RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 6 and 7, 2003

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]

March 6-7, 2003

The Recombinant DNA Advisory Committee (RAC) was convened for its 90th meeting at 1:00 p.m. on March 6, 2003, at the Bethesda Marriott Hotel, Pooks Hill Road, Bethesda, MD. Dr. Theodore Friedmann (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 1:00 p.m. until 3:45 p.m. on March 6 and from 8:30 a.m. until 11:30 a.m. on March 7. The following individuals were present for all or part of the meeting.

Committee Members

W. Emmett Barkley, Howard Hughes Medical Institute
Martha C. Bohn, Northwestern University Medical School
Baruch A. Brody, Baylor College of Medicine
James F. Childress, University of Virginia (via teleconference on Day One only)
Neal A. DeLuca, University of Pittsburgh
Theodore Friedmann, University of California, San Diego
Thomas D. Gelehrter, University of Michigan Medical School
Linda R. Gooding, Emory University
Larry G. Johnson, University of North Carolina, Chapel Hill
Philip R. Johnson, Jr., Columbus Children's Hospital
Terry Kwan, TK Associates
Maxine L. Linial, Fred Hutchinson Cancer Research Center
Bernard Lo, University of California, San Francisco
Madison Powers, Georgetown University
David Sidransky, Johns Hopkins University School of Medicine
Robert D. Simari, Mayo Clinic and Foundation
Diane W. Wara, University of California, San Francisco

RAC Executive Secretary
Stephen M. Rose, Office of the Director (OD), National Institutes of Health (NIH)

***Ad Hoc* Reviewer/Speaker**
Mitchell B. Cohen, Cincinnati Children's Hospital Medical Center

NIH Staff Members
Catherine Barnard, OD
Robert H. Hall, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Laurie Harris, OD
Robert Jambou, OD
Cheryl McDonald, OD
Maureen Mongtomery, OD
Marina O'Reilly, OD
Margarita Ossorio, NIAID
Alexander Rakowsky, OD
Gene Rosenthal, OD
Thomas Shih, OD

[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.

Danilo A. Tagle, National Institute of Neurological Disorders and Stroke, NIH

Others

There were 53 attendees at this 2-day RAC meeting. A list of attendees appears as Attachment II.

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

Dr. Friedmann, RAC Chair, called the meeting to order at 1:00 p.m. on March 6, 2003. Notice of this meeting as set forth in the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on February 28, 2003 (68 FR 9697). This meeting involved the review of three protocols, data management safety information and clinical reports.

Dr. Rose referred the RAC members to the NIH Rules of Conduct and Conflict of Interest notice provided in their briefing materials.

II. Minutes of the December 4-6, 2002, Meeting/Drs. Childress (via teleconference) and Gelehrter

Dr. Gelehrter and Dr. Childress reviewed the draft December 2002 RAC meeting minutes. A few minor changes were suggested, but otherwise the minutes were accurate in content. Dr. Gelehrter recommended that the draft minutes be approved.

A. Committee Motion 1

It was moved by Dr. Brody and seconded by Dr. Gelehrter that the RAC approve the December 2002 RAC meeting minutes. The vote was 17 in favor, 0 opposed, and 0 abstentions.

III. Data Management Report/Drs. Brody, Gooding, Johnson, Simari, and Wara

A. Adverse Events/Dr. Simari

In the reporting period of November 1, 2002, through January 31, 2003, 243 adverse events (AEs) were reported to the OBA, 134 of which were deemed serious. Of the 134 serious adverse events (SAEs), 98 were initial reports. Of those 98 initial reports, 11 were classified as "A1"—serious, unexpected, and possibly associated with the gene transfer. Each of these 11 SAEs was reviewed extensively.

The RAC discussed an SAE from protocol #371, "A Phase I safety study in patients with severe hemophilia B Factor IX deficiency using adeno-associated viral vector to deliver the gene for human Factor IX into the liver." The first research participant in the highest dose cohort presented with elevated transaminases 4 weeks after gene transfer product infusion delivered via the intrahepatic artery. These transaminases were elevated up to eight times the upper limits of normal. Results from week 5 and another test at week 5 plus 2 days showed a slight decline in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) from their highest levels, with other levels remaining within normal limits. At that time, the sponsor received a verbal clinical hold from the U.S. Food and Drug Administration (FDA). Followup information from the sponsor indicated that ALT and AST levels peaked in this research participant between weeks 4 and 5 but returned to normal by week 14. In addition, Factor IX levels were raised to 10 percent (from the previous baseline level of less than 1 percent) for a few weeks beginning at week 2. Such events were not seen in the animal studies that used comparable doses. After extensive study of this incident, the sponsor reported that the etiology remains unclear, but the incident is being called transient and self-limiting.

