous T Cells for Patients with CLL

Principal Investigators: William Wierda, M.D., Ph.D., The University of Texas MD Anderson Cancer Center (MDACC), and Laurence Cooper, M.D., Ph.D., MDACC
Sponsor: MD Anderson Cancer Center
Additional Presenters: Thomas J. Kipps, M.D., Ph.D., University of California, San Diego (UC San Diego)
RAC Reviewers: Drs. Cannon, Kohn, and Ross

A. Protocol Summary

Advanced B-cell malignancies, such as chronic lymphocytic leukemia (CLL), are generally considered incurable using conventional therapies.

One approach to improve patient survival may be to enhance the immune response against the cancer by combining T-cell therapy with chemotherapy. To accomplish this, the investigators have developed a strategy to infuse tumor-specific T cells rendered specific for CLL through expression of chimeric antigen receptor (CAR) that recognizes the molecule ROR1 expressed on CLL cells.

The proposed trial seeks to co-infuse two populations of ROR1-specific CAR+ T cells using a competitive repopulation experiment to determine whether signaling through CD28/CD3-zeta or CD137/CD3-zeta results in sustained T-cell proliferation and thus superior therapeutic potential. This single-center, proof-of-concept human gene trial) evaluates the safety, feasibility, and persistence of adoptively transferred ROR1-specific autologous T cells in research participants with advanced B-lineage CLL after receiving one of three chemotherapy lymphodepleting regimens cyclophosphamide/fludarabine/rituximab; fludarabine/bendamustine/rituximab; bendamustine/rituximab). Two populations of T cells, are rendered specific for ROR1 by electrotransfer, using a Nucleofector device, of *Sleeping Beauty* (SB) DNA plasmids expressing (i) SB11 hyperactive transposase and one of two (ii) transposons coding for CARs, designated ROR1RCD28 and ROR1RCD137, that can activate T cells through either chimeric CD28 and CD3-zeta or CD137 and CD3-zeta endodomain, respectively. Specificity of these CARs for ROR1 is derived from a ROR1-specific mAb.

This study is designed to test the safety and feasibility of using genetically modified T cells in a small number of research participants. In part, this trial will determine which population of genetically modified T cells exhibits superior persistence and thus therapeutic potential. The recipients of these infusions will be monitored closely for signs of toxicity. Data will be collected on how well the tumor-specific T cells function in the body. Additional studies will be necessary to determine the ability of the genetically modified T cells to alter patient survival.

B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novelty of the ROR1 target. Whereas ongoing clinical trials involve the administration of antibodies to ROR1, the fact that this is the first trial to employ a CAR T-cell construct that targets ROR1 deserves further discussion.

Three RAC members provided written reviews of this proposed trial.

Dr. Cannon expressed concern about the novelty of the target, ROR1. There is a known risk associated with the use of novel CAR-engineered T cells whereby the potent T cells so generated can have unexpected adverse consequences against normal cells that express low levels of the target antigen. Therefore, she requested more information about the distribution of ROR1 on normal cells and noted that no prior animal studies were provided to assess safety. An unknown risk exists of a T-cell response being

mounted against a normal host cell expressing even low levels of ROR1, which could be exacerbated by antitumor effects leading to tumor lysis syndrome and cytokine storm; given that risk, Dr. Cannon asked the investigators about monitoring to assess if such a reaction is occurring and what mitigation steps would be taken. She noted with concern that the efficacy data provided was minimal, with no evidence of activity against CLL cells (only cell lines), and the specificity of the cytotoxicity of the engineered T cells against the cell lines was not convincing. Dr. Cannon asked for data to back up the investigators' statement that ROR1 CAR expression in T cells from normal donors and CLL patients resulted in activity against primary CLL B cells.

Dr. Kohn's primary concern was that this protocol is a first-in-human study to target the ROR1 antigen, and he noted that some previous clinical trials targeting novel antigens have encountered unexpected on-target, off-tissue toxicities due to distribution of the target antigen on normal tissues at low levels sufficient to activate these potent T cells. As such, he asked the investigators to provide more information about the distribution of expression of the ROR1 protein, notably what percent of CLL patients express ROR1 on their leukemic cells and what is known about expression of the ROR1 protein on other cells in the body. He asked whether demonstration of ROR1 expression on individual participants' CLL cells would be assessed prior to dosing and whether that expression would be followed serially to look for antigen escape. Because of minimal preclinical data demonstrating efficacy of the anti-ROR1 CAR-transduced T cells, Dr. Kohn requested additional data on efficacy of the ROR1 CARs. He queried as to how the use of different conditioning regimens, likely with different spectra of toxicity, would affect statistical interpretation of the primary endpoint of safety. He requested that the investigators clarify the language concerning the timing of advancing to higher cell dose levels, and wondered whether a plan had been developed to measure cytokines in a timely manner if an apparent cytokine storm syndrome occurs, to possibly guide a targeted intervention. With regard to the informed consent document, Dr. Kohn averred that it was clear and easy to read but was missing a statement about a request for autopsy in case of the research participant's death.

Dr. Ross noted that the potential risks of using ROR1-directed CAR T cells were not discussed in detail, with little preclinical data presented regarding whether ROR1 is expressed in normal tissue and the risk of infusing T cells with the potential to target normal tissue. She asked the investigators to provide data from experimental models regarding the use of either anti-ROR1 antibodies or directed T cells that address ROR1's specificity *in vivo*. She was concerned about the lack of a preclinical model, given the SAE (a fatality) that occurred when CAR T cells directed at ERBB2, which is expressed on normal lung, were used for immunotherapy. Dr. Ross asked whether all patient CLL cells express high levels of ROR1 and, if not, whether the investigators would screen patient cells for ROR1 expression prior to initiating the experimental therapy. She also asked for the rationale for using two different CAR+ T cells for a first-in-human target, and whether the investigators would consider using only a single CAR+ T cell if an SAE occurs. With regard to the informed consent document, Dr. Ross noted that the document first states that "the researchers want to learn if these T cells are effective in attacking cancer cells in patients with CLL"; this statement should be listed as a secondary goal and not a primary goal, as in the protocol. She asked whether the research participants or their insurers should bear the cost of chemotherapy that is part of the investigational study and not part of treatment. In addition, Dr. Ross suggested that the list of possible risks include the recent adverse event reported to the RAC regarding the potential deleterious growth of K562 cells.

C. RAC Discussion

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

- Dr. Fong shared Dr. Ross's reservations about using the combination of the two T-cell populations simultaneously. He asked the investigators to clarify why they chose not to conduct parallel studies, each with one T-cell population.
- Dr. Fong asked the investigators why they are not waiting for the animal studies to conclude before going forward with this clinical study.

- Dr. Kohn recommended that the investigators redesign the protocol so as to conduct at least the low-dose cohort in parallel before combining the two T-cell populations at higher doses. Doing so would provide some initial safety assessment.

D. Investigator Response

1. Written Responses to RAC Reviews

Using the anti-ROR1 monoclonal antibody to screen tissues, the investigators have found high reactivity with neoplastic cells of CLL patients, via immunohistochemistry and flow cytometry. To date, they have screened more than 1,000 patient samples for ROR1 and have discovered that 96 percent of these cases express ROR1, indicating that the prevalence of ROR1 expression in CLL is high. They also have screened normal tissues and tissue arrays for ROR1 with the anti-ROR1 used to generate the CAR; these studies indicate that the expression of ROR1 is highly restricted.

