RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 5-6, 2006

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.]

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

December 5-6, 2006

The Recombinant DNA Advisory Committee (RAC) was convened for its 106th meeting at 12:15 p.m. on December 5, 2006, at the National Institutes of Health (NIH), Building 31-C, Conference Room 10, Bethesda, Maryland. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 12:15 p.m. until 3:15 p.m. on December 5 and from 8:30 a.m. until 4:00 p.m. on December 6. The following individuals were present for all or part of the meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania Medical Center
Stephen Dewhurst, University of Rochester Medical Center
Hildegund C.J. Ertl, The Wistar Institute
Howard J. Federoff, University of Rochester
Ellen E. Grant, HealthNow New York Inc.
Helen Heslop, Baylor College of Medicine
Jeffrey P. Kahn, University of Minnesota
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Nicholas Muzyczka, University of Florida
Glen R. Nemerow, The Scripps Research Institute
Naomi Rosenberg, Tufts University
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities
Scott E. Strome, University of Maryland Medical Center
Richard G. Vile, Mayo Clinic College of Medicine
David J. Weber, The University of North Carolina at Chapel Hill
Lee-Jen Wei, Harvard University

Office of Biotechnology Activities (OBA) Director/RAC Executive Secretary

Amy P. Patterson, Office of the Director (OD), NIH

***Ad Hoc* Reviewers and Speakers**

Natasha Caplen, Ph.D., National Cancer Institute (NCI)
Terry J. Fry, M.D., NCI
T. Jake Liang, M.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Nonvoting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, 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

Jacqueline Corrigan-Curay, OD
Stephen Creekmore, NCI
Kelly Fennington, OD
Linda Gargiulo, OD
Mary Groesch, OD
Robert Jambou, OD
Laurie Lewallen, OD
Maureen Montgomery, OD
Marina O'Reilly, OD
Dina Paltoo, National Heart, Lung, and Blood Institute, NIH
Eugene Rosenthal, OD
Karen Schweikart, NCI
Thomas Shih, OD

Others

There were 79 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 these Minutes.

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

Dr. Federoff, RAC Chair, called the meeting to order at 12:15 p.m. on December 5, 2006. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on November 13, 2006 (71 FR 66180). Issues discussed by the RAC at this meeting included public review and discussion of four protocols, a gene transfer safety assessment board report, and a presentation and discussion of FDA guidance on testing for replication-competent adenovirus (RCA) in adenoviral (Ad) vector-based gene transfer.

Dr. Patterson reminded all RAC members of the rules of conduct that apply to them as special Federal Government employees and suggested that any questions be addressed to the OBA committee management officers.

II. Minutes of the September 20, 2006, RAC Meeting/Drs. Somia and Weber

Dr. Somia noted that the September 20, 2006, RAC minutes were an accurate representation of the meeting. Dr. Weber noted that the minutes were clear and concise and accurately reflected the discussion and decisions of that meeting.

A. Committee Motion 1

It was moved by Dr. Weber and seconded by Dr. Somia that the RAC approve the September 20, 2006, RAC meeting minutes. The vote was 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

III. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Albelda, Federoff, and Heslop

Drs. Albelda and Federoff provided the report for the period of July 26, 2006, through October 10, 2006.

Dr. Albelda stated that 116 adverse events (AEs) were reported during this period, of which 114 were types A or C. Of the new initial reports, 13 were type A, and 6 were followup reports. No AEs required public discussion.

Dr. Federoff stated that the OBA received 16 protocol submissions, of which 12 were not selected for public review at this RAC meeting. Of those 12 protocols not selected, 9 were for cancer, and 1 each were for peripheral artery disease, heart failure, and limb girdle muscular dystrophy; 7 used plasmids; 2 used adeno-associated viral vectors, and 1 each used a pox viral vector, an adenoviral vector, and a herpes viral vector.

During the reporting period, 136 amendments were received by the OBA, of which 31 were site or principal investigator changes, 14 were protocol design modifications, 11 were protocol status changes, 38 were annual reports, 4 were responses to *Appendix M-I-C-1* of the *NIH Guidelines*, and 38 represented other amendments and notifications.

IV. FDA Guidance on Testing for Replication Competent Adenovirus (RCA) in Adenoviral Vector-Based Gene Transfer

Speaker: Daniel M. Takefman, Ph.D., FDA
Moderator: Dr. Vile

Dr. Vile provided background about this emerging issue regarding the level of contamination by RCA that is acceptable with any given clinical usage, including the use of *ex vivo* modified cells, and conditionally replicating oncolytic Ad vectors.

Dr. Takefman explained that the current recommendations with regard to RCA detection are contained in a November 2004 guidance document, "Content and Review of Chemistry, Manufacturing, and Control Information for Human Gene Therapy Investigational New Drug Applications." The current recommendation is that master virus banks and final vector clinical lots should be tested for RCA contamination, and that the maximal level of RCA should be less than 1 in $3x10^{10}$ viral particles, which is "as low as reasonably achievable given current capability." This level was based on data reviewed in current investigational new drug (IND) submissions and on manufacturing capacity, not safety. In July 2001 this recommendation was reviewed by the FDA's advisory committee as well as a working group from academia and industry that was working on Ad vector reference material. Both groups agreed that the recommended level would be achievable by industry and academia.

In terms of how to detect RCA contamination, the FDA asks that assays involve amplification by an *in vitro* culture system followed by a suitable end point assay. For RCA detection, most researchers choose A549 cells as an indicator cell line that is sensitive to support wild-type replication.

The FDA has been somewhat flexible about the level of RCA contamination. If a manufacturer has made a vector lot with contamination slightly above the allowed level, the FDA will, on a case-by-case basis, allow the vector to be used in clinical trials, following internal discussions with the entire review team.

Most vectors have some level of RCA contamination, even though that level is below current FDA recommendations. Therefore, vector doses of $1x10^{14}$ vector particles being administered to human research participants are likely to include up to 2,000 RCAs even when manufacturers are meeting the current FDA recommended level.

Because of confusion about *ex vivo* transduced cells, the FDA does not have requirements for testing cells that are being transduced *ex vivo* with Ad vectors. No lot-release testing for RCA is required, but on a case-by-case basis the FDA will ask the manufacturer to submit qualifying data to show that the cells they are administering are incapable of supporting Ad replication.

The use of oncolytic adenoviruses presents a challenge. While these vectors are designed to preferentially replicate in tumors, the vectors do still replicate at a lower level in normal cells. The level of selectivity should be assayed by determining titer in tumor versus normal cells. To detect wild type contamination, polymerase chain reaction (PCR) based assays are recommended. Dr. Takefman noted that the FDA is currently working on the report of the International Conference on Harmonization Workshop on Oncolytic Viruses to provide useful and harmonized recommendations that will help manufacturers produce oncolytic viruses.

A. RAC Discussion

The discussion following this presentation included issues such as the development of the guidance based on manufacturing ability versus safety, the flexible of the guidance, cytopathic effect (CPE) testing as part of lot-release testing, the difficulties of producing virus lots of consistent quality, and the fact that extensive culturing of secretions from research participants to look for RCA has been expensive but has not produced a positive culture.

In response to questions from RAC members, Dr. Takefman offered the following explanations and elaborations:

- Although the FDA tries to be somewhat flexible if a lot is slightly above the allowable level, the level was deemed achievable for most manufacturers by an *ad hoc* working group from academia and industry. The FDA believes that it was important to set a level that would ensure lot-to-lot consistency as well as vector preparation quality.

- It is hoped that even lower levels of RCA contamination will be seen in the future. The FDA wants to encourage manufacturers to make continued improvements in vector production methods and production of new cell lines that contain minimal helper sequences that, in theory, would help reduce levels of RCA contamination.

- The FDA does not ask for CPE testing for lot-release testing, primarily because it is not an adequately sensitive or precise assay.

- In clinical trials regulated by the FDA, no AE data relating to RCA have been shown.

- Most researchers are not doing infectivity assays but rather PCR-based assays to monitor for vector in serum and saliva. If the FDA requests that in a particular trial participant's secretions be cultured to look for RCA, such a request is not necessarily related to safety but likely to issues related to the licensing of gene transfer products, that is, to environmental assessment and regulatory requirement issues.

- Although the FDA guidance states, "We believe that an appropriate maximum level of RCA contamination would be less than 1 in $3X10^{10}$ virus particles," the FDA encourages investigators who have a lot that does meet this criterion to discuss the matter with the FDA before discarding that lot.

- The FDA's Good Manufacturing Practices concept called "quality by design" states that lot-release testing alone cannot be relied on to ensure product quality; quality must be built into a product and built into a process. Under that concept, manufacturers having a problem with RCA contamination are encouraged to try a different method and/or another cell line that has the minimal helper sequences.

B. Public Comment

Dr. Raj K. Batra, University of California, Los Angeles (UCLA), asked whether the FDA has decided on a specific cell line, other than A549, as a target for RCA. Dr. Takefman responded that the FDA asks only that an applicant supply data showing that the specific cell line has the appropriate level of sensitivity to qualify the RCA assay.