B. Amendments/Dr. Wara

In the reporting period of November 1, 2002, through January 31, 2003, 54 annual updates and 20 amendments that described changes other than site and investigator changes were submitted to the OBA. Dr. Wara discussed several examples from these annual updates and amendments by providing examples from several different protocols.

Protocol #129 is a retroviral vector gene transfer protocol for patients receiving a bone marrow transplant (BMT) for relapsed Epstein-Barr-virus-positive Hodgkin's disease. In their amendment (received in an annual update), the investigators stated that they chose not to revise their consent form in light of the X-linked severe combined immunodeficiency disease (SCID) study in France, because their protocol is much less likely to produce leukemia in participants since it is a marking study. The RAC is uncertain whether it will prove true that marking studies are safer, and the risk of leukemia in these studies may be less acceptable because a marking study has no direct therapeutic benefit to the participant.

Protocol #172 uses high-dose carboplatinum and etoposide followed by transplantation with peripheral blood stem cells to treat germ-line tumors. Because the study is closed to accrual, and the current informed consent document states that insertional mutagenesis is a potential AE, no modifications of the document are planned by the investigators. The RAC recommends that information about the SAE in the French X-linked SCID study should be reported to research participants who participated in similar studies, even though those studies may be closed to accrual. More frequent laboratory testing or clinical visits may be desirable for individuals who have already received retroviral gene transfer in completed studies.

In Protocol #370, an animal AE was submitted by the principal investigator as part of an annual update for a pilot study of gene transfer for individuals with Fanconi's anemia. Myelodysplasias and leukemias resulting from high rates of clonal hematopoiesis occurred in the control animals that received CD34+ cells stimulated *ex vivo* with growth factors but not transduced with the vector. This study raised the possibility that CD34+ cells treated with growth factors *in vitro* may be problematic. The RAC believes that additional work needs to be done to sort out the various relevant factors in this finding.

Protocol #453 is a multicenter, open-label, two-part, dose-escalation study to determine the tolerability of interferon-beta gene transfer in the treatment of recurrent or progressive glioblastoma. An amendment was submitted that revises the exclusion criteria to allow for the enrollment of research participants whose tumor contacts a ventricle wall. The investigators stated that most glioblastoma tumors do contact a ventricle wall, so the current criteria excluded large numbers of potential participants. The RAC expressed concern about modifying the exclusion criteria in this study because similar protocols described an inflammatory response when any injection is placed into the ventricle.

The Gemini study is a long-term safety and persistence study following retroviral gene transfer in identical twins who are discordant for human immunodeficiency virus infection. A total of 28 research participants are enrolled in the long-term, follow-up phase of the study, and investigators recently reported by abstract that participants who received interleukin-2 (IL-2) along with their transduced CD34+ cells have had a longer persistence of transduced cells at higher numbers. This report was offered to the public at this meeting for informational purposes.

Protocol #530 is a Phase II study of tumor necrosis factor alpha (TNFα) gene transfer and radiation for first-line treatment of nonresectable locally advanced pancreatic cancer. Several research participants have experienced significant AEs consistent with high dose TNF-alpha—hypotension, anorexia, fever, and chills—even though only a low amount of product is detected in these participants. This situation merits additional observation and evaluation.

Dr. Wara briefly discussed two large Phase II/III gene transfer studies. Protocol #280 is a trial of chemotherapy alone vs. chemotherapy plus SCH 58500 (Adp53) in newly diagnosed stage III ovarian and primary peritoneal cancer patients with residual disease after surgery. The sponsoring company made the decision to close the study before enrollment was completed due to significant abdominal complications such as peritonitis, adhesion formation, small-bowel obstruction or perforation, and minimal signs of benefit. In contrast, Protocol #366 is a Phase III, open-label, randomized study to compare the

overall survival and safety of biweekly intratumoral administration of RPR/INGN 201 (Adp53) vs. weekly methotrexate in 240 participants with refractory squamous cell carcinoma of the head and neck. This study may be the first Phase III gene transfer study to be completed and reported (expected in 2003). In this study, autopsies are no longer requested because of the low rate of compliance. Dr. Wara wondered whether alternative approaches to autopsy compliance might be sought for all studies.

