RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 17-18, 2008

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

Contents

[Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at www4.od.nih.gov/oba/rac/protocol.pdf.]

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NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

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The Recombinant DNA Advisory Committee (RAC) was convened for its 113th meeting at 8:15 a.m. on June 17, 2008, at the National Institutes of Health (NIH), Building 31-C, Conference Room 6, Bethesda, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:15 a.m. until 4:15 p.m. on June 17 and from 8:10 a.m. until 9:45 a.m. on June 18. The following individuals were present for all or part of the June 2008 RAC meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania
Jeffrey S. Bartlett, Columbus Children's Hospital
Stephen Dewhurst, University of Rochester Medical Center
Hildegund C.J. Ertl, The Wistar Institute *(present on Day 1; via teleconference on Day 2)*
Hung Y. Fan, University of California, Irvine
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University *(present on Day 1; via teleconference on Day 2)*
Ellen E. Grant, HealthNow New York Inc.
Jeffrey P. Kahn, University of Minnesota *(present on Day 1 only)*
Joseph A. Kanabrocki, The University of Chicago
Prediman K. Shah, Cedars-Sinai Medical Center *(via teleconference)*
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities *(present on Day 1 only)*
Scott E. Strome, University of Maryland *(present on Day 1; via teleconference on Day 2)*
Richard G. Vile, Mayo Clinic
David J. Weber, The University of North Carolina at Chapel Hill *(via teleconference on Day 1; present on Day 2)*
Lee-Jen Wei, Harvard University *(via teleconference)*
David A. Williams, Children's Hospital Boston/Harvard Medical School *(present on Day 2 only)*
John A. Zaia, City of Hope

Office of Biotechnology Activities (OBA)

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

***Ad Hoc* Reviewers and Speakers**

Carmelo Cuffari, Johns Hopkins School of Medicine *(via teleconference)*
Raynard S. Kington, OD, NIH
Crystal L. Mackall, National Cancer Institute (NCI), NIH

Nonvoting Agency Representatives

Kristina C. Borror, U.S. Department of Health and Human Services (DHHS)
Daniel M. Takefman, U.S. Food and Drug Administration (FDA), DHHS

[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 Staff Members

Rose Aurigemma, NCI
Chi-Ping Day, NCI
Linda Gargiulo, OD
Laurie Lewallen, OD
Maureen Montgomery, OD
Marina O'Reilly, OD
Gene Rosenthal, OD
Karen Schweikart, NCI
Tom Shih, OD

Others

There were 66 attendees at this 2-day RAC meeting.

Attachments

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

I. Day 1 Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff, RAC Chair, called the meeting to order at 8:15 a.m. on June 17, 2008. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on May 23, 2008 (73 FR 30133). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (a subcommittee of the RAC), public review and discussion of five protocols, and an update of the RAC's Biosafety Working Group's consideration of changes to the *NIH Guidelines* regarding noncontemporary human influenza and highly pathogenic avian influenza viruses.

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as special Federal Government employees.

II. Minutes of the March 11-12, 2008, RAC Meeting/Drs. Bartlett and Kanabrocki

Drs. Bartlett and Kanabrocki noted that some minor edits were needed but requested RAC acceptance of the March 11-12, 2008, RAC meeting minutes.

A. Committee Motion 1

Dr. Albelda moved and Dr. Kanabrocki seconded that the RAC approve the March 11-12, 2008, RAC meeting minutes. The vote was 14 in favor, 0 opposed.

III. Gene Transfer Safety Assessment Board (GTSAB)

RAC Reviewers: Drs. Albelda, Federoff, and Strome

Dr. Albelda reported that of the 23 protocol submissions received by the OBA in the past 3 months, 18 were not selected for public review at this RAC meeting. Of the 18 protocols not selected, 15 are for cancer, 2 are for infectious diseases, and 1 is for monogenic disease.

A total of 169 amendments were reported during this 3-month period, including 62 principal investigator (PI) or site changes, 55 annual reports, 13 *Appendix M-I-C-1* responses, and 39 others (changes in status and protocol design modifications).

Three *Appendix M-I-C-1* reviews were discussed briefly, as was one amendment:

- Protocol #0401-624, A Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD) for Recurrent Malignant Gliomas, was reviewed by the RAC at its March 2004 meeting. Due to the expanded cellular tropism of the vector conferred by the addition of an RGD motif, the RAC requested biodistribution and preclinical toxicology studies as well as studies assessing the contribution of the vector to antitumor immune response; the investigator submitted a detailed report addressing these issues.

- Protocol #0610-810, A Phase I, Open-Label, Rising-Dose Study of the Safety and Tolerability of Single Doses of NUC B1000, an RNAi-Based Therapy for Chronic Hepatitis B, was reviewed by the RAC at its December 2006 meeting. At the time, a concern centered on the appropriate animal model, and the investigators responded that there were no disease models of human chronic hepatitis B virus (HBV) infection. Given that this is a specific nucleic acid-based study agent and not a drug, the PI stated that only human HBV would be an appropriate target; no animal model is available for human HBV. In addition, the investigators provided reasons why transgenic models were not possible. As requested, a variety of studies were performed to examine off-target effects of the RNAi construct; details of these studies are contained in the investigators' response.

- Protocol #0704-846, A Phase I, Dose-Ranging Study to Assess Safety and Distribution of GT-111 in Patients with Advanced Metastatic Cancer, was reviewed by the RAC at its June 2007 meeting. Although the RAC had suggested that the investigators conduct nonhuman primate (NHP) studies at the highest proposed human dose, the investigators responded that NHPs are nonpermissive for the Ad5 replication. It was agreed by local institutional review boards (IRBs) and the FDA that the study would only proceed to the $1x10^{13}$ dose following analysis of the complete monitoring data, data summaries, and analysis of previous cohorts.

- Protocol #763, A Randomized Phase II Trial of Interferon and GMCSF vs. K562 GMCSF Vaccination in Chronic Myelogenous Leukemia, was amended to add two in-depth interviews that will explore participant motivation for trial enrollment and emotions regarding cessation of the gene transfer at protocol-defined endpoints. The first interview will occur prior to dosing, and the second interview will follow completion of the trial.

Dr. Albelda discussed the adverse events (AEs) that were reported to the OBA during this reporting period. A total of 152 AEs were reported from 39 trials, of which the overwhelming majority were unrelated to the gene transfer products. There were 45 initial and followup reports in which the AE was possibly related to the gene transfer products, including 23 new reports and 22 followups from 13 protocols. Overall, the GTSAB reviewed 12 initial and 16 followup AEs from 14 trials, none of which were deemed to merit discussion at this RAC meeting.

IV. Discussion of Human Gene Transfer Protocol #0802-905: Phase I Trial of Intravenous Recombinant Human 4-1BB Ligand Fusion Protein (hIg-h4-1BB-Ls) in Combination with Intratumoral Adenoviral Vector Expressing Human Interleukin-12 cDNA (Adv.hIL12) and Oral Sunitinib Malate in Patients with Metastatic Nonhematologic Neoplasms

Principal Investigators:	Max W. Sung, M.D., and Savio L.C. Woo, Ph.D., Mount Sinai School of Medicine (MSSM)
Additional Presenter:	Shu-Hsia Chen, Ph.D., MSSM
RAC Reviewers:	Dr. Albelda, Dr. Ertl, and Ms. Shapiro
Ad hoc Reviewer:	Crystal L. Mackall, M.D., NCI, NIH

Dr. Strome recused himself from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

Using animal models of colon cancer, the investigators have demonstrated improved tumor response and prolonged survival through the use of a combination of three different agents. The first involves the use of a modified adenovirus (Ad) that expressed interleukin-12 (Adv-hIL-12), a protein that improves the body's natural response against tumor cells. Mouse studies showed that intratumoral injection of an adenovirus vector expressing murine interleukin-12 induced antitumor immunity mediated by natural killer (NK) cells leading to partial tumor regression and survival prolongation. The efficacy and safety of the agent are currently being tested in research participants with colon cancer that has spread to the liver. The second involves intravenous (IV) administration of hIg-h4-1BB-Ls, a laboratory-designed protein that combines two naturally occurring proteins to give rise to an enhanced antitumor response when used with the modified Ad. This protein has not been used previously in humans, and its safety will be tested in monkeys before being used in research participants.

This clinical trial will use a fixed dose of the modified Ad that has been shown to be safe in humans in combination with increasing doses of hIg-h4-1BB-Ls. Once it has been demonstrated that this combination is safe in humans, the investigators will include sunitinib malate (Sutent®), an FDA-approved drug, in the treatment scheme at both half the clinical dose and the full clinical dose. This combination of treatments has been shown to be effective and safe in mouse models of disease.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the protocol's status as the first human trial of the hIg-h4-1BB-Ls agonist, the known severe toxicities seen in a 2006 trial in London that involved agonists of T-cell costimulatory molecules, the failure of the preclinical research in that London trial to predict a safe starting dose, and the safety in humans of the combination of three immunostimulatory molecules.

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

Dr. Albelda noted that this proposed trial is being submitted earlier than most and that much of the key toxicity data have not yet been performed, thus limiting the ability of the RAC to assess potential toxicity in the proposed trial. He asked why the three agents are needed, and about the data to show that the hIg-h4-1BB-Ls cross-reacts equivalently in rhesus macaque cells and in human cells Questions and issues related to clinical concerns included: 1)a request for updates on clinical trials that have used adenoviral vector expressing human interleukin-12 (Adv-hIL-12), 2) discussion of why and how hIg-h4-1BB-Ls stimulation might be similar to or different from the approach used in the TGN1412 trial in which non-human primate studies failed to predict a severe adverse reaction that led to cytokine storm in the first six subjects that received the experimental medication, 3) a summary of toxicities and results of studies using hIg-h4-1BB-Ls agents, 4) justification for the use of a heterogeneous mix of tumors located at various anatomic sites, 5)justification for starting with a combination approach rather than starting by finding the maximal tolerable dose (MTD) of the hIg-h4-1BB-Ls alone, 6)the rationale for the 2-week interval between cohorts, and 7)the rational for not excluding individuals with brain metastases. Dr. Albelda recommended that the investigators add to the informed consent document a discussion of the potential risks of uncontrolled immune activation (a "cytokine storm"), which is what occurred in the TGN1412 trial.