V. Discussion of Human Gene Transfer Protocol #0610-807: A Phase I Trial of Intratumoral Administration of Secondary Lymphoid Chemokine Gene-Modified Autologous Dendritic Cells in Advanced Non-Small Cell Lung Cancer

Principal Investigator:	Steven M. Dubinett, M.D., UCLA
Additional Presenters:	Felicia Baratelli, M.D., UCLA; Raj K. Batra, M.D., UCLA; Sherven Sharma, Ph.D., UCLA; Hiroko Takedatsu, M.D., Ph.D., UCLA; Gang Zeng, Ph.D., UCLA
Presenters by Phone:	Jay M. Lee, M.D., UCLA; Karen L. Reckamp, M.D., UCLA; Antoni Ribas, M.D., UCLA: Robert Suh, M.D., UCLA
RAC Reviewers:	Drs. Grant, Nemerow, Strome, and Vile

Dr. Kahn recused himself from consideration and discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Lung cancer is the leading cause of cancer death in the United States, accounting for more than 160,000 deaths in 2004—more deaths than prostate, colon, pancreas, and breast cancers combined. Lung cancer has a poor prognosis, and the median life expectancy for participants in the trial is six months.

The investigators hypothesize that intratumoral administration of autologous dendritic cells (DCs) that have been gene-modified *ex vivo* by infection with a replication-deficient Ad vector expressing the secondary lymphoid organ chemokine CCL-21 gene can be used to stimulate specific and therapeutic antitumor immunity in participants with advanced lung cancer. This hypothesis is based on extensive studies performed *in vitro* and in nonhuman animal models of lung cancer.

The objectives of the study are to determine (1) the safety and maximum tolerated dose (MTD) of CCL-21 gene-modified DC (Ad-CCL-21-DC) when administered as an intratumoral injection into a tumor site of participants with advanced NSCLC, (2) the local and systemic biologic activity (i.e. generation of anti-tumor immune responses) of Ad-CCL-21-DC when administered intratumorally, and (3) the clinical activity (i.e. reduction in tumor burden) of Ad-CCL-21-DC. The study is designed as a phase I, non-randomized, dose-escalating, multi-cohort trial enrolling participants in a modified 3+3 design.

B. Written Reviews by RAC Members

Four RAC members voted for in-depth review and public discussion of the protocol. Key issues included the (1) The transgene (CCL-21) has not been used in humans, (2) the diverse biological functions of the transgene have safety implications, and (3) there are safety issues related to the presence of replication-competent viruses and the type of assay that should be used to test for them.

Dr. Grant requested that the investigators provide information about the psychological evaluation that the study participants must undergo and the questions asked during the consent process. She was especially concerned about the stress involved in participating in this clinical trial. Dr. Grant also asked the investigators to elaborate on the accommodations they will make for additional behavioral counseling after the study concludes, if a participant requests such counseling.

Noting that the proposed starting dose is based on preclinical studies using murine cells, Dr. Nemerow wondered whether transduced human cells would produce the same levels of the CCL-21 chemokine. Asking for more discussion about the rationale for increasing the dose of DCs tenfold in the second cohort, Dr. Nemerow suggested a more gradual dose escalation because the potential side effects are not yet known. He asked how the concentration of chemokine at 48 hours compared to the amount of transduced human DCs needed to cause toxicity in mice. He also asked what percentage of human DCs would be transduced in the trial and whether the investigators plan to assess the presence of CCL-21 in tumor biopsies, as is planned for other cytokines. He suggested that it might be important to know the amount of RCA that is required to produce a CPE in DC cultures and how that amount of RCA compares with the currently accepted level of RCA for clinical use. Regarding informed consent document issues, he requested that the investigators provide a more quantitative risk assessment for bleeding and/or pneumothorax from the proposed bronchoscopy and highlighted a section in the document about risks associated with injection of DCs that the investigators agreed to eliminate.

Dr. Strome suggested that the investigators should consider adding an exclusion criterion for potential participants with limited pulmonary reserve, and post-study chest x-rays for all participants immediately following discharge. He asked what provisions will be made for participants who develop treatment-related complications (e.g., pneumothorax). He suggested that a single-site injection should be considered, since their proposal to inject participants reproducibly at four different tumor sites is exceptionally difficult. Also the investigators should rank the proposed biopsy studies in order of importance to the overall goals of this clinical trial, so that the same studies can be performed on all participants and evaluate the presence of tumor-specific antigens in the primary tumor biopsy. The investigators should consider phenotype, rather than genotype, when predicting the frequency of a given human leukocyte antigen in different populations, and consider pulsing only a fraction of the final product with the helper protein keyhole limpet hemocyanin (KLH).

Noting that this novel trial is well supported by preclinical studies that show efficacy with few AEs, Dr. Vile stated that his most significant safety concerns centered on the lack of data on the toxicity of the chemokine in humans, and he asked the investigators to provide a rationale for how they believe the murine data are related to human studies. Dr. Vile wondered whether the investigators had looked preclinically at the effects of CCL-21 on recruiting or activating potentially suppressive immune effectors (such as T_{reg} and suppressive DCs) and whether the investigators are concerned about how CCL-21 expression would influence the migration and localization of DCs once they are injected into the tumors. Noting that the investigators plan to pulse the DCs with KLH, he asked whether doing so would interfere with the ability to present tumor antigens from the tumor. Dr. Vile asked the investigators to discuss any available data on how freezing and then thawing the DCs transduced with the Ad-CCL-21 vector might alter DC form and function and chemokine expression. He requested that the investigators discuss the similarity among research participants in the amount of CCL-21 expressed from the injected DCs as well as the investigators' plans for monitoring and controlling differences in the amounts of expressed chemokine.

C. RAC Discussion

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

- Dr. Federoff asked whether a participant would receive an additional dose if that individual had significant bullous change and warranted insertion of a chest tube.

- Dr. Weber suggested three changes to the informed consent document: (1) The investigators should include a full list of the inclusion and exclusion criteria, (2) all references to this procedure as "gene therapy" should be changed to "gene transfer" or some other term that does not imply treatment, and (3) the risks of radiation from computerized tomography (CT) should be described.

D. Investigator Response

Dr. Dubinett explained that the investigators have had previous experience with injecting immune modulators through a bronchoscope, and he noted that individuals would be selected to participate in this protocol only if they did not have an airway compromised by an endobronchial lesion. Lesions that are not an airway tumor then would receive a transthoracic injection. Therefore, in addition to the exclusion criteria for respiratory status, the investigators would not accept into this clinical trial research participants who have either obstructing or near-obstructing lesions in the major bronchi.

To avoid the need to insert multiple chest tubes in study participants, Dr. Dubinett stated that the investigators decided that no research participant with grade 3 or greater toxicity would proceed in the study.

All of the tumors will be assessed by reverse transcriptase (RT)-PCR as part of the immune monitoring. Genotyping will be performed by PCR analysis in the Department of Immunogenetics at UCLA.

Regarding a participant's possible need for a chest tube due to bullous disease, Dr. Dubinett explained that the investigators would use the same criteria as the standard of care in clinical practice—transthoracic access to a tumor would not be used in an individual with bullous disease. He agreed to add language to the protocol and the informed consent document to indicate this.

Although the investigators are most interested in establishing the optimal biological dose, they are not able to state that goal because of the nature of the study and the number of participants in each cohort, according to Dr. Dubinett. From what they know thus far, they do not anticipate that the dosing regimen proposed for this trial will meet the MTD.

All prospective subjects will be offered participation in the Lung Cancer Support Group at the Cancer Center. Upon completion of the study, all subjects will maintain lifetime follow-up with the clinical investigators.

While DC form/function and CCL-21 release does not appear to be affected by freezing and thawing, the clinical protocol is being adapted to freeze the leukapheresis product rather than Ad transduced DCs.

In response to Drs. Strome and Vile's suggestion regarding KLH pulsing, the investigators anticipate pulsing only a fraction of the final product with KLH. To help determine what fraction of DCs should be exposed, they plan on testing DCs for their ability to present specific antigens under these conditions with or without KLH.

Addition of wild type Ad to DCs in culture (5pfu/10^6DCs) did not result in production of replication competent Ad at 72 hours. Issues related to in situ RCA production are further obviated by the changes in AdCCL-21 cGMP vector Lot# L0604006.

Approximately 13% of human DCs are transduced with the 1436 Ad vector particles. The human and murine DCs produced similar amounts of CCL-21 following transduction.

E. Public Comment

Public attendees offered no comments.

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:

<u>Preclinical</u>

- Given the inherent variability among Ad vector production lots, the investigators should consider whether each dose cohort can be normalized so that a more predictable amount of CCL-21 production, as a function of the number of DCs transduced, is introduced at a given dose level.

- Data will be gathered to assess the balance between the induction of an immunosuppressive and immunostimulatory response as it pertains to the biological action of CCL-21. Because the balance may shift toward immunosuppression as CCL-21 levels increase, the investigators should consider additional assays that would provide more detailed immunological characterization, particularly with respect to T-cell populations.

- Given that freeze-thawing of the transduced DCs may adversely affect cell viability, the number of viable and transduced cells should be standardized subsequent to freeze-thawing and prior to administration. The investigators' idea of addressing this issue by limiting the freezing to the leukapheresis product is reasonable.

- The stimulation of the cells with KLH may result in KLH being the primary antigen presented by the DCs, potentially at the expense of tumor antigens. The investigators' proposal to partially pulse the final product with KLH should address this concern.

- The presence of intracellular CCL-21 (fluorescence-activated cell sorter [FACS] analyses) in tumor biopsies should be assessed as will be done for other cytokines.

Clinical

- The Ad serotype 5 antibody status of participants should be documented because it may assist in clarifying the biological responses and be relevant to safety.

