RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 9-11, 2004

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 9-11, 2004

The Recombinant DNA Advisory Committee (RAC) was convened for its 95th meeting at 1:00 p.m. on March 9, 2004, at the Bethesda Marriott Hotel, 5151 Pooks Hill Road, Bethesda, MD. Dr. Diane Wara (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 1:00 p.m. until 4:45 p.m. on March 9, from 8:00 a.m. until 4:45 p.m. on March 10, and from 9:00 a.m. until 2:35 p.m. on March 11. 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
James F. Childress, University of Virginia
Neal A. DeLuca, University of Pittsburgh
David L. DeMets, University of Wisconsin Medical School
Thomas D. Gelehrter, University of Michigan Medical School
Helen Heslop, Baylor College of Medicine
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
Nicholas Muzyczka, University of Florida
Glen R. Nemerow, The Scripps Research Institute
Madison Powers, Georgetown University
Naomi Rosenberg, Tufts University
David Sidransky, Johns Hopkins University
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* Reviewers/Speakers**

Steven T. DeKosky, University of Pittsburgh
Matthew J. During, University of Auckland
Genoveffa Franchini, National Cancer Institute, National Institutes of Health
Theodore Friedmann, University of California, San Diego
Michael G. Kaplitt, Weill Medical College, Cornell University
Nancy M.P. King, University of North Carolina, Chapel Hill *(via teleconference)*
Walter J. Koch, Jefferson Medical College
Suzanne M. Michalek, University of Alabama, Birmingham
Richard G. Vile, Mayo Clinic

[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.

Nonvoting/Agency Representatives

Kristina C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services

Stephanie L. Simek, U.S. Food and Drug Administration (FDA)

NIH Staff Members

Rose Aurigemma, NCI
Elaine Collier, NCRR
Robert Jambou, OD
Mary Joyce, NHLBI
Steven Krosnick, NCI
Laurie Lewallen, OD
Cheryl L. McDonald, OD
Maureen Montgomery, OD
Alexander Rakowsky, OD
Gene Rosenthal, OD
Paul Shehy, NINDS
Thomas Shih, OD
Sonia I. Skarlatos, NHLBI
H. Eser Tolunay, NHLBI
Joseph E. Tomaszewski, NCI
Gisele White, OD
Rosemary Wong, NCI/ RRP

Others

There were 106 attendees at this 3-day RAC meeting. A list of RAC members, *ad hoc* reviewers/speakers, nonvoting/agency liaison representatives, and Office of Biotechnology Activities (OBA) staff members is included as Attachment I. A list of public attendees is included as Attachment II.

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

Dr. Wara, RAC Chair, called the meeting to order at 1:00 p.m. on March 9, 2004. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on February 19, 2004 (69 FR 7773). Issues discussed by the RAC at this meeting included public review and discussion of six protocols, a data management report, update on the RAC Gene Transfer Clinical Trial Design Working Group, update on a gene transfer protocol first reviewed by the RAC in 2001, and an overview of investigator and institutional responses to Appendix M-I-C-1 of the *NIH Guidelines*.

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

II. Minutes of the October 17, 2003, and December 3-4, 2003, RAC Meetings/Former RAC Chair Theodore Friedmann, M.D., University of California, San Diego, and Ms. Kwan

Ms. Kwan noted that the October 17, 2003, RAC meeting was the continuation of the second day of the September 2003 RAC meeting, which was canceled because of a hurricane. Most of the RAC members were present via teleconference for the October 17 continuation meeting. The December 3-4, 2003, meeting was the regular quarterly meeting of the RAC. Ms. Kwan stated that both sets of minutes

accurately reflected their respective meetings and that no changes were required to the minutes of either the October 17 or December 3-4 RAC meetings.

A. Committee Motion 1

It was moved by Ms. Kwan and seconded by Dr. Gelehrter that the RAC approve the October 17, 2003, and December 3-4, 2003, RAC meeting minutes. The vote was 18 in favor, 0 opposed, 0 abstentions, and 0 recusals.

III. Discussion of Human Gene Transfer Protocol #0401-629: A Phase I Dose-Escalation Trial of vvDD-CDSR (Double-Deleted Vaccinia Virus Plus CD/SMR) Administered by Intratumoral Injection in Patients with Superficial Injectable Tumors

Principal Investigator:	David L. Bartlett, M.D., University of Pittsburgh Medical Center
RAC Reviewers:	Drs. Childress, DeMets, and P. Johnson
Ad hoc Reviewer:	Genoveffa Franchini, M.D., National Cancer Institute, NIH

A. Protocol Summary

Oncolytic, replication-selective viruses may hold promise as novel anticancer therapeutics that are designed to destroy tumors. These viruses are engineered to multiply and spread efficiently in cancerous tissue but not in normal tissue. The vvDD-CDSR virus only replicates efficiently in proliferating cells with high nucleotide pools such as cancer cells. However, in animal models, using mice or non-human primates, non-cancerous proliferating cells, such as bone marrow stem cells or cells in the gut, were not detectably infected.

The vvDD-CDSR virus is engineered from the vaccinia virus that was used to eradicate smallpox worldwide. Millions of individuals received vaccinia virus smallpox vaccinations safely, with only rare complications. In addition, more than 90 cancer patients have safely received various vaccinia viruses by intratumoral injection. The vvDD-CDSR virus was modified by taking out viral genes that are critical for viral multiplication in non-cancerous cells, thus making this virus even safer than the wild-type vaccinia virus. The virus expresses two additional genes. The first of these genes encodes for cytosine deaminase (CD), which can convert a safe drug to a toxic drug at the tumor site, thus shutting down viral replication if necessitated by safety concerns. The second gene encodes for the somatostatin receptor (SR), which allows a “tracer” to accumulate wherever the virus is active and allows visualization of the virus’ location in the body through use of an x-ray.

The goals of the main trial are to determine the safety and the maximum tolerated dose (MTD) of the virus in humans. Other goals include determining tumor shrinkage, viral spread in blood, shedding into the urine or throat, and the immune response to the virus. A total of approximately 15 to 25 people will participate in this study. These participants will have injectable superficial tumors that have failed standard treatments and are not curable by surgery or other treatments. Participants will be involved actively in this study for approximately 3 months.

Because this specific virus has not been administered previously to humans, it will be injected directly into the tumor to maximize the potential for safety and efficacy. Local anesthesia will be used as required for pain. One to three tumors will be injected for each participant, and each tumor will be injected four times. If the injected tumors are stable or shrinking, repeat injection cycles will be allowed, up to a total of four cycles administered every 3 weeks. Doses will be escalated among five cohorts using a standard Phase I dose-escalation design, and the planned dose escalation will continue unless severe toxicities warrant halting it.

Safety assessments, including blood testing, adverse event (AE) collection, and physical examinations will be carried out every other day for one week following injection of the vvDD-CDSR virus into the tumors and will be assessed weekly thereafter, through day 28 of the final cycle of injection. The amounts of virus in blood, urine, and the throat will be assessed over time after injection. Viral replication,

gene expression, and inflammatory cell infiltration in tumors will be assessed after injection by obtaining a small piece of tumor tissue before and once after the injection cycle. Tumor shrinkage, if any, and time-to-tumor progression at injected and noninjected tumor sites will be assessed.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Twelve RAC members voted for in-depth review and public discussion of the protocol. RAC reviewers Drs. Childress, DeMets, and P. Johnson and *ad hoc* reviewer Dr. Franchini submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Childress focused his review on the informed consent document, stating that the document itself contained most of the relevant information and was generally clear. He noted some inconsistencies between the informed consent document and the protocol and suggested that the investigators make clear their intention to request an autopsy if death occurs. Requested clarifications included statements about the risks of the altered vaccinia virus, laboratory procedures undertaken during the study, processes for discontinuation of or withdrawal from the study, and pregnancy as an exclusion criterion. Dr. Childress also suggested modifying the language within the informed consent document to remove use of the terms “therapy” or “treatment.”

Dr. DeMets concentrated his review on the design and statistical sections of this protocol. Because they are proposing a classic Phase I dose-escalation design, the investigators will be searching for the MTD; however, because the vaccinia virus will be replicating, the assumptions made in this model may not be correct in this situation. The protocol should be designed to determine the response to the doses given, not to find the MTD. Regarding the statistical review, Dr. DeMets noted that, using the proposed numbers of participants, the investigators could have a theoretical response rate of 20 percent or greater and yet show no clinical response. He suggested that the investigators better define the response rate so as not to miss an actual response and that they think about how resulting data will be used to decide how and when to proceed to Phase II.

Dr. P. Johnson wondered whether different types of tumors would respond differently to the proposed experimental agent. This result could complicate data analysis and reduce the statistical power of the trial. He noted that previous exposure to vaccinia, which is likely in the case of most potential participants, might affect the first dose of vaccinia given in the study and also might diminish or eliminate the effectiveness of the proposed subsequent doses. Dr. P. Johnson asked which viral infections would be screened for and what criteria would be used to select the participants to be studied for vector localization using octreotide scans. He also suggested that the term “patients” not be used when referring to research participants.

Dr. Franchini expressed particular concern about preexisting vaccinia immunity, which would vary among the enrolled participants, stating that such immunity would likely influence the primary end points, the MTD and safety. She noted that a sufficient number of vaccinia-naive, tumor-bearing participants might be necessary to evaluate the true safety of vvDD in humans. Regarding tumor reduction, Dr. Franchini asked whether the tumor cytolytic activity of vvDD had been maintained in nude mice treated with vaccinia immunoglobulin. She also noted the need for clarification of the criteria for inclusion, and the exclusion for human immunodeficiency virus (HIV) infection, more detailed guidelines for treatment, and information regarding the avoidance of household contact with infants and young children by the research participants.

C. RAC Discussion

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

- Dr. L. Johnson asked for clarification of the investigators’ rationale for the vector design, particularly the inclusion of two transgenes that may or may not be useful to the study.

- Dr. P. Johnson suggested that the investigators consider studying a nonhuman animal model with preexisting immunity to vaccinia.