IV. Discussion of Human Gene Transfer Protocol #0301-570: Use of *in vivo* Expression Technology To Identify Virulence Factors and Protective Antigens of *Vibrio cholerae 01*

Principal Investigator:	Carol O. Tacket, M.D., University of Maryland School of Medicine
Additional Presenters:	James B. Kaper, Ph.D., University of Maryland, and Andrew Camilli, Ph.D., Tufts University
Sponsor:	Division of Microbiology and Infectious Diseases, NIAID, NIH
RAC Reviewers:	Drs. Bohn and Johnson, Ms. Kwan, and Dr. Wara
Ad hoc Reviewer:	Mitchell B. Cohen, M.D., Cincinnati Children's Hospital Medical Center

A. Protocol Summary

Cholera is a disease caused by infection with the bacterium, *Vibrio cholerae (V. cholerae)*. Although few cases of cholera are seen in the United States, large outbreaks of this disease have occurred in more than 100 countries in Asia, Africa, and South America, and worldwide, cholera causes 100,000 to 150,000 deaths annually. The main symptom of cholera is watery diarrhea, which can be severe enough that even adults can become dehydrated, go into shock, and die if they are not treated. Other symptoms of cholera include low-grade fever, stomach cramps, nausea, vomiting, and loss of energy.

An ideal vaccine for the prevention of cholera is not yet available. Previous work has resulted in the development of an attenuated live oral cholera vaccine, CVD 103 HgR. This vaccine confers strong protective immunity against experimental challenge with virulent *V. cholerae* 01 after a single dose. Although this vaccine is highly protective in U.S volunteers and has been licensed in several developed countries for protection of travelers to cholera-endemic countries, a recent field trial in Indonesia failed to show efficacy in the native population of a cholera endemic country. The development of attenuated cholera vaccines has been hampered by the fact that *V. cholerae* strains deleted of the CTX genes encoding cholera toxin can still produce varying amounts of diarrhea and other symptoms such as headache, fever, abdominal cramps, and malaise in many individuals. Such symptoms are not seen with the CVD 103-HgR vaccine, probably because this strain colonizes the human intestine at greatly reduced levels compared to ctx-negative strains that are reactogenic and avid colonizers. The ability to construct a strain which colonizes the intestine better and therefore stimulates a more vigorous protective immune response is hindered by the uncertainty as to what bacterial factor is responsible for the reactogenicity of highly immunogenic, colonizing vaccine strains.

This study is an opportunity to identify new *in vivo*-expressed virulence factors of *V. cholerae* and to ascertain new proteins that contribute to the protective immune response. I*n vivo* expression technology will be used to identify *V. cholerae* 01 genes that are expressed in human volunteers. A library of *V. cholerae* 01 genes fused to a promoter-less gene trapping system in *V. cholerae* 01 strain CVD 110, consisting of a pool of 1×10^8 colony forming units in bicarbonate, will be given orally to up to 5 healthy adult inpatient volunteers. Volunteers will reside on a research isolation ward for 9 days after challenge to collect multiple specimens of stool and duodenal fluid for culture. *V. cholerae* isolates will be recovered from the stools and duodenal fluids, and the isolates will be screened for *in vivo* expression of the TnpR recombinase by loss of the *neo-sac*B genes. The identity of the genes expressed *in vivo* will be determined by sequencing.

B. Reviews by RAC Members and Ad Hoc Reviewer

Twelve RAC members voted to review this protocol publicly. Drs. Bohn, Wara and P. Johnson, Ms. Kwan, and Dr. Cohen submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Bohn was concerned about whether the size and composition of the volunteer group was appropriate to identify relevant proteins in an at risk population. She asked how the clones identified in the study would be analyzed and compared to the sera from previous immunity challenge trials or the infant mouse preclinical studies. She requested further discussion of possibly applicable animal models such as the removable intestinal tie-adult rabbit diarrhea model or primate studies to identify proteins expressed in the intestine.

Dr. Wara stated that this study was a well-designed protocol, based on careful science and in an important area. Her major concern was the risk:benefit ratio for the study with healthy volunteers. She also asked if three to five volunteers would provide sufficient genetic diversity given the discrepancy between the results of an earlier vaccine study in the United States and its related field study in Indonesia. She asked how it will be determined which *in vivo* expressed genes are relevant clinically or for antibody production.

Dr. P. Johnson's also asked how the investigators selected the number of subjects and whether that number should be larger due to the possible absence of genetic diversity in such a small group. This is a pilot study being conducted in a well-controlled setting, but investigators will need to conduct a similar trial in research participants at risk in an endemic area. He also asked about how many colonies would be evaluated to gain as much information as possible in this trial.

Ms. Kwan suggested that investigators clarify the nontechnical abstract. She noted that the investigators' responses to Appendix M of the *NIH Guidelines* were listed as "not applicable"; however, a thorough discussion of the preclinical and risk-assessment studies should be included. With regard to selection and payment of volunteers, Ms. Kwan noted the dilemma that the $900 payment is limited compensation for the amount of time volunteers would be sick, but it might still be enough of an incentive for students to be unduly encouraged to participate. The investigators should be aware of this possibility. She suggested that investigators utilize the services of professional test preparers to ensure that the items in the postconsent questionnaire are appropriate. Ms. Kwan also suggested that the number of pure science questions in the questionnaire be reduced and to concentrate on ensuring that the individuals understand how sick they are likely to feel as a result of participation in this study.