- The investigators should clarify the meaning of immunologic findings expected to be collected from the tumor biopsies and outline their value and priority with respect to the stated study goals in the protocol. Specifically, the protocol should describe how these results and their bearing on safety (MTD) will be interpreted in the absence of antigen-specific outcome data.

- The investigators should consider another exclusion criterion to screen for participants with limited pulmonary reserve in whom the risk of inflammation around the injected tumor or pneumothorax would constitute a significant safety concern. The entry criteria with respect to pulmonary status include a requirement that the participant have an FEV1 >1.0 L, a PCO_2 <44mmHg, and a PaO_2 >90 percent on room air. The investigators should exclude participants with a major endobronchial lesions that could result in potential airway compromise. The investigators should describe these additional exclusion criteria in detail in the protocol.

- As an additional safety precaution, post-treatment chest x-rays will be performed for all participants immediately prior to discharge after bronchoscopy or transthoracic CT-guided biopsy.

- The dose-limiting toxicity (DLT) for pneumothorax is defined as NCI Common Toxicity Grade 3 (development of sclerosis or the need for surgery). Given that this is a vulnerable population (particularly those with bullous disease) and that the insertion of a chest tube multiple times would be a morbid event in this population, it may be prudent to consider the insertion of a large bore chest tube to determine DLT. In addition, a transthoracic approach should not be used in a participant with adjacent bullous disease.

- It may not be feasible to conduct all of the proposed tests outlined in Aim 3.2.1 (Tumor Aspirate or Core Biopsy Specimen) given the extremely small size of the biopsies. Moreover, obtaining adequate cell numbers for FACS, immunohistochemistry, and quantitative PCR may not be possible. Thus, the investigators should consider ranking these studies in order of their

importance to the overall goals of the protocol so that the same studies can be performed on all participants.

Ethical/Social/Legal

- The investigators should add a summary to the informed consent document explaining the quantitative risks of the bronchoscopy procedure used to inject the tumors with the transduced DCs, particularly with regard to the risk of bleeding and/or development of a pneumothorax.

- The term "gene transfer" rather than "gene therapy" should be used to minimize the therapeutic misconception problem.

- The investigators should include inclusion and exclusion criteria in the informed consent document.

- Participants who request psychological counseling should be provided such counseling at no charge. In addition, the investigators anticipate that an upfront psychological evaluation of potential participants will be done under a separate protocol by different investigators.

- In describing the increased risk of radiation from the x-rays and CT scans that are required for research purposes, the investigators should describe the risk as a percentage of background radiation in a year.

G. Committee Motion 2

It was moved by Dr. Heslop and seconded by Dr. Weber that the RAC recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

VI. Day One Adjournment

Dr. Federoff adjourned Day One of the December 2006 RAC meeting at 3:15 p.m. on December 5, 2006.

VII. Day Two Opening

Dr. Federoff opened Day Two of the December 2006 RAC meeting at 8:30 a.m. on December 6, 2006.

VIII. Discussion of Human Gene Transfer Protocol #0607-784: A Phase I, Open-Label, Dose-Escalation, Pharmacodynamic Study of Intranodal Injection of Adenovirus-CD154 (Ad-ISF35) in Patients with Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

Principal Investigator:	Januario E. Castro, M.D., University of California, San Diego (UCSD) Moores Cancer Center
Additional Presenters:	Charles E. Prussak, Ph.D., Memgen, LLC; Thomas J. Kipps, M.D., Ph.D., UCSD; William G. Wierda, M.D., Ph.D., The University of Texas M.D. Anderson Cancer Center
Sponsor:	Memgen, LLC
RAC Reviewers:	Drs. Dewhurst, Heslop, and Kodish

A. Protocol Summary

Chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are diseases characterized by accumulation of slowly-dividing, mature-appearing monoclonal B lymphocytes in the blood, bone marrow, and lymphoid tissues. With an average annual incidence of 2.7 persons per 100,000, this

disease is the most common adult leukemia-lymphoma. There is no cure for CLL/SLL. This study proposes to assess the safety and potential clinical benefit of a novel immunostimulatory therapy. Approximately 23 leukemia patients, ages 19 through 28 years, with or without previous chemotherapy, will be enrolled in this study.

Recognizing that CD40/CD40 ligand (CD154) interactions play a critical role in immune activation, the study proposes to treat patients by directly injecting into pathologically enlarged lymph nodes a replication defective adenovirus that induces expression of a functional and stable chimeric ligand of CD40(CD154)-Ad-ISF35. We hypothesize that injection of Ad-ISF35 will lead to expression of CD154 in the injected lymph node tissue and promote immune activation against leukemia-lymphoma cells.

The primary objectives of the study are to determine the safety and maximum tolerated dose of Ad-ISF35 when injected directly into lymph nodes of patients with CLL/SLL. The secondary objectives are to determine and monitor the clinical and biological responses and the pharmacodymamic parameters in patients treated with intranodal injections of Ad-ISF35.

B. Written Reviews by RAC Members

Twelve RAC members voted for in-depth review and public discussion of the protocol. Key issues included the significant immune system risks associated with reengineered immunostimulatory molecules, in particular the possibility of a life-threatening "cytokine storm." In addition, because the Ad-ISF35 vector construct closely resembles the human CD40 ligand, it could bind directly to CD40 on malignant cells and possibly promote their growth.

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

Noting that his review was colored by events occurring in March 2006 in the TeGenero monoclonal antibody trial in the United Kingdom, Dr. Dewhurst raised several questions about the preclinical data and models. He asked for updated information about the ongoing *ex vivo* gene transfer protocol using Ad-ISF35 and for further information about the grade 2 edema and capillary leak reported in the ISF154 trial. He also asked about the affinity of the CD154 (CD40L) proteins encoded by Ad-ISF154 vs. Ad-ISF35 for human CD40, and the cell-surface copy number of the CD154 (CD40L) proteins encoded by Ad-ISF35 in CLL cells exposed to a fixed multiplicity of infection (MOI) of each of the corresponding viruses. Dr. Dewhurst also requested that the investigators explain the rationale for modifying the transgene encoded by Ad-ISF35 so that it lacks the normal matrix metalloprotease cleavage site found in CD154.

Dr. Heslop also asked the investigators to provide an update on the results of the Phase II study and the ongoing clinical trial using this vector to transduce autologous CLL cells (RAC Protocol #0601-757). She also asked the investigators to comment on whether it might be necessary to discuss within the informed consent document the serious AEs from the March 2006 TeGenero study in the United Kingdom. She suggested that tetramer analysis be used to study CTL responses to adenovirus in the research participants.

Dr. Kodish limited his review to the informed consent document. He noted the following concerns:

- In the Study Purpose section, the investigators should include a clear statement about the expectation or lack thereof of benefit to the participant.

- The investigators should include some information about the sponsoring company, Memgen, LLC, which is mentioned without elaboration in the Compensation section.

- The investigators should better explain how leukapheresis fits into the research plan.

- The investigators should reword some of the technical terms to make them more understandable.

- The investigators should refrain from using terms such as “therapy” and “treatment.”

C. RAC Discussion

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

- Dr. Weber suggested adding the exclusion criteria to the informed consent document prior to screening, instead of taking prospective participants to the screening process before checking on exclusion criteria.

- Dr. Ertl noted that it has been shown that preexisting antibodies to Ad5 increases the toxicity, a counterintuitive result that has been observed in gene transfer trials in nonhuman primates. She asked whether the investigators would monitor participants with regard to preexisting antibodies to Ad5 to take that potential safety implication into consideration.

- Dr. Nemerow noted that a drop in platelet counts is a feature of Ad-mediated toxicity that the investigators should watch for and evaluate.

- Dr. Weber asked the investigators how they would ensure that all doses are injected into the lymph node and how they would prevent the possibility that toxicity seen at a higher dose was due to mistaken injection into a vein or artery rather than into a lymph node.

- Dr. Grant suggested that the investigators evaluate the psychological competence of the participants to make sure they are competent to consent.

- Noting that they will likely see very low transduction rates, Dr. Albelda wondered whether the investigators are planning to look for gene transfer or to biopsy tumors to ascertain whether this protocol has succeeded.

- Dr. Strome asked the investigators whether and how they have looked for evidence of lymphoma proliferation with human CD40 ligand molecules. With the fully humanized version of this molecule to be delivered intranodally by Ad vector, there is a possibility of proliferation.

- Dr. Vile noted that if the pharmacodynamic studies show no changes but the trial is safe, the investigators would have no readout at the end of the trial as to whether their procedure had transduced a single cell. Dr. Federoff added that it would be in the interest of the investigators to know not only whether this procedure and the vector will be well tolerated, but also at some point it would be imperative to know whether a transgene had been delivered and expressed.

- Dr. Albelda suggested an addition to this trial that would include dosing to the MTD, then dosing three additional participants by injecting two nodes, one of which would be biopsied to allow for analysis of gene transfer.

D. Investigator Response

Dr. Castro and Dr. Kipps provided an update of the previous related trials. A phase I and II trial used an adenoviral vector expressing mouse CD154, which favorably affected the general rates of disease progression in all treated participants. Most treated participants experienced flu-like symptoms that resolved within one week and none experienced $\geq$ 2 grade hepatotoxicity. Analysis of plasma cytokine levels over the duration of treatment revealed high levels of cytokines typically observed in Th1-type immune responses.

Dr. Wierda presented a summary of the ongoing phase I trial at MD Anderson Cancer Center using Ad-ISF35 to modify CLL cells *ex vivo*. To date five of seven planned research participants have been dosed with no dose-limiting toxicities or SAEs.

