RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 7-8, 2002

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 <www.nih.gov/od/oba/docs.htm>.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]

March 7-8, 2002

The Recombinant DNA Advisory Committee (RAC) was convened for its 85th meeting at 8:00 a.m. on March 7, 2002, at the Bethesda Marriott Hotel, Congressional Ballroom, 5151 Pooks Hill Road, Bethesda, MD 20814. Dr. Theodore C. Friedmann (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 4:50 p.m. on March 7 and from 8:30 a.m. until 2:45 p.m. on March 8. The following individuals were present for all or part of the meeting.

Committee Members

Martha C. Bohn, Northwestern University Medical School and Children's Memorial Institute for Education and Research
Xandra O. Breakefield, Massachusetts General Hospital
Baruch A. Brody, Baylor College of Medicine
James F. Childress, University of Virginia
Theodore C. Friedmann, University of California, San Diego
Linda R. Gooding, Emory University School of Medicine
Larry G. Johnson, University of North Carolina, Chapel Hill
Philip R. Johnson, Jr., Columbus Children's Hospital
Nancy M.P. King, University of North Carolina, Chapel Hill
Sue L. Levi-Pearl, Tourette's Syndrome Association, Inc.
Maxine L. Linial, Fred Hutchinson Cancer Research Center
Robert D. Simari, Mayo Clinic and Foundation
Diane W. Wara, University of California, San Francisco

Executive Secretary

Amy P. Patterson, National Institutes of Health (NIH)

Ad Hoc Reviewers/Speakers

Thomas D. Gelehrter, University of Michigan Medical School
Katherine A. High, Children's Hospital of Philadelphia
Mark A. Kay, Stanford University School of Medicine
Ruth L. Kirschstein, NIH
Brian W.J. Mahy, Centers for Disease Control and Prevention
Richard G. Vile, Mayo Clinic
Thomas R. Ziegler, Emory University Hospital

Nonvoting/Agency Representatives

Kristina Borror, Office of Human Research Protections
Philip Noguchi, U.S. Food and Drug Administration (FDA)
Stephanie L. Simek, FDA

[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 NIH Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

NIH Staff Members

David G. Badman, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Sarah Carr, OD, Office of the Director (OD)
Greg Evans, National Heart, Lung, and Blood Institute (NHLBI)
Kelly T. Fennington, OD
Jay J. Greenblatt, National Cancer Institute
Laurie Harris, OD
Robert Jambou, OD
Bob Lanman, OD
Kathy Lesh, OD
Rebecca P. Link, NHLBI
Cheryl McDonald, OD
Catherine McKeon, NIDDK
Maureen Montgomery, OD
Marina O=Reilly, OD
Penny Powell, OD
Alexander Rakowsky, OD
Steven Rose, OD
Gene Rosenthal, OD
Thomas Shih, OD
Allan Shipp, OD
Sonia I. Skarlatos, NHLBI
Lana Skirboll, OD
Gisele White, OD

Others

Approximately 60 individuals attended this 2-day RAC meeting. A list of attendees appears in Attachment II.

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

Dr. Friedmann, RAC Chair, called the meeting to order at 8:00 a.m. on March 7, 2002. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on February 13, 2002 (67 FR 6728). In addition to public discussion of four protocols, the agenda at this meeting included a report and analysis of the detection of adeno-associated viral vector sequence in research participants' semen, informed consent issues of human gene transfer research, updates from the NIH Office of Biotechnology Activities (OBA), and data management safety information and clinical updates.

Dr. Patterson asked RAC members to note that the NIH Rules of Conduct and Conflict of Interest notice were provided to them in the premeeting materials.

A list of abbreviations and acronyms and their meanings appears in Attachment III.

II. Minutes of the December 6, 2001, RAC Meeting/Ms. Levi-Pearl

Ms. Levi-Pearl, the RAC reviewer, noted that the minutes were an accurate summary of the December 2001 RAC meeting.

A. Committee Motion 1

As moved by Ms. Levi-Pearl and seconded by Ms. King, the RAC accepted the December 6, 2001 minutes by a vote of 12 in favor, 0 opposed, and 2 abstentions by RAC members who had not attended the RAC meeting in December 2001.

III. OBA Updates

A. Roundtable Introduction of New RAC Members/Dr. Friedmann

The RAC members introduced themselves. Dr. Friedmann noted that one member, Dr. Kwaku Ohene-Frempong, Children's Hospital of Philadelphia, was not able to attend this meeting.

B. Final Action on Severe Adverse Event Reporting/Dr. Patterson

Dr. Patterson explained that the final action to enhance the reporting, assessment, and communication of safety information contains four elements: (1) harmonization of the NIH requirements for the scope and timing of reporting of safety information with FDA requirements; (2) ensuring public access to safety information and requirements of applicable statutes that protect confidential commercial information; (3) protection of research participant privacy; and (4) establishment of a safety data assessment board. With implementation of the final action, principal investigators and sponsors need to follow one set of reporting criteria for both the NIH and the FDA. Establishment of the data assessment board will result in an enhanced and systematic analysis of safety data across all trials that will be presented publicly to inform the design and conduct of ongoing and future clinical trials.

The following reporting guidelines and provisions will now apply:

- All SAEs that are nonfatal or non-life threatening, expected, and considered possibly associated with the use of the gene transfer product must be reported within 15 calendar days after sponsor receives the information.

- SAEs that are unexpected and considered possibly associated, fatal or considered life threatening must be reported within 7 calendar days after sponsor receives the information.

- All other adverse events must be reported annually.

- While the FDA's requirements apply to the sponsor, the NIH's oversight pertains to the institution and thereby rests with the PI. The PI is the responsible party for reporting safety information to the NIH; however, the PI may delegate to another party, such as the corporate sponsor, the role—but not the responsibility—of reporting safety information to the NIH.

- The final action does not affect requirements for reporting to institutional review boards (IRB), or funding bodies.

The Gene Transfer Safety Assessment Board's functions will be to analyze safety information across all trials with the goal of recognizing trends as early as possible. The Board's findings, conclusions, and aggregated trend analyses will be reported for public discussion at RAC meetings to inform the design and conduct of ongoing and future clinical trials. The Board will be composed of approximately 15 members with expertise in relevant fields, and 2 RAC members will sit on this Board to provide an intellectual continuum between the deliberations of the Board and the activities of the RAC. The Board will meet in closed sessions and provide periodic summary reports to the RAC. The Board will be staffed by the OBA, and FDA staff will also be involved in its deliberations.

C. Clinical Gene Transfer Database/Kelly T. Fennington, OBA

Ms. Fennington provided an update on the Genetic Modification Clinical Research Information System (GeMCRIS) database. Key features of this relational database include its use of controlled medical vocabularies, a common electronic adverse event reporting format for both the NIH and the FDA, Web-based public access with security measures to protect trade secret and confidential commercial information, and usability for a diverse group of potential users. A demonstration and exhibit will occur at the meeting of the American Society of Gene Therapy in June, and public launch is expected in summer 2002. The next steps for GeMCRIS are to add more controlled vocabularies for more robust analysis, *ad hoc* query capability, a user manual for investigators, and user feedback capabilities.

D. IBCs in a Changing Research Landscape: A Policy Conference—Debriefing/Allan Shipp, OBA

Mr. Shipp reviewed the objectives of the December 7-8 2001 conference, which were to take a fresh look at the expectations, roles, and responsibilities of IBCs and apply these to traditional and nontraditional IBC arrangements; examine the *NIH Guidelines* in light of the new environment; and to provide a professional development opportunity for Institutional Biosafety Committee (IBC) members and staffs. Conclusions from the conference were as follows:

- There is a need for simplified and accessible information about the *NIH Guidelines* and IBC requirements.

- While ensuring biosafety oversight, IBC policies should be flexible to accommodate the changing environment.

- IBC members and staff need more professional development opportunities.

Mr. Shipp reported that OBA staff has already developed a new posting of a portion of the office's web site dedicated to information for and about IBCs. This includes materials from the December IBC policy conference to broaden access. OBA has also undertaken planning of a future professional development conference for late 2002. The policy issues concerning IBCs are being considered on a case-by-case basis in the short term, and will be addressed in the long term as part of efforts to update the *NIH Guidelines*.