- Several RAC members suggested that the investigators consider restricting their study to a single type of tumor.

- Dr. L. Johnson was concerned about the lack of data on the utility of this technique.

- Ms. Kwan requested further discussion about the likelihood that repeated cycles of the vaccinia virus would have an effect considering that the participants may have either pre-existing immunity or develop immunity with the repeated administrations.

- Dr. L. Johnson asked about the possible consequences of expressing SR in nontumor tissue.

- Dr. Simek noted that in a vector submitted for a clinical trial, it is expected that all of the transgenes in the vector serve a function. If a particular transgene does not serve a function for that particular clinical trial, the FDA generally recommends its removal from the vector.

D. Investigator Response

Dr. Bartlett responded with the following information:

- It is not possible to predict with certainty whether the maximum tolerated dose would differ between individuals with or without pre-existing immunity. Dr. Bartlett noted that the researchers would probably have difficulty finding cancer patients without prior vaccinia exposure, but noted they could assess and stratify the research participants by history of exposure or measurable antibodies. He also noted that in a previous clinical trial with vaccinia (Mastrangelo et al., Cancer Gene Therapy 6:409, 1998), all participants were vaccinated with vaccinia prior to receiving a direct intratumoral injection of vaccinia. Five of seven participants responded. Individuals receiving boosts of vaccinia who have previously been vaccinated also demonstrate vaccinia replication in injected skin. A local injection of the virus may be successful at avoiding circulating antibodies and immune cells, allowing for local replication and tumor response.

- Regarding preimmunization status, the investigators have shown that nude mice without a T-cell response respond more fully to the virus than do immunocompetent mice. Response differences in humans will be one of the results of this trial.

- Dr. Bartlett agreed to add a CD4 count to the protocol so that potential participants who are immunosuppressed because of their cancer would be eliminated from participating in this trial. He also agreed to clarify the HIV testing process and to conduct both reverse transcriptase-polymerase chain reaction (RT-PCR) and antibody tests for HIV.

- Dr. Bartlett agreed to include in the protocol the necessary restrictions and precautions regarding research participant contact with young children or others who might be at increased risk if exposed to the vaccinia virus.

- Addressing the RAC members' questions and concerns about the SR gene, Dr. Bartlett clarified that due to cost concerns in this Phase I study, octeotride scans would be used only for participants in the highest dosing group where the likelihood of detecting a difference in the tumors would be greatest. In response to Dr. L. Johnson's concern regarding any possible consequences of expressing the SR gene in non-tumor tissue, Dr. Bartlett noted that to his knowledge there are no effects and that SR present in non-tumor tissue.

- In response to concerns about studying different tumor types in this Phase I study, Dr. Bartlett explained that the preclinical work did not suggest the particular histology is predictive of

response and that all tested histologies showed selective replication and response. The preclinical studies of the virus included subcutaneous tumors of the following histologies: human melanoma in athymic mice, rabbit VX2 tumor, rat sarcoma, human colon cancer, and murine colon cancer. Because the investigators do not expect toxicities would differ by tumor type, they would prefer to include participants with various tumor types as proposed to enhance accrual and to get a sense of responses based on tumor types, which might help inform the design of subsequent trials.

- The investigators' ultimate goal is to utilize the CD gene to convert 5-fluorocytosine to 5-fluorouracil in tumor cells. Mixed responses have been seen *in vivo*; in some cases, prodrug activation shuts down viral replication and can slow the response to virus alone, whereas in other cases, prodrug activation improves the response. Because the CD gene is present in addition to many other proteins that vaccinia produces, Dr. Bartlett reiterated the investigators' belief that it will not be harmful in any way and that it will not enhance the vector's antigenicity.

E. Public Comment

There was no public comment.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- Given that the protocol does not specify that all subjects will have radionuclide imaging, the use of the somatostatin receptor transgene in the vector delivered to all subjects is questionable. The rationale for this element of the protocol and the plan to incorporate the cytosine deaminase transgene in the vector that all subjects will receive should be more fully developed.

- Study cohorts should be stratified according to their baseline immune status. Consideration should be given to preferential inclusion of subjects with subcutaneous tumors, such as melanoma, who may be less likely to have pre-existing immunity to vaccinia, such as younger individuals (age 30 or younger).

- The proposed study design will likely determine the maximum tolerated dose, but given the heterogeneous population, the variability in pre-existing immune status, and the small sample size, it may not accurately assess the activity of the vector. As such, the tumor responses seen in this Phase I safety study, as presently designed, should not necessarily influence the decision to proceed to Phase II testing.

- The informed consent document should provide a detailed description of the precautions and restrictions the subject should adhere to regarding contact with individuals in the same household, particularly those who may have an increased risk of exposure to vaccinia.

G. Committee Motion 2

It was moved by Dr. P. Johnson and seconded by Dr. Lo that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 17 in favor, 0 opposed, 0 abstentions, and 1 recusal.

IV. Update on Protocol #0104-469: Subthalamic Glutamic Acid Decarboxylase (GAD) Gene Transfer in Parkinson's Disease Patients Who Are Candidates for Deep-Brain Stimulation

Presenters: Michael G. Kaplitt, M.D., Ph.D., Cornell University, and Matthew J. During, M.D., Ph.D., University of Auckland
Sponsor: Neurologix, Inc.

(In-depth review and public discussion of this protocol occurred at the June 2001 RAC meeting.)

Dr. During reviewed this protocol and the rationale behind it; he did not provide the preclinical data because those data have been published and presented previously to the RAC. The vector is an adeno-associated virus (AAV), the gene is GAD, the vector production site is the University of Auckland and Neurologix, Inc., and the target is the subthalamic nucleus of the human brain. The design is a Phase I, open-label, dose-escalation, investigator-initiated study. Dr. During stated that the funding source is Neurologix, Inc., and he provided information about the various clinical sites and investigators. Regarding conflict of interest issues, Dr. Kaplitt is an unpaid consultant whose father is an officer and shareholder of Neurologix, Inc.; Dr. During is a paid consultant to Neurologix, Inc., but has no clinical role in the study; and Drs. David Eidelberg, M.D. and Andrew Feigin, M.D. from North Shore Hospital, have no conflict of interest. On the basis of RAC recommendations, a substantial number of changes have been made to the initial proposed protocol.

The first procedure was performed in August 2003. Dr. During described and showed pictures of the procedure and described the demographics of both the screened population and the enrolled participants. Analysis of results to date show no surgical complications, no local inflammation, no fevers or change in laboratory values, no radiographic evidence of toxicity, no study-related AEs, and one serious adverse event (SAE) unrelated to the intervention, which was a result of hospitalization.

Dr. Kaplitt brought to the RAC's attention one process issue: the release of protocol-related documents prior to the preparation of the final protocol. He noted that information about this protocol, based on the initial proposal reviewed by the RAC, was published in *Human Gene Therapy* about 4 months after his group's RAC appearance, which created unnecessary confusion. Dr. Kaplitt requested that the RAC and the OBA consider some way to ensure that protocol-related documents are not released until they are finalized.

A. RAC Discussion

Questions and issues discussed by RAC members and answered by Drs. Kaplitt and During included the following:

- Dr. Bohn asked about the availability of data on neutralizing antibodies to AAV in the study's participants; Dr. During responded that such data are not yet available.

- Dr. Bohn wondered whether any of the imaging data suggest overflow to other areas of the brain. Dr. Kaplitt explained that, based on nonhuman primate studies, no overflow is expected. The investigators are using global positron emission tomography with a physiological marker that monitors glucose utilization as the most effective method of discerning transport out of the local area; those data are still being collected.

- Dr. Bohn requested comments about the lack of a control group, which has been used in other trials for Parkinson's disease. Dr. During explained that this Phase I trial is a dose-finding study and a tolerability-finding study. No claims will be made about efficacy, and the trial is not powered to make such claims. The Phase II study will have a control group, and Dr. During stated that the investigators would seek the RAC's advice about how best to design a control group. Dr. Kaplitt added that the general consensus, to which the investigators eventually agreed, was that the myriad potential confounds to conducting sham surgery in this Phase I study would obviate the desirability of using a control group.

- Dr. DeLuca asked how the conflict of interest issues are being managed, beyond mere disclosure. Dr. Kaplitt responded that the study was designed specifically so that the two neurologists determine whether individuals enter the study and the outcome for participants, and neither of them has a role at Neurologix, Inc. At Dr. Simari's request, Dr. Kaplitt detailed the informed consent process.

- With respect to the process issue discussed by Dr. Kaplitt, Dr. Rose explained that it has been clear from the beginning that protocol submissions made to the OBA for RAC review are public documents. Under the Freedom of Information Act (FOIA) and the Federal Advisory Committee Act (FACA), the OBA cannot withhold initial protocols or parts of protocol submissions except for specific sections that are labeled Trade Secret or Commercial Confidential. The OBA's procedure is to contact PIs when the OBA receives a request for release of material so marked so the PI can contact the requester directly. Dr. Kaplitt suggested that when releasing initial submissions, OBA include wording clarifying that it is an initial submission that has not yet been reviewed or modified. Dr. Rose explained that OBA could not add such wording because PIs—not the OBA—should identify the stage of development and review of their protocols. Dr. Rose, however, said that the initial submissions could be so identified by the submitter at the beginning of the protocol submission. Dr. Robert Jambou, FOIA coordinator for the OBA, explained the legal requirements regarding FOIA and FACA: Any information submitted to a Federal agency is publicly available unless a "commercial confidential" exemption is claimed. Dr. Rose reminded the RAC that the *NIH Guidelines* specifically state that an entire protocol submission cannot be designated as "commercial confidential."

B. Public Comment

Dr. Friedmann asked about the status of the lesioned nonhuman primate studies and what has been learned about the procedure in those animals. Dr. During explained that a collaborative study using primates has not yet been published, and the agreement with the two investigators includes not discussing the study until data analysis is completed and a manuscript is in press. The unilateral Parkinsonian primate model lasted approximately 12 months. No AEs were seen, and preliminary data and behavior analyses indicate a positive effect. The data analysis and publication are scheduled to occur within the next few months.