CAR+ T cells targeting ROR1 have not previously been evaluated in human trials. Therefore, an unknown risk exists of an on-target deleterious autologous T-cell response being mounted against normal host cell(s) expressing even very low levels of ROR1. In addition, on-target T-cell responses could occur due to synchronous activation of T cells recognizing ROR1 in a large bioburden of ROR1+ CLL, which could lead to a variant of tumor lysis syndrome and cytokine storm. These risks are mitigated in five ways by the design of this proposed trial. Dosing-related toxicity is most likely to occur within the first month of infusion.

The investigators provided new data demonstrating specific lysis ability of ROR1-specific T cells. The proposed trial infuses autologous T cells; thus, activation of T cells through endogenous T-cell receptors will not lead to unwanted targeting of normal structures.

Although expression of ROR1 is not part of the eligibility criteria for this trial, it will be evaluated pre- and post-infusion to gauge the therapeutic potential of the ROR1R-CAR-T cells and the potential for the emergence of antigen-negative escape variants.

The rationale for pre-infusion conditioning therapy is to cytoreduce CLL and achieve an *in vivo* environment conducive to persistence (and numeric expansion) of the infused genetically modified T cells. There are no clear data identifying the ideal conditioning regimen nor are there compelling data identifying key factors to direct selection of a specific conditioning regimen. Therefore, the investigators provide conditioning therapies that are likely to have cytoreductive activity and be well tolerated. Given that research participants may have had various prior treatments, three options are necessary to avoid employing a regimen to which a participant is refractory due to prior exposure. Investigators enrolling and dosing participants on this trial are highly experienced and experts in CLL management, and will be in the best position to select an individualized regimen most likely to achieve cytoreduction. All proposed conditioning regimens have been used extensively in Phase II or Phase III trials and in standard practice, and they involve standard FDA-approved agents in safe doses and schedules. Toxicity profiles are well known, and myelosuppression, the most common toxicity with these regimens, is similar and comparable between these regimens at the indicated doses. While cytoreduction is the objective for one course of the conditioning regimen, it is unlikely that a single course of any of these regimens will result in complete or partial remission by the formal response criteria used for CLL.

This trial phases in a multicenter trial to help broaden the appeal of immunotherapy infusing ROR1R-CAR-T cells. Participants will be enrolled at MDACC and UC San Diego once all regulatory obligations have been satisfied and the protocol and informed consent document and process have been approved by the respective IRBs.

If a female participant becomes pregnant prior to or after infusion of her ROR1R-CAR-T cells, she will be removed from the study, no further testing or dosing will occur, and she will be enrolled on the long-term follow-up observational study. Removal will be accomplished in order to assess the primary and secondary objectives of this trial.

Cytokine in the serum will be measured. Research participants will be monitored for symptoms of cytokine release syndrome and will be managed as indicated in the protocol and Appendix F.

The rationale for using two different ROR1-specific CAR constructs is to determine if one of the constructs is associated with superior expansion or persistence following adoptive transfer. This analysis will be determined in correlative laboratory investigations and included as secondary objectives. The primary objective is to assess safety and feasibility by identifying the maximum tolerated dose (MTD) of ROR1-specific CAR+ T cells. Ultimately if there is *in vivo* indication of favorable numeric expansion, augmented effector function, or enhanced persistence favoring one construct over the other, then the superior construct will likely be chosen to move forward in clinical investigations.

Regarding who should bear the cost of chemotherapy that is part of this study, the investigators explained that the conditioning chemoimmunotherapy (CIT) is intended to have cytoreductive activity, given that all options are standard CIT regimens used to treat the disease. Since anti-tumor cytoreduction is intended with the CIT, those regimens will be charged to insurers as a standard treatment regimen.

The investigators agreed to modify the informed consent document as suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Dr. Wierda reiterated that the investigators are starting this trial at a low dose of infused cells. They will monitor the research participants closely and will halt the protocol if they encounter any signal to indicate a problem with cross reactivity. Cross reactivity is not expected, based on Dr. Kipps' antibody data, but the investigators intend to be vigilant about monitoring for it.

With regard to the two CAR constructs to be tested in this trial, Dr. Wierda noted that the investigators would be serially sampling the research participants and will be able to assess the persistent disease and the T-cell population, in the blood and in the bone marrow. The planned correlative studies will look at activation markers on the T cells, will enable the investigators to distinguish between the two populations, and will look at intracellular cytokine levels. The investigators believe they will get a signal from the collected data and thus will be able to make a statement about differing effectiveness between the two constructs.

In response to Dr. Fong's suggestion of conducting parallel studies with the two different T-cell populations, Dr. Wierda stated that the investigators' intent is to determine which construct should move forward into a Phase II trial and further clinical development. With the planned correlative studies, the investigators will be able to assess which construct may be ideal as well as which construct will be more safe. Dr. Cooper added that the basic scientific question is that the investigators do not know which CAR design ultimately gets rid of a given burden of tumor. In addition, after being infused, the T cells will propagate, an ability that, *in vivo*, may or may not be related to the T cells given. There will be significant heterogeneity among research participants if using parallel cohorts; the only way to get rid of that heterogeneity is to use the same population of T cells from one research participant, bifurcate it in the laboratory to put in the different CARs, and then combine them back into the common milieu of a given research participant to make side-by-side comparisons. If the signal is not clear—if either 4-1BB or CD28 does not "win" every time—the investigators may not have a definitive answer, but it is important to provide a chance at a definitive outcome. If the investigators discover which population of T cells persists, then that is the approach they will take forward into the more advanced, large-scale studies.

Regarding the currently ongoing animal studies, Dr. Kipps explained that those studies are using a different type of therapy that uses antibodies as opposed to CARs. However, previous animal studies provide reassurance to move to a Phase I clinical trial. Extensive studies with the University of California, Los Angeles, as part of a consortium grant looked at antibodies that seem to have activity against leukemia and that indicate that hematopoiesis should not be problematic in humans.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- This protocol proposes to use two different second generation CARs, one with a 41BB T-cell signaling domain and the other with CD28. Using two populations may complicate the analysis of an SAE. As this is primarily a safety study, the investigators should consider conducting an initial safety study in a small number of patients evaluating the CARs separately on parallel tracks, perhaps at least at the lowest dose, before moving to a protocol testing competitive repopulation at higher doses.
- The protocol should specifically exclude those patients whose CLL does not express the target antigen (ROR1), as it is likely that the risk/benefit ratio for this patient population would not favor enrollment.
- K562 cells are used in the culture of the T cells. While the K562 cells are irradiated prior to use and are not expected to persist due to immune responses against the cells, a recent SAE in a trial using K562 cells as a vaccine vehicle demonstrated that it is possible for leukemia cells to remain viable after radiation. While the SAE in that protocol involved direct administration of a significant number of irradiated K562 cells to a very immunosuppressed patient, it may be prudent to test an aliquot of irradiated K562 cells for viability if the plan is to irradiate batches of K562 cells for future use.

Ethical, Legal, Social Issues

- In the section of the informed consent document describing the risks of receiving T cells that express a CAR, it is important to include the fact that there can be unexpected and unanticipated consequences, including death.
- The risk of insertional mutagenesis with this technology is unknown. While it is true that, to date, there have been no reports of insertional mutagenesis with integrating vectors in mature T cells and the risk is presumed low, experience with Sleeping Beauty technology is more limited. Therefore, it is important that the consent adequately communicate that the risk of insertional mutagenesis is not known for the Sleeping Beauty technology.