IV. Discussion of Human Gene Transfer Protocol #0201-513: Phase I Study of Intravenous Dioleoyltrimethylammoniumpropane:Cholesterol-*fus1* Liposome Complex (DOTAP:Chol-*fus1*) in Patients With Advanced Non-Small Cell Lung Cancer Previously Treated With Chemotherapy

Principal Investigator:	Charles Lu, M.D., University of Texas M.D. Anderson Cancer Center
Sponsor:	None
RAC Reviewers:	Dr. Breakefield, Dr. L. Johnson, and Ms. King

A. Protocol Summary

Non-small cell lung cancer is the leading cause of cancer-related death in the United States. Patients with advanced lung cancer or lung cancer that has spread to other organs are currently treated with palliative chemotherapy-based treatments. Even the most active chemotherapeutic agents have increased survival by only several months. New therapeutic agents are needed.

The results from the preclinical studies of intravenous delivery of tumor suppressor genes complexed to DOTAP:Chol show reduction of experimental metastases and prolongation of survival in a SCID mouse human lung cancer model. The *fus1* gene has been selected because of its high degree of selective apoptosis for lung cancer cells compared to normal bronchial epithelial cells, its ability to completely inhibit the growth of subcutaneous tumors with intratumoral injection, and its ability to mediate a reduction

in lung experimental metastases comparable to p53. It is likely that this gene will be deleted in the early stages of lung carcinogenesis thus making it an attractive target for all stages of disease. Extensive toxicity studies have been conducted in mice and show that intravenous doses up to 100 µg of the DOTAP:Chol-*fus1* complex are tolerated without toxicity. Mice have received up to six consecutive daily doses of 50 µg without toxicity.

The objectives of this study are to assess the toxicity of DOTAP:Chol-*fus1* administered intravenously; determine the maximum tolerated dose of DOTAP:Chol-*fus1* administered intravenously; assess the expression of *fus1* following IV delivery of DOTAP:Chol-*fus1* in tumor and normal cell biopsies; and assess any anticancer activity for DOTAP:Chol-*fus1*.

B. Reviews by RAC Members

Drs. Breakefield and L. Johnson and Ms. King submitted written reviews, to which the investigators responded both in writing and during this meeting.

Dr. Breakefield noted that the normal function of the *fus1* gene is not known and that questions remain about the potential toxicity of *fus1* to other tissues and the biodistribution of this complex in animals and humans after IV administration. She asked about the transduction of normal lung and tumor cells. She was particularly concerned about liver toxicity and recommended monitoring IL-6 levels as a potential marker for liver damage. The study did not specify any age limitations, but if young participants were to be enrolled, preclinical studies should be performed in younger animals.

Dr. L. Johnson's concerns centered on the novelty of the transgene and questions regarding dosing, toxicity, and the informed consent document. He noted that the data from intravenous infusion studies suggested at least a possibility for significant liver, renal, or cardiac toxicity, which could be linked to volume, rate of infusion, or dose effects. To distinguish these possibilities, he recommended studies in larger animals. To provide a time course of histological changes or other measures of toxicity, the toxicology evaluation should include autopsies performed on mice sacrificed at different time points.

Ms. King discussed needed modifications to the informed consent document to make it more appropriate for a phase I study. She recommended avoiding the usage of "treatment", "gene therapy", and "patient" language. The informed consent document should include a request for autopsy, and Appendix M-III-B-2-c provides suggested language. It was also recommended that the IRB incorporate these changes into their informed consent template. Dr. Borror concurred with Dr. King's comments about the template.

C. RAC Discussion

Several concerns were raised by RAC members in addition to those expressed by the primary reviewers:

Ms. Levi-Pearl asked whether human subjects would be enrolled prior to completing the primate studies. Dr. Bohn asked if laser-capture microdissection would be used in conjunction with quantitative PCR on the biopsy specimens.

D. Investigator Response

In response to concerns about the informed consent document, Dr. Lu explained that the M.D. Anderson Cancer Center requires the use of a template informed consent document that is accessible only online and cannot be changed by investigators. He had already had a discussion with the scientific editor at MD Anderson responsible for the template. He would discuss this with the IRB again based on the RAC recommendations.

Dr. Jack A. Roth, University of Texas M.D. Anderson Cancer Center, explained that the investigators had been in discussion with the FDA for several months regarding a good laboratory practices (GLP) studies in mice and in nonhuman primates that should address many of the issues. Data from these animals will include renal function, liver function, and hematologic serum studies.

In response to Dr. Breakefield's concerns about toxicity, Dr. Roth responded that the GLP studies include 15 animals per group, which should be an adequate number. *In vitro* studies have been performed with lung fibroblasts, and toxicity to other organs is being studied in the animal models.

Dr. Roth stated that the FDA would decide whether research participants could be enrolled prior to completing the primate studies. It was his understanding that the projected primate studies needed to be completed first.

Regarding Dr. Bohn's suggestion about the use of laser-capture microdissection, Dr. Roth responded that the biopsies obtained are quite small because the research participants are not surgical candidates. For such small biopsy specimens, laser-capture microdissection is technically difficult to conduct. The investigators have been able to look at several sections from the biopsies and identify tumor cells.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

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

- The informed consent document is highly technical and should be revised to enhance comprehension by the research participants.
- Because this is a phase I safety trial, the informed consent document should emphasize the investigational rather than therapeutic nature of the study. Throughout the informed consent document, therefore, potentially misleading terms such as "therapy," "treatment," and "patient" should be replaced with terms such as "gene transfer," "intervention," and "research participant." The RAC understands that the informed consent document for this protocol was based on a template provided by the Institutional Review Board (IRB); therefore, the RAC recommends that these changes should be considered for incorporation into the template as well.
- The RAC discussed the scope of the preclinical data and the lack of age limitations in the study design and recommended that the Principal Investigator confer with the IRB about whether the protocol should exclude certain age groups. If any changes are made to the protocol, these should also be included in the informed consent document.
- As highlighted in the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, in gene transfer research, autopsies have the potential to yield information important to guiding future studies. Appendix M-III-B-2-c provides suggested language investigators could adopt when making autopsy requests. The Principal Investigator should advise the IRB of the provisions of the *NIH Guidelines* and urge the IRB to allow the informed consent document to include a request for autopsy.
- The toxicology studies should include measurement of blood interleukin-6 (IL-6) levels as a tool to help monitor inflammation and potential for liver toxicity.
- The RAC stressed the importance of the preclinical GLP studies. As the results become available, to inform the RAC, the data should be submitted to OBA as follow-up information.

G. Committee Motion 2

As moved by Ms. Levi-Pearl and seconded by Dr. L. Johnson, these recommendations were approved by a vote of 13 in favor, 0 opposed, and 0 abstentions.

V. Informed Consent Issues of Human Gene Transfer Research/Dr. Brody and Ms. King

A. Ms. King

Ms. King noted that informed consent documents have been an issue for RAC consideration since human gene transfer studies began. Informed consent documents should function as an educational exchange to inform research participants about the purpose, design, and safety of the protocol. Helpful guidance for the development of informed consent documents is provided in Appendix M-III, and IRBs and PIs should be aware of Appendix M-III as a resource.

Ms. King proposed establishing a RAC working group to develop additional guidance about informed consent. The group could work on drafting a model informed consent document or template for use in gene transfer research and participant information materials. Links to examples of model consent forms, relevant literature, and past RAC discussions about informed consent documents could be added to the OBA web site. A gene transfer policy conference on consent issues would be useful. The working group will report back to the RAC at a subsequent RAC meeting.

Informed consent issues to be considered in the gene transfer field include the appropriate language to describe complex concepts, the over exuberance of the field, and the potential for the therapeutic misconception by the very ill populations of research participants that often enroll in GT trials. Consent documents need to clearly distinguish among different types of potential benefits: direct benefits resulting from receipt of the intervention being studied, collateral benefits resulting from being a research participant (independent of the studied intervention, such as close monitoring or free testing), and aspirational benefits to society, science, and future patients.

Ms. King discussed her own current research, an analysis of consent documents entitled “The Social Construction of Benefit in Gene Transfer Research.” This research is being conducted by telephone interview with investigators, study coordinators, research participants, and IRB chairs or members. It includes an analysis of informed consent documents submitted to OBA and responses to Appendix M. Data entry for the consent document analysis is expected to be completed in May 2002.