V. Day One Adjournment/Dr. Wara

Dr. Wara adjourned the first day of the March 2004 RAC meeting at 4:45 p.m. on March 9, 2004.

VI. Day Two Opening/Dr. Wara

Dr. Wara opened the second day of the March 2004 RAC meeting at 8:00 a.m. on March 10, 2004.

VII. Discussion of Human Gene Transfer Protocol #0401-624: A Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD) for Recurrent Malignant Gliomas

Principal Investigators:	Frederick F. Lang, Jr., M.D., M. D. Anderson Cancer Center, and Charles A. Conrad, M.D., M. D. Anderson Cancer Center
RAC Reviewers:	Drs. DeLuca, Powers, and Wara
Ad hoc Reviewer:	Richard G. Vile, Ph.D., Mayo Clinic

A. Protocol Summary

Each year, approximately 8 of every 100,000 people in the United States are diagnosed with primary malignant brain tumors, representing approximately 2 percent of all diagnosed cancers. Approximately 13,000 Americans die of malignant brain tumors every year, representing about 2 percent of all U.S. cancer deaths. Glioblastoma multiforme accounts for 23 percent of primary brain tumors in the United States and are the most commonly diagnosed brain tumor in adults between the ages of 45 and 74 years. Although rare, glioblastoma is among the most challenging cancers to treat because of the aggressive invasion of normal brain tissue. Despite surgery, radiation, and chemotherapy, the median survival of

patients with glioblastoma multiforme is less than 1 year. Improving this dismal prognosis requires new treatment approaches.

Proteins encoded in the E1 region of adenovirus bind and inactivate tumor suppressor proteins. Adenoviral replication requires inactivation of the tumor suppressor Rb/p16/E2F that forces cells into the cell cycle. Cancer cells also require inactivation of Rb/p16/E2F in order to sustain tumor proliferation. Alterations in the Rb/p16/E2F pathway occur in nearly all malignant gliomas.

The delta-24-RGD vector is a conditionally replicating competent oncolytic adenovirus that selectively kills glioma cells based on the inactivation of Rb pathway in the cell. To achieve this selectivity, adenovirus was genetically modified by deleting 24 nucleotides from the E1a locus. Because the majority of glioma cells have lost Rb function, these cells are permissive for delta-24 replication. However, delta-24 is not capable of replicating in non-dividing cells due to the E1a mutation.

Delta-24 was also modified to enhance its ability to infect tumor cells. Although adenoviruses infect tumor cells by binding to the Coxsackie-Adenovirus Receptor (CAR), most gliomas express low levels of the receptor and are resistant to viral infections. Delta-24's infectivity was improved by inserting an 11 amino acid peptide, called RGD, into the HI loop of the fiber knob of Delta-24. RGD binds $\alpha_v\beta_3$ integrins that are preferentially expressed on tumor cells.

The Phase I protocol proposed is designed to study the safety of administering the delta-24-RGD virus to determine the MTD. Two groups of research participants will be studied in the trial. The first group of participants will have inoperable tumors. A second group of research participants, with operable tumors, will undergo stereotactic injection of the delta-24-RGD virus using a permanently implanted catheter in the center of the tumor. After 14 days, the tumor will be removed surgically, and biological specimens will be evaluated for pathological and molecular changes. By monitoring the participants throughout this study, the Phase I trial will provide basic information about the safety and biological effects of injecting the delta-24-RGD virus into human brain tumors.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Thirteen RAC members voted for in-depth review and public discussion of the protocol. This protocol is similar to other studies using replication-competent adenoviruses, but this is the first protocol involving the addition of an arginine-glycine-aspartic acid (RGD) motif to one of the proteins on the outside of the gene transfer vector coat. Although the apparent enhanced survival of the gene transfer vector is important, few if any biodistribution studies exist to document the mechanism underlying this improvement. Along with other questions regarding the *in vivo* use of tropism-modified vectors, this protocol constitutes a significant expansion of the technology of *in vivo* viral vector delivery. RAC reviewers Drs. DeLuca, Powers, and Wara and *ad hoc* reviewer Dr. Vile submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. DeLuca noted that the preclinical data were well prepared and presented. He asked for more information about the attenuation of the virus, and whether the RGD modification increased transduction of non-tumor cells also. He also asked whether nonhuman primate studies and biodistribution studies had been conducted using the delta-24-RGD virus.

Dr. Powers expressed concern about the increase in risk associated with catheter use. He asked whether any conflicts of interest currently exist. Because the informed consent document discusses therapeutic options, including the option of no therapy, Dr. Powers requested a discussion of the differences in quality of life expected under each option and whether the participants will understand the relevant differences.

Dr. Wara was also concerned about the absence of biodistribution preclinical studies for delta-24-RGD4C. She suggested that each potential research participant be tested for HIV and active hepatitis infection and infected individuals should be excluded from the trial. She asked about the basis of the dose-escalation scheme, and whether all participants in the first group would be dosed and safety determined before any participants in the second group receive the delta-24-RGD4C virus. Dr. Wara also

requested that the investigators include statements in the informed consent document regarding autopsy and acceptable birth control.

Dr. Vile was primarily concerned about how the RGD motif tropism enhancement might affect the toxicity profile of the vector. He suggested that the investigators assume that there will be some degree of replication of the virus in normal cells, especially since this protocol uses a virus in which tropism is intentionally expanded; therefore, this assumption should be evident in the design of the study as well as in the informed consent process. Dr. Vile asked whether the investigators have human data about the expression of alpha vs. beta integrins on normal brain cells around the tumor site and whether binding of virus to the integrins would send a signal to tumor or normal cells that might promote cell division. Because aggressive replication might cause dangerous levels of local inflammation, Dr. Vile asked whether antibody reactivity or brain inflammation might be expected in participants in whom preexisting adenoviral antibodies were present.

C. RAC Discussion

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

- Dr. DeLuca asked the investigators whether they planned to test for changes in the expression or coding sequence of other adenoviral proteins. The virus is attenuated in Rb^+ cells, but he expressed concern about whether other mutations could reduce that attenuation independent of the 24-base-pair mutation. Dr. DeLuca noted that reduction in attenuation has occurred in prior experiments with replication-impaired viruses.

- Ms. Kwan asked whether the institutional review board (IRB) had approved this protocol, as approval was unclear from the investigators' presentation.

- Dr. Vile asked whether the virus had spread anywhere other than in tumor, liver, or blood in nude mice.

- Dr. Wara asked in what animal model(s) the investigators intend to conduct biodistribution studies and whether the investigators plan to complete the biodistribution studies before this proposed human trial commences.

- Kristina C. Borror, Ph.D., Office for Human Research Protections, noted that the informed consent document was overly complex and should be simplified and that the use of "patient" throughout the protocol should be changed to "subject" or "research participant."

D. Investigator Response

Drs. Conrad and Lang and Juan Fueyo, M.D., of the M. D. Anderson Cancer Center, responded with the following information:

- To maximize safety, toxicity information will be analyzed in Group A cohort participants at the proposed dose level before any participants in the Group B cohort are enrolled.

- The investigators will add wording to this protocol stating that the participants will be asked to utilize two different methods of birth control.

- Serum was not collected in the long-term mice studies to test for spread of the virus, and the investigators did not assay all of the organs. In preclinical research using the p53 transgene, the investigators looked in the serum, sputum, and urine for adenovirus, and no viral particles were found. However, an immune response was discovered, and antibody titers to human adenovirus type 5 (Ad5) peaked at about 2 months. Dr. Lang noted that, if the virus gets into the

bloodstream, it would have a tropism for the liver; therefore, the investigators' biodistribution studies will focus on that site.

- The investigators will conduct three biodistribution studies concurrently, using a nonhuman primate model, cotton rats, and nude mice. The FDA requires completion of these studies before they will grant an investigational new drug (IND) application.

- The investigators' IRB has not yet approved this protocol; it will review a resubmitted protocol pending this RAC review. As a result of feedback from the RAC review, many modifications will be made to the protocol and the informed consent document.

- Regarding the question about compensatory mutations, the investigators will use an assay to determine whether the virus has recombined or rearranged. The assay will help determine whether the virus is undergoing rearrangement, but the investigators believe that because the delta-24 deletion is small, the genome of the virus will remain stable.

E. Public Comment

There was no public comment.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- In light of the potential safety implications of the expanded cellular tropism of the Delta-24-RGD vector, biodistribution and preclinical toxicity studies as well as studies assessing the contribution of the Delta-24-RGD to the anti-tumor immune response in the brain should be completed and evaluated before initiation of the protocol. Once completed, the Principal Investigator is invited to submit these results to OBA for presentation to the RAC.

- Additional studies should evaluate the possibility that second-site mutations would allow the Delta-24-RGD virus to replicate more efficiently in Rb+ cells.

- The language in the informed consent document is too complex and should be simplified.

- "Patient" should be changed to "subject", "research subject", or "research participant."

- The statement throughout the informed consent document that the virus "does not replicate" in normal cells should be revised to say that the virus replicates "less efficiently" in normal cells

G. Committee Motion 3

It was moved by Dr. Powers and seconded by Dr. L. Johnson that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

VIII. Discussion of Human Gene Transfer Protocol #0401-625: A Phase I Study of a Tropism-Modified Conditionally Replicative Adenoviral Vector (Ad5-Delta-24-RGD) for Intraperitoneal Delivery in Ovarian and Extraovarian Cancer Patients

Principal Investigators:	Ronald D. Alvarez, M.D., University of Alabama, Birmingham; Mack N. Barnes III, M.D., University of Alabama, Birmingham; and David T. Curiel, M.D., Ph.D., University of Alabama, Birmingham
RAC Reviewers:	Drs. Linial, Powers, and Sidransky
Ad hoc Reviewer:	Richard G. Vile, Ph.D., Mayo Clinic

A. Protocol Summary

Ovarian cancer is the fifth most common cancer among women, excluding nonmelanoma skin cancers. The American Cancer Society estimates that about 25,580 new cases of ovarian cancer will be diagnosed in the United States during 2004; ovarian cancer accounts for 4 percent of all cancers in women. Ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system; it is estimated that 16,090 women will die from ovarian cancer in the United States during 2004.