G. Committee Motion 5

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made but not seconded to approve these recommendations, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XI. Review and Discussion of Human Gene Transfer Protocol #1210-1190: A Phase I Open-Label Study to Assess the Safety, Tolerability and Preliminary Efficacy of LX-1101 (Lymfactin,TM VEGF-C Adenoviral Vector) in the Treatment of Patients with Secondary Lymphedema Associated with the Treatment of Breast Cancer

Principal Investigator: Stanley Rockson, M.D., Stanford University School of Medicine
Sponsor: Laurantis Pharma, Ltd.
Additional Presenters: Kari Alitalo, M.D., Ph.D., University of Helsinki, Finland; Burkhard Blank, M.D., Laurantis Pharma, Ltd.; Alan Boyd, M.D., Laurantis Pharma, Ltd.;

RAC Reviewers: Mark Pegram, M.D., Stanford Cancer Institute; Anne Saaristo, M.D., Ph.D., Turku University Central Hospital, Finland
Dr. Koch, Ms. Mallino, Dr. Strome, and Dr. Zoloth

A. Protocol Summary

Lymfactivin™ (LX-1101) is a gene-based medicine that is under development for the treatment of secondary lymphedema that occurs in some patients following breast cancer treatment. Primary lymphedema is a rare hereditary condition; secondary lymphedema is caused by disease or damage to or blockage of lymph vessels. In the United States, the most common cause of secondary lymphedema is surgery and/or radiotherapy for breast cancer, which results in unilateral lymphedema of the arm. Secondary lymphedema associated with the treatment of breast cancer is a chronic, progressive disease characterized by gross swelling of the affected arm. Long-term accumulation of fluid and proteins in the tissues leads to inflammation and eventual scarring of tissues. Affected areas may feel tender and sore, and loss of mobility or flexibility can occur. In rare cases, long-term, severe lymphedema can also place the patient at risk of developing lymphangiosarcoma, a rare and highly aggressive form of soft tissue cancer. It is a disabling and disfiguring condition severely affecting a patient's quality of life and psychological status.

No medicinal products are approved to treat secondary lymphedema associated with the treatment of breast cancer. The current approach consists mainly of non-drug treatment, usually massage and exercise, sometimes in combination with compression garments and, in rare cases, surgery such as liposuction. These methods have been shown to be effective in reducing lymphedema but do not address the root cause of secondary lymphedema—the damage to the lymphatic vessels caused by surgery and/or radiotherapy. Additionally, these methods, with the exception of surgery, are mostly long-term and time-intensive treatments, and whatever treatment is proposed, the possibility of a cure remains questionable. There is consequently a need to develop more effective treatments for lymphedema in breast cancer survivors.

Lymph node transfer without growth factor therapy is currently being used as an experimental treatment for lymphedema and the preliminary results have shown that it seems to provide at least some benefit for some patients. However, it is essential for the survival and function of the transferred lymph node that connections are formed between it and the lymphatic system. The experimental data seems to show that not all transferred lymph nodes form these connections into the existing lymphatic vasculature. If this happens, the transferred lymph node may lose the special architecture it needs to function and may fail to survive.

Vascular endothelial growth factors (VEGFs) are involved in all types of vascular growth and are capable of directly inducing the growth of blood or lymphatic vessels. VEGF-C is a vascular endothelial growth factor that is crucial for the development and growth of lymphatic vessels, and VEGF-C protein is naturally made in small amounts by human cells. Using a gene transfer approach to increase the amount of VEGF-C in the area around the lymph node at the time of transfer has been shown in animal models to improve the survival and maintain the architecture of transferred lymph nodes.

Modified adenoviruses have been used in numerous clinical trials to facilitate gene transfer. LX-1101 is a non-replicating, adenoviral vector created to express the human VEGF-C gene. Infected cells in and around the transferred lymph node will act as 'factories' producing VEGF-C at a higher level for a few days. During surgery, a flap of tissue containing healthy lymph nodes will be removed from the subject's lower abdominal area. The LX-1101 will be injected into the tissue flap before it is transferred into the axillary region of the subject's affected arm, thus reducing the subject's systemic exposure to LX-1101. This procedure will be performed in conjunction with breast reconstruction surgery, and will take place after a minimum of 1 year has elapsed since the breast cancer treatment and provided no evidence exists for tumor recurrence or any other malignancy. Research participants will receive only one experimental treatment with LX-1101.

Laboratory studies in mice and pigs have demonstrated that transient and localized expression of VEGF-C increased the number of lymphatic vessels in the region of, and connected to, the transferred lymph node, and improved the survival of the node and preserved its internal architecture. In these studies, the adverse effects seen, such as bruising and temporary fluid accumulation around the site of the surgery, were similar in all animals operated on whether they were treated with LX-1101 or they were controls. Tissues from a study in domestic pigs showed that the DNA from LX-1101 was undetectable after 60 days, with the exception of pigs injected intravenously to increase their systemic exposure to the drug. In these animals, which were given a dose of LX-1101 10-fold higher than the maximum dose that could be given to humans, a very small amount of DNA still remained in the spleen only. Following this work, it was concluded that LX-1101 was safe in a large animal model, and that it had been shown in animal models of lymph node transfer to be effective in improving the survival and architecture of transferred lymph nodes. In this planned Phase I dose escalation and expansion study, dosing will not exceed a dose 10-fold lower than the highest dose tested in animals.

The planned clinical study of LX-1101 will be the first in humans and will enroll up to 24 subjects. The main objectives of the study are to evaluate the safety of the experimental drug Lymfactin™ (LX-1101); to test for any harm, discomforts, or side effects associated with its use and whether these change at different doses; and to determine which dose will be recommended for further study. In the first part of this two-part study, up to three different doses will be tested in three to six research participants per dose. The dose recommended for further investigation on the basis of the results from the first part of the study will be the tested in six to nine additional participants.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. Key issues included the novel use of the VEGF-C transgene. Given the limited success to date of gene transfer using other VEGF forms to treat different vascular diseases, discussion was deemed to be warranted with regard to the risks and benefits of using an autologous transplant of gene-modified abdominal wall tissue to promote the development of effective lymphatic channels in a large area of the arm.

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

Dr. Koch suggested that the investigators either provide preclinical data comparing lymph node transplantation alone versus transplant plus VEGF-C or that they conduct a Phase I trial to collect that data in humans. He noted that the preclinical efficacy data in the pig model was neither statistically significant nor robust, thus making a clinical trial in humans premature if it is based solely on that data. He queried as to whether any parameter, even in the mouse, was responsive to a dose effect. Dr. Koch asked the investigators to comment on any potential cardiovascular effects of VEGF-C gene delivery, especially any electrocardiogram (ECG) abnormalities or abnormal blood pressure noted in mouse or pigs. He asked the investigators to clarify the data presented regarding expression in the pigs, and to elaborate as to whether the pigs were treated with the product proposed for this clinical trial or with a different vector. Dr. Koch requested that the informed consent document include more detail and description about the purpose of the computed tomography scan before enrollment and dosing.

Ms. Mallino focused her review on the informed consent document. She asked the investigators to clarify whether research participants needed to be free of active cancer for at least one year or for at least two years, as both timeframes were specified. Clarification was also needed regarding the participant's availability for annual contact and followup. She noted that some minor changes to the informed consent document would add emphasis and ease of reference for the participant. Ms. Mallino requested that the investigators provide a rationale for the differences between participant eligibility as presented in the protocol and those presented in the informed consent documents.