Dr. Brody

Dr. Brody identified three other informed consent issues he believed were in need of guidance from the RAC:

1. Increasingly, protocol reviewers and research participants want more information about financial conflicts of interest involving investigators and institutions.

2. With research participants who are dying, the informed consent process should include a discussion of alternatives that involve palliative care rather than further interventions.

3. Regarding autopsy requests, pathologists often will not perform an autopsy if the family does not approve the procedure, even when the research participant had previously consented to it. Therefore, it is important to share the consent document with the family in these situations. If families deny autopsies, much important information about gene transfer will be lost.

C. RAC Discussion

Dr. Childress underscored that IRBs are interested in streamlining the consent process, educating investigators, and developing a more uniform consent model. The RAC could provide guidance on issues unique to GT research.

Dr. Wara expressed her concern that there be synergy between the approach taken by the participant's primary care physician and the language used in the consent process, especially regarding the discussion of death and autopsies.

Dr. Brody agreed that emphasis needs to be placed on the informed consent process and not just the document. For example, research participants should be given enough time to think about research participation.

Dr. Breakefield noted that informed consent documents act as protection for institutions and investigators.

Ms. Levi-Pearl discussed the need for collaboration among the various agencies that oversee research protections and that are in a position to tutor IRBs at the local level.

D. Public Comment

Shirley M. Clift, Cell Genesys, discussed a problem created by the FDA requirement for long-term followup. She noted that a disconnect is often created by language in the consent document that refers to follow-up for 15 years even for research participants with advanced cancer who may not be expected live beyond a few months.

E. Formation of a Working Group

Dr. Friedmann requested volunteers for a working group that would report to the RAC at the June 2002 meeting with a work plan for proceeding on this issue. The following individuals volunteered: Dr. Brody, Dr. Childress, Ms. Levi-Pearl, and Dr. Wara; there also will be one designee each from the FDA and the Office for Human Research Protections. Ms. King and Dr. Brody will serve as co-chairs for this working group.

VI. Detection of Adeno-Associated Virus Vector Sequence in Research Participant Semen: Followup

Presenters:	Mark A. Kay, M.D., Ph.D., Stanford University School of Medicine, and Katherine A. High, M.D., Children's Hospital of Philadelphia
RAC Discussants:	Drs. Breakefield, Childress, and P. Johnson and Ms. King

A. Presentation

At the December 2001 RAC meeting, the RAC and *ad hoc* consultants discussed the detection of DNA sequences from an adeno-associated virus (AAV) vector in the semen of the first research participant enrolled in a Phase I safety study using AAV to deliver the gene for human Factor IX into the liver of research participants with severe hemophilia B (Factor IX deficiency). The data from this study were presented and analyzed, and the potential significance of these data for the risk of vertical transmission—germ-line gene transfer—was discussed.

Dr. High presented recent data from a rabbit model to study the kinetics of vector clearance as a function of dose after intravascular delivery of vector. Total semen analysis at the time points of 7, 15, 22, 38, 44, 50, 73, and 87 days indicated that animals receiving a midlevel dose were positive for 3 weeks after injection and that high-dose animals were beginning to clear vector from their semen starting at 7 weeks. The rabbit model results indicated that following intravascular injection, white blood cells from the buffy coat and total semen were positive for vector sequence in a dose dependent manner and that vector sequences clear from total semen over time. Except for one time point in which the samples were likely contaminated, vector sequences had not been detected in motile sperm by PCR.

Dr. Kay updated the RAC on the progress of the clinical trial since December 2001. On the basis of discussion at the RAC December 2001 meeting, the informed consent document was modified. In December 2001, data from the first research participant were available only to week 10 post-injection. Additional information to week 24 indicated normal liver function and blood chemistry results, and no change in coagulation (including Factor IX levels). Vector sequence had cleared from semen samples from several weeks in a row out to 24 weeks. A second participant received vector on Jan. 29, 2002, and vector sequence was transiently detected in saliva, serum, stool, and urine. Analysis of total semen detected vector sequence in all three samples at week one and one of three samples at week four. The total semen sample from week eight will be fractionated. Additional research participants may be enrolled only if the motile sperm fraction is negative.

B. RAC Discussion

Dr. P. Johnson commended Drs. High and Kay for quickly producing new data since the December 2001 RAC meeting. He reviewed the issues raised at the December 2001 RAC meeting and summarized the status of each:

- *Duration of the clinical hold and the continued enrollment of research participants.* This issue was addressed by the FDA. A second research participant has been enrolled and is proceeding through the protocol.

- *Assay of the semen samples.* Drs. High and Kay updated the RAC on the relevant data as noted above.

- *Evaluation of the informed consent document.* Dr. Kay discussed how the document had been modified in response to the recommendations made at the Dec. 2001 RAC meeting.

- *Likelihood of germ-line gene transfer.* Although vector shedding is occurring, the likelihood of transduction of motile sperm appears low. Vector shedding is thought to be dose-dependent, but the length of time vector shedding will continue is not known.

Dr. Breakefield asked whether nonhuman primates were being studied. Dr. High responded that the investigators had attempted to study semen from the nonhuman primates but had difficulties obtaining adequate samples. Currently no data are available from nonhuman primates, but further analysis is anticipated.

Dr. Breakefield asked about the possibility of horizontal transmission of vector in semen to female partners and whether the half life of AAV vectors in semen is being determined. Dr. High noted that this question will be addressed as their collaborators at Avigen develop an infectivity assay to test collected rabbit materials. In addition, Phillipe Moliere of France conducted infectious titering of body fluids

following vector injection in nonhuman primates. According to his data the material remains infectious in body fluids for 3 days.

Ms. King asked whether any children had been born to research participants following gene transfer. She suggested that the RAC consider the issues associated with this possibility including the type of follow-up needed, the requirement for long-term contraception use, and how to obtain consent for the follow-up of offspring. Dr. Childress agreed that the RAC should be at the forefront in thinking about these issues and recognizing that risk extends beyond the participant.

Dr. Noguchi and Dr. Simek both stated that the FDA recognizes the difficulty of including in an informed consent document a provision requiring that children of a research participant be tested for vector transmission. Dr. Wara cited the experience in the pediatric HIV field in following children born to HIV infected mothers who received treatment to decrease perinatal transmission. This field took two years to develop a plan, but then was successful in both obtaining consent and following the children.

Dr. Brody suggested an NIH request for proposals to develop animal models to study germ line transmission by a wide range of vectors.

In response to Dr. Friedmann's question as to whether germ line transmission could be accomplished *in vivo* by using high concentrations of vector, Dr. Kay explained that this is being investigated. In these experiments, AAV will be mixed with mouse semen or sperm and then used to fertilize female mice *in vitro.* Fetuses will be examined for vector integration. However, two major issues with this study involve questions about whether it will yield statistically significant data and the limitations of using a mouse model.

As an interim step to developing guidance, Dr. Patterson suggested that a working group of the RAC with input from the NIH, FDA, and investigators develop a series of hypothetical case studies using different vector systems. She suggested that the working group discuss specific vector systems in specific clinical settings and develop recommendations for guidance.

C. Next Steps

Drs. Friedmann and Patterson will discuss the points raised during this RAC discussion and will report back to the RAC regarding suggested next steps. Dr. Friedmann asked Drs. High and Kay to keep the RAC informed of their ongoing research.

VII. Discussion of Human Gene Transfer Protocol #0201-514: Transduction of the Upper and Lower Airway Epithelium in Healthy Subjects by an AAV2 Vector That Encodes Human Placental Alkaline Phosphatase

Principal Investigators:	Moira L. Aitken, M.D., University of Washington, and A. Dusty Miller, Ph.D., Fred Hutchinson Cancer Research Center
Sponsor:	None
RAC Reviewers:	Drs. Brody, Gooding, and L. Johnson

Dr. Friedmann noted that the subject population has changed from normal volunteers ("healthy subjects") to individuals who have cystic fibrosis (CF).