Dr. Cohen summarizing the need for additional insights into the protective immune responses to *V. cholerae* and the properties of the human challenge model. He stated that interpretable data should result from a study of a limited number of volunteers who would be carefully screened prior to enrollment. He noted that the mouse model might not correlate with *in vivo* gene expression in humans due to factors such as infectious dose, incubation time, and host differences. He suggested that the study include volunteers with blood group O because they are more at risk for severe cholera. He requested clarification of the rationale for the number of colonies to be analyzed.

C. RAC Discussion

The following questions and concerns were raised during the RAC discussion of the protocol:

- Dr. Sidransky suggested that collecting samples from the entire gastrointestinal system might not provide the requisite information; sample collection should be concentrated in the stomach or the duodenum.
- Dr. Sidransky suggested that investigators sample as many clones as possible from the research participants, rather than limiting to 20 the number of clones to be taken for analysis.
- Dr. Brody expressed concern about study withdrawal because, although participants would technically be allowed to withdraw, they would have to remain in the isolation ward until they took a course of tetracycline and their stool culture no longer showed evidence of *V. cholerae* bacteria. He

suggested that wording in the informed consent document more accurately reflect the actual process of withdrawal from a study such as this.

- Dr. Friedmann noted that there are likely to be occasional situations in which a research participant must not only withdraw from the study but also leave the isolation ward, for example, the death of a relative. In those cases, directly observed drug therapy, in which the participants return to the clinic each day to have their drug-taking witnessed, would be appropriate.
- Commenting on the pay scale issue, Dr. Brody stated that participants receiving $900 for their part in this study may be paid too little because it may be considered exploitive to pay what amounts to approximately one-half of the minimum wage for time spent.

D. Investigator Response

Dr. Kaper agreed that the issue of diversity is valid. The investigators will compare the *in vivo* expressed proteins to specimens from 30 years of stored sera from a wide range of individuals. In addition, collaborations with researchers in Bangladesh and Calcutta will allow testing of promising antigens to be extended to those populations.

Regarding the issue of animal models, Dr. Kaper discussed a long history of attempts to use a primate model for cholera, stretching back to the 19th century. He explained that the standard of testing for recombinant cholera vaccines is adult humans. The infant mouse model has provided some useful information in terms of colonization aspects of *V. cholerae,* but the model has not proven useful for predicting protective immunity in humans.

In response to Dr. Sidransky's suggestion of analyzing and banking more than 20 clones per participant, Dr. Kaper explained that investigators are planning to collect at least 500 clones, analyze an initial 20 clones, and freeze the remainder of the stool or duodenal samples.

Responding to the questions about genetic diversity, Dr. Camilli stated that the purpose of the pilot study is to validate the approach using only three to five healthy U.S. volunteers. In the study, the investigators will identify the genes expressed in all the volunteers and focus on the small subset of genes that might be broad-spectrum antigens. Dr. Camilli expressed hope that this small study would be followed by a larger, more genetically diverse study that would include volunteers from cholera-endemic populations. Dr. Tacket explained that in a previous study, 7 of 10 volunteers developed diarrhea in response to administration of the organism, yet all 10 of those volunteers shed the organism in their stool. Results from this prior study indicate that whether or not research participants develop diarrhea, the *V. cholerae* bacteria can be recovered from serial fecal cultures; therefore, three to five volunteers should be adequate for this study. There was not a statistical rationale for the choice of three to five volunteers, so Dr. Tacket asked the RAC for suggestions for the appropriate number of volunteers.

Regarding the pay scale, Dr. Tacket noted that there may be people in the surrounding community (Baltimore, Maryland) for whom $900 is coercive, but there are also people for whom such payment is appropriate. As she interviews potential research participants, Dr. Tacket will try to make decisions about the effect of the amount of money on the decision to participate and enroll potential participants accordingly.