A discussion of the potential for immune activation leading to the production of cytokines that could contribute to some of the SAEs observed in the TeGenero trial will be included in the informed consent form along with the other modifications suggested by the reviewers.

In response to Dr. Dewhurst's question regarding the cell surface copy number of CD154/Cd40L proteins encoded by Ad-ISF154 vs. Ad-SF35, transduction experiments with the vectors in CCL cells showed higher levels of CD154 transgene expression in cells transduced with Ad-ISF35.

Studies of the ligand-receptor affinity of the proteins encoded by Ad-ISF35 or Ad-ISF154 have not been performed due to the complexities of evaluating interactions that could be affected by differences in avidity, receptor-ligand multimerization, and ligand valence. Functional studies have indicated that the ligand encoded by Ad-ISF35 is able to induce CD40-ligation resulting in up regulation of immune-co-stimulatory molecules on leukemia B cells in a fashion similar to the ligand encoded by Ad-ISF154.

ISF35 cannot be cleaved by matrix metalloproteases that cleave ISF154. ISF35 cannot be released readily from the plasma membrane or generate a soluble molecule capable of effecting CD40-ligation, minimizing the risks of developing the systemic toxicity caused by soluble CD154.

Dr. Kipps explained that the generalized grade 2 edema and capillary leak was observed in the same research participant following the second infusion. This type of AE was not observed among the other 17 research participants receiving Ad-ISF154 or those receiving Ad-ISF35.

Dr. Kipps confirmed that the investigators will monitor all the cytokines that are released systemically and that looking for changes in cytokine profiles will be an important part of participant monitoring.

Noting that this trial will include advanced-stage participants, Dr. Kipps stated that the investigators are acutely aware that the participants are already immune compromised and therefore are beginning the dose escalation at a level that could be considered conservative, a schema different from that employed by many chemotherapy trials.

Dr. Castro explained that the protocol includes a section that describes how the doses are calculated and that takes into account the three-dimensional volume of the lymph node to be injected. Volumes have been selected carefully so as not to exceed more than one-third of the lymph node and to prevent potential risk.

Dr. Kipps explained that it has not been possible to address directly the concern regarding induction of proliferation with CD40 ligand because the investigators have not been able to culture leukemic cells *in vitro*; something is impairing the expression. They have been able to drive high levels of expression of the transcript in CLL cells but cannot get the protein expressed, nor have they been able to see activation of immune costimulatory molecules or death receptors. In large measure, that result is likely explained by the induction of p21, which is very strong with CD40 ligation due to the induction of p53 and p73. Regarding the possibility of mass increase in size, he explained that other trials that have attempted to activate immune response occasionally have resulted in expansion of some of the tumor masses before shrinking begins. Tumor-mass effect will be monitored in this clinical trial. With a total of 22 participants in the other trials who have been injected with up to $3x10^9$ CD40 ligand-expressing cells, some increase in leukemic cell counts and lymph node size would likely have been seen if leukemic cell proliferation had been induced. The investigators will measure lymph node size distal to as well as at the site of injection.

In response to the suggestion of several RAC members that needle-aspiration biopsy of the injected lymph node be done, the investigators declined to include this procedure in their Phase I trial for reasons that included reduction of overall safety of the trial, lack of usable information, potential morbidity (hematoma formation and infection), and participant discomfort with the procedure.

In response to Dr. Albelda's suggestion to add three participants at the MTD level and biopsy one of their lymph nodes, Dr. Kipps stated that such a trial extension could be covered in future studies. He stated

that the investigators would prefer not to complicate or compromise the primary safety study, but they realize that this suggestion would be a natural extension to the protocol at some future point.

E. Public Comment

Public attendees offered no comments.

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:

Scientific/Medical/Study Design Issues

- To quantify the dosage more accurately and generate a body of data that may be useful in determining subsequent dosing, it is important to assess transgene delivery to the nodes as well as dose and expression levels. However, since it will be difficult to measure transduction efficiency directly without performing a biopsy, another approach may need to be developed.

- The plan to monitor cytokine responses and any shifts in patterns of cytokine release may be a useful safety measure given the risk of triggering an acute immune response (a grade 2 edema and capillary leak developed in a participant in Protocol #0601-757). However, because host factors also play a role in immune response, cytokine response measurements may be of limited utility in guiding dose determinations or adjustments across an entire cohort or subsequent cohorts.

- Cytotoxic T-lymphocytic responses to adenovirus should be evaluated, for example, by tetramer analysis.

- In the preclinical studies using the mouse model, the cause of cell elimination may have been due at least in part to murine immune responses to the human ligand, an unlikely effect when humanized CD40 ligand and autologous cells are being used in the research participants. Intensive monitoring for any evidence of progression of disease, including the size of lymph nodes at the injection site and at peripheral sites, is important. Nodal biopsy also would be useful in characterizing the cell population, nodal architecture, and presence of any inflammatory infiltrate.

- The main objective of the protocol is to determine the MTD. Ultimately, a complete safety assessment requires an analysis of the effects of both the Ad vector and transgene expression. Feasible approaches for measuring transgene expression are needed, and the appropriate timing of those measurements should be carefully determined. Having these data in hand during the evaluation of tolerability will allow toxicity due to transgene expression to be distinguished from other factors and shed important light on the safety and efficacy of (a) given dosage(s).

Ethical/Legal/Social Issues

The informed consent document should be modified in the following ways:

- The benefits section should clearly state that the purpose of the protocol is to assess safety and that direct benefits cannot be expected.

- The investigators should describe the relevance to the protocol of the severe AEs experienced by participants in the TeGenero monoclonal antibody study.

- In the Optional Procedures section, the term "treatment" could mislead prospective participants about the potential study benefits, and the investigators should replace it with other wording such as "in order to participate in this study."

- The investigators should clarify the wording "potentially dead of the local skin," which is not clear.

- The investigators should define the term "leukapheresis" and provide an explanation about how it fits into the research plan.

- The investigators should clarify the sponsoring company's role in the study.

G. Committee Motion 3

It was moved by Dr. Albelda and seconded by Dr. Grant that the RAC recommendations, summarized orally by Dr. Federoff to include a variety of preclinical, clinical, and consent form issues, be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 16 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IX. Discussion of Human Gene Transfer Protocol #0610-813: A Pilot Study of Genetically Modified Haploidentical Natural Killer Cell Infusions for B-Lineage Acute Lymphoblastic Leukemia

Principal Investigator:	Wing Leung, M.D., Ph.D., St. Jude Children's Research Hospital
Additional Presenters:	Dario Campana, M.D., Ph.D., St. Jude Children's Research Hospital; and Arthur W. Nienhuis, M.D., St. Jude Children's Research Hospital
RAC Reviewers:	Drs. Albelda and Ertl and Ms. Shapiro
Ad hoc Reviewer:	Terry J. Fry, M.D., NCI, NIH

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

A. Protocol Summary

Acute lymphoblastic leukemia (ALL) is the most common form of cancer in children. Cure rates are about 80 percent in children but only about 40 percent in adults. Some subgroups of ALL patients are resistant to currently available treatments, including those with unfavorable genetic abnormalities, infant ALL, relapsed ALL, and refractory ALL.

Modern frontline therapy for patients with B-lineage ALL is based on intensive administration of multiple drugs. Persistent leukemic cells that are resistant to chemotherapy cause leukemia relapse. In patients with relapsed disease, response to the same drugs after relapse is generally poor, and dosages cannot be further increased without unacceptable toxicities. For most patients, particularly those who relapse while still receiving first line therapy, the only therapeutic option is hematopoietic stem-cell transplantation (HSCT). For patients who relapse after transplant or who are not eligible for transplant, there is no other available therapeutic option.

There is mounting evidence that natural killer (NK) cells have powerful antileukemic activity. In patients undergoing allogeneic HSCT, several studies have demonstrated NK-mediated antileukemic activity. NK cell infusions in patients with primary refractory ALL or multiple-relapsed ALL have been shown to be well tolerated and void of graft-versus-host disease effects.

NK cell cytotoxicity is most powerful against acute myeloid leukemia cells, whereas their capacity to lyse ALL cells is generally low and difficult to predict. The investigators have developed a novel method to redirect NK cells toward CD19, a molecule highly expressed on the surface of B-lineage ALL cells. In this method, NK cells are first expanded from peripheral blood by co-culture with the irradiated K562 cell line modified to express membrane-bound interleukin (IL)-15 and 41BB ligand. This modified cell line

promotes vigorous and selective growth of NK cells. Then, the expanded NK cells are transduced with a signaling receptor that binds to CD19. NK cells expressing these receptors show powerful antileukemic activity against CD19+ ALL cells *in vitro* and in a nonhuman animal model of leukemia.

This study will assess the safety of infusing genetically modified NK cells into research participants who have chemotherapy refractory or relapse B-lineage ALL. In this same cohort, the investigators intend to study the *in vivo* lifespan and phenotype of genetically modified NK cells and explore the efficacy of NK cells in individuals with B-lineage ALL.

B. Written Reviews by RAC Members

Five RAC members voted for in-depth review and public discussion of this protocol. Key issues included the use of a complex transgene contained in a retroviral vector that has the potential to cause insertional mutagenesis and the need to discuss additional ethical considerations because children will be enrolled. Three RAC members and one *ad hoc* RAC member provided written reviews of the proposed study.