A. Protocol Summary

CF is the most common autosomal recessive genetic disorder in persons of northern European ancestry. It occurs once in 3200 live births. Although the median life span has increased from 18 years in 1972 to 32 years in 1998, CF remains a life-threatening disease with the majority of patients dying from respiratory failure. Recent improvements in longevity are attributed to better anti-pseudomonal antibiotic therapies and the multi-discipinary care provided by specialized centers. However, conventional

treatments for CF lung disease that do not replace the dysfunctional CFTR are clearly unsatisfactory and make therapies introducing the corrected gene an appealing possibility.

CFTR is believed to have many functions in the epithelial cells including chloride ion secretion, modulation of sodium channels, and transporting ATP. How defects in CFTR lead to disease manifestations in CF remains unclear, but there are several hypotheses. These include the airway hypertonic periciliary fluid inactivates defensins thereby allowing airway infection; the inflammatory mechanism in CF is upregulated with absence of inhibitor factor; and hyperabsorption of sodium leads to dehydration within the airway and alters the rheological properties of mucus. The gene responsible for CF spans 250 kb and produces a 6.5 kb transcript that encodes CFTR.

An AAV vector with a strong promoter derived from Rous sarcoma virus to improve the efficiency of gene expression will be used in this study. The use of a reporter gene rather than CFTR is proposed for several reasons. First, CFTR is normally expressed at very low levels, and antibody detection of CFTR encoded by transfer vectors is unreliable. Second, functional assays for CFTR function in the lung are complex and unreliable for detecting low level expression of CFTR. The human placental alkaline phosphatase (hpAP) reporter gene will allow facile and sensitive detection in both the nose and lung. Third, an AAV vector expressing reporter genes will provide a simpler assay for functional gene expression to help determine whether AAV-mediated gene transfer can achieve 5-10 percent transduction efficiency estimated to be required for correction of the CFTR defect. Fourth, the manufacture of an efficient AAV-CFTR vector is not a trivial undertaking and possibly should not be pursued if the initial clinical trials of AAV2-AP show a low transduction rate.

In the proposed clinical trials, transduction by vectors with AAV2 capsid will be studied. Concurrently, animal studies with AAV6 vectors will be completed. Subsequent clinical trials will test vectors with capsid proteins from AAV6. Results in mice indicate that vectors having AAV6 capsid proteins transduce airway epithelial cells much more efficiently than those with AAV2 capsid proteins. It also appears that AAV6 vectors are less able to stimulate an immune response, an advantage for repeat transduction in humans. However, because much more is known about the safety profile of AAV2 vectors, study of both vectors in humans starting with AAV2 is proposed.

The rationale for going directly into participants with CF rather than involving healthy volunteers is because healthy lung tissue does not have the same airway barriers to vector transduction such as mucus and proteolytic enzymes. In addition, this population may potentially benefit in the future from the development of an effective vector to deliver CFTR to the airways.

B. Reviews by RAC Members

Drs. Brody, Gooding, and L. Johnson submitted written reviews, to which the investigators responded both in writing and during this meeting.

Given the preclinical data indicating AAV6 vectors may have advantages over AAV2 vectors, Dr. L. Johnson questioned the choice of AAV2 vectors for the clinical trial. He was satisfied that some useful information could be learned from a clinical trial with an AAV2 vector with a stronger promoter and a marker gene that had not been used in previous studies of AAV2 vectors in humans. He was concerned about possible toxicities associated with ectopic expression of AP. Regarding the proposed study population, he was concerned about the effective exclusion of women, but supported the change to CF participants. The informed consent document should be less definitive about this trial leading to a treatment for CF and should address the risks associated with bronchoscopy.

Dr. Gooding questioned the rationale for not conducting non-human primate studies of AAV6 vectors before choosing a vector for the clinical trial. Because many AAV serotypes are found naturally in nonhuman primates, a primate model could be useful to study safety and compare efficacy of different AAV serotypes. The change in participant population was of concern because immune responses to the vector may prevent participants from receiving AAV vector gene transfer again. She questioned the use

of a marker gene rather than analyzing CFTR expression by RT/PCR. Dr. Miller responded that RT/PCR could not be used to determine transduction levels. Also because of the difficulty in constructing AAV CFTR vectors, information could be obtained more quickly by comparing marker vectors to determine the AAV serotype to be used for the CFTR vector.

Dr. Brody also suggested starting with larger animals or primates to study AAV6 vectors further. He questioned whether the study design would allow sufficient statistical analysis to justify the use of a control group. He asked whether women could be included in the study if contraception were required. He also asked about the exclusion criteria referring to a history of substance abuse and how participant confidentiality would be protected.

C. RAC Discussion

Dr. Friedmann suggested a matrix approach for further animal studies comparing the different serotypes of vector and the higher capacity vectors generated by homologous recombination before selecting an optimized vector for human use.

Dr. Noguchi noted that to exclude a participant group such as women requires quantifying the greater risk for that group. Dr. Miller responded that the risk is from the potential antibody response to human placental AP which is limited to women because men do not have a placenta. Dr. Noguchi noted that the risk of an antibody response may not be limited to the placental tissue, and he encouraged the investigators to determine the risks associated with AP expression in other tissues and the immune response in both men and women.

Dr. L. Johnson clarified the references to his published data suggesting that the CF defect could be corrected by CFTR expression in only 5-10 percent of cells. Because it is not know whether the sodium or chloride transport defect needs to be corrected, it may be necessary to correct all the cells instead.

Dr. Breakefield suggested that in addition to the exclusion of potential research participants with adenoviral infections, individuals with active herpes infections should also be excluded.

Dr. Bohn recommended that more basic research be performed to determine the safety and efficacy of vectors with different serotypes, and promoters and the high capacity vectors before proceeding into humans with an optimized CFTR vector.

D. Investigator Response

Dr. Miller suggested that the question of whether AAV2 works well in humans has to be addressed in clinical studies. While animal models can be useful in testing safety, human trials will be needed to test efficacy of AAV2 vectors in humans. Subsequent trials will study AAV6 or other vectors and compare the results to those obtained with the AAV2 vectors.

In response to whether a requirement for using contraception would enable women capable of reproduction to participate, Dr. Aitken stated that investigators were concerned about the antibody response to the AAV and to AP. The antibodies to AP are likely to be permanent and the effect of an antibody response on placental tissue and a future pregnancy is not known.

Dr. Miller explained the rationale for the change to a CF participant population. Since the goal is to provide therapy to CF patients, using a CF epithelium model at a Phase I stage of investigation is the most useful model available. Transduction levels achieved in research participants with CF may differ from that in normal volunteers because the epithelium of CF participants is damaged, perhaps allowing virus to get into cells more efficiently. On the other hand, the higher levels of mucus in CF lungs may interfere with transduction. Healthy volunteers are being considered as good models for young children with CF. Dr. L. Johnson noted that studies during the past few years have shown that bacteria colonize a large percentage of the airways of CF children even before the age of 1 year, and that high levels of cytokine production exist in those airways.

In response to a question from Dr. Friedmann, Dr. Miller explained that the target cells are the bronchial epithelium of the small airway.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

Dr. Friedmann summarized the following RAC recommendations, and observations:

- Noting the limitations of AAV2 vector transduction of airway epithelium, some members recommended that large animal studies be conducted on the AAV6 and the higher capacity vectors to identify an optimized vector to be used in the clinical studies. The current preclinical and clinical experience with AAV2 derived vectors was considered to be sufficient to proceed with the protocol in cystic fibrosis research participants as proposed. The principal Investigators, in consultation with their Institutional Review Board (IRB), were asked to reconsider the selection of the vector, and to notify the RAC through OBA of the outcome of the discussion.
- The Principal Investigators' decision to change the study population from healthy volunteers to participants with cystic fibrosis was supported.
- It was suggested that the Principal Investigators, in consultation with the IRB, re-evaluate the basis for the exclusion criteria. Potential toxicities as the result of human placental alkaline phosphatase (AP) gene transfer in both sexes should be considered and the following other questions addressed: What is the expression level of AP in tissues other than the placenta? Would there be any long-term immunological effects from the generation of antibodies to AP? Would an antibody response have the potential to affect the placenta or fetus jeopardizing future pregnancies?
- A Data and Safety Monitoring Board should be established to review the safety and efficacy data after completion of the studies in the nasal epithelium (Part A) and before commencing the studies in the bronchial epithelium (Part B). If no gene expression is detected in the nasal epithelium, the merits of proceeding to studies in the lower airways should be carefully considered because the likelihood that an antibody response to the vector will be generated still exists.
- The informed consent document should discuss more fully and clearly all the risks associated with the intervention, including the risk of death associated with bronchoscopy.
- The protocol and informed consent document should clarify that this is a pilot observational study and not powered for statistically valid comparisons with the control group.
- The protocol excludes participants who have used substances of abuse within 30 days prior to screening for possible enrollment. Given that information about illicit behavior may be obtained, the confidentiality of information collected during screening should be protected.