Regarding the issue of participant withdrawal, Dr. Tacket noted that investigators can't physically detain participants who no longer wish to participate. Such individuals would be given a course of Cipro (ciprofloxacin hydrochloride), because it is a longer lasting antibiotic than tetracycline, and then sent home, with instructions to return to the clinic each day to have their drug-taking witnessed. She explained that the concept of quarantine no longer applies in Baltimore City. Although at one time quarantine allowed legal detention of research volunteers, other infection control measures and universal precautions are now used to protect public health. Dr. Tacket agreed to consider Dr. Brody's suggestion to alter the informed consent document to accurately reflect the withdrawal procedure for participants who refuse to remain in the isolation ward.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann noted that few questions or issues remain and stated the following RAC recommendation:

- In order to garner as much information as possible about the genes isolated by the selection process, investigators should characterize and identify the gene inserts from as many *V. cholerae* colonies as is technically feasible.
- The procedures developed for screening and selecting healthy volunteers are commendable. Considering the long isolation period required by the protocol and the serious discomfort participants may experience during that time, it is indeed difficult to establish an appropriate level of financial compensation. Under these circumstances, the extensive and repeated interviews that are used to evaluate potential research participants understanding of, and motivation for, participation will be critical to ensuring appropriate subject selection. In addition, the section of the informed consent document explaining the isolation process should be carefully reviewed and consideration given to emphasizing the need to remain in the isolation unit until tests show that the participant is no longer infected with *V. cholerae* bacteria.

G. Committee Motion 2

It was moved by Dr. Cohen and seconded by Dr. Sidransky that this recommendation expressed the comments and concerns of the RAC. The vote was 16 in favor, 0 opposed, and 0 abstentions. (Dr. Childress did not vote, as he was no longer participating by teleconference.)

V. Public Comment

Ms. Eve Lapin shared her views supporting the continuation of gene transfer studies attempting to find treatments for X-linked rare diseases, despite recent SAEs in the X-linked SCID study in France. Her family has been affected by adrenoleukodystrophy (ALD), commonly called "Lorenzo's oil disease." Two of her three sons have ALD. By the time their eldest son was diagnosed, the disease had progressed beyond the stage where there is an effective treatment. Through genetic screening, their second son was found to have the same mutation, and he received the standard treatment, bone marrow transplantation (BMT). However, BMT for ALD has a high complication rate including morbidity from the associated conditioning chemotherapy and graft-vs-host disease. Ms. Lapin expressed concern that certain types of gene transfer could be relegated to situations where there are no other therapeutic options. While allogeneic BMT is the standard of care for many diseases, it is a risky and difficult treatment. There is room for improvement in the acceptable treatment, and other treatments should be explored. Parents of children who have rare fatal diseases do not expect gene transfer to be able to solve all problems, but it is a promising avenue to explore. While she acknowledged the importance of preclinical research, she urged the gene transfer field to pursue a parallel track in which clinical applications are encouraged.

VI. Day One Adjournment/Dr. Friedmann

Dr. Friedmann adjourned the first day of the March 2003 RAC meeting at 3:45 p.m. on March 6, 2003.

VII. Day Two Opening Remarks/Dr. Friedmann

Dr. Friedmann opened the second day of the March 2003 RAC meeting at 8:30 a.m. on March 7, 2003.

VIII. Discussion of Human Gene Transfer Protocol #0301-564: Phase Ia/b Trial of Second-Generation Designer T Cells in Adenocarcinoma

Principal Investigator:	Richard P. Junghans, Ph.D., M.D., Beth Israel Deaconess Medical Center/Harvard Medical School
Sponsor:	None
RAC Reviewers:	Drs. Friedmann, L. Johnson, and Powers
Ad hoc Reviewer:	None

(Ms. Kwan recused herself from discussion of this protocol.)

A. Protocol Summary

The protocol focuses on the development and application of chimeric immunoglobulin-T cell receptors (Ig TCR) for cancer therapy. The molecules are fusion products of antibody (Ab) binding domain with the zeta signaling chain of the TCR. When the new chimeric TCR is expressed in recipient T cells, the resulting "designer T cells" are redirected to attack tumors expressing the surface antigen (Ag) recognized by the Ab. In this protocol the specificity of Ab against carcinoembryonic antigen (CEA) will be combined with the cytotoxic potency of T cells in an immune therapy against colorectal cancer. The strategy is designed to bypass a major drawback of cancer immunotherapy approaches, which is that most tumor antigens are normal self proteins to which the patient is immunologically tolerant. In a phase I clinical trial with 1st generation designer T cells, there were preliminary indications of efficacy but limited duration of response. *In vitro* experiments suggested that activation induced cell death (AICD) contributed to lack of *in vitro* persistence of the designer T cells. The strategy was redesigned to add CD28 co-stimulation (signal 2) upon tumor contact via an IGCD28TCR to prevent AICD. These 2nd generation designer T cells with signal 1 + 2 were shown to overcome AICD and instead undergo accelerated proliferation on contact with CEA+ tumor targets and sustain tumoricidal activity. In the proposed clinical trial, participant T cells will be transduced with a retroviral vector expressing anti-CEA IgCD28TCR to create 2nd generation designer T cells. These cells will be returned to the participant by intravenous administration and the participant followed for toxicity and anti-tumor response.