Dr. Albelda asked the investigators to provide preclinical data showing the effects of the modified NK cells in an immunodeficient mouse model engrafted with human leukemia and to explain why the modified NK cells don't affect normal B cells expressing CD19. Regarding the involvement of a pediatric population for this protocol, he asked the investigators to review both the treatment algorithm for treating B-lineage lymphoma and the rationale behind their belief that the risk of a second malignancy due to insertional mutagenesis is low enough in this proposed study to justify the use of this approach in children. Dr. Albelda also requested that the investigators discuss the potential toxicity issues of using IL-2 in children. Noting that the investigators propose to use preparations with as low as 10 percent CD19 positivity, a low level of acceptable transduction, Dr. Albelda asked the investigators to justify using cells in which up to 90 percent would not have the CD19 transgene and suggested that the investigators consider making this criterion more stringent.

Dr. Ertl asked whether a trial like this had ever been conducted in individuals with B-lineage ALL. She asked whether NK or T cells could become activated or proliferate in response to CD19 ligation. In response to the investigators' statement that transduced NK cells have no proliferation advantage in immunodeficient mice used as leukemia models, Dr. Ertl asked whether IL-2 was used in these studies, whether similar studies were conducted in immunocompetent mice, and whether the NK cells were transduced with mouse CD19. Noting that CD19 is universally expressed on B cells, including early B-cell precursors, she asked how the investigators plan to address the effect of treatment on B cells.

Ms. Shapiro asked that the investigators justify including children concurrently with adults as participants in this protocol by discussing the following issues: whether/how the target disease and the prevalence of the target disease differ in children compared with adults, whether and how standard treatment and response to standard treatment differ in children, and whether and how anticipated response to the protocol intervention is likely to differ in children. In addition, Ms. Shapiro offered a detailed critique of the various informed consent documents, as follows:

- The investigators should modify the Phase I and Phase II informed consent documents to appropriately protect the confidentiality of recipients when participant-donors request information about the results of the study.

- The investigators should clarify the payments of costs associated with research-related injury.

- In the document for NK cell recipients who are younger than 18 years of age, the investigators should change the language regarding an older child's assent to indicate that that child's failure to assent would be taken into account.

- The investigators should further explain the extent, if any, of potential xenotransplant infectious disease risk that may exist when small amounts of mouse proteins and iron particles are added to donor blood cells.

- The investigators should make clear how much time will elapse after termination of study participation before they will notify participants about new information learned during the study and about significant findings related to the gene transfer agent.

- The investigators should ensure that the age of assent is consistent throughout the informed consent documents.

- The investigators should state the costs of follow-up treatment more explicitly in the various informed consent documents, specifically, who pays for return visits to St. Jude and who bears the cost of blood draws for replication-competent retroviral (RCR) testing conducted at other medical facilities.

Dr. Fry noted that one of the safety features of this protocol is that the cells will not persist because of immunologic rejection, which may not occur if participants are dosed after haploidentical stem-cell transplantation from the same donor; therefore, the investigators should add eligibility criteria to address this concern. Because the listed eligibility criteria are broad, he wondered whether all relapsed ALL patients would be eligible and whether matched unrelated donor availability constitutes an exclusion. Dr. Fry asked whether the transduced receptor would be able to overcome the inhibitory signal that might be expressed for the infused NK cells and whether toxicity attributable to IL-2 would be dose limiting. Regarding the secondary end point for the trial, Dr. Fry asked the investigators to discuss how they plan to distinguish a response to the conditioning regimen from an efficacy response to the infused NK cells.

C. RAC Discussion

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

- Dr. Nemerow wondered how the MOI for the transduction experiments compares with the French X-linked severe combined immune deficiency study and whether it would be possible to use a lower MOI to achieve the same level of transduction.

- Regarding the consent process, Dr. Kodish asked the extent to which alternatives to the proposed protocol would be offered to older children and their families.

D. Investigator Response

Dr. Campana explained that this will be the first trial to investigate the use of NK cells expressing anti-CD19 receptors. However, the investigators had carefully considered the issue of including children in the trial in the belief that the potential for benefit or relative risk cannot be adequately addressed in adults because of the differences in prevalence and biology between childhood and adult ALL. ALL is the most common form of cancer in children, constituting approximately one fourth of childhood malignancies. The incidence of ALL is highest between the ages of 2-4; it decreases during later childhood, adolescence, and adulthood. Treatment protocols for children and adults with ALL are similar but produce cure rates typically higher than 80% in children versus 30%-40% in adults, a discrepancy partly due to the different frequency of genetic subsets between the two patient populations. Despite treatment success for childhood ALL, leukemia that is refractory to chemotherapy occurs in a proportion of children. These predominantly include leukemias with adverse genetic abnormalities, and leukemias that recur while patients are receiving chemotherapy or after hematopoietic stem cell transplantation. For patients with these types of ALL, there is no proven curative therapy. The options that are typically discussed by the primary attending physician with the family include participation in Phase I/II studies, donor lymphocyte infusions (for patients who relapse after transplant and the donor is available), palliative chemotherapy or hospice care. Participation in the NKCD19 protocol will not preclude receiving the other types of care later.

Dr. Campana explained that NK cells become activated in response to CD19 ligation but do not significantly proliferate. T cells do proliferate in response to CD19 ligation via the receptor. However, the

number of T cells transduced with the receptor should be low, because under the culture conditions used, T cells do not significantly proliferate. Moreover, a T cell depletion step is included before infusion of the NK cell product.

In response to Dr. Ertl's question regarding the effect on B cells, the investigators responded that in addition to monitoring the CD19+ blood cell count, they will measure the Ig levels once a month and will give the participants IVIG if their IgG level is lower than 500 mg/dl. They noted that conventional chemotherapy alone profoundly reduces humoral immunity in children with ALL, and CD19+ B-cell progenitors become undetectable after only two weeks of remission induction chemotherapy.

The risk of insertional mutagenesis in NK cells should be lower because NK cells, in contrast to hematopoietic stem cells, have a limited life-span and self-renewal potential. Experiments were performed in which NK cells from four donors were transduced with a retroviral vector and then stimulated with pulses of the K562-mb15-41BBL cell line. NK cells expanded but invariably underwent senescence and died after 3-6 months of culture. Also the risk of malignancy developing from the transduced NK cells should be low due to the fact that donor NK cells will be eliminated by the resurgent cellular immunity of the recipient after the effects of the transient immunosuppression caused by the conditioning regimen have ceased.

Regarding the potential toxicity issues of using IL-2 in children, both Dr. Campana and Dr. Leung stated that a relatively low dose level is proposed for the trial. Capillary leak syndrome is one of the major potential risks. However, six patients with acute myeloid leukemia received identical conditioning and IL-2 before infusion of primary, unmodified NK cells, and none developed toxicities above grade II.

In response to Dr. Albelda's comment regarding the low transduction level, the criteria was made more stringent and the minimum expression of anti-CD19 receptors on NK cells was raised to 30%. From the preclinical studies, transduction efficiencies should be well above this threshold.

In response to Dr. Fry's questions about the eligibility criteria, the criteria were made more stringent and will include only those patients refractory to chemotherapy or those who relapse after transplant. These patients would not be eligible for transplant at the investigators' institution. Also another haploidentical family donor for adoptive NK cell therapy will be used if a patient has leukemia relapse after haploidentical stem cell transplantation.

Regarding how the investigators plan to distinguish a response to the conditioning regimen from a response to the NK cells, Dr. Campana explained that they will monitor the presence of leukemic blast in the blood before and during the conditioning regimen and after NK administration to provide an idea of response to the leukemic cell population. Participants with circulating blasts will have nearly real-time measurement of their leukemic burden. To maximize detection sensitivity, the investigators plan to use both flow cytometry and PCR to track the leukemic cells.

Expressing doubt that a lower MOI would produce the same level of transduction, Dr. Campana explained that the MOI used is 4 to 6, and it is used in participants who already have very aggressive leukemia. Dr. Nienhuis further explained that mature lymphocytes—in contrast to immature lymphoid progenitors—are not susceptible to the clone dominance that often precedes transformation.

Dr. Nienhuis stated that the investigators will assure participants that St. Jude will bear the costs directly related to participation in this protocol and any complications that may occur as a consequence of this protocol.

E. Public Comment

Public attendees offered no comments.

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:

Scientific/Medical/Study Design Issues

- The protocol involves the administration of haploidentical NK cells that have been transduced with a murine retroviral vector expressing a chimeric receptor consisting of the single-chain variable region of a murine anti-CD19 monoclonal antibody and signaling domains of 4-1BB and CD3 zeta. Several aspects of this process raise safety issues. Because the transduction uses an MOI of 4 to 6, multiple vector integration events may occur in the transduced cells. The CD3zeta signaling domain of the chimeric receptor is a T-cell proliferation signal; therefore, T-cell contamination and the potential for T-cell transduction are of concern. To address these concerns, it will be important to quantitate the number of residual T cells in the final product, minimizing them and T-cell transduction as much as possible. It will also be important to be especially vigilant in followup monitoring for T-cell transduction and proliferation events.

- According to the protocol, modified NK cells to be used for infusion include preparations with transduction efficiency as low as 10 percent of NK cells expressing anti-CD19 receptors. This threshold is too low; at a minimum, the acceptable transduction efficiency of NK cells should be 30 percent.

- After participants' immune systems have recovered from the intervention, as measured by the level of B cells, children should be revaccinated against childhood infections following standard procedures.

- The investigators should include in the protocol a description of how participants will be prioritized for participation.