Dr. Ertl asked the investigators to provide data from the Phase I trial of the Adv-hIL-12 vector. She noted that the recombinant protein has not been tested in humans and that it is conceptually similar to the construct used in the TGN1412 trial that caused a severe and unanticipated cytokine storm in all research participants. As such, Dr. Ertl suggested that the extensive preclinical toxicity testing might not predict toxicity in humans and that it would be important for the investigators to conduct a preclinical toxicity study for each of the combinations proposed. She further suggested that preclinical toxicity studies

should focus on NHPs, and that the mode of action among the three drugs should be assessed in more depth. Dr. Ertl suggested excluding all individuals with chronic infections or a history of allergic reactions or autoimmunity and monitoring, including frequent serum collection during the initial 48 hours to screen for an elevation of proinflammatory cytokines. She stated her strong belief that each of the three components in this trial should first be tested individually in humans and then tested in combinations of two before all three components are combined.

Ms. Shapiro asked the investigators to provide results of the tissue cross-reactivity experiments using relevant nonmalignant tissues from macaques and humans, because ensuring use of the most appropriate preclinical species is a critical ethical consideration in estimating the safe starting dose in humans. In addition, she offered six critiques of the informed consent document, including contradictory and vague statements about blood samples, possibly misleading statements about the dose given in the animal models, the need to clarify the risk of leakage of the viral vector from the liver into the abdomen, and the need to clarify the limits of coverage for research-related injury by defining "short-term hospitalization and professional attention, if these are required."

Although acknowledging that multimodality therapy may be needed to amplify the immune response to sufficient levels as to be clinically meaningful, Dr. Mackall noted that this trial uses two agents that have had only limited studies (Adv-hIL-12) or no studies (hIg-h4-1BB-Ls) as single agents in humans, making it impossible to predict the therapeutic index. She stated that single-agent data using hIg-h4-1BB-Ls in humans would be essential to interpret the risk for substantial toxicity when combined with systemic levels of IL-12, especially given that the NHP is not a perfect preclinical model. Dr. Mackall explained the importance of careful assessment of the data from the NHPs with regard to the effect of hIg-h4-1BB-Ls on Ad immune responses when Adv-hIL-12 is added. Given sunitinib's challenging side-effect profile when administered as a single agent, she suggested that this complex regimen be investigated in NHPs and/or that the clinical trial begin with a more conservative dose escalation. In addition, Dr. Mackall suggested that the informed consent document clarify that, although sunitinib is an FDA-approved agent with a known toxicity profile, the toxicities may be new or enhanced by the combination immune-based therapy proposed for this trial.

C. RAC Discussion

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

- Dr. Ertl requested that the investigators begin first by establishing the MTD hIg-h4-1BB-L alone as is being done for the advenoviral vector in a Phase I trial. When the two agents are then combined the initial dose used for the combination should be one log below the MTD for each agent when used alone. A similar dose reduction should be made in the initial cohort that receives all three drugs together, the hIg-h4-1BB-L, adenoviral vector containing IL-12 and sunitinib. The investigators agreed to do so.

- Dr. Ertl asked the investigators for additional specific information about how they have defined the MTD. She noted that the MTD in one participant could be a fatal dose in the next participant, since individuals react unevenly because their immune systems are extremely heterogeneous depending on genetics.

- Dr. Mackall cautioned that perhaps not all grade 3 toxicities should be considered dose-limiting toxicities (DLTs) in this trial. For example, a grade 3 fever probably should not be a DLT on this study because one of the goals of this study is to induce immune activation. The investigators should consider which grade 3 toxicities would be DLTs and whether other Grade 3 toxicities would only be considered DLTs if they persisted for a predetermined time period.

- Dr. Mackall reiterated the concerns expressed in her written review about the use of sunitinib. Sunitinib inhibits many kinases, not merely the c-kit kinase that is the target of this study. She asked whether the investigators had considered more c-kit-specific kinase inhibitors, for example, Gleevec. She noted that sunitinih has a number of side effects of sunitinib include rashes on

hands and feet with blisters and substantial gastrointestinal (GI) toxicity, and few individuals can tolerate 28 days of continual dosing.

- Dr. Ertl suggested that the investigators discuss with the FDA the possibility of adding to the exclusion criteria list individuals with neutralizing antibodies to the Ad at titers higher than 100.

- Dr. Takefman clarified that the FDA does not require lifelong followup for any product. Although there exists long-term followup guidance that outlines a 15-year followup procedure, this protocol might not be required to carry out 15 year follow-up under that guidance.

- Dr. Federoff summarized several RAC members' concerns related to the complex immunopolypharmacy of this three-agent protocol—how to incrementally add another agent and the criteria for evaluating the toxicity of that added agent. He reiterated that the investigators should construct the safest approach that will likely be successful as additional agents are added to the clinical study.

D. Investigator Response

1. Written Responses to RAC Reviews

Explaining the need for three reagents, the investigators stated that the three reagents serve different purposes in activating and improving the tumor-bearing host's immune response regarding innate immunity, adoptive immunity, and reverse immune tolerance through the action of sunitinib.

Although using a heterogeneous mixture of tumors might make interpretation of data difficult, the investigators explained that preclinical studies have shown that the toxicity and efficacy are quite similar in different tumor models such as colon and breast cancer and melanoma.

Regarding the NHP studies, the human 4-1BB ligand, when used at a dose of 10ug/ml *in vitro* gives rise to similar levels of proliferation using PBMCs isolated from human and Rhesus monkey blood. The reported human and monkey proliferation experiments were done individually. However, the investigators plan to perform dose response curves of hIg-h4-1BBLs in the T cell proliferation assays using human and rhesus monkey PBMCs in a side-by-side experiment.

As a result of several RAC reviewers' concerns about starting this trial with a combination approach, the investigators agreed to add a separate Phase I clinical trial with dose escalation of the hIG-h4-1BB-Ls alone. The entry dose will depend on the results observed in the preclinical pharm/tox studies in tumor-bearing mice and normal rhesus monkeys, which will be set at 0.01 times the MTD, with escalation doses at half-log increments.

The investigators agreed to exclude potential participants who have brain metastases.

Regarding the addition of sunitinib to the combination experimental regimen, the investigators provided data showing that (1) pharmacological disruption of c-kit receptor signaling through the use of sunitinib can prevent the accumulation of myeloid-derived suppressor cells in the spleen, bone marrow, and tumors isolated from tumor-bearing mice; (2) treatment with sunitinib prevents T-cell anergy in tumor-bearing mice; and (3) treatment with sunitinib significantly improves long-term survival in mice treated with Adv.mIL-12 + 4-1BB immune activation therapy.

Although the recombinant protein proposed for this trial has not been tested in humans and is conceptually similar to the anti-CD28 construct used in the TGN1412 clinical trial in the United Kingdom (U.K.) that caused a severe cytokine storm, the investigators stated that they do not expect the same adverse effects as were observed in that trial. They vowed to take extensive precautions to maximize participant safety, including treating participants at each dose cohort sequentially and not concurrently and administering to participants a small bolus of the fusion protein prior to continuing with the full dose. In addition, the 4-1BB ligand proposed by the investigators and the ligand used in the U.K. trial are

structurally and functionally different. TGN1412 is a "super" co-stimulatory molecule; it does not require CD3 costimulation and can induce T cell proliferation on its own. The proposed 4-1BB ligand fusion protein requires co-stimulation with CD3 on T cells to have proliferative effect, and is a natural ligand. The molecular structure and Fc-Ig structure are also different from that contained in TGN1412. The proposed 4-1BB ligand does not have the Fc receptor binding site, which can nonspecifically bind to macrophages or dendritic cells and induce an inflammatory cytokine storm.

The investigators agreed to include several early timepoint toxicity studies in the NHPs to ensure that cytokine levels stay within a safe range. Volume permitting, the investigators also agreed to use a maximal dose in the NHPs that is at least five times the maximal anticipated human dose.

The investigators agreed to exclude potential participants with autoimmune diseases, because this protocol could activate the immune system.

The investigators agreed to monitor participants' serum proinflammatory cytokine levels at frequent and early timepoints during the initial 48 hours of this trial.

The investigators agreed to revise the protocol to change references to "anaphylactic reactions" (that would eliminate participants from continuing their participation in the trial) to read "hypersensitivity reactions." Participants who developed grade 3 or 4 hypersensitivity reactions would be eliminated from additional participation.

Since IL-12 will activate natural killer cells to kill the tumor cells and release tumor antigens and viral antigens, the investigators expect that the experimental treatment will induce both antiviral and antitumor immune responses. Preclinical efficacy studies indicated that IL-12 expression persisted for 7 to 10 days.

Because of concerns about sunitinib's side-effect profile, the investigators agreed to revise the informed consent document to make clear to participants that toxicities may be new or enhanced by the combination immune-based experimental therapy. In addition, they agreed with one RAC reviewer's recommendation to implement a more conservative dose escalation scheme for sunitinib, starting with 25 mg per day and progressing to 37.5 mg per day and then 50 mg per day.

Once the tissue cross-reactivity studies using relevant nonmalignant tissues from macaques and humans have been performed, the investigators agreed to provide those results to the OBA.

To correct contradictory wording, the investigators agreed to revise the informed consent document to indicate that participants may withdraw their consent at any time, at which time all stored biological samples would be destroyed and lifelong followup would be discontinued.

The investigators agree to clarify in the clinical protocol that there will be a two-week interval between patients within a cohort and a four-week interval between cohorts, with the interval commencing the day after administration of the last trial agent.

2. Responses to RAC Discussion Questions

Dr. Sung stated that, for any serious adverse reaction (e.g., anaphylactic shock), the data and safety monitoring board (DSMB) would be notified.

Regarding research participant characteristics, Dr. Sung stated that these individuals would be on study for 57 days, with followups after that time. These individuals will have good performance status (measured by the Karnofsky scale at 70 percent and higher), will not be terminally ill, and will be anticipated to survive at least 16 weeks beyond the end of the protocol.

Although the investigators would prefer to start dosing at a biologically active dose, Dr. Woo explained that, because of the trial design and the study not being powered to examine efficacy endpoints, the only dose level it will be possible to determine from this study would be the MTD.

In response to concerns regarding the statement about financial compensation in the event or a research related injury in the informed consent document, Dr. Sung agreed to go back to the IRB and ask permission to include a more defined time period associated with the compensation from the institution. Participants should be covered by MSSM for severe injuries caused by toxicity that require prolonged hospitalization.

Dr. Sung explained how the investigators arrived at the MTD for this protocol. In their Phase I trial of IL-12 alone, the investigators have used the same MTD as in the combination trial—Grade 3 or more toxicity with the exception of the elevation of the activated partial thromboplastin time.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- The toxicology data are based on studies in a tumor-bearing mouse model. However, the interpretation of any long-term toxicity will likely be complicated by a potential tumor's effects on the animals' physiology and early deaths due to tumors. The investigators should consider toxicology studies in nontumor-bearing animals. Preclinical studies to assess the efficacy of the experimental treatment in the same mouse model would complement the toxicology data. In addition, toxicology studies should be conducted in "outbred" mice because their immune systems might provide a more relevant model of how the experimental agents will behave in humans.