B. Reviews by RAC Members

Drs. Friedmann, L. Johnson, and Powers submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Friedmann expressed the following concerns: 1) the need for more information about the efficiency of transduction, and number of integrations events; 2) the growth advantage of the transduced cells and IL-2 dependence; 3) potential use of self-inactivating (SIN) vectors or vectors with silencer or suicide elements or with controlled gene expression; 4) the lack of large animal studies; 5) the potential for the transgene to have unexpected oncogenic properties; 6) monitoring of participants for evidence of clonally expanding T cells; and 7) inclusion of information in this protocol about the X-linked SCID experience from the Paris study.

Dr. L. Johnson noted that a major issue has been the possibility of insertional mutagenesis, given the survival advantage induced by this transgene. He asked about the preclinical data supporting longer survival and proliferation of the transduced T-cells. He was also concerned about subjecting participants to two tissue biopsies within a 10-day period, and suggested increasing the interval between biopsies. The criteria for repeat administration of the gene transfer should be based on objective measures, and autopsies offered to all participants. Safety considerations included the absence of specific plans to evaluate integration events and the need include information about the SAEs in the X-SCID trial in the informed consent document.

Dr. Powers limited his review to the informed consent document. Two ethical issues initially caused concern: (1) the need to supplement the informed consent document to discuss the significance of the second SAE in the French X-SCID trial involving a retroviral vector and (2) the need to remove references

to “treatment” or “therapy” in the document. Dr. Powers noted that both issues were addressed adequately in the revised document received in mid-February 2003.

C. RAC Discussion

The following questions and concerns were raised during the RAC discussion of the protocol:

- Dr. Brody stated that plans for long-term monitoring of patients should be included in the informed consent document, although he was concerned that discussion of a 10-year follow-up visit could raise participants’ expectations inappropriately. He suggested language changes to the informed consent document long-term follow-up section such as: “The Federal Government requires long-term blood drawing after the study is completed. The blood may be drawn by your primary physician.” Removing the timetable for follow-up would remove the suggestion of extended life expectancy.
- Dr. Borror suggested simplifying complex words in the informed consent document, for example, “persistence,” “interim,” and “detrimental.” She noted that the medical and scientific matters were described well.

D. Investigator Response

With regard to Dr. L. Johnson’s comment that the protocol language did not clarify which research participants might be eligible for repeat administration of the gene transfer, Dr. Junghans agreed to provide more specific criteria.

In response to Dr. Friedmann’s concern about characterizing the initial transduction and integration events similarly to that being conducted for all current X-linked SCID research participants, Dr. Junghans pointed out that unlike the X-SCID trial, mature T cells were being transduced and the participants eligible for the study are not expected to survive three years. Dr. Junghans explained that the investigators plan to store lymphocyte samples from participants, taken at the various time points currently required by the FDA. If leukemia should develop in a participant, the archived samples could be used to analyze clonality and integration sites.

Regarding potential oncogenic effects of the transgene, the zeta chain and CD28 cytoplasmic domain were already endogenously expressed in the cells. The proliferation rate of the transduced cells was identical to that of untransduced cells and unlike the X-SCID experience, there is no selective growth advantage for the transduced T cells.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendations, suggestions, and comments:

- The criteria for repeat administration of engineered T cells should be clarified and be based on objective, defined clinical measures rather than left to the discretion of the investigators. Repeat administration may complicate the study design and the subsequent interpretation of the data. The investigators, therefore, could consider alternatives such as the submission of protocol amendments requesting single subject exemptions or a separate modified protocol specifically designed to include the evaluation of multiple administrations
- To obtain vital information about the safety and efficacy of gene transfer, all research participants should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. All subjects should be asked to advise their families of the request and of its scientific and medical importance.

- To help enhance the readability and accessibility of the informed consent document, the documents should be revised to include language more readily understandable to potential participants. For instance, simpler words for concepts such as 'persistence", 'interim", and "detrimental" could be used.
- The section of the informed consent document describing long-term follow-up should be modified to avoid any suggestion that participation in the study could result in an increased life expectancy.

G. Committee Motion 3

It was moved by Dr. Brody and seconded by Dr. Bohn that these recommendations expressed the comments and concerns of the RAC. The vote was 15 in favor, 0 opposed, and 0 abstentions. Ms. Kwan did not vote because she had recused herself from discussion of this protocol.