Ovarian cancer is a deadly disease in need of new treatment paradigms. Previous trials have investigated the utility of using cold viruses such as adenoviruses that exert their antitumor activity by selectively replicating in infected ovarian cancer cells and causing these cells to burst. These initial trials demonstrated limited clinical activity, which in part might be attributable to the inability of these conditionally replicative adenoviruses (CRADs) to achieve efficient cancer cell infection.

Investigators at the University of Alabama, Birmingham, and the M. D. Anderson Cancer Center have developed a novel infectivity-enhanced CRAD called Ad5-delta-24-RGD that has been shown to achieve dramatically enhanced antitumor activity in laboratory models of ovarian cancer. The investigators hypothesize that, by virtue of the enhanced tumor cell infection achieved with this infectivity-enhanced CRAD, an enhanced therapeutic effect in women with ovarian cancer may be realized.

This proposal is a human gene transfer protocol for ovarian and extraovarian cancer patients with persistent or recurrent disease, for whom no curative therapies exist. This Phase I protocol will determine the MTD and the spectrum of toxicities encountered with intraperitoneal delivery of the Ad5-delta-24-RGD virus in women with recurrent ovarian cancer, determine the biologic effects encountered with intraperitoneal delivery of the Ad5-delta-24-RGD virus in women with recurrent ovarian cancer, and determine the immunologic response generated against the Ad5-delta-24-RGD virus when administered intraperitoneally to women with recurrent ovarian adenocarcinoma. The investigators anticipate that this clinical trial will establish the safety of this novel reagent and provide an indication of the efficacy of this approach in women with ovarian cancer.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Thirteen RAC members voted for in-depth review and public discussion of the protocol. Although similar to other studies using replication-competent adenoviruses, this protocol involves the addition of an RGD motif to one of the proteins on the outside of the gene transfer vector coat, which is intended to direct the gene transfer vector to specific tissue sites. Few if any biodistribution studies exist to document the mechanism underlying the resulting increased efficiency of gene transfer vector delivery and/or increased efficacy against the target tumor cells. RAC reviewers Drs. Linial, Powers, and Sidransky and *ad hoc* reviewer Dr. Vile submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Linial's main concern related to the lack of biodistribution and toxicity studies in nonhuman animals. She queried whether further work had been done to characterize the virus in ovarian cell tissue culture to explain the poor replication at low multiplicity of infection, whether high neutralizing antibody titers would be an exclusion criterion, and what is known about the distribution of alpha and beta integrins in normal ovary vs. malignant cells. Dr. Linial noted that several portions of the informed consent document were in need of alteration for increased clarity, including the criteria for removal from the study; she also stated that the investigators should make clear in the informed consent document that the virus to be used in this study is modified and has not yet been tested in humans.

Dr. Powers requested that references to "patient" and "treatment" throughout the informed consent document be changed so that readers of this document do not infer that some form of treatment is being offered. The entire section on risks and discomforts should include fewer sweeping reassurances and vague references and more specifics on the risks and discomforts of the side effects that might be

expected, along with the scientific bases for those expectations. Dr. Powers asked whether any physicians or administrators have financial interests in this research.

Dr. Sidransky asked that the peritoneal aspirate and/or the biopsy material be assessed for baseline genetic status and integrins, that RT-PCR assays be considered for viral deoxyribonucleic acid (DNA) in whole blood and serum, that manufacturing quality control and preclinical safety studies in nonhuman animals be completed and further evaluated before proceeding with the human trial. He stated that ovarian cancer is not a malignancy for which clinical response measures are readily available. He also asked whether there are any commercial ties to this new vector and, if so, the relationship of all investigators to such an entity.

Dr. Vile noted that this group of investigators has extensive experience with the proposed viral type as well as with the disease to be studied. His most important concern stemmed from the small amount of data relating to the expected consequences of using a tropism-expanded virus in humans; the protocol as submitted stated that data on the "biodistribution and toxicity animal studies will be provided when available," which is not adequate because these data are critical to an informed assessment of the protocol. Dr. Vile noted that the murine model does not adequately reflect the human clinical situation for ovarian cancer in that it lacks an immune component and in that the murine tissues are poor substrates for viral replication if any should escape the tumor; relevant studies previously performed in the murine model should clarify this problem.

C. RAC Discussion

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

- Dr. P. Johnson asked the investigators for their rationale for conducting nonhuman primate studies.

- Dr. Vile queried whether the RGD vector infects human activated T cells or activated macrophages or immune cells. He noted that infiltrating immune cells that are activated and replicating might act as a source to distribute the virus to other locations like the spleen.

- Dr. Simari asked for clarification of the difference between the Rapid Access Intervention Development (RAID) program and the National Gene Vector Laboratories (NGVL).

D. Investigator Response

Drs. Alvarez and Curiel responded with the following information:

- The investigators share the RAC members' concerns about the issues of hepatic distribution and toxicity to the liver and plan to address that question in their planned safety studies.

- Recognizing the inadequacy of murine models with respect to gauging the hepatotoxicity of candidate CRADs, the investigators recently piloted the use of fresh primary tumor or normal tissue processed using a tissue slicer that allows maintenance of tissue in three-dimensional configuration in explant culture. Using this process, they can obtain a replication differential that parallels what would be anticipated in humans if there were ectopic localization. This procedure is new, but the investigators will look at the delta-24 RGD using this assay. Dr. Curiel offered the RAC members a copy of a manuscript describing this new technique to gauge CRAD hepatotoxicity.

- The investigators will amend the informed consent document to modify the issues about the ONYX-015 activity in ovarian cancer patients. Another addition to the informed consent document will be made to clarify that the product has not been tested in humans and that the dosing will be based on toxicity and biodistribution studies.

- The investigators understand the importance of monitoring neutralizing antibodies, determining whether there is an association between the presence of neutralizing antibody and the ability of cells to be transfected, and observing toxicity and/or clinical effects. If such an association is uncovered, it may become an exclusion factor in subsequent trials.

- Modifications in the informed consent document requested by Dr. Powers will be incorporated when the safety studies in nonhuman animals have been completed, and the investigators will bring the improved document to the RAC for review along with the results of those safety studies.

- The investigators have conducted preliminary studies that immunized mouse models with both unmodified viruses and RGD-modified viruses; these studies demonstrated that both viruses induce an immune response. The investigators subsequently looked at the effect of neutralizing antibodies to inhibit transfection of both the unmodified adenoviruses and the RGD-modified viruses using the serum from animals preimmunized with both vectors; results indicated that transfection was inhibited but with a lesser response to the RGD-modified vector than to the unmodified adenoviral vector. The manuscript summarizing these findings is currently in preparation.

- In concert with the RAID program, the investigators are looking at what would be the appropriate toxicology and biodistribution studies to conduct with nonhuman primates, since vector replication is somewhat limited in the nonhuman primate model. Discussion with the FDA is currently under way regarding whether such studies will be required.

- The RAID program has been extraordinarily helpful in moving this trial forward as well as with the manufacturing issues and the toxicology and safety study design. In previous studies using other vectors, the NGVL has assisted in a similar fashion. The RAID mechanism has helped for gene therapeutics and other pharmaceuticals, whereas the NGVL is specifically for gene therapeutics.

E. Public Comment

Dr. Borror stated that the informed consent document contained complex language that should be simplified.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- In light of the potential safety implications of the expanded cellular tropism of the Delta-24-RGD vector, biodistribution and preclinical toxicity studies, with particular attention to potential hepatotoxicity, should be completed and evaluated before initiation of the protocol. Once completed, the Principal Investigator is invited to submit these results to OBA for presentation to the RAC.

- Additional studies should evaluate the possibility that second-site mutations would allow the Delta-24-RGD virus to replicate more efficiently in Rb+ cells.

- Discussion of clinical efficacy as a secondary endpoint throughout the protocol, including the consent form, should clearly state the difficulty in measuring and evaluating tumor burden in thse patients.

- The language in the informed consent document is too complex and should be simplified.

- Please clarify the risk:benefit sections to include a statement that this is the first human use of this product. A statement should be added regarding the potential for increased replication of this

product due to the expanded tropism of the virus. Include those risks identified in the upcoming biodistribution studies.

G. Committee Motion 4

It was moved by Dr. Sidransky and seconded by Dr. Lo that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 17 in favor, 0 opposed, 0 abstentions, and 0 recusals.

IX. Discussion of Human Gene Transfer Protocol #0311-614: First Time in Human Safety Study of *Streptococcus mutans* Lactic Acid-Deficient Effector Strain (A2JM) Administered in Conjunction with Twice-Daily Dose of D-Alanine Mouthwash in Healthy Adult Male Subjects for Replacement Therapy as an Aid in the Protection Against Dental Caries

Principal Investigator:	Constance E. Stone, D.M.D., University of Florida
Additional Presenters:	Jeffrey D. Hillman, D.M.D., Ph.D., Oragenics, Inc.; Robert Zahradnik, Ph.D., Oragenics, Inc.; and Michael J. Rosenberg, M.D., M.P.H., Health Decisions
Sponsor:	Oragenics, Inc.
RAC Reviewers:	Drs. Barkley and Gelehrter and Ms. Kwan
Ad hoc Reviewer:	Suzanne M. Michalek, Ph.D., University of Alabama, Birmingham

A. Protocol Summary

Despite the availability of safe and effective dental caries prevention measures-including daily oral hygiene procedures, community water fluoridation, and professional use of topical fluoride and dental sealants-tooth decay remains a major health problem estimated to afflict 5 billion people worldwide. Approximately $40 billion was spent in the United States in the year 2003 on dental caries, a figure that represents 5 percent of the total national health care costs. An increasing body of evidence has associated oral infections with systemic diseases, such as cardiovascular disease, and roughly 10 million disability days are lost to dental caries each year.