Dr. Strome noted that VEGF trials for vascular disease have had little to no success, although the proposed product in animal models has shown some improvement in lymph node survival. As such, he requested more preclinical information about experimental animal numbers, limb measurements recorded, time interval to recording, and that the results were sustainable, plus the amount of swelling in

the affected arm relative to the normal arm at entry, the surgical complications, timing post-surgery of the initial swelling, and the rate of progression. He strongly suggested that patients receiving post-surgical radiation therapy not be included in this Phase I trial absent data from similarly irradiated animals that showed no more-serious, post-surgical complications. Dr. Strome suggested that the investigators use circumferential measurements to evaluate results of the intervention, and not inject even small amounts of fluid anywhere on the arm. Regarding entry criteria, Dr. Strome noted that, in the United States, many reconstructions are done at the time of extirpation or within several months thereafter; the investigators propose waiting 1 year, which he believed would pose quality-of-life issues for the research participants. He asked the investigators to explain why they propose to restrict entry to patients who do not smoke. Given the potential issue of increasing lymphedema because of the surgery as well other complications if the graft fails to be beneficial, Dr. Strome suggested using only cohort 2 and cohort 3 because an impact seems less likely with cohort 1 based on the animal data presented. With regard to adverse events, Dr. Strome was adamant that wound healing issues should not be excluded from reporting, as the investigators proposed. He suggested that the statistical analysis should be prepared by an independent entity and that all information regarding SAEs should be forwarded from all sites directly to the chosen investigator, and he expressed concern about the sponsor's right to delay publication submission for 90 days. Dr. Strome asked why the proposed level of scar removal was necessary, especially the lysis of scar tissue around vessels and motor nerves, which could lead to serious complications. In addition, he suggested several clarifications within the informed consent document, including that the risk of nerve injury should be discussed and post-operative pain and its potential duration should be stated more clearly.

Dr. Zoloth focused her review on the ethical considerations of this proposed trial. She suggested that the investigators include a notation that the treating doctors would be paid for accumulation of research participants, if that is the case. She asked the investigators to clarify discussion of how participants can withdraw from the study, noting that they can only withdraw from "being studied" but cannot undo the surgery or genetic intervention they have already received. Acknowledging the proprietary nature of the experimental product, Dr. Zoloth nonetheless expressed concern about incomplete data, especially a reference to significant failure of transplantation without VEGF. She stated that the nature of the risk associated with this experimental treatment was not fully explained in the informed consent document, noting that gene transfer with unknown or unexplained risk would be attempting to treat a disease that in most cases is deeply frustrating and painful but is not usually life-threatening.

C. RAC Discussion

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

- Dr. Strome stated that he would like the investigators, before proceeding to a clinical trial in humans, to gather data from an animal model that is irradiated, showing in that setting that they can get new lymphatic vessels that are substantive and meaningful.
- Reiterating that there is no animal model for this disease because it is solely a human disease, Dr. Zoloth suggested the investigators clarify this point in the informed consent document and in the decision-making process, and that references to the results of animal experiments related to this trial be re-characterized accordingly.
- Dr. Fong suggested the need for further clarity in the informed consent document with regard to potential residual cancer that remains in a local area within the patient, which could be stimulated by the local growth factor that will be inserted there.
- Dr. Hammarskjöld noted that the vector described in the protocol is a first-generation adenovirus vector with E1E3 deleted, which has been problematic in previous studies. She expressed concern that this vector behaves potentially very differently in animals than it behaves in humans.
- Dr. Hammarskjöld asked the investigators whether they intend to determine the duration of VEGF expression and how that correlates to preexisting immunity to the vector.
- A poll of the RAC reviewers for this trial indicated that some serious concerns remained. Dr. Zoloth opined that more work is needed to answer the questions raised at today's RAC meeting and she would prefer to review this protocol again after those concerns were addressed. Dr.

Strome expressed concern about the fact that there has been no real success to date in humans and the potential exists to do harm, particularly with infection, if graft failure occurs.

- Dr. Badley requested discussion about the association between VEGF and breast cancer disease progression. Significant literature exists, most of it observational, that links VEGF levels to disease recurrence and widespread metastases. Breast cancer is notable for late recurrences, so the fact that there is no evidence of disease at two years is not an adequate threshold. He expressed concern about giving VEGF to subjects who may have subclinical disease without the benefit of long-term followup. As such, he suggested two approaches: (1) testing this intervention in a non-breast cancer population first, recognizing the difficulties associated with accrual, and (2) measuring with long-term followup the rate of disease recurrence in dosed individuals.
- Dr. Ornelles noted several low risks, including increased levels of adenoviral receptors in breast cancer cells, and the association in mouse models of VEGF-C with increased metastases that, in combination, increase the overall level of risk. Although each individual risk is extremely small and unlikely to occur, he suggested that a different vector choice might be preferable.

D. Investigator Response

1. Written Responses to RAC Reviews

The porcine model of autologous lymph node transplantation is adequate and appropriate in view of these theoretical considerations:

- Useful experimental models for human chronic lymphedema are not readily available and existing models do not address the concerns that surround this novel therapeutic approach.
- Autologous lymph node transfer for human breast cancer associated lymphedema has already been incorporated into clinical practice in the United States (albeit with limited successful outcome) and therefore does not require additional evaluation in an animal model.
- The porcine model used, while not specifically quantitatively addressing the lymphedema outcomes, successfully demonstrates the remarkably improved engraftment and viability in this simulation of the autologous transplanted lymph node.

Preclinical studies have been conducted in mice that compared lymph node transfer alone versus transfer plus administration of the VEGF-C gene therapy. Lymphatic drainage also improved considerably in mice that received AdVEGF-C transduced lymph nodes as compared with control-treated transplants. Closer inspection of the VEGF-C treated lymph nodes *in situ* showed that they had formed both afferent and efferent connections with the host lymphatic vasculature. Chimeric lymphatic vessels were also observed that were composed of endothelial cells from both the donor and the recipient in close proximity to these nodes. It was not possible to repeat this work in the pig model due to the anatomical configuration of the lymphatic system in pigs.

During the early design discussions, the investigators considered including a group of research participants to receive lymph node transplantation alone as part of the Phase I study. However, because the primary objective of this study was to determine the safety and tolerability of the AdVEGF-C and given that this study would be the first time this product would be given to humans, the investigators believed it would be inappropriate to include a group of research participants that received lymph node transplantation alone. Including such a group was deemed more appropriate in any subsequent Phase II clinical study.

There is no truly identical animal model available that is equivalent to the human condition of lymphedema. It was not the objective of the pig studies to establish successful lymph node engraftment in the animals because this can only be tested in humans. The use of VEGF-C in the pigs was mainly done to demonstrate that VEGF-C would grow new lymphatic vessels and over an equivalent area to that in humans. The pig studies were also performed to confirm that VEGF-C in comparison to VEGF-D was the most appropriate growth factor to use in this situation. The pig model was also used to confirm the route of administration of the product (injection via the perinodal route). As the pigs used in the model do not actually develop lymphedema as it occurs in humans, it is not possible to determine such outcomes as

changes in limb size and progression of controls, as well as long-term improvements in those measurements.