G. Committee Motion 3

It was moved by Dr. L. Johnson and seconded by Dr. Brody that these recommendations expressed the views of the RAC and would be included in the letter to the investigators. The vote was 12 in favor, 0 opposed, and 1 abstention.

VIII. Day One Adjournment/Dr. Friedmann

Dr. Friedmann thanked the participants and adjourned the first day of the March 2002 RAC meeting at 4:50 p.m. on March 7, 2002.

IX. Day Two Opening Remarks/Dr. Friedmann

Dr. Friedmann opened the second day of the March 2002 RAC meeting at 8:30 a.m. on March 8, 2002.

X. Remarks of NIH Acting Director/Ruth L. Kirschstein, M.D., NIH

Dr. Kirschstein, NIH Acting Director, discussed the establishment, history and continuing importance of the RAC. She noted that the RAC reviews scientific, medical, legal, social, and ethical issues and has served as a model for fostering public discussion of important scientific and ethical issues. New provisions enacted by the NIH will ensure that the RAC continues to monitor, in a timely manner, the safe and ethical conduct of recombinant DNA research. Dr. Kirschstein commended RAC members for being willing to serve many volunteer hours and offered thanks for their service on behalf of the American public.

XI. Swearing In of New RAC Members/Dr. Kirschstein

Dr. Kirschstein administered the oath of office to new RAC members, who had already signed the written version of the oath.

XII. Discussion of Human Gene Transfer Protocol #0112-512: Phase I Study of Human Growth Hormone-Releasing Hormone Expressed by a Plasmid DNA Myogenic Vector in Patients With Cancer Cachexia

Principal Investigators:	Malcolm K. Brenner, M.D., Ph.D., and Uday R. Popat, M.D., Baylor College of Medicine
Sponsor:	None
RAC Reviewers:	Dr. P. Johnson, Ms. King, and Dr. Simari
Ad Hoc Consultants:	Thomas D. Gelehrter, M.D., University of Michigan Medical School; Richard G. Vile, Ph.D., Mayo Clinic; and Thomas R. Ziegler, M.D., Emory University Hospital

A. Protocol Summary

Complications of cancer—such as weakness, weight loss (cachexia), and anemia—are present in more than half of affected patients. Other clinical manifestations include loss of appetite, muscle wasting, loss of fatty tissue, and fatigue, resulting in poor quality of life. In addition, cachexia may prevent therapy from being given and may be a direct cause of death.

Growth hormone-releasing hormone (GHRH) stimulates the synthesis and secretion of growth hormone from the anterior pituitary, which in turn stimulates insulin-like growth factor-1 (IGF-1) production. These molecules have previously been used to treat conditions associated with metastatic cancer, but side effects in long-term therapy (associated with protein peaks and troughs) may be detrimental. A gene transfer approach may overcome the primary limitation (short half-life in serum) to GHRH use. In a study of adult mice, GHRH was efficiently regulated long-term in a mifepristone specific system and induced increased weight, lean body mass, bone mineral density, hemoglobin, and hematocrit levels. In large animal studies with pigs and dogs, long-term expression of GHRH consistently produced significant physiologic changes in weight, body composition and had a positive effect on protein and bone metabolism, with no discernable adverse effects.

In this Phase I study, the researchers propose to inject increasing amounts of DNA-expressing GHRH to determine the safety of this approach in research participants with advanced cancer. This study will utilize an inducible system (using the Gene Switch) to control GHRH gene expression via an inducer, oral mifepristone.

B. Reviews by RAC Members and Ad Hoc Reviewers

Dr. Brody abstained from voting. Dr. Breakefield, Ms. King, and Dr. P. Johnson submitted written reviews, as did *ad hoc* consultants Drs. Gelehrter, Vile, and Ziegler, to which the investigators responded in writing and during this meeting.

Dr. Simari expressed concerns about the ability of the preclinical animal studies conducted to date to predict the proposed use of this inducible gene transfer system in the clinical study. None of the animal studies combined all of the elements to be used in the human trial. For example, some animal studies looked specifically at the ability of a plasmid vector system to express the GHRH transgene, but did not use an inducible system. Another study used the inducible system, but did not include the proposed GHRH transgene/plasmid system. He also asked about the effect of mifepristone use in cancer patients.

Dr. Gelehrter requested more information about the preclinical studies of the regulated expression system. He asked about the specificity of the skeletal muscle actin promoter, and the leakiness and induction of expression of the transgene by MFP. Since IGF1 is a putative growth factor for various cancers, he expressed concern that if the proposed gene transfer is successful, there may be the possibility that increased IGF levels will actually cause enhanced cancer growth.

Dr. P. Johnson noted the lack of data on the potential efficacy of the proposed approach in humans. He expressed concern about the possible effects of the GHRH/GH/IGF family on tumors, and the unexplained excessive mortality reported in critically ill patients receiving high dose rhGH treatment. He also asked about the potential for this approach to be used for other types of chronic cachexia (e.g., AIDS) or to be misused for enhancement purposes (e.g., bodybuilders).

Ms. King requested more information on cancer cachexia, discussion of the use of mifepristone as the inducer, rationale for the selection of research participants, and the consent monitoring process. Noting that the informed consent document was in need of considerable revision, Ms. King provided specific suggestions to assist in that process including replacing “patient” and “treatment” with “research participant” and “experimental intervention.” She also requested that the investigators consider whether this gene transfer, if ultimately shown to have sufficient desired effects, could be applicable to cachexia due to other causes such as AIDS, the late stages of other diseases, or anorexia.

Dr. Vile asked why the investigators chose this particular inducible system. He requested discussion of the likely effects of long-term administration of mifepristone on the research participants’ general health, and on tumor progression. Regarding the inducible system, he asked about the choice of the relative ratios of plasmid doses, the kinetics of induction, and possible immune response to non-human or fusion proteins. Since the clinical protocol proposes using a two plasmid inducible system with oral MFP as the inducer and injection into the *gluteus maximus* of participants with tumor burden, Dr. Vile suggested studying an animal model that included all these components.

Dr. Ziegler noted that published data on the use of GHRH, GH, and IGF-1 in *in vivo* cancer models and human cancer patients were not well outlined in the protocol. He also noted that the investigators do not yet appear to have experience with the proposed system in species other than mice without cancer. He suggested that the inclusion/exclusion criteria control for type of cancer, tumor burden, ongoing treatment and underlying nutritional status in this pilot study. Dr. Ziegler suggested that a nutritionist be involved in the design of this study and that the nutritional intake of research participants be monitored.

C. RAC Discussion

Ms. King pointed out that the investigators stated that they submitted their proposal to the RAC prior to extensive review by their IBC or IRB because they believed the RAC now preferred to review protocols at an earlier stage of development. Ms. King noted that recent changes in the timing of protocol submission was meant to allow investigators to choose one of several pathways, including submitting the protocol to the RAC and the IRB at the same time.

Ms. King expressed concern about Dr. Popat's recruitment of his own patients in this study because he indicated that many patients of his could benefit from this treatment." She noted that this trial is not a treatment but is a Phase I study. Ms. King also suggested that investigators consider use of a consent monitor.

Dr. Gelehrter asked about the regulation of the gene switch in the pig model, particularly evidence that expression can be turned off, which is a safety issue.

Dr. Gelehrter asked whether a midsize or large-animal model with cancer is available that could be used to more closely approximate the human situation. Dr. Linial suggested the feline leukemia model in which lymphomas or sarcomas can be induced by injecting newborn kittens with virus.