- The investigators should gather preclinical data to shed light on the expected duration of the sunitinib-induced reduction in myeloid-derived suppressor cells and T-regulatory cells in the bone marrow and in peripheral blood after the final sunitinib treatment.

- Before initiating the clinical study, the investigators should conduct preclinical studies in an animal model to determine whether 4-IBB can activate memory T cells without ligation of the CD3 molecules of the T-cell receptor. If this does occur, it could have toxic effects and these data could further inform the determination of the appropriate dose to use in the clinical study.

- In the ongoing NHP studies, blood samples should be drawn from the animals prior to administration of the study agents to ensure that sufficient baseline blood samples are available for additional tests that may be necessary.

Clinical/Trial Design Issues

- Initial participants are to receive 4-IBB alone. Once the MTD of 4-IBB is determined, participants are to receive the combination of 4-IBB and Adv-hIL-12. The MTD of the Adv-hIL-12 vector is to be determined through an ongoing clinical trial in participants with metastatic colon cancer. The primate being used in the NHP studies is not able to develop a tumor that can be injected with the Adv-hIL-12; therefore, these studies will not provide data on the safety of the combination of Adv-hIL-12 and 4-IBB, particularly immune system effects. As a precautionary measure, when the combination is used, the starting dose should be at least one log below the MTD of each agent alone. This approach should be repeated once the MTD for the two agents together is established and the sunitinib is to be added; that is, the starting dose for 4-IBB and Adv-hIL-12 when used in combination with sunitinib should be one log below the MTD for both agents when

used together. This stepped approach may help minimize the risk of toxicities due to combining immunoreactive entities.

- The dose-escalation trial that will determine the MTD of the Adv-hIL-12 vector should exclude participants with high titers of preexisting neutralizing antibodies to Ad because the neutralizing antibodies will make it more difficult to detect toxicity from systemic circulation of the vector.

- The study design defines toxicities of grade 3 and above as DLTs. However, there may be some grade 3 toxicities that should not automatically be categorized as DLTs because they may be an expected physiologic response to an immune-mediated agent, for example, lymphopenia or fever. As such, it might make sense to consider some grade 3 toxicities to be DLTs only if they persist beyond a certain expected timeframe.

- Anaphylactic reactions should be analyzed by the study's DSMB on an urgent basis.

- Sunitinib was chosen due to its ability to inhibit c-kit, a tyrosine kinase receptor. Inhibition of c-kit has been shown to block expansion of myeloid-derived suppressor cells and prevent tumor-specific T-cell anergy. Although combining sunitinib with 4-IBB and Adv-hIL12 has been shown in animal models to enhance tumor regression, sunitinib is not the most specific c-kit inhibitor and can induce broad tyrosine kinase inhibition. In addition, sunitinib has considerable toxicities, including bleeding and GI and dermatological toxicities. The protocol should address other c-kit inhibitors that were considered and why sunitinib was chosen over a tyrosine kinase inhibitor with more specificity.

Ethical/Legal/Social Issues

- The statement in the informed consent document that participants with research-related injuries will receive short-term hospitalization and professional attention at no cost is ambiguous and should be clarified. For example, what are the limits of the institution's policy of addressing research-related injuries (e.g., how many hospital days would be covered)?

G. Committee Motion 2

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Ertl moved and Dr. Albelda seconded the motion that the RAC approve these summarized recommendations. The vote was 13 in favor, 0 opposed, 0 abstentions, and 1 recusal.

V. Discussion of Human Gene Transfer Protocol #0804-917: A Phase IIa Randomized, Placebo-Controlled, Double-Blind, Multicenter, Dose-Escalation Study to Evaluate the Safety, Tolerability, Pharmacodynamics, and Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis

Principal Investigator:	Kim L. Isaacs, M.D., Ph.D., The University of North Carolina at Chapel Hill
Additional Presenters:	Bernard Coulie, M.D., Ph.D., and Lothar Steidler, Ph.D., ActoGeniX NV
Sponsor:	ActoGeniX NV
RAC Reviewers:	Drs. Federoff, Kahn, and Zaia
Ad hoc Reviewer:	Carmelo Cuffari, M.D., Johns Hopkins School of Medicine *(via teleconference)*

Drs. Albelda, Strome, Vile, and Weber recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic or relapsing immune activation and inflammation within the GI tract. Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of idiopathic IBD. In healthy individuals, tight regulation of the mucosal immune system prevents excessive inflammatory responses towards normal intestinal bacteria. The crucial role of the immunomodulating cytokine Interleukin-10 (IL-10) in this process is demonstrated by the observation that IL-10 knockout mice spontaneously develop enterocolitis but not when kept under germfree conditions. The latter suggests that, in the absence of the immunomodulatory effects of IL-10, an unrestricted intestinal inflammatory response develops towards normal enteric antigens.

The rationale for the proposed Phase 2a study is based on observations, which indicate that hIL-10, delivered locally at the colonic site of inflammation using engineered L. lactis bacteria may not only be more efficacious than systemically administered IL-10 but might also ensure a more favorable safety and tolerability profile. In the proposed Phase 2a clinical trial, non-pathogenic, living L. lactis bacteria (designated L. lactis sAGX0037), engineered to synthesize and secrete hIL-10 in the GI tract, will be administered to UC patients. In L. lactis strain sAGX0037, hIL-10 expression is driven by a specific lactococcal promoter sequence, resulting in a 4-fold increased expression of hIL-10 in vitro (as determined by ELISA on bacterial supernatant) compared to the L. lactis strain used in the earlier Phase 1 trial (Thy12).

The proposed Phase IIa clinical trial will enroll 60 UC patients who are experiencing a moderately active stage of disease. AG011 will be administered both orally (capsules) and rectally (enema); participants will receive either one of the three doses of AG011 or placebo during 28 days. Vital sign measurements and blood samples will be taken at each clinical visit. At several clinical visits, either a brief or an elaborate physical examination will be conducted. Before and after the experimental treatment period, colon biopsies will be taken during endoscopic investigations, and stool samples will be collected and analyzed.

The blood samples, physical examinations, and a stool diary will be used to evaluate safety, tolerability, pharmacodynamics, and efficacy parameters. The stool samples will monitor the excretion of AG011 to validate the environmental containment measures and to study efficacy parameters. The levels of cytokines also will be studied, and the colon biopsy samples will be studied to evaluate the efficacy of AG011.

B. Written Reviews by RAC Members

Nine RAC members voted for in-depth review and public discussion of the protocol. Key issues included the use of a novel vector, the transgene, and disease indication.

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

Dr. Federoff asked the investigators to discuss why they are proposing a Phase IIa trial, given that the study agent has not been studied in a Phase I setting with UC patients using the rectal route of administration. Regarding participants being allowed to receive up to a 20-mg dose of prednisone or its equivalent, Dr. Federoff requested clarification of how this corticosteroid dose-level limit had been established, how the blunted stress response caused by the corticosteroid would be managed, and how the investigators would manage concerns about potential opportunistic infections. He asked the investigators to provide the rationale and data for selection of the twice-daily dosing, for concurrent oral and rectal administrations, and for the proposed dose levels, escalation, and duration. Noting that data from a similar trial revealed that the bacterial burden was elevated at days 4 and 6 of that 7-day trial, Dr. Federoff requested a detailed rationale for timing parameters concerning measurements of bacterial burden, endoscopic analyses, and biopsy and biomarker studies. He asked for the data from the 4-week monkey toxicology study. Dr. Federoff also asked whether systemic absorption of IL-10 is affected by the extent of colitis and, if so, whether and how the calculation of the no-observable-adverse-effect level (NOAEL) as extrapolated from normal mice would be influenced.

Since the exclusion criteria included use of certain treatments for UC, Dr. Kahn asked whether the investigator expected that potential participants may taper off their existing therapy in order to enroll in the trial. Dr. Kahn asked the investigators to clarify the participant recruitment process and to explain how the interests of participants would be protected if their physicians also serve as study investigators who have an interest in recruitment into this placebo-controlled trial. Regarding the informed consent document, he requested clarification of the phrase "your study doctor" and the mixed roles of doctors and researchers encountered throughout the document and asked that the investigators separate the role of the participant's physician from that of the study physician. Dr. Kahn asked that descriptions of the laboratory tests be included in the informed consent document, that mention of a request for autopsy be included, and that the second signature box for legal representatives be removed since it is unnecessary.

Dr. Zaia asked whether AG011 had been tested in the mouse model of UC, whether and to what extent it is possible to eradicate AG011 from lab animals after infection, and for justification of the starting and escalating doses of AG011. Regarding the design of the clinical protocol, he requested details (dose and AEs) of the Phase I study conducted in CD, asked whether the investigators plan to monitor systemic immunologic effects, and suggested storage of peripheral blood mononuclear cells (PBMCs) in case of a later need to assess the effect on immune function. Regarding the informed consent document, Dr. Zaia suggested adding an explanation that this study is the first use of this agent in humans, removing the words "these are not expected to occur" following the list of potential risks, and adding the statement that immunosuppressive effects could cause altered host resistance to viral and other infections.

Dr. Cuffari expressed concern that both UC patients and their physicians might taper off components of their existing therapy (i.e., immunosuppressants and biologics) to participate in or recruit for this clinical trial; he suggested that an objective oversight committee detached from the sponsor should be constituted to deal with this anticipated ethical issue. Noting that the sample size might be too small to allow for meaningful conclusions to be drawn from this trial, Dr. Cuffari asked whether this drug should be studied in a Phase I trial and how the treatment arms had been determined. He suggested that individuals with indeterminate colitis be excluded from this clinical trial and asked for clarification of all aspects of the physician's role within the informed consent document.

C. RAC Discussion

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

- Dr. Cuffari asked how the investigators had measured bioavailability of the IL-10 transgene and whether immunofluorescent studies were conducted to look at the tissue in either humans or animal models. Doing so may be particularly important because colonic transit is much more accelerated in patients with active UC.

- Dr. Ertl asked whether any abnormalities were observed in the results of the mucosal response testing in mice and monkeys.

- Dr. Ertl also asked the investigators whether any of the animals in the preclinical studies or the research participants in the Phase I trial had developed antibodies to the bacteria.

- Regarding measurement of the transgene, Dr. Albelda suggested that the investigators build into the protocol specific measures to indicate whether the bacteria are making IL-10, how much IL-10 they are making, and where that IL-10 is located.