Researchers have known for approximately 50 years that tooth decay is an infectious disease and that the principle etiologic agent is an indigenous flora called *Streptococcus mutans (S. mutans*). *S. mutans* sits on the tooth surface and converts sugar into lactic acid. The lactic acid dissolves the mineral that compromises the tooth surface. There is also a clear correlation between the onset of tooth decay leading to the breakdown of the normal anatomy of the tooth surface, which allows for impaction of food and debris in the gums, and the development of periodontal disease. Years ago, Pasteur considered the possibility that naturally occurring bacterial interactions could be exploited to prevent and cure diseases caused by certain pathogens. In recent times this hypothesis has been developed into a therapeutic approach called replacement therapy. The bacterial organism used in replacement therapy is called an "effector strain."

A2JM is a naturally occurring *S. mutans* strain, originally isolated from a human subject, which has been genetically modified to reduce the pathogenic potential of and increased the colonization potential of *S. mutans*. A2JM has also been genetically modified to be completely dependent on environmental D-alanine for growth. Instead of lactic acid, A2JM makes the neutral compounds ethanol and acetone in amounts comparable to other microorganisms that colonize the human oral cavity. Preclinical studies suggested that A2JM is well suited to serve as an effector strain in the replacement therapy of dental caries.

The purpose of this Phase I study is to test the safety of A2JM with D-alanine mouthwash in healthy subjects. The design of this study is intended to minimize risk by examining safety in a small group of volunteers (and transfer to their spouse/partners) with a short duration of exposure to A3JM as well as establish the utility of the antiseptic mouthwash chlorhexidine for the eradication of A2JM in humans.

Steps to minimize the environmental impact of human study with *S. mutans* A2JM include development of a D-alanine dependent bacteria and use of an eradication procedure anticipated to be able to kill A2JM. The removal of the D-alanine mouthwash will prevent the permanent establishment of A2JM while the use of the antiseptic mouthwash chlorhexidine is expected to eliminate any remaining A2JM. A single application of A2JM will be tested because once implanted, proliferation is expected to establish colonization over time. Eradication will be studied both in the presence and absence of the D-alanine mouthwash to ascertain whether chlorhexidine can eliminate A2JM even in the presence of D-alanine.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Twelve RAC members voted for in-depth review and public discussion of the protocol. Novel aspects of the protocol included the concept of replacing normal flora with a novel, genetically altered mutant microbe and the rationale using normal volunteers in a clinical trial for preventive therapy. RAC reviewers Drs. Barkley and Gelehrter and Ms. Kwan and *ad hoc* reviewer Dr. Michalek submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Barkley asked the investigators to explain why the study population is limited to healthy males between the ages of 21 and 35 years and whether they expect healthy females in this age group to respond similarly. He requested further explanation of the rationale for a 7-day regimen, particularly whether it would be sufficient to assess the safety and tolerability of A2JM colonization or to address the secondary objectives of horizontal transmission and genetic stability of A2JM. He asked whether the investigators had studied horizontal transmission by housing noninfected sentinel rats with infected rats, and what the data are regarding the eradication of A2JM from the oral cavities of infected rats. Dr. Barkley also asked if there had been an eradication phase following previously conducted human studies using a different strain of *S. mutans*. He asked the investigators to describe the protection measures to be used by health care workers when applying the A2JM to the oral cavity of a research participant.

Dr. Gelehrter focused his review on the effects of this therapy on the flora of the oral cavity. He asked whether all humans harbor *S. mutans*, the effect of mutacin 1140 production on the *in vivo* growth of other oral organisms in addition to wild-type *S. mutans*, the effect of replacing wild-type *S. mutans* with A2JM on other components of the oral flora, and the anticipated long-term effects on the composition of oral flora due to 2 months' application with chlorhexidine antibacterial mouthwash. Dr. Gelehrter also requested that the investigators explain how they propose to assess the genetic stability of the replacement organism at the end of the experimental period.

Ms. Kwan also questioned the rationale for excluding females from this study. She suggested that the investigators define the term "stable relationship" because, if they are concerned about horizontal transmission, they should recognize that people with stable relationships might have other sexual partners. Given this possibility, and the concern for assessing horizontal transmission, she suggested the investigators consider interviewing the prospective participant apart from the participant's known spouse/partner. Ms. Kwan also inquired whether the participants would need to adhere to any special hygiene precautions. She questioned whether there would be any expected persistence of the A2JM if the subject or partner declined to continue the study and also stopped the chlorhexidine treatments. She noted that there was some inconsistency between the animal data and the informed consent document with respect to the persistence of the A2JM bacteria after cessation of D-alanine administration. She suggested the informed consent document be reworded to be less adamant that the bacterial colonization would cease if the D-alanine mouthwash were withdrawn. She also questioned whether the alcohol produced as a metabolic byproduct of the altered *S. mutans* would be significant enough to alter a person's Breathalyzer test result.

Dr. Michalek also questioned the study's gender limitation. She asked how the bacteria would be applied to the interproximal spaces and whether a 7-day experimental period is sufficient time to establish the effectiveness of A2JM to colonize the tooth surfaces of the participants or to assess horizontal transmission. She asked the investigators how probable is it that a spouse/partner would become colonized with A2JM, as well as the probability of colonization in a participant receiving a challenge of A2JM but no D-alanine mouthwashes. She asked the investigators what effect chlorhexidine treatment

for 30 to 90 days would have on the indigenous oral microflora. She asked for clarification of who would be doing the microbiological analysis of the saliva samples for levels of total bacteria, total S. mutans, and total A2JM. Also she asked who would be assessing the genetic stability of the A2JM isolates from subjects and what this would involve.

C. RAC Discussion

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

- Dr. DeMets requested further explanation of the criteria for choosing 16 participants and asked whether that number would help achieve the investigators' goals in this protocol.

- Dr. Gelehrter asked whether the minimal infectious dose could be determined and whether it was dependent on the indigenous oral flora in each individual research participant.

- Dr. Wara questioned whether the increased stringency of hygiene likely to be practiced by the participants for the first seven days after application of the study agent would complicate the assessment of horizontal transmission.

- Dr. Lo asked whether echocardiograms should be used to screen for unrecognized valvular disease to further mitigate the possibility of bacteremia or endocarditis in potential participants. He also noted that the informed consent document should more clearly delineate the exclusion of subjects with gingival impairment and the fact that there is a very low, but not non-existent, risk of serious infection such as bacteremia or endocarditis.

- Dr. Gelehrter asked about the relationship of dental caries to periodontal disease and whether dental caries is a problem typically of the pediatric age group. He also expressed concern about replacing the normal flora, however pathogenic, with the proposed slightly altered flora.

- Dr. L. Johnson asked whether both the research participant and his partner would receive the D-alanine rinse.

D. Investigator Response

Drs. Hillman, Rosenberg, Stone, and Zahradnik responded with the following information:

- The investigators clarified that the gender limitation was at the request of the FDA, and the age limitation is to facilitate enrollment. The investigators believe that A2JM will not behave differently in females or other age groups. The rationale for limiting the study to males was based on the large body of evidence indicating that most children acquire S. mutans from their mothers during a window of infectivity between ages two and four years. Once safety data was obtained, the study population would be expanded.

- In response to Dr. Gelehrter's questions, Dr. Hillman explained that in prior studies in which mutacin-producing strains were implanted into the mouths of both animals and humans, there was no measurable effect on other organisms that are likely to occupy the same sort of habitat as *S. mutans*. The human studies followed subjects for 15 years.

- Dental caries used to be a disease with childhood onset, but with the introduction of fluoride in dentifrices and municipal water supplies, the epidemiology of dental caries shifted to a disease that can have onset at later ages. It is not unusual for teenagers and young adults to get their first decayed tooth, but most children have experienced their first cavity by age 18 or 19.

- A number of correlations exist between dental caries and periodontal disease. The anatomy of the tooth structure is important in protecting the gums from debris. Unrepaired tooth decay correlates with an increase in periodontal disease in both humans and animals.

- The proposed protocol is a first-in-humans study of this particular agent; therefore, it is an exploratory study to be conducted in a small number of research participants. If the safety profile determined in the small study allows, the investigators will gradually expand the number of research participants and the length of the studies.

- In response to Ms. Kwan's question, the investigators explained that *S. mutans* generates alcohol in the range of three orders of magnitude below the detection capability of the breathalyzer test.

- Rats that are colonized with A2JM and fed D-alanine in drinking water will quickly reach a steady-state level colonization. If the D-alanine is removed from the drinking water, the levels of A2JM fall off quickly to low but measurable numbers. The persistence of A2JM after withdrawal D-alanine is likely due to the fact that rats are coprophagic and D-alanine may have come from fecal sources. This animal behavior limits how well the rat model can serve to inform the design of human studies.

- Dr. Hillman noted that there is not a number that can be determined to be the minimum pathogenic dose of S. mutans. However, the presence of 1×10^6 *S. mutans*/ml of saliva is associated with greater risk of developing tooth decay. Below 1×10^3 *S. mutans*/ml, the risk of tooth decay is minimal. Between 1×10^4 to 1×10^5 *S. mutans*/ml, risk increases but is dependent also on other factors associated with the likelihood of developing decay.

E. Public Comment

No comments were received from the public.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- If eradication of the *Streptococcus mutans* Lactic Acid-Deficient Effector Strain (A2JM) is successful after the proposed seven day treatment period, a second study with a longer treatment period prior to eradication might provide more meaningful safety information about the risks of horizontal transmission.

- The risk of bacterial endocarditis is remote, but since it would be a serious adverse event if it did occur, a complete cardiovascular evaluation, with an echocardiogram if indicated, should be included as part of the subjects' baseline physical examinations.