Dr. Noguchi noted that the FDA might require additional studies looking specifically at the use of MFP since it is presently labeled specifically for certain indications and not in combination with the proposed biological agent. He also voiced concerns similar to those of Dr. Gelehrter about the proposed length of dosing and dosage of MFP.

D. Investigator Response

Regarding the use of mifepristone in patients with cancer, Dr. Brenner explained that mifepristone has been used to treat patients with progestin-responsive cancer, although the drug is not labeled for use in that way in the United States. There is no evidence that mifepristone induces tumor growth. Dr. Ruxandra Draghia-Akli, Baylor College of Medicine, summarized the data in the literature, stating that mifepristone has been administered long term as a contraceptive and as a treatment for the complications of Cushing disease and progestin-responsive tumors.

Dr. Brenner explained that the level of IGF-1 is reduced when mifepristone is withdrawn, returning IGF-1 almost to baseline by 7 days and to baseline by 14 days.

Dr. Draghia-Akli explained that the investigators have attempted, without success, to find appropriate large-animal models. Dr. Brenner stated that the issue of finding reasonable animal models for human cancers is a generic one in cancer research.

In response to Dr. Ziegler's concerns about monitoring nutritional intake, Dr. Draghia-Akli commented that the investigators have established a collaboration with nutritionists for the purpose of this trial. Nutrition advice will be offered to research participants, including the advisability of supplementation. During the trial, data regarding nutrition will be collected for each participant.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

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

- The Principal Investigators are urged to expand upon their already completed animal models before proceeding with human studies. The following suggested preclinical studies were suggested:

  - Since none of the preclinical studies to date have used all of the components proposed for use in the study subject population, an animal model should be developed that includes each of these. This would include the inducible system, injection into a muscle comparable to the gluteus maximus, daily use of Mifepristone (MFP) for a length of time comparable to the proposed study, and with the animal being tumor-bearing.
  - The pharmacokinetics of the inducible system, including the "leakiness" of the system in its non-induced state and the length of time needed to reverse the system, should be studied further.
  - The potential of a host immune response to the non-human proteins expressed in this system should be evaluated further.
  - More specific studies analyzing the impact of either MFP or GHRH on tumor growth and spread should be considered.
- Due to the issues raised by studies to prove or disprove a cause and effect relationship between elevated growth hormone levels and the increased risk of developing certain types of cancers, subjects with colon or breast cancer should be excluded from this study.
- In order to more fully examine the effect of the GHRH on the cachectic state, the following are suggested:
  - The inclusion/exclusion criteria should be modified to more clearly describe the research participants. This will help ensure a more homogeneous study subject group
  - Adequate nutritional monitoring, including the potential use of controlled or monitored diets, should be considered. The Principal Investigators should consult with nutrition specialists to develop a specific nutrition monitoring plan.
- The following potential regulatory issues that will need to be addressed in future meetings with the FDA should be considered:
  - Since the study proposes to use MFP for a new indication and at a dose and duration that are not approved, the need for additional safety studies of MFP in this setting should be discussed with the FDA.
  - Since the protocol will employ a double plasmid system, the rationale for the choice of the 10:1 ratio, and the safety and efficacy of different potential ratios should be more completely documented.
- In regard to the informed consent document, the following should be considered:
  - Replace "patient" with "research participant" and "gene therapy" with "gene transfer".
  - The Benefits section should clearly convey that the protocol is a phase I safety study with no expected benefit to the research participants.
  - The use of a consent monitor may be helpful to enhance participant understanding that this is a phase I safety study and that this gene transfer intervention is not intended to cure the research participant's cancer.
- The risks of MFP to the research participant should be more clearly explained and efforts made to prevent inadvertent use by other household members through special labeling of the bottle.

G. Committee Motion 4

It was moved by Dr. Simari and seconded by Dr. P. Johnson that these recommendations be included in the letter to the investigators. The vote was 12 in favor, 0 opposed, and 1 abstention.

XIII. Data Management Report/Drs. Simari and Wara

A. Summary Statistics

Dr. Simari reported that the total number of protocols submitted to OBA since 1998 grew to 505. Seven were submitted during the period between the December 2001 RAC meeting and February 14, 2002. Four were selected for public review. The 505 protocols were categorized as follows:

- 319 were for cancer (200 were for immune modulation).

- 55 were for monogenic diseases (CF and hemophilia were the most numerous).
- 38 were for infectious diseases (37 were for human immunodeficiency virus [HIV]).
- 50 were for other diseases (including 37 for cardiovascular disease).
- 40 were for gene-marking protocols.
- 3 were for other protocols (including healthy "normal" research participants).

B. Adverse Events

Of the 119 adverse event reports submitted to the OBA during the December 1, 2001, to February 1, 2002 reporting period, Dr. Simari reported that 93 were initial reports and 26 were followup reports. Twelve events were deemed serious, possibly associated, and unexpected ("Type A"), and information about them was provided to RAC members. Dr. Simari discussed three of these events.

- In protocol #346, a phase II trial for advanced coronary artery disease with severe angina using an adenoviral vector expressing vascular endothelial growth factor (VEGF), a research participant developed a myocardial infarction (MI), which led to the development of congestive heart failure, renal failure, and cardiogenic shock. The participant recovered with aggressive treatment. The event was attributed to either the study agent or the procedure. Five other SAEs possibly associated with the agent or procedure have been reported in this protocol.
- Follow-up information on an SAE from protocol #412, a phase III trial of an adenoviral p53 vector, suggested that the SAE was more likely due to the research participant taking multiple medications for management of intractable pain rather than to the investigational agent.
- In Protocol #481, a phase I trial of a HSV-1 vector for malignant glioma, the first research participant enrolled in the study experienced an SAE that was initially considered associated with the surgery to debulk the tumor and deliver the gene transfer product. Further analysis led the principal investigator to reclassify the event as possibly associated with the gene transfer product. Additional information on this SAE is expected at the June 2002 RAC meeting.

C. Additional Discussion

Dr. Wara discussed an amendment to Protocol #337, which was a request by the PI for single-subject exemption of a research participant who had been enrolled in two prior gene transfer studies. The request was reviewed by Drs. Mickelson and Friedmann and by the OBA staff. One concern was the global issue of enrolling research participants in two or more gene transfer studies, which could result in difficulties in determining the role, if any, of a specific gene transfer agent in both safety and efficacy. The request was approved, the research participant was enrolled, and followup is pending. Dr. Wara suggested that this issue may be a subject for additional discussion by the RAC.

Dr. Wara summarized the interesting results of four studies in twins where one was HIV+ and one HIV-negative. The studies were sponsored by the National Institute for Allergy and Infectious Diseases (NIAID). In these studies, lymphocytes from the HIV-negative twin were transfused into the HIV+ twin, with transduction of the T-lymphocytes by means of a retroviral vector occurring in 3 out of the 4 studies. In the first two studies, persistence of transduced cells in the HIV+ twin was noted after 5 years had elapsed, a potentially interesting result that merits followup by the RAC. The NIAID proposes enrolling research participants from all four studies in one long-term study named "Gemini." Dr. Wara suggested that the RAC follow the outcome of this long-term followup study.

D. Public Comment

Tyler Martin, Valentis GeneMedicine, clarified that for the sponsor of Protocol #481 is MediGene, not GeneMedicine as had been reported.

XIV. Discussion of Human Gene Transfer Protocol #0201-515: Gene Therapy of Progressive Glioblastoma Multiforme Using a Replication-Defective HSV Multigene Vector NUREL-C2: A Phase I Clinical Trial To Determine the Maximum Tolerable Dose of Vector in Combination With Ganciclovir and Radiosurgery

Principal Investigators:	L. Dade Lunsford, M.D., and Joseph C. Glorioso, Ph.D., University of Pittsburgh School of Medicine
Other Investigators:	Neal A. Deluca, Ph.D., University of Pittsburgh; Ian F. Pollack, M.D., University of Pittsburgh; Ajay Niranjan, M.B.B.S., M.S., M.Ch., University of Pittsburgh Physicians; Anita M. Trichel, D.V.M., Ph.D., University of Pittsburgh
Sponsor:	None
RAC Reviewers:	Drs. Breakefield, Childress, Linial, and Wara
Ad Hoc Consultant:	Richard G. Vile, Ph.D., Mayo Clinic

A. Protocol Summary

Malignant glial tumors are uniformly difficult to treat and are generally fatal within 2 years. Approximately 8,000 cases are diagnosed annually in the United States. A two-stage, Phase I clinical safety trial is proposed in which a guided needle will be used to directly inoculate into the brain tumor replication-defective herpes simplex virus (HSV) gene transfer vector, NUREL-C2. NUREL-C2 expresses four transgenes: (1) HSV-infected cell protein zero (ICP0), (2) thymidine kinase (TK), (3) connexin 43, and (4) tumor necrosis factor alpha (TNF-α). ICP0 has been shown to arrest tumor cell division and enhance transgene expression; TK activation of ganciclovir (GCV) kills infected cells; connexin 43 enhances bystander killing of untransduced neighboring tumor cells; and TNF-α provides an additive effect on TNF-α sensitive tumor cells, enhances killing of TNF-α resistant cells through an intracellular mechanism and improves the effects on concurrent radiosurgery. The combination multigene, GCV, and radio-surgery maximize tumor destruction while sparing surrounding normal neurons.