- Dr. Zaia expressed additional concern about the physician's conflict of commitment between patient and protocol and suggested that the investigators be more specific about the relationship between participant and physician.

- Dr. Somia asked whether the investigators had encountered any gene transfer to other flora in the gut.

D. Investigator Response

1. Written Responses to RAC Reviews

Although AG011 has not been tested in mouse models of colitis, the active component in AG011, the hIL-10 secreting *Lactococcus lactis* (*L. lactis*) bacterium sAGX0037, efficiently reduces intestinal inflammation in mice with anti-CD40-induced colitis in multiple pharmacodynamic experiments.

To define the starting dose of this Phase IIa clinical trial, the sponsor used as a reference the dose tested in the previous Phase I study evaluating Thy12 that showed safety and tolerability. The proposed starting dose is equal to the dose tested in the Phase I study corrected for the fourfold increase in hIL-10 expression level by AG011. The dose-escalation scheme proposed in the protocol reflects a standard escalation scheme. The highest dose is approximately 85-fold lower than the NOAEL based on the mouse toxicology study and 60-fold lower than the NOAEL determined in the monkey toxicology study.

In the Phase 1 study with Thy12, only one dose, given in two oral administrations, was tested. The major AE reported was flatulence which possibly related to the inclusion of milk powder in the formulation, which will not be included in the current formulation. There were no SAEs reported. Four subjects reported exacerbation of CD after completion of use of the study agent.

Regarding the possibility of eradicating AG011 from laboratory animals after infection, the investigators explained that *L. lactis* is a noncolonizing, nonpathogenic, gram-positive bacterium that cannot invade cells or tissues and does not cause infection. Its residence time in the intestine is determined by intestinal transit as it moves along with the fecal stream. AG011 is susceptible to a number of antibiotics directed against gram-positive organisms.

Although systemic immunologic parameters will be monitored in a subset of participants at screening and after the last dose of the experimental treatment, the investigators agreed also to store PBMCs for assessment at a later time.

The investigators agreed to include in the informed consent document a sentence that specifies the theoretical possibility of altered host resistance to viral and other infections. In the unlikely event of such an occurrence, dosing with AG011 will be stopped, and the infection will be treated with the standard care for that infection.

The sponsor's primary rationale for a Phase IIa UC trial with two routes of administration is that regulatory authorities have stated that additional Phase I studies in healthy volunteers are not necessary and would unlikely provide additional data on local toxic effects in the gut in individuals with UC. Second, the sponsor believes the proposed agent will be safe and well tolerated in the context of the proposed Phase IIa clinical trial, based on data related to administration of hIL-10 alone and of hIL-10 secreted by *L. lactis*. In addition, the safety and tolerability of both administration routes have been studied in a relevant-toxicity species (monkey), and the shift of indication from Crohn's disease (Phase I study) to UC (proposed Phase IIa study) is not expected to influence the extrapolation of safety and tolerability data from one participant population to the other.

Corticosteroids are immunosuppressive and theoretically could produce additive or synergistic effects with interleukin-10. However, extensive experience exists with co-administration of these agents. Systemic interleukin-10 therapy was administered in multiple clinical trials of CD and ulcerative colitis while patients continued corticosteroid therapy. No increase in infection was observed. AG011 is designed to deliver hIL-10 locally in the gut lumen. In animal models with up to 85-fold higher doses than the highest proposed clinical dose, there was no detection of IL-10 systemically.

The investigators explained that the exclusion criterion of "active psychiatric problems" is meant to exclude potential research participants with major psychiatric disorders that would interfere with obtaining

informed consent or that would not allow participants to adhere to the research protocol. The sponsor agreed to specify the psychiatric disorders that would be associated with these problems.

No studies or data on the shedding of *L. lactis* in UC patients are available. L. lactis is a non-colonizing bacteria present in some dairy foods and travel through the GI tract of healthy individuals at the same speed of an inert marker. The investigators assume similar kinetics in UC patients, depending on the transit time of the patients.

Regarding dose duration, the investigators explained that this study is designed as a staged, sequential, dose-escalation study with an experimental treatment duration of 4 weeks for all dose groups. In the Phase I study, participants were dosed for only 7 days, and disease exacerbation occurred after withdrawal of the experimental treatment in 4 out of 10 participants; based on animal studies, a dosing duration exceeding 14 days is posited to establish a longer lasting immunomodulatory action. In addition, based on clinical study practice in UC, 4 weeks is the minimal period to evaluate healing of the colonic mucosa by endoscopy performed before and after dosing. The safety of AG011 has been evaluated in mouse and monkey toxicology studies.

Fecal samples for measurement of bacterial exposure and excretion will be taken at the start of the study before dosing, after 1 week of dosing, and 7 days after intake of the last dose (at which time no living bacteria are expected to be secreted via the feces). These timepoints allow for determination of bacteria exposure at the expected steady state (1 week timepoint). Endoscopy will be performed at the start of the study, before the first dose, and at the end of the study after the last dose; the rationale for this timing is driven by UC clinical practice, in which a treatment effect on endoscopy may be expected after a minimum of 4 weeks of treatment.

Systemic absorption of hIL-10 is not affected by the degree of colitis. The investigators based this conclusion on experiments with healthy mice as well as mice with induced colitis, which showed that hIL-10 was not absorbed in the systemic circulation.

Although significantly increased numbers of monocytes were reported for the low-dose and mid-dose male mice treated in the preclinical toxicology studies, the investigators stated that they do not believe these results were related to the treatment because this observation was made neither in the female mice that were treated nor in the high-dose male mice.

The sponsor does not expect that participants will taper off components of their existing UC therapy, in particular immunosuppressants and biologics. Assuming the participant is stable and in remission, the participant's physician will not likely taper off medication that is beneficial. In addition, the immunosuppressives and biologics are indicated for severe UC, whereas only individuals with moderate disease activity are eligible for inclusion in this clinical trial.

Responding to concerns about the role of a participant's physician in this protocol, the investigators stated that references to the study doctor, the local investigator, and the participant's physician indicate the same person. In the investigators' view, any potential conflict of interest is remedied by the informed consent document and the inclusion and exclusion criteria, which protect participants against coercion into participation. They did agree with Dr. Cuffari's suggestion that an independent, objective committee should be constituted to address ethical issues.

Regarding anticipated difficulty in recruiting participants, the sponsor agreed that newer treatment options such as biologics may make recruitment to this protocol more difficult. However, these new drugs are not uniformly effective and may result in serious side effects. AG011 may be a viable alternative to these new drugs based on its anticipated safety and tolerability profile and mode of action through local release of hIL-10.

The sponsor agreed to exclude potential participants with indeterminate colitis.

2. Responses to RAC Discussion Questions

The investigators agreed to specify in the informed consent document the theoretical possibility of altered host resistance to viral and other infections.

Dr. Coulie explained that one of the major hurdles faced by the investigators is how to measure exposure to IL-10 when it is not systemically available. IL-10 is rapidly degraded in serum and even more rapidly in the gut lumen.

Regarding delineating individuals with CD from those with UC, Dr. Coulie explained that each potential participant's medical history must clearly identify UC. If there is any doubt in the medical history as to whether that individual has CD or UC (or if one or the other cannot be determined), the investigators will not include that individual in this study.

Dr. Coulie responded to Dr. Ertl's questions that no studies had been done to assess whether the gene transfer would affect the immune response to a mucosal infection. In the mouse and monkey studies, no antibodies to the transgene or the bacteria were detected. Research participants in the Phase I trial were not tested, but the investigators plan to do this testing in humans during the proposed protocol.

Regarding measuring transgene expression, Dr. Coulie explained that transgene expression cannot be measured in humans without removing the entire colon, which is not an option. In the mice and monkeys, the investigators have removed the colon and have used surrogate markers to correlate the amount of bacteria vs. the IL-10 produced *in vitro* and *in vivo*. However, for research participants, the only measures are indirect measures of IL-10 receptor activation; these pharmacodynamic parameters will be evaluated in a subset of participants. In addition, stool samples will be evaluated using polymerase chain reaction (PCR).

Dr. Steidler stated that gene transfer was not observed in any other flora in the gut.

E. Public Comment

Dr. Borror noted that the use of "agent" as a medication or treatment should be prefaced with "experimental" or "investigational." She also noted that some of the language in the informed consent document was too complex and technical and would not be understandable to all participants.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Clinical/Trial Design Issues

- Phase I studies of the study agent, sAGX0037, have not been conducted, and the rationale for proceeding with a Phase II study design is inadequate. Although conceptually similar to the clinical study of CD, Protocol #0804-917 differs from the Thy 12 study in a number of important ways. In addition to focusing on UC, the stage of the disease and the dosing regimens are different. The promoter is also significantly more potent and is expected to affect a fourfold increase in expression of hIL-10. Furthermore, the rationale for the addition of the rectal administration is based primarily on the observation from the Thy 12 study that little of the oral form reached the distal colon, which is the primary site of the disease. For these reasons, the study should be redesigned as a Phase I/II study.

- UC patients receiving less than 20 mg of prednisone or its equivalent per day may enroll in this trial. This dose level of corticosteroid is suppressive to the hypothalamic-pituitary-adrenal axis and is associated with impaired stress responses. As such, a clinical plan should be developed for the management of immunosuppression in the event of a local or systemic infection.

- In the preclinical toxicology studies in mice, changes in food and water consumption were observed, including a statistically significant increase in water consumption. Blood tests to monitor for changes in electrolytes, particularly those that would be related to excessive free water consumption, should be added to the protocol.

- The biopsy done at the second endoscopy should include a measurement of transgene expression in the mucosa.

- Depression and anxiety, which often are seen in UC patients, may be secondary to the disease. "Active psychiatric problems" is an exclusion criterion to participation. However, the protocol should define more specifically the types of psychiatric problems that are of concern as well as what constitutes an "active" problem.

- The inclusion criteria should be more objective so that a uniform population of participants with a definitive diagnosis of UC is enrolled. Based on the current criteria, a participant with indeterminate colitis, which may actually be CD with predominant colon involvement, could be enrolled. Requiring biopsy results in addition to a clinical history would help achieve this goal.

- Participants are instructed to contact the investigators if "any significant change in health status occurs." The term "significant" is vague. Additional guidance should be provided to help participants understand when they should be seen for an unscheduled visit. Including examples might be helpful.

- Investigators who also serve as participants' physicians can have role conflicts during the course of the study, particularly when decisions need to be made about continued participation of a participant who experiences a clinical event short of a serious adverse event (SAE). Specific stopping rules during the 4 weeks when the study agent is being administered can help manage the investigator/physician role conflicts, and they should be added to the protocol.