In the preclinical animal studies, the dose levels were selected to ensure saturating levels of VEGF-C locally. In the mouse studies, the animals typically received a dose of VEGF-C equivalent to 5×10^{10} virus particles (vp). In the pig studies, the majority of the animals received a dose of 1×10^{11} vp and a few animals received a dose of 1×10^{10} vp. However, it was not possible to demonstrate a significant dose-related difference in the responses seen in these studies. Very little spillover occurred into the systemic circulation or other sites of the body, and adenovirus was quickly eliminated from blood and overall expression was transient, lasting maximally for about two weeks.

No adverse effects beyond a slight down regulation of blood pressure have been noted in mice receiving VEGF-C. In addition, no adverse cardiovascular effects were noted in either the preclinical pig studies or the toxicology and biodistribution study that also involved pigs. During the toxicology and biodistribution study, the animals' ECG readings were monitored during the surgical procedure with concurrent AdVEGF-C administration, and no abnormalities of note were reported; longer term ECG monitoring has not been performed. The study sponsors are not aware of any specific concerns related to an effect of VEGF-C on cardiovascular function, but VEGF-A, which induces a more pronounced down regulation of blood pressure, has not been a problem in numerous human gene transfer trials.

The majority of patients who develop lymphedema will have been irradiated during treatment for their breast cancer. If the investigators exclude these patients, it will be difficult to find research participants for this Phase I clinical study. During the surgical procedure that will accompany the administration of LX-1101, all irradiation-related scar tissue will be excised and replaced with healthy lymph node and fat tissue.

Regarding inclusion criteria, a limb volume ratio of at least 110 percent is accepted in the medical literature as a suitable definition for the presence of lymphedema in an at-risk population. When validated through the documentation of abnormal bioimpedance spectroscopy, this becomes a reliable, serially quantifiable variable that is suitable as an inclusion criterion. Along with serial volumetry and bioimpedance spectroscopy, serial quantitative assessment of quality-of-life through validated instruments will be an additional endpoint measure of treatment efficacy. While radionuclide scintigraphy is useful to demonstrate the functional deficit in lymphedema, it provides only a qualitative assessment of lymphatic function that will be difficult to assess following treatment; since the lymphoscintigram is often abnormal in latent, preclinical disease, it is reasonable to expect that post-treatment changes may not be visualized, even if the clinical treatment outcome is favorable. While posing no threat to patient safety, lymphoscintigraphy is expensive, cumbersome, and uncomfortable to the patient; therefore, the sponsor is considering whether or not to include it as a functional measure in this study.

In discussions with the FDA, it has already been agreed that patients recruited 2 years after completion of treatment for breast cancer will typically have already undergone breast reconstruction. Therefore the procedure that will be carried out in this study will not include concurrent breast reconstruction surgery.

Excluding patients who smoke has been requested by the surgeons who will be performing the study and the experts in the field who were consulted about the study design. The collective view was that smoking would affect the wound healing process and, therefore, recruitment will be limited to non-smokers or research participants willing to give up smoking prior to surgery.

The proposed doses of AdVEGF-C are 1×10^9 vp, 1×10^{10} vp, and 1×10^{11} vp. The dose escalation design involves a 3+3 design whereby at each dose level, providing no dose-limiting toxicities occur, it will only be necessary to dose three research participants at each level before dosing more participants at the maximum tolerated dose. Therefore, the investigators anticipate that only three participants will be dosed in cohort 1, the lowest dose level of 1×10^9 vp. The investigators recognize that this dose is unlikely to be a therapeutic dose in all participants; however, because this is a first-in-human study, using this dose as a starting dose is considered appropriate from a safety perspective.

In discussions with the FDA, the investigators agreed to collect and monitor data relating to wound healing complications.

The sponsor's right to delay publication of the trial results for a maximum of 90 days is necessary to allow for a review of the results in relation to the intellectual property rights of the drug product and is the usual practice in studies with a patentable drug. In practice, review is unlikely to take the full 90 days and therefore any publications will be approved for submission earlier than the time given.

In lymphedema patients, the surgeons have commented that they nearly always find dense ("wood-like") scar tissue next to the axillary vessels. When that scar is removed, they instantly notice lymphatic leak into the axilla. If that scar tissue is not removed (and lymphatic channels are not opened), the remaining scar tissue may restrict the regrowth of the new lymphatic vessels into the axilla. The surgeons agree that axillary scar removal needs to be performed by an experienced microsurgeon to prevent any further complications; that will be the process during this study.

The eligibility criteria in the informed consent document and in the final protocol should be identical: Research participants must have been two years free of recurrence of their breast cancer since completion of cancer treatment prior to entry into this study.

The risks of nerve injury will be added to the informed consent document as requested, together with statements relating to post-operative pain and duration. Details of the pain control protocol also will be included. The final informed consent document will be written to correspond with the final protocol for the clinical study, and the principal investigator and the sponsor will accept and account for all modifications suggested by the RAC reviewers.

2. Responses to RAC Discussion Questions

Regarding the relationship of the animal studies to the human experience, Dr. Rockson explained that one issue that needs to be considered is whether preexisting radiation in the mouse model will affect the biological processes that might respond to the VEGF-C versus the impact of radiotherapy on the potential development of lymphedema. The animal model does not provide the opportunity to assess the impact of the therapeutic intervention on disease resolution, but it does provide the opportunity to study the biology of the response to the adenoviral vector delivered growth factor. With regard to developing a sustained lymphedema, he noted the difficulty, even with radiation, of generating an injury that the animal will sustain as long as the chronic lymphedema in humans. Administering enough radiation to allow the lymphedema to persist has at least a 50 percent mortality rate in the treated animals, creating an impractical laboratory assessment. Therefore, the investigators are trying to learn what they can from the animals about the behavior of the vector and its effect on node viability, rather than using animal models to determine whether this approach will help reverse the pathology of lymphedema.

Dr. Blank stated that, since the investigators submitted the protocol, they have tightened the exclusion criteria to exclude patients with more-advanced tumor stage at the time of initial treatment and initial surgery. Therefore, research participants in this study are unlikely ever to have received axillary radiotherapy.

Dr. Rockson provided background about the natural history of breast cancer lymphedema. It is fairly well established based on observational criteria that the natural tendency of lymphedema is universally to progress, in two basic forms. In some patients, a progressive hydrostatic accumulation of interstitial fluid remains responsive to physical measures that contain the fluid but do not reverse the pathology. Concurrently, the second pathology is progressive adipogenesis that manifests as a significant increase in the subcutaneous adipose layer in the limb and that behaves physically and by appearance identically to the hydrostatic edema, except it is unresponsive to physical measures. If the investigators segregate patients who show progressive increases in limb volume, some of those increases will be based on adipose tissue and potentially would not respond to the proposed intervention. The investigators are not limiting potential participants to those experiencing a 10 percent incremental limb volume; that is the

lower limit of what is acceptable for entry into this trial. If a patient presents with 30 percent or 40 percent incremental volume, she would still be considered.

With regard to the percent of lymphedema indicating the seriousness of the disease, Dr. Rockson said that he has not seen any grade of lymphedema that is modest enough to not cause serious effects in patients. He described patients who have the milder forms as being so fearful of causing exacerbations by their activities that they become hermits; they are afraid to use the limb and they leave it hanging for fear that it will become larger than it is already. Because of the implication of progression, the impact of mild disease becomes as severe as the impact for those individuals with advanced disease.

Dr. Boyd explained that the investigators will look at participants' immune status and will assess their antibody status beforehand and measure the antibody response afterward. They will not be entering participants based on their preexisting adenovirus immunity because this trial is targeted at a general population and it is inappropriate to exclude patients with preexisting immunity.