NUREL-C2 will be used in a phase I dose escalation clinical trial to evaluate the safety of this multifaceted treatment applied to participants with recurrent, progressive glioblastoma multiforme (GBM). Two consecutive cohorts of 8-12 participants with recurrent GBM will receive stereotactic injections of NUREL-C2 into the brain tumor. Participants with large resectable tumors will be recruited in the first cohort. The vector will be injected into the tumor mass and the tumor resected 2-4 days later and evaluated for the presence of vector genomes, transgene expression and evidence of inflammation. At the completion of tumor resection, the same dose of vector contained within 8 aliquots will be injected into the residual tumor margin. Following vector injections intravenous GCV will be administered for 14 days. Participants with unresectable tumors (average tumor diameter < 4cm) will be recruited in the second cohort. These participants will receive vector intratumoral injections according to the same dose escalation schedule, followed by GCV treatment for 14 days and gamma knife radio-surgery within 48 hours of virus inoculation. The size and metabolic activity of all tumors will be followed by serial MRI and Positron Emission Tomography (PET) Scans, respectively. Participants will be enrolled in groups of 3, with each group receiving successively larger doses (10^7, 10^8, and 10^9 PFU) of HSV vector. This study will be to determine the maximal tolerated dose (MTD) of vector and the nature of possible dose limiting toxicity (DLT) associated with either arm of the treatment protocol. The secondary objective is to determine and document the responses.

B. Reviews by RAC Members and Ad Hoc Reviewers

Drs. Breakefield, Childress, Linial, and Wara submitted written reviews, as did *ad hoc* consultant Dr. Vile, to which the investigators responded in writing and during this meeting.

Dr. Breakefield noted that the main issue in this protocol is the potential toxicity of the vector in the brain and how to monitor this toxicity. She requested information about the toxicities of TNF-α and ICP0 and the role of ICP0 in reactivation of latent HSV-1. Dr. Breakefield asked that the investigators supply data to support their hypothesis that the four therapeutic genes are synergistic. She emphasized that the nonhuman primate studies are critical to obtaining an index of the toxicities that might be seen in research participants, particularly in relation to an immune inflammatory response. Dr. Breakefield also suggested that investigators monitor HSV-1 antibody levels over time as a measure of immune response.

Dr. Childress expressed concern about whether the preclinical research had established a threshold of safety that would minimize risks to research participants. He requested data regarding viral status and transgene expression on the five macaque monkeys that received the study vector and also asked the investigators to provide additional analyses of safety studies of other HSV-based vectors.

Dr. Linial asked how the NUREL vectors differed from HSV vectors tested in prior safety studies, as well as why NUREL-C2 was selected rather than NUREL-C. She asked which promoters would be used to express the trangenes and about the tumor specificity and persistence of expression. She requested more information about the potential toxicity of ICP0 in neurons or other cells and the efficacy of the gancyclovir-TK bystander effect in humans.

Dr. Wara asked for more information on the modifications that render the HSV vector replication defective and the differences between NUREL-C and NUREL-C2. Because connexin 43 was described as increasing intercellular communication, the possibility of increased adverse effects by the other transgenes should be considered. She requested that the investigators provide any available additional information on the beneficial or adverse effects of the combination of the four proposed transgenes. Regarding the informed consent document, Dr. Wara suggested, and Dr. Childress concurred, that with respect to any benefits of study participation, the consent document be rephrased to state, “It is not likely that the participant will benefit from participation in this study.” She also asked the investigators to clarify how potential participants will be recruited into the study

Dr. Vile asked why this combination of transgenes was selected. He expressed concern about the lack of preclinical data on the efficacy and safety of the NUREL-C2 vector and wondered whether more extensive *in vivo* experiments on the NUREL-C2 vector might be worthwhile. He suggested that toxicity issues be addressed further with regard to the ICP0 protein, the effects of inflammatory reactions in the brain, and the specificity of NUREL-C2 for tumor cells as opposed to normal brain tissue.

C. RAC Discussion

Dr. Wara questioned how TNF-α enhances the effect of radiotherapy.

Dr. Linial asked how far from the injection site expression of viral DNA or RNA was detected in the *in situ* hybridization studies. She also asked whether vector sequence had been detected in normal tissue in the animal models and, if this occurred in humans, whether there might be damage to associated normal tissue.

Dr. Bohn and L. Johnson suggested that TUNEL (terminal deoxynucleotide transferase-mediated dUTP nick-end labeling) staining might help clarify the proapoptotic function of TNF-α.

D. Investigator Response

Dr. Glorioso replied that tunnel staining had not been conducted during the toxicology studies but would be done in both the rodent and primate studies, using clinical grade vector.

Dr. Glorioso agreed with Dr. Breakefield that it is important to know the immune status of research participants—with respect to antibody responses to potential transgene products and to the virus—and that the protocol could be amended to accomplish collection of those data.

In response to Dr. Wara's query about TNF-α enhancing the effect of radiotherapy, Dr. Lunsford stated that the exact mechanism is unclear, but cytokines may extrapolate the ionization effect of radiation. In animal models, TNF appears to be an effective way to enhance the radiation response and increase the long-term survival of the animals.

Regarding the *in situ* hybridization experiments, Dr. Glorioso explained that some vector distribution was detected in the tumor mostly at the tumor periphery. This suggests that the TNF-α and radiation synergy may be due to an attack on the vascular bed in the tumor region.

In response to Dr. Linial's concern about effect on normal tissue, Dr. David Fink, University of Pittsburgh, explained that mild damage to normal tissue would be very difficult to assess in rodents and primates; subtle brain dysfunction could be assessed only in humans in a slow and careful phase I study.

E. Public Comment

No public comments were offered.

F. RAC Recommendations

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

- In order to gain a more thorough understanding and evaluate the inflammatory and toxic effects of the vector, the investigators should consider performing additional immunohistochemical staining on the brain tissues. Using tissue already obtained from the non-human primate models, the investigators should determine the extent of apoptosis and demyelination, perform staining for specific populations and subpopulations of lymphocytes, and perform *in situ* hybridization and RT-PCR to assess the extent of vector genome distribution. Additionally, the investigators should consider performing these studies in non-human primates using clinical grade vector and following the animals for longer periods of time to observe for any signs or evidence of demyelination.

- Since the supporting studies submitted for this protocol were performed using NUREL-C and NUREL-C2 will be used in this clinical trial, consideration should be given to conducting additional experiments with NUREL-C2 to ascertain whether there are any significant differences in the outcomes given differences in the two agents.

- In order to monitor the immune response after vector administration, anti-HSV-1 antibody levels in the research participants should be measured at baseline and re-measured over the course of the study. These levels could help in understanding the effect of the humoral immune response on amelioration or potentiation of the inflammatory response in the brain.

G. Committee Motion 5

It was moved by Ms. Levi-Pearl and seconded by Dr. Bohn that these recommendations expressed the recommendations of the RAC. The vote was 8 in favor, 0 opposed, and 0 abstentions.

XV. Adjournment/Dr. Friedmann

After thanking RAC members, Dr. Friedmann adjourned the meeting at 2:45 p.m. on March 8, 2002.

[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.]