Ethical/Legal/Social Issues

- The protocol raises ethical issues because there may be no direct benefits to the participants, and moreover, they will be required to forgo other therapeutic options for the duration of the trial. In addition, some investigators may also serve as participants' physicians. Given the potential for role conflicts, recruitment to this trial should be delegated to an entity independent of the sponsor in those cases where treating physicians are also trial investigators.

- A word such as "study," "experimental," or "investigational" should be added before the word "agent" every time it is used in the informed consent document.

- The informed consent document should inform participants that in the event of their death, no matter the cause, a request for an autopsy will be made of their families to obtain vital information about the safety and efficacy of gene transfer. Participants should be asked to inform their families that such a request will be made and why it is scientifically and medically important. See "Request for Autopsy" in *Appendix M-III-B-2-c* of the *NIH Guidelines* and also see *NIH Guidance on Informed Consent for Gene Transfer Research* at the OBA Web site (http://www4.od.nih.gov/oba/rac/ic/appendix_m_iii_b_2_c.html).

G. Committee Motion 3

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Zaia moved the motion that the RAC approve these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 4 recusals.

VI. Certificates of Appreciation for RAC Member Service to NIH

Presenter: Raynard S. Kington, M.D., Ph.D., OD, NIH

Dr. Kington thanked the following RAC members who were rotating off the committee for their service to the NIH: Dr. Albelda, Dr. Dewhurst, Dr. Grant, Dr. Vile, and Dr. Weber. Each received a certificate of appreciation.

VII. Discussion of Human Gene Transfer Protocol #0804-913: A Phase I Study of BikDD Therapy in Advanced Breast Cancer

Principal Investigator:	Gabriel N. Hortobagyi, M.D., The University of Texas M.D. Anderson Cancer Center (UTMDACC)
Additional Presenters:	Joe Ensor, Ph.D., and Mien-Chie Hung, PhD., UTMDACC
RAC Reviewers:	Dr. Dewhurst, Ms. Shapiro, Dr. Strome, and Dr. Wei

AND

Discussion of Human Gene Transfer Protocol #0804-914: A Phase I, Open-Label, Dose-Escalation Study to Assess the Safety and Tolerability of the BikDD Nanoparticle in Patients with Advanced Pancreatic Cancer

Principal Investigator:	Milind Javle, M.D., UTMDACC
RAC Reviewers:	Drs. Dewhurst, Grant, Strome, and Wei

Dr. Kirchhoff recused himself from consideration of Protocol #0804-913 and Protocol #0804-914 due to a conflict of interest.

A. Summary of Protocol #0804-913

Research into the biologic behavior of breast cancer uncovered a number of molecular signaling processes involved in growth, proliferation, invasion, and metastasis. While some of these processes are present in normal cells, their manifestation under pathologic conditions leads to loss of normal cellular control mechanisms. Whereas in normal cells there is ample redundancy of signaling pathways, in transformed cells dependence on a single or a few signaling pathways is not uncommon. Because cancer is commonly a product of genetic changes (mutation, deletion, or overexpression), the concept of cancer gene therapy is to reverse these genetic changes and consequently achieve a therapeutic effect by introducing, in most cases, a single gene.

Bik, is one of the pro-apoptotic BH3-only proteins, and has recently been recognized as an essential stimulator of apoptosis. A mutant *Bik* was shown to have even greater pro-apoptotic effect. Preclinical studies using a liposomal delivery vector indicate that SN liposome-*pcDNA/BikDD* inhibited tumor growth about 50% in human cancer models and significantly increased the survival rates. Preclinical toxicology studies have been completed. The only subclinical effects reported were inflammation of the lungs, liver necrosis, elevation of liver enzymes, and thrombocytopenia. This will be the first human trial with this compound.

The objectives of this trial include determining the toxicity and tolerance of escalating doses of BikDD liposomes, the MTD of BikDD liposomes, the optimal biologically active dose (OBAD), and the *in vivo* biologic activity of BikDD liposomes. In addition, the investigators propose to evaluate tumor response.

B. RAC Members' Written Reviews of Protocol #0804-913

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the proposed systemic delivery of a potent proapoptotic gene that appears to have potential for systemic

toxicity, the need for additional discussion of the safety profile of the IV liposomal vector given the toxicity that has been noted in other clinical trials, and the special concern stemming from the use of a plasmid encoding a constitutively active proapoptotic gene under the control of a cytomegalovirus (CMV) promoter that is active in many cell types.

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

Regarding the overall approach, Dr. Dewhurst asked why the investigators are not proposing to use the composite promoter (CT-90) that selectively expresses in breast cancer cells and results in a more favorable gene expression profile, an approach that is proposed for Protocol #0804-914. He asked for information about the cause of death in the three mice exposed to a dose of 2.4 mg/kg of the study agent. Dr. Dewhurst requested that the investigators discuss the rationale for proposing that the maximal possible dose in research participants be below the minimal effective dose to achieve a biological response in mice. He suggested that cardiac safety monitoring be integrated into the followup analysis of the participants because IV delivery of liposomes containing BikDD might result in accumulation of deoxyribonucleic acid (DNA) in the lung or heart and suggested that the risks section of the informed consent document include a reference to potential cardiac toxicity. Dr. Dewhurst also asked the investigators to clarify how this study might enroll up to 24 participants, given a cohort size of 2 and the use of up to 4 dose levels, and the reasons for the different dose-escalation and toxicity assessment approach in this protocol compared with Protocol #0804-914.

Ms. Shapiro asked the investigators to justify restricting participants' opportunity to receive chemotherapy while on the study. Focusing on the informed consent document, she noted several instances of wording that would support a therapeutic misconception. Regarding the statement on page seven of the informed consent document that "no-cost treatment for research-related injuries is unavailable," Ms. Shapiro stated that ethical analysis suggests that such treatment should be provided; participants should receive no-cost medical treatment if there are injuries as a result of their research participation.

Dr. Strome asked about the potential benefit of this experimental approach in individuals whose tumors have not lost *Bik* expression, specifically whether expression of the mutant transgene enhances the ability of cells with a full complement of *Bik* to undergo apoptosis. He asked the investigators to explain why potential participants who are on chemotherapy would be excluded from this study, from the perspective of ethics as well as the possibility that chemotherapy might potentiate the proapoptotic effects of transgene expression. Given the recognized heterogeneity within tumors, Dr. Strome suggested that the proposed measure of biologic activity—based on apoptosis in the biopsy specimens—would be difficult or impossible to interpret. In addition, he suggested that the investigators consider removing the 4-week interval assessment as a criterion for administering an additional cycle, because tumor change would be difficult to assess within such a short time period.

Dr. Wei stated that he that the continual reassessment methodology (CRM) design proposed for this study was unclear and requested access to the protocol's statistician to clarify the design. Noting that the choice of the doses should be dependent on the posterior distribution, he pointed out that four fixed doses are proposed. Dr. Wei also asked for clarification of why the investigators were discussing the OBAD as well as the MTD.

C. RAC Discussion of Protocol #0804-913

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

- Dr. Strome asked whether the investigator who consents the participant would also be that individual's doctor. He suggested that, when the investigator is the participant's physician, the participant should see another physician who is not involved in the study to hear about therapeutic options that might be considered standard of care.

- Dr. Strome suggested that the investigators change the wording for inclusion criteria from the potential participant having failed a single chemotherapeutic agent for breast cancer to having failed standard-of-care therapy as defined by the investigators.

- Dr. Albelda suggested that the investigators address the biodistribution of IV liposomes into the tumors by employing needle biopsies using PCR and molecular tests.

- Dr. Vile asked what percentage of cells within a tumor must be transduced to achieve a therapeutic effect.

- Significant discussion ensued regarding toxicity issues, bystander effects, and nonspecific targeting related to the use of the proposed CMV promoter rather than the CT-90 BC-specific promoter. Drs. Albelda, Dewhurst, Ertl, Federoff, Flint, and Strome noted that Protocol #0804-913 and Protocol #0804-914 share the same transgene product, but the vector in Protocol #0804-914 would be expected to be transcriptionally regulated to express in the pancreatic cancer (PC) cell type, whereas Protocol #0804-913 proposes to use a ubiquitously expressed vector. The RAC members asked why the investigators are not proposing to use a BC-specific vector that may be available in the near future. Discussion of this question led to a unanimous recommendation that this protocol should go forward with a BC-specific promoter.

D. Investigator Response for Protocol #0804-913

1. Written Responses to RAC Reviews

Regarding the use of the CMV promoter in this proposed trial, the investigators explained that their CMV-BikDD toxicity and safety studies were completed in early 2005, before the CT-90 study, and they have been trying to initiate a Phase I trial using this CMV promoter since that time. In the intervening 3 years and following toxicity and safety studies on CT-90, the investigators developed a versatile expression vector (the "VISA vector"), which can boost gene expression in cancer cells but still remain nearly silent in normal cells, while retaining its anticancer activity with virtually no toxicity (in the PC cell model). The investigators believe the VISA system can be applied to BC, and they are currently testing this possibility using multiple BC-specific promoters, including CT-90. It is likely that the BC-VISA vector will work better than the CT-90 vector. Therefore, to facilitate the benefit of this approach to humans, the investigators are proposing this Phase I trial using CMV-BikDD, for which a complete toxicity and safety study has already been conducted and for which a regulatory package exists. Although the investigators are aware that they could wait until the improved vector is developed, they believe that such a vector could be delayed indefinitely, that a better system will always be in development, and that patients will receive more benefit from initiating this clinical study now while researchers continue to improve the therapeutic strategy in the laboratory.

The exact cause of death in the three mice given the dose of 2.4 mg/kg of CMV-BikDD during the single-dose study is unknown. However, at that dose level, toxicity was encountered in the liver and thymus: acute coagulative necrosis in the liver, splenic atrophy and necrosis, lymphoid infiltrates in the portal triads in the liver, atrophy of the thymus, interstitial inflammation in the lung, and thrombocytopenia.

This Phase I clinical trial is focused on studying toxicity in humans. As such, the investigators plan to start with a baseline electrocardiogram (EKG). If a participant develops symptoms that suggest cardiac or pulmonary toxicity, the treating physician would be able to order all clinically appropriate tests for that symptom. Twenty-one research participants with nonsmall-cell lung cancer have received this liposome in an ongoing trial at the UTMDACC and no cardiac toxicity has occurred to date. However, as an additional safety measure, the investigators agreed to add required EKGs with every 8-week restaging.