IX. General Retroviral Vector Questions/Dr. Rose

Dr. Rose reported on a synopsis of the questions that all Principal Investigators of retroviral vector gene transfer protocols will be asked to answer in all protocol submissions to be considered by the RAC. When completed, these questions will be posted on the OBA Web site. The draft questions are:

1. Is there information on the number of independent integration events in the transduced cell population?

2. Is there information, from *in vitro* or *in vivo* studies, on the potential growth advantage of transduced T cells (or hematopoietic or any cell population transduced by a retroviral vector) and for their potential *in vivo* selection? Do they show any proliferative advantage *in vitro* or in animal reconstruction studies?

3. Is there any plan to use vectors that express the transgene conditionally? Are there any plans to use SIN retroviral constructs to incorporate silencer elements? Are there any plans to include suicide elements in the vectors?

4. Do investigators plan to carry out any large-animal studies to test long-term effects and potential tumorigenicity?

5. Is there evidence for the potential transforming and oncogenic properties of the transgene? How do cells behave *in vitro* and *in vivo* when they express this transgene? Are there any clues on this point from transgenic mice overexpressing the transgene?

6. How do investigators propose to monitor the research participants and detect clonally expanding cells?

7. Will the consent process include discussion of the appearance of T-cell leukemia in Dr. Fischer's study of X-linked SCID?

8. How are investigators monitoring subjects in similar ongoing trials, if any? Have they updated the informed consent process to reflect new knowledge of the emergence of leukemia in the Paris study? What about trials that have been completed—how do investigators propose getting appropriate information to those research participants?

9. Is there information on the existence of transduced cells in the circulation of participants in any of these ongoing trials? Is there information on how many transduced cell clones and how many integration sites exist in those participants?

After discussion, the members of the RAC agreed that the answers to these questions are important to inform their deliberations and all PIs should address these in their protocol submissions.

X. Discussion of Human Gene Transfer Protocol #0212-563: Administration of Peripheral Blood T Cells and Epstein-Barr-Virus-Specific Cytotoxic T Lymphocytes Transduced To Express GD-2-Specific Chimeric T-Cell Receptors to Patients With Neuroblastoma

Principal Investigator:	Heidi Russell, M.D., Texas Children's Hospital, Baylor College of Medicine
Presenters:	Helen E. Heslop, M.D., Texas Children's Cancer Center, Baylor College of Medicine; Martin A. Pule, Baylor College of Medicine; and Claudia Rossig, M.D., Baylor College of Medicine
Sponsor:	None
RAC Reviewers:	Drs. DeLuca, Linial, and Lo
Ad hoc Reviewer:	None

(Dr. Brody recused himself from this discussion.)

A. Protocol Summary

Neuroblastoma is a childhood cancer with about 500 new cases every year in the United States. While children with localized neuroblastoma often can be cured, children with widespread disease are much harder to treat and often die from their disease. One way to treat neuroblastoma is to redirect the patient's cellular immune system to GD-2, a disialoganglioside expressed at high levels by most neuroblastomas and expressed at low levels on normal tissue. A chimeric T-cell receptor (TCR) that recognizes GD-2 was generated by linking the variable domains of an anti-GD-2 monoclonal antibody to the signaling portion of human CD3-zeta. When retrovirally transduced human T-cells express the chimeric receptor, the T cells kill GD-2 expressing targets. A major barrier to use of chimeric TCR transduced T-cells, however, is the lack of full activation and subsequent persistence *in vivo* partly caused by lack of co-stimulation. The investigators plan to overcome this deficit by transducing EBV-specific T-cells (EBV CTLs). More than 50% of neuroblastoma patients are EBV seropositive and earlier studies with EBV CTLs has shown persistence and function of the cells *in vivo* for several years. The investigators hypothesize that transduced EBV CTLs will be activated and persist in response to EBV-positive cells when stimulated through their native TCR. These bi-specific cells will also kill neuroblastoma cells recognized by their GD-2 targeted chimeric TCR. The investigators plan to fully test this hypothesis by simultaneously infusing both GD-2 chimeric TCR peripheral blood T-cells and transduced EBV CTLs to EBV seropositive patients with advanced neuroblastoma. A small difference in the non-coding region of the retroviral construct used to transduce the different cell types will allow differential tracking by real-time quantitative PCR and therefore allow tracking of persistance.

B. Reviews by RAC Members

Drs. DeLuca, Linial, and Lo submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. DeLuca asked about the available preclinical safety data particularly from similar studies in which there is signal transduction from both the chimeric and natural TCR combined with the potential for insertional mutagenesis induced by the vector. He suggested more frequent monitoring with follow-up testing after the first year. Dr. DeLuca also suggested that the language in the informed consent document regarding the X-linked SCID trial could be simplified by using the wording used by the NIH: "Gene transfer was a cause of both leukemias."