Regarding long-term followup, Dr. Pegram opined that, in this Phase I trial, there is not much utility in long-term followup because of the small sample size. The investigators will exclude the high-stage, high-risk patients. It is unlikely that a nonrelapse patient would be converted into a relapse patient with this vector; if a relapse occurs, then, by definition, micrometastatic disease was present that ultimately would have relapsed anyway. A survivor would not be converted into a nonsurvivor based solely on VEGF-C exogenous administration.

In response to general concerns expressed by RAC members about including research participants who were experiencing "only" a 110 percent increase in limb volume, Dr. Rockson proposed to remove the bioimpedance criterion and increase the minimum limb volume ratio to 120 percent. He cautioned about going higher than 120 percent because of the scarcity of patients. He also suggested specifying that, after therapeutic intervention, the patient should have no change in limb behavior with overnight recumbency. He noted that this altered characterization of potential research participants represents an advanced stage of lymphedema.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Preclinical Issues

- If future protocols will enroll research participants who received direct radiation to the axilla during breast cancer treatment, additional animal studies should be performed that involve radiation to the axilla to demonstrate efficacy after radiation and to evaluate the potential for postsurgical complications (e.g., motor nerve injury, wound breakdown, cellulitis, increased lymphedema) in an axilla scarred by direct radiation.

Clinical and Trial Design Issues

- Given the possibility of an adverse outcome (for example, increased lymphedema or even that VEGF-C could promote the growth of cancer cells) and the fact that Phase I studies often lead to no direct benefit to research participants, the risk/benefit assessment favors enrollment of the more severely affected research participants. The inclusion criteria of the protocol should be modified to limit enrollment to those research participants with at least Stage II or III lymphedema, with a limb size of at least 120 percent of the volume of the contralateral arm regardless of the bioimpedance data, and who experience no overnight recovery in edema after therapeutic intervention.

- Given the potential for VEGF to promote tumor growth, research participants enrolled in this trial should be followed long term to obtain information on reoccurrence of their cancer.
- Because of disease-specific exclusion criteria, the research participant population to be enrolled is unlikely to have received radiation directly to their axilla. However, there should also be a specific criterion to exclude such patients, as direct radiation to the axilla could increase the possibility of scarring from the procedure. Furthermore, the animal data supporting this approach did not include an irradiated axilla model.
- The surgical procedure for the transplant of the abdominal pad will involve removal of all scar tissue, including around the nerves. It can be difficult to distinguish scar tissue from nerve tissue during surgery, and removing such scar tissue might increase the risk of neuronal injury. The protocol should be amended so that perineural scar tissue is not resected unless there is also a need to resect the nerve for a therapeutic reason (i.e., the lymphedema is associated with pain). Surgical complications should be included in reports of SAEs to the OBA.

Ethical, Legal, Social Issues

- For this Phase I study, the final version of the informed consent document should incorporate the comments raised in the written reviews by the RAC members, including the proposed modifications to the statements regarding potential for benefit and more explicit description of the risks of the procedure. In particular, potential research participants need to understand that the risk of the gene transfer is unknown and may include such serious complications as the VEGF-C promoting tissue growth, including any residual cancer cells in the axilla. This is particularly important given that lymphedema, while certainly a debilitating condition, is usually not a life-threatening one.
- One of the difficulties in developing this therapeutic approach is the lack of an animal model that accurately reflects the physiology of human lymphedema. The consent should explicitly explain the limitations of the available animal models.
- The informed consent document should state explicitly that the investigators will receive financial payments related to their recruitment of research participants into this study, and the statement that there is no direct benefit to the investigators should be removed.
- In the informed consent document statement regarding withdrawal from the study, it is important to clarify that, although a research participant may withdraw from further testing, the surgical flap and the effects of the adenoviral vector expressing VEGF-C are likely to persist.

G. Committee Motion 6

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made but not seconded to approve these recommendations, and the RAC approved these summarized recommendations by a vote of 14 in favor, 0 opposed, 2 abstentions (Drs. Fost and Zoloth), and 0 recusals.

XII. Review and Discussion of Human Gene Transfer Protocol #1209-1182: Autologous Activated T Cells Transduced with a Third-Generation GD-2 Chimeric Antigen Receptor and iCaspase9 Safety Switch Administered to Patients with Relapsed or Refractory Neuroblastoma (GRAIN)

Principal Investigators: Chrystal Louis, M.D., M.P.H., Texas Children's Cancer Center, Baylor College of Medicine, and Malcolm Brenner, M.B., Ph.D., Center for Cell and Gene Therapy, Baylor College of Medicine

Additional Presenters: Gianpietro Dotti, M.D., Baylor College of Medicine

RAC Reviewers: Drs. Badley, Fong, and Fost

A. Protocol Summary

Neuroblastoma (NB) is one of the most common malignant tumors of childhood. Because children with high-risk disease continue to have poor outcomes despite intensive therapy, new treatment strategies are required. Researchers have shown that some patients with NB can benefit from immunotherapy as the disease appears susceptible to monoclonal antibodies targeting a structure commonly expressed by the tumor called GD2 antigen and to T-cell immune responses elicited by tumor vaccines. Therefore, the investigators have constructed an artificial receptor, called a chimeric antigen receptor (CAR), that can recognize the GD2 antigen on tumor cells. It combines the ability to recognize the tumor with the capacity to signal T cells to kill the tumor cells. Placing these CARs into T cells allows them to target and kill GD2+ tumors like NB.

The investigators have modified CARs so they can make T cells more efficient and can last longer in the body. This was accomplished by adding to the ζ -chain endodomain two additional costimulatory components called CD28 and OX40. To make sure these enhanced cells are safe, the investigators also added a safety switch that can be triggered by an injection of a drug that is harmless to normal cells but kills, in a few hours, more than 90 percent of cells with the enhanced safety features. This safety switch has been successfully tested in children treated with another type of T-cell therapy and should allow the investigators to kill the T cells quickly if problems develop.

Research participants with relapsed/refractory NB who enroll in this trial will receive an infusion of autologous T cells expressing the tumor targeting receptor (GD2-CAR.CD28/OX40 ζ) with the safety switch (inducible caspase9 [iC9]) gene. Research participants who have no evidence of toxicity and who have stable disease or better at the time of disease evaluation will be eligible to receive an additional dose of T cells. Research participants who experience toxicity thought to be caused by the T cells will be given a dose of medication that activates the safety switch and kills the modified T cells that were infused.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of this protocol. Key issues included a novel co-stimulating domain in a pediatric population. While many protocols employ second-generation CARs, using either CD28 or 4-1BB in combination with CD3 ζ , only six of the 64 CAR protocols registered with OBA contain three co-signaling domains and these have all used CD28 in combination with 4-1BB. Although OX40 and 4-1BB are in the same family of tumor necrosis factor receptors, to date OX40 has not been used in a clinical trial. Children with relapsed or refractory NB have a very poor prognosis, but the risks and benefits of proceeding in a Phase I trial with a novel CAR deserve further discussion.