The investigators chose the CRM method for this trial, as opposed to the 3+3 method, because the 3+3 method would result in an imprecise toxicity rate estimation given the small number of participants in the trial at each dose level. The CRM allows flexibility to specify a target toxicity, and the MTD (as chosen by the CRM) will be the dose that is estimated to be closest to achieving the desired toxicity rate. In

addition, all toxicity information is shared among the different dose levels of the CRM. As developed by the UTMDACC, the CRM software employs a stopping rule to protect against the possibility that even the lowest dose is too toxic.

Since the BikDD gene is a more potent inducer of apoptosis than the wild-type *bik*, the investigators expect that it will benefit participants who still retain the *bik* gene in addition to those who have lost it. Therefore, they noted that it would not be necessary to incorporate *bik* expression as an entry criterion.

Regarding the exclusion of participants who are on chemotherapy, the investigators explained that most participants in these Phase I trials have resistant disease that has progressed after multiple lines of chemotherapy. Therefore, adding a chemotherapy that the individual most likely has already received might add to toxicity without benefit. A Phase I trial is designed to examine the toxicity dose and schedule of administration of the new study drug by itself.

Although tumor change might be difficult to assess at the 4-week interval assessment, the investigators stated the importance of participants being seen and examined by a physician at least every 4 weeks to assess toxicity and tolerability of the study drug. Restaging imaging studies are proposed for every 8 weeks, which is standard in metastatic, advanced BC patients on any systemic regimen.

Participants will be considered to be benefiting if they have stable or decreasing disease on restaging and if the experimental treatment is tolerated without significant toxicity. Although efficacy is not a primary endpoint in a Phase I trial, the investigators stated their belief that it would be inappropriate not to allow a participant to continue if she were receiving benefit. If a participant's disease is no longer measurable, she could continue until the disease progresses or until two cycles have been completed after a complete response.

The investigators agreed to change wording in the informed consent document to remove any indication that the study drug is a "treatment."

Regarding no-cost treatment for research-related injuries, the investigators explained that the UTMDACC is unable to guarantee no-cost treatment of such injuries while continuing to be able to develop and conduct clinical trials. This study will be monitored closely for side effects and toxicities, and any individual who meets the eligibility criteria as outlined in the protocol can participate; thus, the investigators believe they are observing both nonmaleficence and justice in this clinical trial.

2. Responses to RAC Discussion Questions

Dr. Hortobagyi explained that the treating physicians and the collaborators in the clinical trial are the same individual. The UTMDACC has developed a standard-of-care list of priorities for BC management as well as a list of priorities for clinical trials, and all participating physicians are expected to follow that set of guidelines.

Dr. Hortobagyi stated that the investigators do not have preclinical safety or toxicity data about this agent in combination with chemotherapy. Therefore, it would be risky to include participants who are receiving other drugs because there is no information about the interactions with those other drugs.

Regarding measuring biological activity, Dr. Hortobagyi explained that the investigators anticipate that they will not be able to obtain biopsies on every participant because, although most participants will accept a biopsy, all will not. Heterogeneity exists in these tumors, and depending on where the needle is inserted, the extracted sample might not be representative of the entire tumor volume. At the conclusion of this trial, the investigators expect to know the MTD and the pattern of major toxicities, if any. They expect that they may or may not know the optimal biological dose and that additional trials might be necessary to obtain that information.

Regarding the possible use of a BC-specific vector, Dr. Hortobagyi stated that the investigators have discussed this issue a number of times. He explained that, at some point in time, the decision must be

made about whether to take an agent to the clinic. It would likely take 3 to 5 years to take the BC-specific promoter associated with BikDD to the clinic. He estimated that the cost of going through the preclinical evaluation, preclinical toxicity, and generation of DNA with a new vector would be approximately $1 million, similar to the cost to date. As there is no commercial venture involved in this protocol, its funding would be dependent on the NIH.

E. Public Comment for Protocol #0804-913

Dr. Borror noted that the description of the study drug in the informed consent document did not mention gene transfer or how it works and was uninformative or misleading in several instances; she stated that the description in Protocol #0804-914 was clearer. In addition, she expressed confusion about the optional procedures for biopsies and suggested that it would be helpful to state how these optional procedures are different from the required procedures.

F. Summary of Protocol #0804-914

Pancreatic cancer is one of the most chemotherapy-resistant of solid tumors in humans for which novel and effective strategies are needed. The *Bik* (*Bcl-2* interacting killer) gene is a member of the pro-apoptotic *BH3* family that has the potential of targeting cancer effectively due to its broad binding range and affinity for the anti-apoptotic *bcl-2, bcl-xl, bcl-w and Mcl-1*. A mutant *Bik* gene (*BikDD*) has been developed that has strong binding affinity to these anti-apoptotic proteins. Preclinical studies have also demonstrated that systemic delivery of the *BikDD* gene by liposome complexes significantly inhibited the growth of human breast cancer *in vivo*. The investigators have developed an expression vector "VISA" (VP16-GAL4-WPRE integrated systemic amplifier) and identified the cholecystokinin type A receptor (CCKAR) promoter as a pancreatic cancer-specific promoter. The modified CCKAR-VISA (*C*-VISA) composite is engineered to systemically target transgene expression in pancreatic cancers *in vivo*. Systemic administration of C-VISA driven *BikDD* in DNA:liposome complexes repressed pancreatic tumor growth, metastases and prolonged survival in xenograft and syngeneic orthotopic mouse models of pancreatic cancer without toxicity. The protocol will explore the C-VISA *BikDD* plasmid encapsulated in DOTAP:Cholesterol liposome-based nanoparticle (*BikDD* nanoparticle) in a phase I clinical trial for patients with advanced pancreatic cancer. The phase I study will include subjects with advanced pancreatic cancer who have received one standard therapy (such as gemcitabine), have adequate laboratory values and performance status (ECOG 0-1). Dose levels planned are 0.04, 0.05 and 0.06 mg/kg. If Maximal Tolerated Dose (MTD) is not reached at 0.06 mg/kg, further dose escalations will occur by 33% dose increments until the MTD is reached. Dose escalation will be as per standard 3+3 design. Tumor biopsies will be obtained before and on day 24 of study drug administration and analyzed for apoptotic index (AI), caspase-3 and *BikDD* mRNA expression. Optimally effective biological dose (OBAD) will be determined using these results. Tumor reassessment using radiological studies will be conducted periodically and treatment will continue until toxicity or progressive disease.

G. RAC Members' Written Reviews of Protocol #0804-914

Eleven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the novel approach using systemic delivery of a potent proapoptotic gene that, despite the PC-specific promoter, might have the potential for systemic toxicity, and the need for more discussion of the safety profile of the IV liposomal vector, due to toxicity seen in other clinical trials.

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

Dr. Dewhurst asked for information about the cause of death in the three mice exposed to a dose of 2.4 mg/kg of the study agent. He requested that the investigators discuss the rationale for proposing the maximal possible dose in research participants to be below the minimal effective dose to achieve a biological response in mice. Dr. Dewhurst suggested that cardiac safety monitoring be integrated into the followup analysis of the participants, because IV delivery of liposomes containing BikDD might result in accumulation of DNA in the lung or heart, and suggested that the risks section of the informed consent document include a reference to potential cardiac toxicity. Dr. Dewhurst also asked the investigators to

clarify how this study might enroll up to 24 participants, given a cohort size of 2 and the use of up to 4 dose levels.

Dr. Grant requested that the investigators ask potential participants during screening about any natural remedies—in addition to medications—they are taking. In addition, she suggested adding a sentence about assessing potential participants' emotional health (e.g., "Your emotional health will be assessed to ensure that you are able to partake in the study requirements.") because hallucinations and confusion are two of the potential side effects of this protocol.

Dr. Strome asked about the potential benefit of this experimental approach in individuals whose tumors have not lost *bik* expression, specifically whether expression of the mutant transgene enhances the ability of cells with a full complement of *bik* to undergo apoptosis. He asked the investigators to explain why potential participants who are on chemotherapy would be excluded from this study, from the perspective of ethics as well as the possibility that chemotherapy might potentiate the proapoptotic effects of transgene expression. Given the recognized heterogeneity within tumors, Dr. Strome suggested that the proposed measure of biologic activity—based on apoptosis in the biopsy specimens—would be difficult or impossible to interpret. In addition, he suggested that the investigators consider removing the 4-week interval assessment as a criterion for administering an additional cycle, because tumor change would be difficult to assess within such a short time period.

Noting that the investigators propose to use a standard dose-finding scheme with MTD criteria, Dr. Wei asked how that scheme plus the investigators' stated interest in the OBAD could be considered simultaneously in this trial. He also wondered why Protocol #0804-913 proposes using a more elaborate Phase I design, whereas this protocol proposes using the standard design, especially since both protocols are being proposed by the UTMDACC.

H. Investigator Response for Protocol #0804-914

Written Responses to RAC Reviews

The exact cause of death in the three mice given the dose of 2.4 mg/kg of CMV-BikDD during the single-dose study is unknown. However, at that dose level, toxicity was encountered in the liver and thymus: acute coagulative necrosis in the liver, splenic atrophy and necrosis, lymphoid infiltrates in the portal triads in the liver, atrophy of the thymus, interstitial inflammation in the lung, and thrombocytopenia.

The starting dose for this proposed study is 0.04 mg/kg. The doses for the animal toxicity studies, when converted to the human equivalent, yield a no-observable-effect level (NOEL) of 0.025 mg/kg, a NOAEL of 0.10 mg/kg, and a minimal effective dose of 0.0625 mg/kg. In this study, the minimal effective dose will be reached at dose level 3, which is significantly below the NOAEL. Therefore, this study is likely to reach effective dose levels that may not be toxic.

Twenty-one participants with nonsmall-cell lung cancer have received this liposome in an ongoing trial at the UTMDACC; no cardiac toxicity has occurred to date. However, the investigators agreed to revise the protocol to require that EKGs be repeated before each new cycle and at the time the participant discontinues study drug administration.

Since the BikDD gene is a more potent inducer of apoptosis than the wild-type *bik*, the investigators expect that it will benefit participants who still retain the *bik* gene in addition to those who have lost it. Therefore, they noted that it would not be necessary to incorporate *bik* expression as an entry criterion.

Regarding the exclusion of participants who are on chemotherapy, the investigators explained that most participants in these Phase I trials have resistant disease that has progressed after multiple lines of chemotherapy. Therefore, adding a chemotherapy that the individual most likely has already received might add to toxicity without benefit. A Phase I trial is designed to examine the toxicity dose and schedule of administration of the new study drug by itself.