- As written, the protocol now requires the prospective participant and his spouse/partner to be interviewed together to discuss the risks of horizontal transmission via intimate contact. Since the interviews will include a discussion of whether they meet the inclusion criterion of being in a stable relationship, it is advisable to interview the subject and partner separately to discuss whether there are risks of exposure via intimate contact to other individuals outside the recognized partnership.

- The informed consent document should state that the *Streptococcus mutans* Lactic acid-deficient Effector Strain (A2JM) may persist even after the D-alanine mouthwash is discontinued and what the consequences of this might be for the subject and close contacts.

- The informed consent document should more clearly state that gingival impairment is an exclusion criterion for this study.

G. Committee Motion 5

It was moved by Dr. Gelehrter and seconded by Ms. Kwan that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

X. Update on the RAC Gene Transfer Clinical Trial Design Working Group

Presenters: Dr. DeMets; Nancy M.P. King, J.D., University of North Carolina, Chapel Hill *(via teleconference)*, and Cheryl L. McDonald, M.D., NIH OBA

Dr. DeMets noted that, in addition to previous teleconferences, the Working Group held its first face-to-face meeting on Feb. 12, 2004 at the OBA.

The group began with a review of background articles largely focused on statistical issues, from which they identified key study design elements and issues to be further discussed by the statisticians in the group. The statisticians will then have a teleconference among themselves to further develop these ideas and formulate an action plan for addressing them.

Some aspects of gene transfer trials that differ from other clinical trials are the following: gene transfer trials may have limited preclinical data and are in the very early stages of clinical development when submitted for RAC review; standardization of the vector itself may be difficult; in certain instances, such as with tumor vaccines, dose levels may be difficult to compare; toxicities may be delayed; and often the design issues are not well discussed within the protocols. Also, gene transfer protocols may be designed to apply to a wide range of diseases but one design may not fit all. One issue facing the group that has been touched upon at previous RAC meetings is the question of what does “maximum tolerated dose” mean in the setting of gene transfer where the assumption of an increasing dose-response relationship may not hold true. Dr. DeMets invited members of the RAC to submit to the group any references they think might be helpful and relevant.

Dr. DeMets noted that this summer, an intern may assist the group’s work plans, especially by reviewing the design and monitoring plans of recent protocols and by formulating a checklist or other document to help investigators compose these portions of the protocol. The group will continue to meet by teleconference and communicate via email as their work product evolves. Working Group Co-Chair, Ms. King noted that the group would like to come up with a product that would help investigators design the trials to address relevant question(s) and to help IRBs, IBCs, and the RAC as well.

A. RAC Discussion

Dr. DeMets and Ms. King both noted that ethics and study design are intertwined. If the study isn’t designed properly, or doesn’t ask the right question then that raises ethical issues. One possibility for inclusion in the group’s final document is a table of confidence intervals for sample sizes and estimated event rates. This might serve as a guide for investigators and reviewers to better understand what a study is able to show at a proposed sample size.

Dr. L. Johnson stated that the statistical concerns raised by Dr. DeMets are extremely difficult for investigators to answer, and he hoped that the statistical community would be able to render assistance.

Ms. Kwan stated that producing a helpful checklist must include not only statistical parameters but also information to help investigators determine whether they have stated the research question correctly.

Dr. Lo noted that one of the difficulties in assessing gene transfer protocols is considering whether the underlying principals and assumptions employed in the design of traditional studies are applicable to

gene transfer trials. The nature of the disease under study, the risks, and the potential benefits of the gene transfer study may not be the same as those considered in the more traditional study designs and this may lead to different questions being asked in gene transfer trials.

XI. Day Two Adjournment/Dr. Wara

Dr. Wara adjourned the second day of the March 2004 RAC meeting at 4:45 p.m. on March 10, 2004.

XII. Day Three Opening/Dr. Wara

Dr. Wara opened the third day of the March 2004 RAC meeting at 9:00 a.m. on March 11, 2004.

XIII. Data Management Report/Drs. L. Johnson, Simari, and Wara

Dr. Simari reported that 18 protocols had been submitted to the OBA since December 2003, 12 of which were not selected for public review. Of the 12 trials not selected for public review, ten were for cancer, one was for cardiovascular disease, and one was for a monogenic disorder (neuronal ceroid lipofuscinosis). Regarding vector usage, three used pox vectors, three employed plasmid vectors, two employed adenoviral vectors, and one each used ribonucleic acid transfer, a retroviral vector, an AAV vector, and a herpes virus vector.

The OBA tabulated data and provided background information on AEs during the past three months; a total of 162 AEs were reported, 143 of which were considered serious type A or type C events. The majority of events were considered type C. A total of 7 protocols were classified as initial type A events, all of which were reviewed in detail by the OBA staff and the RAC's Data Management Group.

Dr. Simari summarized one of the serious AEs, from 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. This protocol was publicly reviewed because it was the first use of intravenous DNA liposomes to target non-small cell lung cancer. The investigators reported two research participants with AEs, both of whom had similar symptom complexes, including being admitted to the hospital with grade 2 fever, generalized body aches, chest pain, dysuria, palpitations and hemoptysis, and grade 3 lymphopenia 1 day following infusion of the gene transfer product. Both individuals exhibited similar symptom complexes, and both symptom complexes resolved with supportive care. The investigators stated that this mild-to-moderate lymphopenia had been seen in other participants within the study and suggested that they are conducting additional nonhuman primate studies to sort out the relevance of this symptom complex to the study product.

Dr. Wara reported that the OBA received 57 annual updates or substantial amendments and 54 site or PI changes. She briefly discussed amendments reported from two protocols: #9908-337, Transduction of CD34+ Cells from the Umbilical Cord Blood of Infants or the Bone Marrow of Children With Adenosine-Deaminase-Deficient Severe Combined Immunodeficiency (ADA-Deficient SCID), and #0110-503, A Single Dose-Escalation Study to Evaluate the Safety of the Nasal Administration of CFTR-001, a Gene Transfer Vector, to Participants with Cystic Fibrosis (CF).

Protocol #9908-337 is being conducted at Children's Hospital of Los Angeles and at the NIH. The FDA has taken this study off clinical hold, and the investigators at Children's Hospital are interested in enrolling one participant into the study. This potential participant has late onset ADA-deficient SCID, and the participant and the family have opted not to proceed with either of the two usual therapies because of the potential side effects and the lack of a matched donor. The participant would be enrolled and dosed by the co-PI at the NIH, and the protocol has been amended to allow enrollment of this individual.

Dr. Wara reported on the conclusion of Protocol #0110-503; which was not initially selected for in-depth and public RAC review. A total of 12 adult participants with mild-to-moderate CF were enrolled. The study used a novel technology in which the transgene was compacted into DNA nanoparticles. The primary end points were safety and tolerability, and the secondary end points included serial nasal potential differences. Three dose groups were studied, and partial corrections were seen in 8 of 12 participants; these corrections were transient and lasted up to 6 days. There were no reportable AEs, and Dr. Wara reported that the RAC Data Management Group believes that the major issue that remains to be examined is whether this product can show actual clinical improvement in addition to the corrections seen in this study.

During the past 3 months, the OBA received seven substantial responses to Appendix M-I-C of the *NIH Guidelines*, two of which were extensive. Dr. Wara publicly commended the two investigators who submitted the extensive responses. Dr. Paul Sieving, Director of the National Eye Institute, NIH, submitted a detailed response to numerous RAC recommendations regarding Protocol #0304-575, a Phase I study of NT501, an implant of encapsulated human NTC201 cells releasing ciliary neurotropic factor in patients with retinitis pigmentosa. Also commended was Dr. Elizabeth Jaffee, M.D., Johns Hopkins University School of Medicine, who submitted a complete response to RAC recommendations regarding Protocol #0304-578, "A Phase I vaccine safety and chemotherapy dose-finding trial of allogenic granulocyte macrophage-colony stimulating factor secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin."

XIV. Overview of Investigator and Institutional M-I-C-1 Responses

Presenter: Cheryl L. McDonald, M.D., NIH OBA

At the December 2003 RAC meeting, the RAC requested a compilation of current data about the responses to the M-I-C-1 reporting requirements relative to the publicly reviewed protocols. Dr. McDonald explained that in October 2000 the *NIH Guidelines* were amended to add post-enrollment reporting requirements, with "enrollment" defined as the process of obtaining informed consent from a participant.

Starting with Protocol #0009-411 and ending with Protocol #0310-611, representing the timeframe from December 2000 through December 2003, a total of 200 protocols were submitted to the OBA, of which 43 received in-depth review and public discussion by the RAC. Formal responses were submitted by 11 of those 43, and some form of partial response was submitted by 4 of the 43; thus, a total of 15 of 43, or 35 percent, of the protocols reviewed in depth by the RAC provided some form of response.

On the basis of the 15 protocols with some form of response, analysis indicated that RAC recommendations were generally well received, detailed responses to the RAC recommendations were supplied in most cases, most of the RAC recommendations were implemented in some form, and if a RAC recommendation was not implemented, a sound rationale was provided for the incongruity. Examples of responses to RAC recommendations included the following: Additional preclinical studies were designed, additional biodistribution and immunologic assessments were included, enhanced and clearer criteria for data review by a data and safety monitoring board (DSMB) were implemented, and many of the suggested changes to the relevant informed consent documents were made. The most common reasons that RAC recommendations were not implemented were as follows: The IRB would not allow the suggested language changes in the informed consent document, discussions with the FDA led to a different protocol design often with a variation of RAC-suggested changes, and the PI or the sponsor believed that more preclinical work had been conducted than had been presented to the RAC.

Dr. McDonald noted that receiving timely feedback is a complex and ongoing issue. She noted in summary that in general, RAC recommendations are well received and addressed. Often the RAC recommendations are incorporated into the final clinical protocol.

A. RAC Discussion

Dr. McDonald and Alexander Rakowsky, M.D., explained that, approximately one-third of the PIs of publicly reviewed protocols have submitted a partial or complete response stating that enrollment in the trial has begun.