Dr. Linial asked whether investigators had considered using a self-inactivating vector with a cellular promoter to control transgene expression rather than the LTR. She also asked whether the long-term follow-up in the HIV study cited was of sufficient length to provide information about the safety of the vector. Dr. Linial asked whether the use of *Herpesvirus papio* virus infections in rhesus macaques might be a valid animal model for this study.

Dr. Lo asked whether any of the investigators have a financial interest in the company. The informed consent document should refer to gene "transfer" rather than "therapy" and include palliative care as an alternative to participation rather than no treatment. He wished to discuss the procedures being used to explain the study to children in a developmentally appropriate manner for obtaining their assent.

C. RAC Discussion

The following questions and concerns were raised during the RAC discussion of the protocol:

- Dr. Friedman requested a summary of how previous retrovirus-based marking studies had helped in the design of this study. He asked about the potential long-term effects in marking studies that might come from an insertional mutagenic event, or from potential effects of reporter transgenes, for example, an autoimmune response to a transgene.
- Dr. Wara asked whether the child-life specialist would work with the child throughout research participation.

D. Investigator Response

In response to Dr. Friedmann's marking question, Dr. Heslop stated that the retrovirus-based marking studies had proven helpful in the design of the clinical study by allowing investigators to learn about the persistence of the cytotoxic T lymphocytes, their function, and the locations to which they home. Previous marking studies may have had less risk than current studies because they were performed in the early and mid-1990s, when there was a lower efficiency of gene transfer. An immune response to expression of the neomycin resistance gene had not been observed in any of their studies, but she was aware of a report of an immune response to marked mesenchymal cells that resulted in eradication of the adoptively transferred cells.

Dr. Pule responded to Dr. Linial's suggestion of using rhesus macaques as an animal model by explaining that the most useful model would involve a latent EBV infection allowing for reactivation of EBV CTLs. While there are many macaque herpes viruses, none involve a latent infection, thus they do not mimic the situtation in the human trial.

In response to Dr. DeLuca's query about the long-term *in vitro* studies, Dr. Rossig explained that investigators have not seen any enrichment or positive selection of the transduced cell population over several months.

Dr. Russell discussed obtaining consent from child participants. She noted that most of the children diagnosed with neuroblastoma are younger than 2 years old, so they would not be consented directly for any of their chemotherapy. By the time they reach recurrence and participation in this trial, most of these children will still be younger than 10 years of age. On the basis of the individual child's cognitive development, investigators will attempt to explain the study, including the process of injection, how the child will receive the drug, and some of the side effects. However, most of the explanation of the trial itself is provided to the parent(s) or legal guardian(s) of the child. In most cases, these explanations will include the presence of a child-life specialist or social worker. Child-life specialists are trained in child development and understanding, and they assist in explaining to the child what the child can expect from the clinical trial process.

In response to Dr. Wara's question, Dr. Russell explained that the Texas Children's Cancer Center supports up to four full-time child-life specialists, supporting the children not only in gene transfer trials but also in other areas. Most of the child-life specialists will meet their clients at diagnosis and will be with them throughout the course of their treatment, acting as a patient advocate in regard to clinical trials and at other times.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendation:

- Because this is a phase I safety trial, the informed consent document should make clear that this is not a therapeutic study and should emphasize the investigational nature of the study. Throughout the document, therefore, potentially misleading terms such as "therapy", "treatment", and "patient" should be replaced with terms such as "gene transfer", "intervention", and "research participant." Additionally, and if your IRB agrees, since the study will enroll primarily young children, the document should be written for the parent/legal guardian who will be providing permission for the child's enrollment. As such, change "you" to "your child" wherever it appears.

G. Committee Motion 4

It was moved by Dr. Powers and seconded by Dr. Gelehrter that the recommendation shown above expressed the comments and concerns of the RAC. The vote was 15 in favor, 0 opposed, and 0 abstentions. Dr. Brody did not vote because he recused himself from discussion of this protocol.

XI. Closing Remarks and Adjournment

Dr. Friedmann thanked participants and adjourned the meeting at 11:30 a.m. on March 7, 2003.

[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.]

_____
Stephen M. Rose, Ph.D.
Executive Secretary

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

Date:

_____
Theodore Friedmann, M.D.
Chair

Attachment I

RECOMBINANT DNA ADVISORY COMMITTEE

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Attachment III
Abbreviations and Acronyms

AE	adverse event
ALD	adrenoleukodystrophy (also known as Lorenzo's Oil disease)
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMT	bone marrow transplant
EBV	Epstein-Barr virus
FDA	U.S. Food and Drug Administration
IL-2	interleukin-2
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, National Institutes of Health
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
SCID	severe combined immunodeficiency disease
SIN	self-inactivating
TNF	tumor necrosis factor
V. cholerae	*Vibrio cholerae*