Although tumor change might be difficult to assess at the 4-week interval assessment, the investigators stated the importance of participants being seen and examined by a physician at least every 4 weeks to assess toxicity and tolerability of the study drug. Restaging imaging studies are proposed for every 8 weeks, which is standard in metastatic, advanced PC patients on any systemic regimen.

Regarding the protocol design, Dr. Javle explained that the investigators have more experience with a 3+3 design than with the CRM design suggested by Dr. Wei. The design of this protocol was reviewed and approved by the FDA.

I. Public Comment for Protocol #0804-914

No public comments were offered.

J. Synopsis of RAC Discussion and RAC Observations and Recommendations for Protocol #0804-913

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

Safety Concerns

- The *BikDD* plasmid uses a CMV promoter for transgene expression that is not specific for BC cells and has known off-target toxicities. The plasmid is to be delivered in liposome-based nanoparticles by IV infusion. Such liposome nanoparticles are known to accumulate at high levels in normal tissues, including lung and heart, in addition to the targeted tumor cells. Such off-target expression of a potent proapoptotic transgene has significant safety risks, and the CMV promoter should not be used in this clinical study. To proceed with the protocol, a more specific promoter should be used. According to the reports in the literature[2], a plasmid using a more specific CT-90 promoter that is selectively expressed in BC cells is under development. Favorable preclinical data on its safety profile are already available, and once additional toxicological data are obtained, this more specific plasmid could be ready for clinical evaluation.

Clinical/Trial Design Issues

If the study proceeds with a more specific promoter, the following additional points should be considered:

- There are a number of chemotherapeutic options for advanced BC. Chemotherapy might also potentiate the proapoptotic effects of transgene expression. However, if the decision is made to exclude patients on chemotherapy, then it should be clear from the protocol that only participants who either have failed or have refused additional standard chemotherapy for metastatic BC might be enrolled in the trial.

- The measure of biological activity of the proapoptotic *BikDD* transgene will be based on the apoptosis index in the biopsy specimen. Given the heterogeneity in tumor tissue, using a single biopsy specimen as a measure of biological activity is unlikely to yield reliable data. It would be more accurate to characterize this endpoint in a descriptive term rather than as a measure of transgene biological activity. The tumor samples should be used to detect the presence of the vector DNA sequences in the tumor and to assess whether transgene expression has occurred.

- Although the protocol indicates that the CRM design will be used to determine the MTD, it is not clear that the CRM design is being followed. Conceptually, the CRM can be accomplished by assuming a parametric form of the toxicity rate related to the dose, with a few unknown

[2] Day CP, Rau KM, Qiu L, Liu CW, Kuo HP, Xie X, Lopez-Berestein G, Hortobagyi GN, Hung MC. Mutant Bik expression mediated by the enhanced minimal topoisomerase llalpha promoter selectively suppressed breast tumors in an animal model. *Cancer Gene Ther* 2006 Jul; 13(7):706-19. Epub 2006 Mar 3.

parameters. A prior distribution is first assumed for those parameters and then is updated with the data sequentially. With each new data point, the potential toxicity rate based on the new posterior for the next dose is integrated until the MTD dose is obtained. It is not clear, however, that the proposed CRM design is based on these design principles. For example, it is not clear why the fixed 33-percent escalation rule, which is more often used in the 3+3 design, is being used. A more explicit discussion of the statistical assumptions underlying the proposed CRM design should be included in the protocol.

Ethical/Legal/Social Issues

- In cases where the investigator is providing clinical care to the prospective participant, an oncologist who is not directly involved in the trial should be the primary physician to administer the consent process. This is especially important because participants in this study may not be eligible for alternative therapies. An oncologist who is not directly affiliated with the trial may be best able to ensure that the participant understands the implications of enrolling in this trial *in lieu* of pursuing alternative therapies.

K. Committee Motion 4

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Flint moved and Ms. Shapiro seconded the motion that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

L. Synopsis of RAC Discussion and RAC Observations and Recommendations for Protocol #0804-914

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

Clinical/Trial Design Issues

- The measure of biological activity of the proapoptotic *BikDD* transgene will be based on the apoptosis index in the biopsy specimen. Given the heterogeneity in tumor tissue, using a single biopsy specimen as a measure of biological activity is unlikely to yield reliable data. It would be more accurate to characterize this endpoint in a descriptive term rather than as a measure of transgene biological activity. The tumor samples should be used to detect the presence of the vector DNA sequences in the tumor and to assess whether transgene expression has occurred.

M. Committee Motion 5

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Somia moved that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VIII. Day 1 Adjournment

Dr. Federoff adjourned Day 1 of the June 2008 RAC meeting at 4:15 p.m. on June 17, 2008.

IX. Day 2 Call to Order and Opening Remarks

Dr. Federoff, RAC Chair, opened Day 2 of the June 2008 RAC meeting at 8:10 a.m. on June 18, 2008.

X. Discussion of Human Gene Transfer Protocol #0804-922: Adoptive Immunotherapy for CD19+ B-Lymphoid Malignancies Using *Sleeping Beauty* Transposition to Express a CD19-Specific Chimeric Antigen Receptor in Autologous *Ex Vivo* Expanded T Cells

Principal Investigators:	Laurence J.N. Cooper, M.D., Ph.D., and Partow Kebriaei-Tabari, M.D., UTMDACC
Additional Presenter:	Perry Hackett, Ph.D., University of Minnesota
Sponsor:	Maurie Markman, M.D., UTMDACC
RAC Reviewers:	Drs. Fan, Vile, Weber, and Williams

Dr. Somia and Dr. Kirchhoff recused themselves from consideration of this protocol due to conflicts of interest.

A. Protocol Summary

Most patients with advanced B-lymphoid malignancies undergoing autologous hematopoietic stem-cell transplantation (HSCT) without obtaining a complete remission (CR) have no curative treatment. To augment the immune response against CD19+ malignancies, an immunotherapy protocol has been developed using infusions of CD19-specific autologous T cells transduced by the Sleeping Beauty transposon system to express a chimeric antigen receptor (CAR) The T cells, obtained by steady-state leukapheresis, are rendered specific for CD19 by electrotransfer of two *Sleeping Beauty* (*SB*) DNA plasmids expressing (i) *SB*11 transposase and (ii) transposon coding for CAR, designated CD19RCD28, that can activate T cells through chimeric CD28 and CD3-ζ endodomain upon binding cell surface CD19 using a scFv derived from a mouse monoclonal antibody. The *SB* system is analogous to retrovirus as the *SB* transposon is similar to a provirus, with the use of plasmid internal and direct repeats similar to viral long terminal repeats that are recognized by plasmid transposase similar to viral integrase. The transduced T cells will be expanded by co-culture with irradiated K562 cells engineered to express CD19 and co-stimulatory molecules and then infused into research participants following autologous HSCT.

The primary objectives of the study are to assess the safety, feasibility, and persistence of the T-cell infusions. The secondary objectives are to assess the (i) immune response against transgenes, (ii) homing potential of the adoptively transferred T cells, (iii) ability of low-dose IL-2 to improve survival of the infused T cells, and (iv) disease response.

B. Written Reviews by RAC Members

Eight RAC members voted for in-depth review and public discussion of the protocol. Key issues included the first use of the *Sleeping Beauty* (*SB*) Transposon System™ (SBTS™) in a gene transfer clinical trial and safety issues regarding the potential of insertional mutagenesis of the SBTS™ compared with other integrating retroviral vectors.

Four RAC members provided written reviews of this proposed trial.

With regard to the SBTS™, Dr. Fan asked whether binding sites in human DNA exist for the *SB* transposase and whether it is possible for the *SB* transposase to mobilize or rearrange the cellular DNA of the transduced T cells. He requested additional information about the duration of persistence of the transposase and expressed concern that the *SB* transposon could induce tumorigenicity, given that modified *SB* transposons have been used to discover new oncogenes in mice. Dr. Fan asked the investigators to discuss whether progression toward monoclonality or a shift in clones occurs during the selection of the CD19-specific T cells.

Focusing his comments on the use of the SBTS™, Dr. Vile asked the investigators to further address several issues, including (1) expansion of the evidence for their statement that “Transposon-induced deletions and rearrangements do not apparently contribute to *SB*-induced cancer, and *SB* transposition does not cause genome-wide genotoxicity”; (2) the possibility that cotransfection of the proposed two plasmids might induce recombination such that the transposase gene might acquire integration signals

and become integrated in rare T cells; (3) whether inclusion of a suicide gene might be beneficial in case the expanded T cells reinfused in participants were to acquire transformed phenotypes and therefore need to be removed; (4) whether *in vivo* animal studies are warranted to determine the toxicity of the SBTS™ on the T cells; and (5) additional discussion of the advantages of the SBTS™ compared with the use of retroviral transduction in this T-cell population.

Dr. Weber asked the investigators to expand their discussion of the infectious risks associated with rituximab to include viral reactivation and suggested inclusion of a table describing the timing of safety tests and a description of the power of this study design to detect SAEs. Dr. Weber suggested that the investigators revise the action plan for research participant management in the event positive surveillance cultures are encountered and that an infectious disease consultation should be obtained earlier in the evaluation and should not be considered optional.

Dr. Williams suggested that the background section on insertional mutagenesis should be updated to indicate that IL-2 would be given to only a subset of research participants, which is currently made clear only in the statistics section. Regarding preclinical issues, he asked whether the efficiency transduction of the target population using electroporation is adequate to generate sufficient numbers of T cells to allow successful prediction of dose targets. Data on integration sites are measured in mouse cells only, so Dr. Williams requested comparable data on integration site preference in human T cells. Regarding clinical issues, he suggested that the investigators measure residual disease as an entry criterion and wondered whether, based on FDA guidance, the investigators could pursue shorter followup for participants with no evidence of gene transfer. Dr. Williams stated that it is not clear that the number of participants would allow determination of the secondary endpoint of IL-2 efficacy or what biological, biochemical, or molecular endpoints would be used to make that assessment.

C. RAC Discussion

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

- Dr. Weber noted in the informed consent document the repeated use of “gene therapy” as opposed to the more appropriate “gene transfer,” a preference for having two separate informed consent documents rather than trying to integrate the document for minors into the document for adults, the absence of an assent form, the need to include a list of acceptable methods of birth control for males, the need to describe some of the procedures in lay language, removal of the discussion of efficacy because this protocol is only meant to measure safety, and the need to divide the list of side effects of drugs by frequency and severity.

- Dr. Weber expressed concern about the excessive risk involved in requiring two DLTs (instead of the more usual one) at each dose level before escalation of the protocol would be halted.