Dr. DeLuca stated that, because institutional biosafety committees (IBCs) review approved protocols on a yearly basis, they could be educated about the M-I-C-1 requirements, and they could remind the investigators of the need to submit the M-I-C-1 response.

Dr. McDonald explained that the submission of annual reports is determined by the date on which the IND was granted, while the response to M-I-C-1 is predicated on enrolling a first participant. Because they have not yet enrolled any participants, the investigators for some protocols that have been reviewed publicly by the RAC have not yet submitted an M-I-C-1 response.

Dr. Wara requested a brief update as part of the data management report at each RAC meeting; she posited that it would be useful for RAC members to know the volume of M-I-C-1 responses. Dr. McDonald agreed to provide that information.

XV. Discussion of Human Gene Transfer Protocol #0401-623: A Phase I/II Dose-Escalating Randomized Controlled Study to Assess the Safety, Tolerability, and Efficacy of CERE-110 (Adeno-Associated Virus [AAV]-Based, Vector-Mediated Delivery of Beta-Nerve Growth Factor [NGF]) in Subjects with Mild to Moderate Alzheimer's Disease

Principal Investigator:	David A. Bennett, M.D., Rush University Medical Center
Additional Presenters:	Zoe Arvanitakis, M.D., Rush University Medical Center; Roy Bakay, M.D., Chicago Institute of Neurosurgery and Neuroresearch; Raymond T. Bartus, Ph.D., Ceregene, Inc.; Jeffrey M. Ostrove, Ph.D., President, Ceregene, Inc.; Mark H. Tuszynski, M.D., University of California, San Diego
Sponsor:	Ceregene, Inc.
RAC Reviewers:	Drs. Bohn and Lo
Ad hoc Reviewer:	Steven T. DeKosky, M.D., University of Pittsburgh

A. Protocol Summary

Alzheimer's disease (AD) is the most common cause of dementia, afflicting approximately 4.5 million Americans. AD patients suffer a devastating decline in cognition and quality of life, and the disease represents a significant social and financial burden to society. The current standard-of-care medications for AD, the cholinesterase inhibitors (ChEIs), alleviate symptoms by augmenting cholinergic function; however, although ChEIs improve the function of remaining cholinergic neurons in the basal forebrain, they do not prevent the death of these neurons. It has been posited that protecting cholinergic neurons from degeneration and death, as well as enhancing their vitality, might slow the course of cognitive decline in AD.

Although it has been recognized for almost 20 years that neurotrophic proteins such as NGF can both improve function and prevent cholinergic neuronal death in experimental animals, a practical and safe method for delivering NGF to these neurons in humans does not yet exist. CERE-110 is a gene transfer vector engineered from an AAV in which all of the AAV genes have been removed and replaced with the gene for NGF.

The proposed clinical trial will investigate the safety and efficacy of the administration of CERE-110 to the basal forebrain region containing the nucleus basalis of Meynert (NBM) in participants with AD. The Phase I portion of the study will evaluate six participants to establish the safety of CERE-110 at two different doses. The blinded Phase II portion, which will evaluate 30 participants, will continue to examine the safety of CERE-110 and is also designed to provide a preliminary indication of the effectiveness of CERE-110 for AD.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Twelve RAC members voted for in-depth review and public discussion of the protocol. This protocol proposes to use an AAV-based, vector-mediated delivery of NGF in research participants with AD. The most relevant previous study for AD registered with the OBA involves the infusion of genetically modified cells to provide the same transgene, NGF, to the brain. The other CNS gene transfer studies registered with the OBA involve other disease models such as Parkinson's disease and Canavan's disease. The RAC does not have sufficient follow-ups on any of these studies—regarding either the viral vectors or the transgenes—to assess whether the approach is appropriate and safe. RAC reviewers Drs. Bohn and Lo and *ad hoc* reviewer Dr. DeKosky submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Bohn expressed concern regarded whether it is ethical to deliver a growth factor gene to the brain in the absence of a means for turning off the gene should adverse effects ensue over time; data from Protocol #9906-322, the prior NGF study, might assist in dealing with this concern. She was also concerned about the lack of information on the outcome of participants in Protocol #9906-322, and she stated that it appeared premature to undertake the current study without having this information available. Dr. Bohn asked about the volume of vector to be injected and whether it would be standardized by dilution of various vector stocks. She requested more information about the data to support the statement that the adverse effects of NGF protein administration are specific to ventricular administration and can be avoided by direct administration to the brain parenchyma. She asked whether bilateral rather than unilateral injections of AAV-NGF are necessary and constitute the best protocol design considering the surgical risk. She also suggested modifications to the informed consent document, primarily dealing with the irreversibility of gene implantation and surgical risks.

Dr. Lo asked how the investigators will determine whether a participant is currently capable of giving consent, and what the investigators would do if a participant loses decision-making capacity and does not want to participate in follow-up measurements. He suggested that, because of the possibility of mental deterioration coupled with the lengthy follow-up period, participants should designate a surrogate who would make decisions for them in the future if needed. He asked what level of efficacy, as well as safety, do the investigators believe would justify including placebo surgery in a Phase II trial. He expressed concerns about the lack of measures to reverse overexpression of NGF in the brain or other tissue. He was also concerned about the possibility of the reactivation of herpes zoster, which should be mentioned in the informed consent process. He asked how the investigators plan to deal with participants who experience claustrophobia induced by magnetic resonance imaging; and under what circumstances would the investigators break the double-blind code.

Dr. DeKosky was concerned about the nature of the change occurring in the cholinergic circuitry in the presence of the enzyme. Noting that enzyme presence prevents the death and increases the vitality of neurons, he asked whether the investigators believed that the enzyme might restore the natural physiologic activity of the neurons. Regarding the risk of overexpression in a system that cannot be turned off, Dr. DeKosky suggested that the investigators specify an action plan to deal with the possibility of a cholinergic overload produced by successful transgene expression. Dr. DeKosky asked about the rate of daily secretion of NGF and the estimated amounts of NGF that might be produced by the vector. He noted that the investigators are targeting the most manipulable system for improvement of cognition but that many other biological abnormalities could limit improvement.

C. RAC Discussion

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

- Dr. DeLuca asked for clarification of how much NGF is considered safe—how much NGF is made *in vivo* relative to how much NGF is made with the AAV system.

- Dr. Bohn asked whether the investigators would be screening participants for neutralizing antibodies to AAV.

- Dr. Lo discussed the importance of making clear to surrogates the nature and extent of their role. He suggested that the investigators word the informed consent document and process as explicitly as possible.

- Dr. DeKosky asked how overexpression of NGF might result in behavioral toxicity.

D. Investigator Response

Drs. Ostrove, Bennett, Bartus, and Tuszynski responded with the following information:

- Persistent *in vivo* expression is seen in the aged monkey brain for up to 1 year, as evidenced by results from a study of the proposed vector in 25-year-old rhesus monkeys. No toxicity was evident in the brain on either Nissl stain or cellular markers for the cholinergic system, so there is no evidence of overstimulation of the cholinergic neurons or death of the cells in this region in the brain. Regarding sprouting, the *in vivo* approach shows greater diffusion of the NGF vector itself than with an isolated cell graft, so sprouting is not seen in the NBM. There were no adverse effects on cognitive function in the three aged monkeys comparing the preoperative and postoperative behavioral states, and there were no effects of nontargeted NGF delivery, no weight loss, and no evidence of pain in these primates.

- This gene transfer trial proposes to use a vector with an unregulated transgene. The investigators stated that they would prefer to use a vector with controlled gene expression, if one were ready for use in human trials.

- NGF is not a new molecule, and a tremendous amount is known about NGF in the brain. NGF has a positive effect on cholinergic neurons that are degenerating, there is no evidence in 20 years of study that high levels of NGF in the parenchyma cause pathology of those neurons, and no evidence exists to indicate that sprouting is undesirable in cholinergic neurons. All the data indicate that high concentrations of NGF produce no harm; when problems do occur, they occur relatively quickly, and such changes have been empirically linked to misdirected or nontargeted NGF.

- Sprouting in this system with NGF does not appear to do any harm. Cholinergic sprouting in the cortex likely represents a restoration of the morphology of the neurons and may be responsible for some of the reported functional benefit.

- At the end of a Phase II trial, participants who underwent the sham surgery would be offered an opportunity to have the actual procedure if evidence of efficacy was shown. The company has agreed to finance those surgeries.

- Regarding screening participants for AAV antibodies, the investigators will draw samples but will not use the results for exclusion from the study. Two of the aged monkeys tested positive for AAV antibodies and showed no detectable differences from monkeys that did not have AAV antibodies. Those monkeys also showed good NGF expression.

- To address the concern about cholinergic hysteria, the investigators have given hundred fold greater NGF levels to young rats and young monkeys, stimulating the system far in excess of what is intended in humans. To the extent to which symptoms in nonhuman animals are observable, none were seen. It is unclear, however, whether or not an animal is a good model for humans in this regard. The investigators intend to treat study participants with an anticholinergic if cholinergic hysteria is worse than any benefit the participant experiences.

E. Public Comment

Dr. Borror noted that, overall, the informed consent document was clear and well written. She suggested that the section titled "What Is Gene Transfer?" should be deleted because it is uninformative and because gene transfer is described in sufficient detail elsewhere. Because female participants are required to be postmenopausal, the notation about urine pregnancy testing also should be deleted from the informed consent document.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- Concerns about potential risks to research subjects remain due to the lack of a rescue strategy in the protocol. The proposed research could possibly result in cholinergic overactivity in cortical and other areas innervated by the basal forebrain cholinergic nuclei and there is no means of reversing the innervation. While the presentation of toxicity data from protocol 322 (which involved the ex vivo manipulation of autologous fibroblasts) and the presentation of preclinical data utilizing a lentiviral vector were interesting, it is not possible to directly compare these data with the proposed use of AAV-NGF. Thus, additional safety and toxicity data in non-human primate brains at time points both shorter and longer than three months should be gathered using the specific product to be used in this research study.