…/s/…

Amy P. Patterson, M.D.
Executive Secretary

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

Date: …/s/…

Theodore Friedmann, M.D.
Chair

Attachment I
Committee Roster

RECOMBINANT DNA ADVISORY COMMITTEE

CHAIR

FRIEDMANN, Theodore, M.D.
Professor of Pediatrics
Director
Human Gene Therapy Program
Whitehill Professor of Biomedical Ethics
Center for Molecular Genetics
School of Medicine
University of California, San Diego
Mail Stop Code 0634
9500 Gilman Drive
La Jolla, CA 92093-0634
(858) 534-4268

BOHN, Martha C., Ph.D.
Director
Neurobiology Program
Department of Pediatrics
Northwestern University Medical School
Interim Co-Director
Children's Memorial Institute for Education and Research
Suite 209
2300 North Children's Plaza
Chicago, IL 60614-3363
(773) 868-8052

CHILDRESS, James F., Ph.D.
Kyle Professor of Religious Studies
Professor of Medical Education
University of Virginia
Cocke Hall, Room B-10
Charlottesville, VA 22903-4126
(434) 924-3741

BREAKEFIELD, Xandra O., Ph.D.
Professor of Neurology
Molecular Neurogenetics Unit
Massachusetts General Hospital
Building 149
13th Street
Charlestown, MA 02129-2000
(617) 726-5728

GOODING, Linda R., Ph.D.
Professor of Immunology
Department of Microbiology and Immunology
Emory University School of Medicine
O. Wayne Rollins Research Center, Room 3107
1510 Clifton Road
Atlanta, GA 30322
(404) 727-6065

BRODY, Baruch A., Ph.D.
Leon Jaworski Professor of Biomedical Ethics
Director
Center for Medical Ethics and Health Policy
Baylor College of Medicine
1 Baylor Plaza
Houston, TX 77030-3498
(713) 798-3503

JOHNSON, Larry G., M.D.
Associate Professor of Medicine
Division of Pulmonary Diseases and Critical Care Medicine
Cystic Fibrosis/Pulmonary Research and Treatment Center
University of North Carolina, Chapel Hill
Campus Box 7248
Thurston-Bowles Building, Room 7123A
Chapel Hill, NC 27599-7248
(919) 966-7052

JOHNSON, Philip R., Jr., M.D.
Professor of Pediatrics
President
Children's Research Institute
Columbus Children's Hospital
Room W-591
700 Children's Drive
Columbus, OH 43205-2696
(614) 722-2735

KING, Nancy M.P., J.D.
Professor
Department of Social Medicine
University of North Carolina, Chapel Hill
Wing D
Campus Box 7240
Chapel Hill, NC 27599-7240
(919) 962-1136

LEVI-PEARL, Sue L.
Director
Medical and Scientific Programs
Tourette's Syndrome Association, Inc.
4240 Bell Boulevard
Bayside, NY 11361-2861
(718) 224-2999, ext. 222

LINIAL, Maxine L., Ph.D.
Member
Division of Basic Sciences
Fred Hutchinson Cancer Research Center
University of Washington
1100 Fairview Avenue, North
Seattle, WA 98109-4417
(206) 667-4442

OHENE-FREMPONG, Kwaku, M.D.
Professor of Pediatrics
Director
Comprehensive Sickle Cell Center
Division of Hematology
Children's Hospital of Philadelphia
34th Street and Civic Center Boulevard
Philadelphia, PA 19104-4901
(215) 590-3423

SIMARI, Robert D., M.D.
Associate Professor of Medicine
Director
Bruce and Ruth Rappaport Program in
Vascular Biology
Mayo Clinic and Foundation
Member
Molecular Medicine Program
200 First Street, SW
Rochester, MN 55905-0002
(507) 284-3727

WARA, Diane W., M.D.
Professor of Pediatrics
University of California, San Francisco
Room M-679
505 Parnassus Avenue
San Francisco, CA 94143-3466
(415) 476-2865

EXECUTIVE SECRETARY

PATTERSON, Amy P., M.D.
Director
Office of Biotechnology Activities
Office of the Director
National Institutes of Health
Suite 750
MSC 7985
6705 Rockledge Drive
Bethesda, MD 20892-7985
(301) 496-9838

Attachment II
Attendees

Moira L. Aitken, Fred Hutchinson Cancer Research Center
Kevin Alleman, American Association for the Advancement of Science
Philippe Bishop, FDA
Kathy Bochinksi, Novartis
Malcolm K. Brenner, Baylor College of Medicine
Jeffrey W. Carey, GenVec
Mike Christini, private citizen
Shirley M. Clift, Cell Genesys
Laura Corvette, FDA
John R. Cutt, Novartis Pharmaceuticals Corporation
Karen H. Darcy, Genetic Therapy, Inc.
Joann C. Delenick
Neal A. Deluca, University of Pittsburgh
Srdjan Djurovic, City of Oslo Ullevaaj University Hospital
Ruxandra Draghia-Akli, Baylor College of Medicine
Jeff Edelson, Therion Biologics Corporation
Brooke Esteves, Wyeth Genetics Institute
David Fink, University of Pittsburgh
Joseph C. Glorioso, University of Pittsburgh School of Medicine
Bambi Grilley, Baylor College of Medicine
Beverly L. Harding, University of Pittsburgh
Vaughn B. Himes, Targeted Genetics Corporation
Scott C. Jenkins, *The Blue Sheet*
Lin Ji, University of Texas M.D. Anderson Cancer Center
Ruth Ryan Lessard, Introgen Therapeutics, Inc.
Alec Liacouras, CSR/NIH
Zhifeng Long, Genetic Therapy, Inc.
Charles Lu, University of Texas M.D. Anderson Cancer Center
L. Dade Lunsford, University of Pittsburgh School of Medicine
Jill Glasspool Malone, Gene Delivery Alliance, Inc.
Robert Malone, Management Systems Designers, Inc.
Nancy S. Markovitz, FDA
J. Tyler Martin, Sr., Valentis
Natalia Martin, FDA
Kevin McCleary, Stellar Systems
Maritza McIntyre, FDA
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Andra E. Miller, Biologics Consulting Group
Jim Morris, Genzyme
Bentley J. Moyer, Valentis
Ajay Niranjan, University of Pittsburgh Physicians
Phil Pendergast, Ohio State University
Anne M. Pilaro, FDA
Ian F. Pollack, University of Pittsburgh
Uday R. Popat, Baylor College of Medicine
Dietmar P. Rabussay, Genetronics, Inc.
Rajagopal Ramesh, University of Texas M.D. Anderson Cancer Center
Gyu-Seek Rhee, Korea FDA
Jack A. Roth, University of Texas M.D. Anderson Cancer Center
T. Shimada, Ambience Awareness International, Inc.
Louis C. Smith, consultant
Lisa A. Speicher, Wyeth Genetics Institute

Richard Sublett, Introgen Therapeutics, Inc.
Daniel M. Takefman, FDA
Anita M. Trichel, University of Pittsburgh
Ruth S. Turner, Genzyme Corporation
Christopher S. Yun, PRA International

Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
AP	alkaline phosphatase
CF	cystic fibrosis
CFTR`	CF transmembrane conductance regulator
DNA	deoxyribonucleic acid
DOTAP: Chol-*fus1*	dioleoyltrimethylammoniumpropane:Cholesterol-*fus1* liposome complex
FDA	U.S. Food and Drug Administration
GeMCRIS	Genetic Modification Clinical Research Information System
GH	growth hormone
GHRH	growth hormone-releasing hormone
GLP	good laboratory practices
HIV	human immunodeficiency virus
HSV	herpes simplex virus
IBC	institutional biosafety committee
ICP0	infected cell protein 0 (zero)
IGF-1	insulin-like growth factor-1
IRB	institutional review board
IV	intravenous
MRI	magnetic resonance imaging
NIAID	National Institute for Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NUREL-C2	completely inactivated HSV gene transfer vehicle (vector)
OBA	Office of Biotechnology Activities (formerly ORDA, Office of Recombinant DNA Activities)
OD	Office of the Director
PCR	polymerase chain reaction
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
TK	thymidine kinase
TNF-α	tumor necrosis factor-alpha
Type A reports	serious, possibly associated, and unexpected adverse event reports
VEGF	vascular endothelial growth factor