- Dr. Albelda requested that the investigators take an aliquot of the sample to be administered to each participant and continue culturing it until the cells could no longer be cultured or until a clone arose. Although such an event is not likely to occur, he stated his belief that the investigators would be reassured by an evaluation of the clonality of the transduced cells.

D. Investigator Response

1. Written Responses to RAC Reviews

There is no evidence that the *SB* transposase binds to human DNA and no human or mouse sequences in GenBank are identical to the SB transposase binding site. Although some integrases can cause recombination of host DNA, no comparable activity has ever been observed for *SB* transposons when individual transposons have been introduced into mammalian genomes.

It is not known how long the *SB11* transposase persists after T cells have been electroporated with the SBTS™. The investigators cultured T-cell genomic DNA for at least 21 days following electroporation to

evaluate for the presence of integrated *SB11* cDNA. The investigators stated that T cells that have been cultured for less than 21 days will not be infused. The toxicity of excessive expression of SB11 activity would likely limit long-term expression.

The investigators explained that the majority of genetically modified and cultured T cells do not reveal detectable integrated *SB11* transposase. Although a small number of cells might take up the transposase gene into their genomes, expression is unlikely. However, even prolonged expression does not necessarily lead to further transposition, because the ratio of transposase to transposon must be maintained. It appears that dominant-negative forms of the transposase are made, which limits long-term function. If the *SB* transposase is expressed, the cells likely will be immunogenic and thus eliminated.

Although nondirectional integration of any given gene can pose the risk of activating tumor genes or silencing tumor-suppressor genes, the risk from insertional mutagenesis would appear to be less than using gamma-retrovirus due to less frequent integration into transcriptional units or regulatory regions. The SB system used for gene transfer is designed differently from those systems used for oncogene discovery in transgenic mice expressing transposase in their cells.

Because there is no evidence that the *SB* transposase interacts with repetitive elements in the human genome, the investigators do not expect that the transposase will induce rearrangements due to recombination among natural repetitive elements in the genome.

Inclusion of a suicide gene in the expression cassette would likely lower the overall rates of *SB* gene delivery and transposition due to the increase in transposon cargo size. Also the presence of immunogenic viral antigens, such as HSV-TK, would likely lead to inmmune-mediated clearance of the transduced T cells. Alternative nonimmunogenic suicide systems are not widely available. Therefore, in the event of serious toxicity after infusion, the investigators plan to administer steroids, which have been used previously to curtail toxicity from infused T cells such as melanoma-specific T cells.

The advantages of the SBTS™ include its being a nonviral system that can be prepared cleanly, stored stably, and obtained at lower cost with greater reliability, all of which are important if a technology is to be used widely. In addition, the SBTS™ will integrate less frequently into transcriptional units and/or their regulatory motifs compared with the retroviral vectors commonly used, and *SB* is not apt to recombine with any endogenous elements due to its evolutionary origin (fish rather than mammal).

The long-term followup protocol will be modified in the future so that monitoring will cease for participants without evidence of gene transfer one month or more after infusion of genetically modified T cells.

With such a small sample size, the investigators noted that this trial would not have sufficient statistical power to detect differences in survival among participants. However, they intend to use summary statistics to describe the survival of infused T cells with and without IL-2. Survival of T cells will be measured by flow assays and functional assays.

2. Responses to RAC Discussion Questions

Dr. Hackett explained the difference between the gene transfer transposon and those used for discovery of oncogenes by causing tumors. The essential difference is that the gene transfer transposon is designed so that its transgene is expressed with minimal consequence to the genome as a whole. The transposons proposed for this study consist of a promoter driving a single gene of interest; while the transposon used in oncogenesis studies contains a splice acceptor and poly A site. Integration of that transposon into an intron will disrupt expression of the cellular gene.

Regarding the inclusion of safety (“suicide”) genes, Dr. Cooper explained that the investigators have decided not to use selection/suicide genes, primarily because doing so may confound an objective of the study, which is to look for the persistence of T cells. The chimeric receptor-modified T cells are expected to be long-lived in these participants, however, if the suicide genes are used, the transduced T cells may be cleared by an immune response to the suicide gene product. At present, the technology for

nonimmunogenic selection suicide approaches is not available. The plan is to use steroids to ablate an adverse response.

Dr. Kebriaei-Tabari agreed that the investigators would better define "DLT" for this trial. As proposed, the DLT is defined as an SAE greater than grade 3; he offered to consider redefining the DLT as an SAE greater than grade 2 and to consider whether the SAEs are reversible in terms of evaluating the severity.

E. Public Comment

No public comments were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Preclinical Issues

- As *in vivo* models become available, studies should be conducted to determine the potential for the SBTS™ to cause insertional mutagenesis leading to T-cell malignancy in transduced cells.

- Cultures of transduced T cells should be serial-passaged for a period longer than 28 days to evaluate for clonal expansion.

Clinical/Trial Design Issues

- The investigators should retain and culture an aliquot of the transduced T cells to evaluate the clonality of the transduced cells.

- The protocol currently permits dose escalation when one of three participants experiences a DLT at a specified dose level. However, this approach raises the possibility that a similar DLT could occur in participants in the next dose cohort. Since the clinical significance of DLTs varies, the protocol should define the type and duration of a DLT that would require enrollment of additional participants in the same dose cohort to assess the safety of that dose.

Ethical/Legal/Social Issues

- The investigators should make the following changes to the informed consent document:

    - Change the term "gene therapy" to "gene transfer."
    - Use the term "investigator" when referring to the physician-scientist conducting the trial and "your doctor" when referring to the participant's personal physician.
    - Simplify the language used to describe procedures and provide definitions for all technical terms (e.g., B cell, T cell, GMP, CT, and PET).
    - Use trade names in addition to the generic names of medications (e.g., Tylenol and acetaminophen).
    - Provide data on the severity and frequency of adverse reactions associated with each drug used in the protocol.
    - Outline acceptable methods of birth control for men who enroll in the trial.

- The investigators should use separate informed consent documents for research participants and parents/guardians who are consenting for minor children. An assent document should be developed for minor children.

G. Committee Motion 6

Dr. Federoff summarized the comments and concerns of the RAC to be included in a letter to the investigators and the sponsor. Dr. Fan moved that the RAC approve these summarized recommendations. The vote was 17 in favor, 0 opposed, 0 abstentions, and 2 recusals.

XI. *NIH Guidelines for Research Involving Recombinant DNA Molecules*: Noncontemporary Influenza and Highly Pathogenic Avian Influenza

Presenter: Dr. Corrigan-Curay

A. Presentation

Dr. Corrigan-Curay provided a brief update on the current task of the RAC's Biosafety Working Group (BWG) to provide biosafety and containment guidance for recombinant research with noncontemporary human influenza virus H2N2, fully reconstructed 1918 H1N1 influenza virus, and highly pathogenic avian influenza virus H5N1. She discussed the public health impact of influenza, with approximately 20,000 excess deaths in the United States per year and pandemics such as the 1918 influenza pandemic, which caused more than 600,000 deaths in the United States and 20 million to 40 million deaths worldwide. Research on viral virulence mechanisms and development of vaccines and antiviral drugs are public health priorities; however, it is equally important that such research be performed under appropriate containment to protect the health of researchers and the public. She reviewed the guidance on determination of risk group (RG) classifications and biosafety levels (BL) currently in the *NIH Guidelines*. All human influenza viruses are classified as RG2; however, a risk assessment for noncontemporary human influenza viruses and HPAI H5N1 would be expected to determine that a higher level of containment is appropriate. She also reviewed the guidance provided by the USDA and in the Biosafety in Microbiological and Biomedical Laboratories, which describes BL3 containment with specific enhancements.

The BWG is being asked to determine the appropriate RG designations for noncontemporary human influenza strains and HPAI and whether additional biosafety guidance should be provided in the *NIH Guidelines* for research involving recombinant viruses containing sequences from these influenza strains?

To accomplish this, the BWG will be reviewing data on the virulence and availability of preventive or therapeutic measures for noncontemporary strains of human influenza (including H2N2 and 1918 influenza virus) and HPAI H5N1. The group will recommend RG classifications based on the best available data, and develop additional biosafety recommendations for recombinant research with noncontemporary strains of influenza and HPAI, including chimeric viruses, in keeping with other guidances and the emerging data.

B. RAC Discussion

Noting that the current *NIH Guidelines* focuses on the mechanics of what happens in the laboratory, Dr. Weber expressed concern that the level of guidance regarding the occupational health aspects of how to monitor laboratory workers is not nearly as detailed. He suggested that the *NIH Guidelines* add specific guidance on how to monitor lab employees when they get sick as well as on issues about employees' personal protective equipment.

XII. Closing Remarks and Adjournment

Dr. Federoff thanked the RAC members and the OBA staff and adjourned the meeting at 9:45 a.m. on June 18, 2008.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, actions are not considered final until approved by the NIH Director.]

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

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

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

Date: _____

_____
Howard J. Federoff, M.D., Ph.D.
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Attachment I
Recombinant DNA Advisory Committee Roster

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Attachment III
Abbreviations and Acronyms

Ad	adenoviral, adenovirus
Ad.IL-12	adenovirus expressing interleukin-12
Adv-hIL-12	adenoviral vector expressing human interleukin-12
AE	adverse event
BC	breast cancer
BWG	Biosafety Working Group (a subcommittee of the RAC)
CD	Crohn's disease
CMV	cytomegalovirus
CRM	continual reassessment methodology
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
EKG	electrocardiogram
FDA	Food and Drug Administration, DHHS
GI	gastrointestinal
GTSAB	Gene Transfer Safety Assessment Board
HBV	hepatitis B virus
hIL-10	human protein interleukin-10
hIg-h4-1BB-Ls	human 4-1BB ligand fusion protein
HPAI	highly pathogenic avian influenza
IBD	inflammatory bowel disease
IRB	institutional review board
IV	intravenous
L. lactis	*Lactococcus lactis*
MSSM	Mount Sinai School of Medicine
MTD	maximal tolerable dose
NCI	National Cancer Institute, NIH
NHP	nonhuman primate
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NOAEL	no-observable-adverse-effect level
OBA	Office of Biotechnology Activities, NIH
OBAD	optimal biologically active dose
OD	Office of the Director, NIH
PBMC	peripheral blood mononuclear cell
PC	pancreatic cancer
PCR	polymerase chain reaction
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RG	Risk Group
SAE	serious adverse event
SB	*Sleeping Beauty* gene
SBTS™	Sleeping Beauty Transposon System™
UC	ulcerative colitis
U.K.	United Kingdom
UTMDACC	The University of Texas M.D. Anderson Cancer Center