- If a patient has had a relapse of disease after stem-cell transplantation, the same stem-cell donor should not be used again. A different member of the family who is also haploidentical will ensure that the infused NK cells do not persist.

- Because the conditioning regimen itself may have salutary effects, it will be important for the investigators to screen peripheral blood for the presence of leukemic blast cells throughout all stages of the procedure (i.e., before, during, and after conditioning and after the NK cell infusion). A sensitive assay such as flow cytometry and PCR should be used.

Ethical/Legal/Social Issues

During the protocol review, a number of recommendations were made about the informed consent document. The investigators indicated during the RAC meeting that all of the recommended changes would be made. Those comments were attached to the RAC letter, which also included two more highlighted comments that were reiterated during the RAC meeting.

- The statement "whether or not your child takes part in this study is entirely up to you" gives the impression that the participant's wishes are not considered and that the assent process is not meaningful. The investigators should modify the consent document to clarify that the child's wishes will not be disregarded.

- The investigators should clarify in the consent form whether participants will be responsible for costs that are directly related to participation in the study, including the cost of follow-up visits and the cost of blood draws for RCR and other tests performed at outside medical facilities.

G. Committee Motion 4

It was moved by Dr. Weber and seconded by Dr. Kodish that the RAC recommendations, summarized orally by Dr. Federoff to include a variety of preclinical, clinical, and consent form issues, be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 15 in favor, 0 opposed, 0 abstentions, and 1 recusal.

X. Discussion of Human Gene Transfer 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

Principal Investigator:	Robert G. Gish, M.D., California Pacific Medical Center
Submitter:	C. Satishchandran, Ph.D., Nucleonics Inc.
Additional Presenters:	Catherine Pachuk, Ph.D., Nucleonics Inc., Patrick Romano, M.D., M.P.H., University of California, Davis
RAC Reviewers:	Drs. Dewhurst, Kahn, and Kirchhoff
Ad hoc Reviewers:	Natasha Caplen, Ph.D., NCI; and T. Jake Liang, M.D., NIDDK, NIH

A. Protocol Summary

Approximately 5 percent of the world's population is infected with hepatitis B virus (HBV), which is transmitted through sexual contact, intravenous (IV) drug use, and perinatal exposure of newborns to maternal HBV-infected blood. Although most adults infected with HBV recover spontaneously, 5 to 10 percent develop persistent infections; 90 percent of infants infected at birth develop chronic infections. As a result, more than 300 million people worldwide are chronically infected with HBV, and there are approximately 1 million chronically infected persons in the United States.

Liver pathology associated with HBV infection is primarily immune-mediated and is thought to be due to cell-mediated immune responses directed against infected hepatocytes. Treatments that target antigen expression as well as viral replication offer a significant advantage over existing treatment options because they decrease the visibility of infected hepatocytes to the immune system, thereby decreasing the severity of chronic inflammation/hepatitis and secondarily preventing the development of HCC. Such therapies have the potential to be beneficial for healthy carriers as well, a subset of patients who are at risk of developing chronic active HBV. In addition, a subset of patients exhibiting nonimmune-mediated liver pathology caused by large HBV surface antigen overexpression is also likely to benefit from treatment.

Therapies employing ribonucleic acid interference (RNAi) have the potential to inhibit viral replication and down regulate antigen expression simultaneously by a mechanism of action different from that of currently available therapies. RNAi-based strategies also have the potential to prevent the selection of escape mutants. NUC B1000 is designed to target four separate regions of the viral genome.

The initial phase of this protocol will focus on the evaluation of basic safety in research participants with mildly to moderately active disease. This Phase I study is expected to delineate the safety of the NUC B1000 over a dose range of two orders of magnitude to assess the potential, if any, for an immunologically based serious adverse response.

B. Written Reviews by RAC Members

Thirteen RAC members voted for in-depth review and public discussion of the protocol. Key issues included the fact that the shRNA-expressing plasmid administered via IV injection has not been tested in humans, and the current understanding of the safety of RNAi gene transfer in humans is limited. Other issues that need further discussion include the rationale for using a short-lived vector in a chronic disease, lack of a suitable HBV animal model, evidence from preclinical data that the plasmid can migrate

to other organs including heart and lungs, the safety of the DNA complex particularly at the highest dose, and potential toxicities to the liver, particularly given that the participants have chronic liver disease.

Dr. Dewhurst expressed fundamental reservations about whether the preclinical data support this proposed protocol. The product has been tested in "highly artificial," small-animal models, and he asked the investigators to explain why they had not used more biologically relevant models. Dr. Dewhurst noted that, although this protocol is a safety trial, benefit has not been established by the preclinical data; because the investigators propose to use the most plasmid DNA ever delivered to humans, the standard to proceed should be high. He noted that the investigators conclude a strikingly high effectiveness of RNAi against all targetable messenger RNAs and asked the investigators to explain why they believe they can achieve such results when other researchers report considerably lower knockdown values. The safety issues raised by Dr. Dewhurst included preclinical mice data that showed a significant loss of body weight and enlargement of the spleen, and he asked whether the investigators had examined the effect of their product on cellular microRNA (miRNA) levels and diversity. In addition, Dr. Dewhurst requested updated information on the effect of the experimental product on cultured hepatocytes and further elaboration on how the investigators propose to evaluate the effects of the product on innate and adaptive immunity in mice. With regard to the informed consent document, Dr. Dewhurst noted that the Risks section of the document equates results from mouse studies with expected outcomes in humans, which may inappropriately encourage participants to believe that the experimental product is safe. He reiterated a compelling need for at least one biologically relevant animal (preferably a nonhuman primate) model to test the safety and efficacy of IV delivery of this plasmid.

Dr. Kahn limited his review of this protocol to the informed consent document, noting that consent documents are stand-ins for the full process of informed consent. He asked whether there was sufficient information and detail for potential participants to understand what they are being asked to participate in and whether the investigators had provided sufficient protection of their rights and interests as research participants, in general noting a lack of description of the experimental agent and what participants would be expected to do. Dr. Kahn noted a number of overstatements of the potential treatment aspect and also pointed out the need to make clear that this experimental agent is not an antiviral and how it is different. Other points discussed by Dr. Kahn regarding the informed consent document included the following:

- The investigators have not provided enough information regarding the rising-dose aspect of the proposed trial.
- The investigators should discuss autopsy in the document.
- The Compensation section includes statements about research injury, which should not be placed under that heading.
- Followup is shown as lasting for 9 months, but only 6 months of antivirals are to be provided; the investigators should explain or fix this discrepancy.
- The 9-week blood draw is not listed on the calendar/chart in the document.
- Dr. Kahn suggested several wording changes to clarify and better organize the document.

Dr. Kirchhoff suggested that some other animal model intermediate between mouse and human should be explored. He explained that duck or woodchuck models have been used for HBV; although the woodchuck model might require some developmental work, it would make a positive contribution to the field that would allow other researchers to use that model in the future. Regarding the informed consent document, Dr. Kirchhoff suggested that the investigators reduce the amount of medical/scientific jargon by testing the entire text with people who are not scientifically knowledgeable. *[Dr. Kirchhoff did not submit a written review of this protocol; his comments were provided orally at this RAC meeting.]*

Dr. Caplen requested the experimental data supporting the statement that NUC B1000 is effective against multiple genotypes and drug resistant mutants. She suggested that the agent be tested in an HBV expressing model system. She requested discussion of the clinical implications of the 1% transduction of target cells observed in the mouse model, and the off-target effects on other mRNAs and microRNAs. She noted that for the IV injection delivering the plasmid DNA, the proposed doses are large in relation to previously conducted gene transfer trials. It is not likely that a mouse model will be sufficient to establish that there will be no immune responses in humans at the doses proposed for this clinical trial. Other models may need to be investigated.

Dr. Liang explained that his major concern was whether the efficacy shown in the protocol submission materials justifies a human clinical study. The experimental product appears to work well *in vitro*, and the data show that it is effective in that setting, but the *in vivo* data are not convincing. He suggested the study of transgenic mice expressing HBV. He suggested further study of immune responses including detection of anti-DNA antibodies, inflammatory responses to CpG sequences and induction of an interferon response. Dr. Liang also expressed concern about the potential integration of the plasmid DNA and noted that the animal studies do not parallel what is likely to be seen in humans. He also expressed several minor concerns about the informed consent document, including that the duration of treatment should be spelled out for participants; it likely would be much longer than the 6 months of standard antiviral therapy offered by the sponsors of this trial.

C. RAC Discussion

After the RAC reviewers offered their reviews, Dr. Federoff summarized and prioritized the concerns expressed by the reviewers: the adequacy of the preclinical model to study the efficacy of this approach, the effect on miRNAs, the types of immune responses that might be elicited, and 9 months of followup but provision of antivirals for only 6 months.

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

- Dr. Dewhurst expressed concern about the investigators using a single-dose study when the long-term strategy is for multiple inoculations.

- Because, according to the investigators, the transgenic mouse model appears to be difficult to use, Dr. Ertl suggested the investigators to use large nonhuman mammals for some of the preclinical biodistribution studies.

- Dr. Caplen agreed that, at the end of this study, the investigators will have information about whether the expression of short-interfering RNAs (siRNAs) in a small number of hepatocytes has any untoward effects. However, she expressed concern that this study does not include a direct test to show that siRNA reached the hepatocytes.

- Dr. Weber expressed concern that the planned small numbers of participants coupled with rapid dose escalation could result in missing serious AEs, including death, an acknowledged problem with Phase I trials in general.