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

Dr. Badley requested that the investigators present and discuss preliminary data in a xenogeneic severe combined immunodeficiency disease (SCID) model of this novel construct, specifically data regarding the effect of the transduced T cells on tumor size and behavior, cytokine profiles, off-target effects including off-target effect on neurons that express GD2, and transduced T-cell persistence. Noting that a major safety consideration of this construct is the presence of the iC9 suicide gene, he asked the investigators to provide *in vitro* and *in vivo* data in the xenogeneic SCID model demonstrating that this gene is functional and induces T-cell death following homodimerization with AP1903. Dr. Badley also requested to review data on the CD28/OX40 combination being used in third-generation CARs in human studies. He asked for an explanation of the rationale for using the proposed dose escalation of 1×10^7 , 1×10^8 , and 2×10^7 cells per kg as well as clarification of the planned duration of followup and the plans for monitoring for insertional mutagenesis of the retroviral vector in the T cells. Because a principal endpoint of this study includes safety as well as cytokine and chemokine levels in patients who receive the CAR T cells, Dr. Badley suggested excluding patients who have ongoing systemic infections of sufficient severity that systemic anti-microbial therapy is required.

Dr. Fong expressed concern about a major issue in the study design—when AP1903 should be given. The investigators propose that AP1903 be given when a Grade 3 or greater toxicity attributed to the iC9-GD2 T-cell infusion occurs, which he believed is problematic from two standpoints: (1) For some Grade 3 toxicities it may be too early to give AP1903 and could decrease the chance of seeing tumor response, and (2) If a research participant receives AP1903 for a Grade 3 toxicity, it is unclear how this person would be considered for MTD analysis. As such, Dr. Fong suggested using the criteria for MTD as criteria for AP1903 administration and inserting an allowance for clinical judgment of the investigators to administer AP1903 sooner. Dr. Fong noted that the investigators believe that AP1903 will eliminate “more than 90 percent of the injected T cells within 30 minutes of drug administration”; however, if significant toxicity occurs, many activated T cells could remain. Given that scenario, he asked the investigators to explain why additional doses of AP1903, if needed, would be administered at 48-hour intervals, given that the serum half-life of AP1903 is less than 30 minutes. Dr. Fong asked for clarification about the schedule for additional dosing for participants who demonstrate neither dose-limiting toxicity nor progressive disease at the end of the 6-week evaluation period. He also requested discussion about what would happen if transduced T-cell production fails, whether extra cells after the initial three doses would be stored long term for possible future reinfusion, why the dose escalation is a log-level escalation from 1×10^7 to 1×10^8 , and why each cohort consists of only two research participants at each dose level rather than the traditional three.

Dr. Fost noted that this application is well written and includes a lucid and candid informed consent document. He asked whether children are being recruited because of the difficulty of recruiting a sufficient number of adults or for some other reason. He requested further information about the three participants in the prior anti-GD2 study who “had their disease go away” and about the 14 (out of 28) research participants who will not be dosed in this protocol according to the informed consent document. Dr. Fost suggested that the informed consent document include additional guidance about how the antibody “may react with brain cells.” In the section on potential benefits, the first sentence appears to read more optimistically than is warranted for Phase I studies; therefore, it should be reworded using a statement to the effect that clinical benefit is not the purpose of the study and is not likely. He noted that no assent form was provided for the children, and that reference was made to a Baylor Data Monitoring Plan without further information regarding the nature of that plan.

C. RAC Discussion

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

- Dr. Wooley asked the investigators to clarify information in the protocol about the retrovirus production and about the master cell bank.
- Dr. Fost asked whether Baylor has a standardized assent process. He noted that an assent line appears in the informed consent document but suggested that a more robust policy should be in place that requires talking to the child in child-appropriate language about what is going on and asking reasonable questions about whether he or she wants to go forward, not relying on the child to ask questions and raise concerns.

D. Investigator Response

1. Written Responses to RAC Reviews

Available xenograft models for NB poorly reflect the human disease for purposes of this proposed therapy. The tumor lines that will consistently grow in mice are long-term tissue culture adapted lines, have different distribution, and do not induce T-cell trafficking to targeted cells; hence there is no persistence, proliferation, or activation of the CAR T cells. The human CAR-GD2 T cells do not react with mouse neurological tissue.

Data demonstrating that the iC9 gene is functional and induces T-cell death following homodimerization with AP1903 are provided in publications and have been established in humans, and were summarized in

a presentation at the March 2012 RAC meeting. Prior to administration of the human final product in this proposed trial, the activity of the iC9 gene will be revalidated in cells transduced with the final clinical-grade construct.

The proposed dose escalation scale should be stated as “per m²” and not “per kg.” The investigators based this dose escalation on their prior studies of adoptive T-cell therapy for viral disease and for malignancy and on their own and other groups’ studies of CAR T cells. They observed no significant toxicities attributable to infusions of T cells at these doses but have obtained significant disease responses, including complete and sustained remission. The investigators use a modified continual reassessment method for T-cell studies because the dose response/toxicity curves are shallow.

The investigators confirmed that patients with significant intercurrent infections would be excluded from participation in this study.

The words “toxicity attributed to the iC9-GD2 T-cell infusion” are intended to allow appropriate clinical judgment that will be essential to avoid unnecessary treatment with the dimerizing drug. The investigators will discuss with the FDA about using the criteria for MTD as criteria for AP1903 administration and including allowance for clinical judgement of the investigators to administer AP1903 sooner.

The investigators explained that they have chosen the regimen for rescue from toxicity based on three considerations:

- There are no Phase I data for multiple administrations of dimerizing drug. As a compromise, the FDA agreed to a 48-hour interval to allow clearance of the initial dose.
- This proposal was agreeable because the critical issue is not the half-life of the dimerizing drug but the half-life of the processes that are set in motion by caspase 9 dimerization. Ninety percent clearance of iC9-expressing cells occurs within 30 minutes, but a further half log to one log clearance occurs during the ensuing 24 hours. The investigators prefer to retain the residual 1 percent of cells because those cells may be able to re-expand and repopulate as anti-tumor effector cells, potentially without further toxicity if tumor burden is reduced.
- There exists no evidence that immediate retreatment with dimerizing drug will remove additional cells because the populations that are spared are those with the lowest iC9 expression. Because transgene expression at least partially correlates with the degree of T-cell activation, the investigators believe they may need to wait 48 hours or more for the residual T cells to change their activation status. In the event there is significant toxicity and unexpectedly limited removal of T cells by a single dose of dimerizing drug, the investigators will discuss with the FDA whether an earlier second dose can be given in addition to following the protocol guidelines for steroid administration.

Initially, the interval between experimental doses will be at least six weeks to allow for assessment of toxicity and disease response. Repeat dosing is reserved for participants with stable disease or clinical response and who have shown no added toxicity after adoptive transfer. Repeat dosing for any participants who have the combination of high CAR T-cell levels and a major tumor response would be decided using the clinical judgment of the study investigators in conjunction with the research participant and parent(s).

If release criteria for the transduced T cells are not met, the investigators will attempt to remake the cells if the research participant’s condition permits. If the yield of cells is less than the intended dose level and because the dose-response curve in most T-cell therapies is relatively flat, the investigators intend to notify the FDA and dose the research participant at the level for which sufficient cells are available. If that level is below the lowest dose level, that participant will be considered a protocol exception and he/she would not be counted toward dose escalation.

The investigators intend to store all cells made until they are administered or the research participant dies.

The median age of diagnosis for high-risk NB is 23 months; therefore, almost all patients are children or adolescents. Only a small number of adults are diagnosed with the disease each year. The investigators explained that they do not wish to exclude adults from participating in this protocol but noted that NB is designated as a pediatric cancer.