- Considering that the pathogen receptors of humans are differently distributed from those in mice and recognizing different patterns on plasmid DNA, Dr. Ertl asked whether the investigators have used a human cell type such as DCs to assess whether the vectors elicit an innate response.

- Dr. Strome expressed concern about the inclusion of participants who are doing well but have a long-term risk and for whom the potential benefit (based on this clinical trial) is so low as to be almost zero.

- Dr. Kahn requested further clarification about the mismatch between the length of followup and the length of time participants will receive antiviral therapy.

D. Investigator Response

Regarding relevant animal models, Dr. Pachuk stated that the two major models for HBV are mouse and chimpanzee models. The chimp model most closely resembles human infection; however, chimps do not develop disease and their scarce availability limits their use. Studies were attempted using transgenic mice in which the HBV genome is integrated into the mouse genome. The transgenic mice, however, showed inconsistent and large variations in viral expression and replication and in serum HBsAg levels.

The 1% transduction of hepatocytes was determined by Q-PCR analysis, however, efficacy studies indicated a >20% reduction of serum HBsAg. This discrepancy is under investigation. The reduction in HBsAg produced by infected cells may allow patients to overcome the specific immune anergy and exhaustion of T and B cells. Also, repeated dosing should progressively increase the number of transduced cells.

Regarding the higher than routinely reported levels of siRNA knockdown observed, the data indicate the antigen mRNAs, viral replication intermediates, and viral titer are reduced to undetectable levels. While the investigators were not able to comment on the discrepancy with other reported results, they did note that inhibition is dose-dependent.

Based on sequence data, NUC B1000 is predicted to be active against greater than 95% of known HBV genotypes/serotypes and to be active against all known drug resistant mutants associated with small molecule therapies. In experiments, NUC B1000 had similar levels of activity against the ayw and adw serotype and the lamivudine drug-resistant mutant.

The studies in which loss of body weight and spleen enlargement were observed in mice did not involve administration of NUC B1000. In the study, excipient was administered alone. The dose was 2,000-fold higher than that proposed for use in the clinical study.

As a predictor or "off-target" effects by the NUC B1000 shRNAs, alignments were made with known mouse and human miRNAs, and two of the shRNAs have some homology to published miRNAs. Cell culture and animal studies were also used to identify specific and non-specific stress responses, effects on cell viability, and cytotoxicity. Dr. Romano explained that *in vitro* safety studies have been conducted to examine different cell types for the induction of various double-stranded RNA, IFN response to gene products, and induction of various proinflammatory cytokines. The investigators have tried to anticipate all the potential agonists that the experimental plasmid product would produce when it encounters a cell and then chose cell types that had the most robust responses to those agonists. NUC B1000 did not appear to cause off-target responses that impacted significantly on cell viability or pathology in animals within the limits of the studies.

Dr. Romano noted that the investigators have used serum analysis to look at the production of proinflammatory cytokines and the production of IFNs in mice given a dose of 5 mg/kg, the maximal human dose projected for this proposed trial. No induction of proinflammatory cytokines has been detected.

Dr. Gish remarked that the investigators would like to conduct liver biopsies on these participants during or just shortly after dosing, but obtaining consent for a liver biopsy has become extremely difficult in recent years.

E. Public Comment

Timothy M. Block, Ph.D., President and Chief Scientific Advisor of the Hepatitis B Foundation, professor at Drexel Medical School, and a member of the scientific advisory board of Nucleonics Inc., stated that the Foundation is excited about this pioneering technology. He discussed the need for new therapeutic approaches, particularly because available therapies for treating HBV are not usable for half of the people

infected with HBV—nearly 200 million individuals worldwide who are infected with this disease. Dr. Block also discussed the difficulty of working with transgenic models.

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:

Scientific/Medical/Study Design Issues

- Given concerns about the appropriateness of the nonhuman animal models, questions were raised about the adequacy of the preclinical data from safety and efficacy perspectives and whether they provide sufficient justification to advance to clinical studies. This issue is of particular concern because the highest dose cohort will be higher than any dose previously used in a plasmid-based gene transfer trial and in light of the potential efficacy given the low levels of hepatocyte transfection observed in the mouse studies. In addition, the data from the transgenic mouse model study should be submitted to the OBA.

- A nonhuman primate would be a more relevant animal model to use in assessing the safety of the product and establishing appropriate dose level escalations. This animal model is more likely to be predictive of off-target effects of siRNAs.

- Additional efficacy studies in biologically relevant nonhuman animal models, including an HBV-expressing model system, should be considered. The additional studies should be conducted in appropriate models and include:

    - Assessments of transfection levels and the specificity for target cells.

    - Evaluation of the potential adverse effects of RNAi on cellular RNA pathways.

    - Evaluation of the immune responses to RNAi.

- Quantitative measures should be developed to assess the activity of short-hairpin RNAs (shRNAs) against HBV type 1.

- The potential integration of the plasmid DNA should be assessed in the context of inflammation and cellular regeneration, which can promote DNA integration. This model more closely approximates the *in vivo* situation of chronic hepatitis.

- Although there are inherent limitations to the assay, microarray expression profiling is considered by some to be useful in studying the effect of the shRNAs on nontargeted human cellular genes. These data could be linked with bioinformatic and pathway-based analysis of potential miRNA-like interactions.

- As further data is gathered on the biodistribution of the vector, it will be important that the approaches used are also relevant for the clinical study.

- The role of "dicer-like" activity is not clear and should be clarified.

- Given the relatively large doses to be administered, the two hour minimal monitoring period following plasmid administration may not be sufficient and should be reconsidered.

- Additional monitoring should be considered. One visit is scheduled in the third week after the initial plasmid administration. In addition to the follow-up phone call within 48 hours and a visit at

three weeks, a visit at one week should be considered. Blood tests to monitor for acute reactions, which could be missed by Week 3, should be performed.

- Potentially adverse immunological reactions should be monitored, including assays for anti-DNA antibodies, especially in the repeated-dose cohort.

Ethical/Legal/Social Issues

- The informed consent document states that the sponsor will provide standard antiviral (nucleoside analog) therapy for 6 months after the experimental phase if the participant's physician considers it necessary. The consent form also should make clear that the standard therapy is typically administered for much longer than 6 months and that premature cessation of treatment is not recommended. This information is particularly important for prospective participants who do not have insurance or cannot afford to the cost of therapy. In addition, the therapy should be provided for at least the duration of the followup period, that is, for 9 months rather than 6 months.

- The following changes should be made to the informed consent document:

    - Add an explanation of the product, NUC B1000, in lay terms, including its composition, doses, expected effects of siRNA, and any adverse reactions, such as inflammatory responses, that are expected.

    - Separate the discussion of compensation for research injury from the discussion of costs directly related to study participation.

    - Clarify that study participation will be discontinued if participants need additional treatment for complications of the disease and that a referral to their treating physician will be made.

    - Describe the second blood draw at 9 weeks.

G. Committee Motion 5

It was moved by Dr. Kirchhoff and seconded by Dr. Grant that the RAC recommendations, summarized orally by Dr. Federoff to include a variety of preclinical, clinical, and consent form issues, be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 11 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XII. Closing Remarks and Adjournment/Dr. Federoff

Dr. Federoff thanked the participants and adjourned the meeting at 4:00 p.m. on December 6, 2006.

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

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Amy P. Patterson, M.D.
RAC Executive Secretary/OBA Director

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and 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.
Chair
Recombinant DNA Advisory Committee

Attachment I
Recombinant DNA Advisory Committee Roster

Chair

FEDEROFF, Howard J., M.D., Ph.D.
Senior Associate Dean for Basic Research
Director
Center for Aging and Developmental Biology
School of Medicine and Dentistry
University of Rochester
Room 1-9619
Box 645
601 Elmwood Avenue
Rochester, NY 14642

Members

ALBELDA, Steven M., M.D.
Professor of Medicine
Pulmonary, Allergy, and Critical Care Division
Department of Medicine
University of Pennsylvania Medical Center
Biomedical Research Building II/III
421 Curie Boulevard
Philadelphia, PA 19104

DEWHURST, Stephen, Ph.D.
Professor
Department of Microbiology and Immunology
University of Rochester Medical Center
Box 672
601 Elmwood Avenue
Rochester, NY 14642

ERTL, Hildegund C.J., M.D.
Professor of Pathology and Laboratory Medicine
Leader
Immunology Program
The Wistar Institute
Associate Faculty
School of Medicine
University of Pennsylvania
3601 Spruce Street
Philadelphia, PA 19104

FLINT, Jane, Ph.D.
Professor
Department of Molecular Biology
Princeton University
Lewis Thomas Laboratory, Room 234
Princeton, NJ 08544

GRANT, Ellen E., Ph.D., LCSW-R
Vice President, Behavioral Health, Wellness and Health Promotion
HealthNow New York Inc.
1901 Main Street
P.O. Box 80
Buffalo, NY 14240-0080

HESLOP, Helen, M.D.
Professor of Medicine and Pediatrics
Center for Cell and Gene Therapy
Baylor College of Medicine
MC 3-3320
6621 Fannin Street
Houston, TX 77030

KAHN, Jeffrey P., Ph.D., M.P.H.
Maas Family Chair in Bioethics
Director
Center for Bioethics
University of Minnesota
Boynton Health Service Building, Room N504
410 Church Street, SE
Minneapolis, MN 55455-0346