In the prior anti-GD2 study in which three research participants exhibited response, one participant remains disease free at greater than five years and another at greater than three years post infusion. The third participant relapsed after three months.

The informed consent document states that “about 28 people will take part in the study; about 14 will be treated.” The investigators explained that the 14 untreated people will be research participants whose consent will be obtained for blood draw to make the cells but in whom a suitable product will not result or who will become ineligible for, or decline, the experimental treatment. These numbers represent the investigators’ worst-case scenario, and they expect the ratio will be closer to 18:10.

Although the GD2 antigen is present at low levels on some neural tissues, the investigators encountered no central nervous system or other neurological symptoms in their previous human studies of GD2-CAR T cells (other than pain at the site of tumor necrosis). No mouse models are available to address potential (though as yet unseen) neurotoxicity with GD2 CAR. The investigators explained that any guidance about the risk that the antibody could react with brain cells would therefore be speculative and potentially misleading.

The investigators stated their belief that the informed consent document as written reflects the outcome in prior studies of GD2 antibody and GD2 CAR T cells, even though the latter were used in a Phase I study. Of 11 research participants with measurable disease, five had tumor necrosis or complete tumor regression, which was sustained in two participants. Therefore, the informed consent document realistically reflects the potential benefits or lack thereof.

Regarding an assent form, the investigators explained that the policy of the Baylor College of Medicine IRB is that assent is obtained for each child who is capable of providing assent based on age, maturity, and psychological state. Generally, they recommend that patients older than six years and younger than 18 years provide assent to participate in a research study. Two mechanisms are suggested for documenting assent: (1) use of an age-appropriate assent form or (2) inclusion of the following statement in the informed consent document: “When you sign this, you also note that your child understands and agrees to take part in this study according to his or her understanding.” Using either mechanism, signature of the legally authorized representative is also required. The investigators chose to utilize the second mechanism for documenting assent in this study.

2. Responses to RAC Discussion Questions

With regard to the preclinical data for the GD-2 derived chimeric antigen receptor, Dr. Dotti explained that the investigators found *in vitro* that the CD28 costimulation from this chimeric antigen receptor is not sufficient to provide IL-2 production by the T cells upon the engagement with the antigen. OX40 by itself also is not sufficient. Only the combination CD28-OX40 allows the T cells to proliferate in response to the antigen. Preclinical experiments in a SCID mouse model in melanoma, in which the investigators infused T cells with this chimeric antigen receptor, resulted in tumor regression and no toxicity in the mice.

Regarding the PG13 producer cell line, Dr. Dotti explained that PG13 stably expresses gag, pol, and the Gibbon Ape Leukemia virus (GALV) envelope, and the vector is stably transfected.

Dr. Louis clarified that the assent line included in the informed consent document is what the Baylor IRB has deemed minimally necessary. There is no standard process for how an assent is conducted.

E. Public Comment

Dr. Smith clarified that, by regulatory requirement, an institution's IRB must look at the proposal for and documentation of assent. Therefore, with regard to RAC concerns about the assent process, she explained that presumably the IRB will review the assent process for this protocol. In addition, Dr. Smith noted that the informed consent document is long, is highly technical, and uses a high level of English; she was concerned that children—and possibly even parents—would not be able to understand it.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

Clinical and Trial Design Issues

- Currently the investigators propose that AP1903 will be given when there is a Grade 3 or greater toxicity (as measured by the CTCAE) attributed to the GD2 CAR T-cell infusion. For some Grade 3 toxicities, it is probably too early to give AP1903 and premature administration may decrease the chance of seeing tumor response; for example, a patient with known thrombocytopenia whose platelets drop below 50,000/ μ L. Although the goal is to allow for investigator discretion, the investigators should consider outlining more specific parameters for administration of AP1903 so as to avoid premature administration of the drug.
- The current protocol states that the second dose of AP1903 will be given after 48 hours. This is based on half-life of the drug. The investigators should reconsider whether there should be an absolute limitation on redosing earlier in the case of a severe reaction to the cells that does not resolve after a single dose and life-threatening toxicities continue that are not responsive to steroids or other interactions. It may be helpful to model more frequent dosing in an animal model to understand the risks and benefits of earlier redosing.
- The data monitoring committee (DMC), which will oversee this study, is chaired by the Director of Research at Baylor University. As the Chair of a committee often has considerable influence, this could compromise the independence of the DMC, or at least create the appearance that it is not a truly independent DMC. Consideration should be given to appointing an independent chair who does not have conflicting allegiances.

Ethical, Legal, Social Issues

- The informed consent document references a previous study using a GD2 CAR that was slightly different than the one to be tested in this study, and includes information on the clinical response in three patients, stating, “three patients had their disease go away.” To be more informative, the consent document should provide additional details on how long those patients have been in remission and state, in particular, that one of those patients relapsed after 3 months.
- Although many patients will be too young to provide assent, for those who can assent a specific assent document and process should be developed.
- This product is very complex, and while it is obvious that much thought has already gone into developing a readable consent form, there is still room for improvement by simplifying some of the technical language.

G. Committee Motion 7

Dr. Fong summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. A motion was made but not seconded to approve these recommendations, and the RAC approved these summarized recommendations by a vote of 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XIII. Closing Remarks and Adjournment

Dr. Fong thanked the RAC members and the OBA staff and adjourned the December 2012 RAC meeting at 2:35 p.m. on December 5, 2012.

(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.)

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

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

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

Date: _____

Yuman Fong, M.D.
Chair
Recombinant DNA Advisory Committee

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

Chair

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LIAISON REPRESENTATIVE

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**Attachment II:
Public Attendees**

Kristin Bausch, parent
Deborah Chambers, parent
Matthew Chambers, parent
Shirley Clift, Sangamo BioSciences
Catherine Gaynor, parent
Vincent Gaynor, parent
Cameron Gephardt, father
Matt Hiley, parent
Rosemary Hilson, grandparent
Bipalendu Jena, MDACC
Stephen Kaler, NIH
Xiaubin Lu, FDA
Andrew Marsh, ZIOPHARM Oncology, Inc.
Jennifer Sloan, parent
Jennifer Swann, parent
Sarah Turnbull, parent
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Attachment III: Abbreviations and Acronyms

AAV	adeno-associated virus
BSL-1	biosafety level 1
CAR	chimeric antigen receptor
CIT	chemoimmunotherapy
CLL	chronic lymphocytic leukemia
CTCAE	Common Terminology Criteria for Adverse Events
CTLs	cytotoxic T lymphocytes
DCs	dendritic cells
DMC	data monitoring committee
ECG	electrocardiogram
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
GTSAB	Gene Transfer Safety Assessment Board
HSV	herpes simplex virus
IHC	immunohistochemistry
IND	investigational new drug
IRB	institutional review board
irRC	immune-related Response Criteria
MDACC	The University of Texas MD Anderson Cancer Center
MTD	maximum tolerated dose
N0	no known lymph node involvement
N1	T1 tumors with nodal involvement
NB	neuroblastoma
NIH	National Institutes of Health
<i>NIH Guidelines</i>	<i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
OHRP	Office of Human Research Protection, NIH
PFS	progression-free survival
qPCR	quantitative polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
SCID	severe combined immunodeficiency disease
SMA	spinal muscular atrophy
SMN	survival motor neuron
SRC	Safety Review Committee
TAA	tumor-associated antigen
TNBC	triple-negative breast cancer
Tregs	regulatory T cells
VEGFs	vascular endothelial growth factors
vp	virus particles