KIRCHHOFF, Louis V., M.D., M.P.H.
Professor
Department of Internal Medicine (Infectious Diseases) and Epidemiology
University of Iowa
Bowen Science Building, Room 4-403
51 Newton Road
Iowa City, IA 52242

KODISH, Eric D., M.D.
F.J. O'Neill Professor and Chair
Department of Bioethics
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195

MUZYCZKA, Nicholas, Ph.D.
Professor
Department of Molecular Genetics and Microbiology
Director
Powell Gene Therapy Center
College of Medicine
J. Hillis Miller Health Science Center
University of Florida
Room R1-191
1600 Archer Road
P.O. Box 100266
Gainesville, FL 32610-0266

NEMEROW, Glen R., Ph.D.
Professor
Department of Immunology
The Scripps Research Institute
10550 North Torrey Pines Road, Room R214
La Jolla, CA 92037

ROSEN, Michael R., M.D.
Gustavus A. Pfeiffer Professor of Pharmacology
Professor of Pediatrics
Department of Pharmacology
Director
Center for Molecular Therapeutics
Columbia University
630 West 168th Street, PH7W-321
New York, NY 10032

ROSENBERG, Naomi, Ph.D.
Professor
Department of Pathology
School of Medicine
Tufts University
Jaharis Building, Room 512
150 Harrison Avenue
Boston, MA 02111

SHAPIRO, Robyn S., J.D.
Professor and Director
Center for the Study of Bioethics
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53266

SOMIA, Nikunj V., Ph.D.
Assistant Professor
Department of Genetics, Cell Biology and Development
Molecular Genetics Institute
University of Minnesota, Twin Cities
Jackson Hall, Room 6-160
321 Church Street, SE
Minneapolis, MN 55455

STROME, Scott E., M.D.
Professor and Chairman
Department of Otorhinolaryngology-Head and Neck Surgery
University of Maryland Medical Center
Suite 500
16 South Eutaw Street
Baltimore, MD 21201

VILE, Richard G., Ph.D.
Consultant
Department of Molecular Medicine
Mayo Clinic College of Medicine
Guggenheim Building, 18th Floor
200 First Street, SW
Rochester, MN 55905

WEBER, David J., M.D., M.P.H.
Professor of Medicine, Pediatrics and Epidemiology
Division of Infectious Diseases
Schools of Medicine and Public Health
The University of North Carolina at Chapel Hill
Burnett-Womack Building, Room 547
Campus Box 7030
Chapel Hill, NC 27599-7030

WEI, Lee-Jen, Ph.D.
Professor
Department of Biostatistics
School of Public Health
Harvard University
677 Huntington Avenue
Boston, MA 02115

OBA Director/RAC Executive Secretary

PATTERSON, Amy P., M.D.
Director
Office of Biotechnology Activities
Director
Recombinant DNA Program
Executive Secretary
Recombinant DNA Advisory Committee
Office of Science Policy
Office of the Director
National Institutes of Health
U.S. Department of Health and Human Services
Suite 750
MSC 7985
6705 Rockledge Drive
Bethesda, MD 20892-7985

Ad Hoc Reviewers/Speakers

CAPLEN, Natasha, Ph.D.
Head
Gene Silencing Section
Office of Science and Technology Partnerships
Office of the Director
Center for Cancer Research
National Cancer Institute
National Institutes of Health
U.S. Department of Health and Human Services
Building 37, Room 3128B
37 Convent Drive
Bethesda, MD 20892

FRY, Terry J., M.D.
Staff Clinician
Pediatric Oncology Branch
National Cancer Institute
National Institutes of Health
U.S. Department of Health and Human Services
Building 10, Room 1-3940
10 Center Drive
Bethesda, MD 20892

LIANG, T. Jake, M.D.
Chief
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services
Building 10, Room 9B16
MSC 1800
10 Center Drive
Bethesda, MD 20892-1800

Nonvoting Agency Representatives

National Science Foundation

HARRIMAN, Philip, Ph.D.
Program Director
Division of Molecular Biosciences
National Science Foundation
Room 565
4201 Wilson Boulevard
Arlington, VA 22230-1859

U.S. Department of Agriculture

JONES, Daniel D., Ph.D.
National Program Leader/Biotechnology
Cooperative State Research, Education, and Extension Service
U.S. Department of Agriculture
Waterfront Center, Room 3444
800 Ninth Street, SW
Washington, DC 20024

MCCAMMON, Sally L., Ph.D.
Science Advisor
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Unit 98
4700 River Road
Riverdale, MD 20737

U.S. Department of Commerce

LEVIN, Barbara, Ph.D.
Project Leader
Biotechnology Division
National Institute of Standards and Technology
U.S. Department of Commerce
MSC 8311
100 Bureau Drive
Gaithersburg, MD 20899-8311

U.S. Department of Energy

DRELL, Daniel W., Ph.D.
Biologist
Life Sciences Division
Office of Biological and Environmental Research
U.S. Department of Energy
SC-72
19901 Germantown Road
Germantown, MD 20874-1290

U.S. Department of Health and Human Services

Office for Human Research Protections

BORROR, Kristina C., Ph.D.
Director
Division of Compliance Oversight
Office for Human Research Protections
U.S. Department of Health and Human Services
Tower Building, Suite 200
1101 Wootton Parkway
Rockville, MD 20852

U.S. Food and Drug Administration, Office of Cellular, Tissue, and Gene Therapies

TAKEFMAN, Daniel M., Ph.D.
Acting Chief
Gene Therapy Branch
Division of Cellular and Gene Therapies
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM-720
1401 Rockville Pike
Rockville, MD 20852-1448

U.S. Environmental Protection Agency

FREDERICK, Robert, Ph.D.
Program Manager
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
MC 8623D
401 M Street, SW
Washington, DC 20460

MILEWSKI, Elizabeth, Ph.D.
Senior Biotechnologist
Office of Prevention, Pesticides, and Toxic Substances
U.S. Environmental Protection Agency
East Tower, Room 625
MC 7201
401 M Street, SW
Washington, DC 20460

Liaison Representative

FAYL, Gilbert, Ph.D.
Secretary of External Affairs
European Academy of Sciences and Arts
Brussels, Belgium

Attachment II
Public Attendees

Felicia Baratelli, University of California, Los Angeles (UCLA)
Raj K. Batra, UCLA
Timothy M. Block, Hepatitis B Foundation
Trevor Broadt, NCI Frederick/SAIC Frederick, Inc.
Andrew Byrnes, FDA
Dario Campana, St. Jude Children's Research Hospital
Januario E. Castro, Moores UCSD Cancer Center
Allyne Cheifet, Nucleonics Inc.
Theresa Chen, FDA
Charles Coates, Memgen
Steve Comisky, Nucleonics Inc.
Steven M. Dubinett, UCLA
Robert G. Gish, California Pacific Medical Center
Douglas Grum, SAIC Frederick, Inc.
Mike Havert, FDA
Thomas J. Kipps, UCSD
Jay M. Lee, UCLA *(via teleconference)*
Wing Leung, St. Jude Children's Research Hospital
David McCallus, Nucleonics Inc.
Andra E. Miller, The Biologics Consulting Group
Jim Mule, H. Lee Moffitt Cancer Center & Research Institute
Arthur W. Nienhuis, St. Jude Children's Research Hospital
Catherine Pachuk, Nucleonics Inc.
Karyol Poole, SAIC Frederick, Inc.
Dexter Poon, SAIC Frederick, Inc.
Charles E. Prossak, Memgen Pharmaceuticals
Karen L. Reckamp, UCLA *(via teleconference)*
Antoni Ribas, UCLA *(via teleconference)*
Patrick Romano, University of California, Davis
C. Satishchandran, Nucleonics Inc.
Donna R. Savage, Capital Consulting Corporation
Sherven Sharma, UCLA
Robert Suh, UCLA *(via teleconference)*
Hiroko Takedatsu, UCLA
Robert Towarnicki, Nucleonics Inc.
William G. Wierda, The University of Texas M.D. Anderson Cancer Center
Sixun Yang, FDA
Jason Yovandich, SAIC Frederick, Inc.
Gang Zeng, UCLA

Attachment III
Abbreviations and Acronyms

Ad	adenoviral, adenovirus
AE	adverse event
ALL	Acute lymphoblastic leukemia
CLL	chronic lymphocytic leukemia
CPE	cytopathic effect
CT	computerized tomography
DC	dendritic cell
DHHS	U.S. Department of Health and Human Services
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
FACS	fluorescence-activated cell sorter
FDA	U.S. Food and Drug Administration
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HSCT	hematopoietic stem cell transplantation
IFN	interferon
IFNα	interferon alpha
IL	interleukin
IND	investigational new drug
IV	intravenous
KLH	keyhole limpet hemocyanin
miRNA	microRNA
MOI	multiplicity of infection
MTD	maximal tolerable dose
NCI	National Cancer Institute
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NK	natural killer
OBA	NIH Office of Biotechnology Activities
OD	Office of the Director, NIH
PCR	polymerase chain reaction
RAC	Recombinant DNA Advisory Committee
RCA	replication-competent adenovirus
RCR	replication-competent retroviral, replication-competent retrovirus
RNA	ribonucleic acid
RNAi	RNA interference
RT-PCR	reverse transcriptase PCR
shRNA	short-hairpin RNA
siRNA	short-interfering RNA
SLL	small lymphocytic lymphoma
UCLA	University of California, Los Angeles
UCSD	University of California, San Diego