- Concerns about potential risks to research subjects remain for the proposed study regarding the potential levels of transgene expression given the absence of efficacy data using the direct delivery of AAV NGF to aged primates. To address this concern, extending the interval between dose escalations from one month to three months in this Phase I safety should be considered.

- The cognitive assessment data from Protocol 322 is relevant and could affect the conduct of this protocol. As such, and after the data have undergone appropriate peer-review, you are asked to submit them to OBA for presentation to the RAC.

- Since the presentation and discussion at the RAC meeting focused exclusively on the Phase I component of the proposal, we encourage the Principal Investigator to present the Phase II component before initiation of this phase of the study.

- Formalize the designation by the research subject of the "study partner" who will assume responsibility for decision about the subject's continued participation in the study if the subject loses decision-making capacity.

- Add to the informed consent document the potential complication of reactivation of Herpes zoster.

- The informed consent document and process should discuss how the results of phase I will inform the final protocol and conduct of phase II. Key issues that pertain only to the phase II trial, such as randomization to shame surgery, should nonetheless be included in the informed consent document for phase I to provide subjects with an understanding of what they may encounter if they participate in phase II.

G. Committee Motion 6

It was moved by Dr. Bohn and seconded by Dr. L. Johnson that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 11 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XVI. Discussion of Human Gene Transfer Protocol #0401-622: Adenylyl Cyclase VI Gene Transfer for CHF (Congestive Heart Failure)

Principal Investigator: H. Kirk Hammond, M.D., University of California, San Diego
RAC Reviewers: Drs. DeMets, L. Johnson, Lo, and Simari
Ad hoc Reviewer: Walter J. Koch, Ph.D., Jefferson Medical College

A. Protocol Summary

The proposed study is a randomized, double-blind, placebo-controlled, single-dose study to evaluate the safety, tolerability, and clinical effectiveness of ascending doses of human adenovirus-5 (E1/E3-deleted, replication incompetent) encoding adenylyl cyclase type VI (Ad5.AC_{VI}) in subjects with congestive heart failure. The vector will be delivered by intracoronary injection in a solution that contains nitroprusside to increase gene transfer efficiency.

Dilated systolic heart failure, when present with the activities of daily living or during rest (Class III and Class IV symptoms), is associated with a 3-year survival of 50%-a statistic that indicates a worse prognosis than many cancers. Certain newer medications have shown statistically significant prolongation of life, but even with the use of these, the disease is progressive and associated with substantial short-term mortality.

In addition to high mortality, dilated systolic heart failure is associated with reduced left ventricular contractile function, increased left ventricular chamber dimensions, reduced ejection fraction, and elevations in filling pressures of the left ventricle and pulmonary artery wedge pressure. In addition, a hallmark of CHF is intolerance to exercise and elevations in plasma levels of norepinephrine and B-type natriuretic peptide (BNP). This proposed study will assess exercise tolerance, hemodynamic measurements (via right heart catheterization), and serial serum measurements of norepinephrine and BNP levels as reflections of the overall status of the CHF.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Twelve RAC members voted for in-depth review and public discussion of the protocol. This protocol proposes to use an Ad5 vector encoding a novel human AC_{VI} in a new CHF patient population for which alternative therapies exist. RAC reviewers Drs. DeMets, L. Johnson, Lo, and Simari and *ad hoc* reviewer Dr. Koch submitted written reviews, to which the investigators responded in writing and during this meeting.

Regarding the statistical methods section of the protocol, Dr. DeMets requested clarification of how the sample would be determined and what test statistic would be used to compare exercise tolerance. He asked the investigators to provide additional references regarding sample size issues. Dr. DeMets also asked for further explanation of the statement that participants who are to receive cardiac transplantation should be withdrawn from the study. He also stated that statistical methods and sample size should be added to the protocol and requested a better definition of dose-limiting toxicity.

Dr. L. Johnson noted that the design of this protocol is different from many Phase I/II protocols reviewed by the RAC in that it appears difficult to separate the Phase I and Phase II components. In the proposed study, dose, efficacy, and safety are to be evaluated concurrently at all doses of the vector. Dr. Johnson noted that this approach will limit the evaluation of safety at the lower doses because of inadequate power, and placebo participants might be placed at unnecessary risk from procedures. He asked whether the investigators propose a future multiple-dosing scheme for this vector and what AEs or magnitude of changes in cardiac or liver enzymes would warrant discontinuation of the protocol or recruitment of additional participants to a specific cohort. Dr. L. Johnson also requested the establishment of formal criteria for the inclusion of individuals with coronary artery disease and cardiomyopathy. He noted that the exclusion of women from cardiovascular trials in the past may have contributed to inequities in detection and treatment of cardiovascular disease in women and requested that the investigators consider including women of childbearing potential who agree to use contraception.

Preliminary data and efficacy concerns included a request for a summary of data or background studies on the use of nitroprusside in humans, whether basal levels of cyclic adenosine monophosphate (cAMP) were increased *in vivo* and what is known about the toxicity of chronically elevated basal levels of intracellular cAMP, and whether toxicology and biodistribution studies have been completed with the proposed vector. Dr. L. Johnson also noted that the informed consent document and responses to Appendix M needed to address long-term follow-up.

Dr. Lo questioned whether the exclusion criteria should be amended to add the exclusion of left main coronary artery disease or the equivalent where revascularization is indicated. Also, noting that adenovirus-mediated inflammation of the heart is a theoretical risk, he questioned whether persons with CHF caused by myocarditis should be excluded. He noted under the "alternatives" section, the informed consent document should mention the possibility of enrollment in other experimental trials. He questioned whether the statistical plan had accounted for unequal group sizes in the power calculations and how multiple analyses of the data by the DSMB might affect the power calculations. Dr. Lo asked whether examination of the sperm for possible unintended germ-line expression of the transgene might be useful.

Dr. Simari asked what determinants would be used to define dose-limiting toxicity and how dose escalation parameters will be determined. He asked several questions regarding the proposed use of intracoronary nitroprusside, including the logistics of delivery and chemical compatibility with the viral vector, and noted that if nitroprusside were to be used, the use of Viagra® (sildenafil) should be excluded. Dr. Simari noted that the proposed clinical population is very heterogeneous and the investigators should consider how this range of underlying myocardial status will affect gene transfer, distribution, and safety. Given the possible range of cardiac uptake, the investigators should consider the use of myocardial biopsy as a means to study transgene expression in the myocardium.

Dr. Koch questioned the selection of the patient population, the risks of the catheterization procedure, and whether using the study agent as a bridge to transplant or as a molecular adjunct to a ventricular assist device might allow the added benefit of having access to heart tissue to assess myocardial expression. He suggested that it would be helpful to study the effect of adenylyl Cyclase (AC) over-expression with beta-adrenergic receptor blockers or angiotensin converting enzyme inhibitors to look for any potential additive effects of an AC gene transfer. He questioned if perhaps this study should have a comparator arm of nitroprusside (NTP) alone in the intracoronary infusion in order to assess any contribution it is making to cardiac function. He noted that there should be monitoring for extra-cardiac transgene expression, such as in the lungs or liver. He asked for discussion of what could potentially happen if AC activity was detected in other organs. He also asked for clarification of how AC over-expression could lead to any c-AMP-independent effects.

C. RAC Discussion

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

- Dr. Simari questioned whether the viral delivery would be affected by the distribution of viable myocardium.

- Dr. Koch asked whether all subjects would be on beta-blockers or not and how that would affect the assessments in the study.

- Dr. Simari noted that a right heart catheterization is standard of care for patients with left ventricular dysfunction, and that it is a Class I indication for patients with heart failure to get coronary angiography at some point in their clinical course.

- Ms. Kwan suggested that the RAC's recommendations include a specific comment suggesting that the IBC and the IRB look carefully at the amount of detail included in the protocol to ensure sufficient specificity and detail for evaluation.

- Dr. Phil Johnson noted that he does not consider adenovirus to be a vector for prolonged, sustained gene expression and that the heart is not an immune-privileged organ. He postulated that there is a point at which doses of adenovirus sufficient for gene expression would actually lead to an inflammatory immune response in the myocardium.

- Dr. Borror noted that the language in the informed consent document is complex and should be simplified. She also suggested clearer wording regarding the risks involved in the use of placebo.

D. Investigator Response

Dr. Hammond responded with the following information:

- Dr. Hammond clarified that the study agent would not be administered into an occluded artery.

- There is a single intracoronary catheter but it screws onto a manifold and this manifold has multiple ports that will allow for the concomitant administration of the NTP and the virus which are chemically compatible.

- Dr. Hammond agreed with Dr. Simari that from a scientific standpoint, a myocardial biopsy would be informative. However, he noted that there would be increased risks with this procedure, and for those subjects who had received placebo this might not add any extra information or benefit.

- In response to the suggestion that the two lower doses may not need a placebo comparison, Dr. Hammond noted that this study is designed to get aggregate placebo data at the end of the study to compare to the Dose 5 group.

- Although limiting enrollment in this protocol to individuals who have contraindications to beta-blockers is scientifically plausible, to do so would likely create recruiting difficulties. Since this is primarily a safety trial, enough useful information can be gleaned from participants who may or may not be taking beta-blockers.

- Toxicology biodistribution studies with the vector proposed for this study have not yet been conducted. The investigators propose to conduct those studies with the actual clinical product as soon as it is provided by Cornell University. The product will be given to pigs via intracoronary administration and biodistribution data will be collected and submitted to the FDA in the IND.

- Toxicity-related stopping rules will be delineated for this protocol to the best of the investigator's ability to define them *a priori*.

- In response to the concerns of immune-mediated toxicity as raised by Dr. Phil Johnson, Dr. Hammond noted that the adenoviral doses proposed for this trial are below the virus particle per gram of tissue ratios reported in the literature.

E. Public Comment

There was no public comment.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- Preclinical toxicology and biodistribution studies using the same vector and transgene to be used in humans should be completed prior to initiation of the clinical protocol.

- The lack of specific endpoints defining dose-limiting toxicities or pre-determined stopping rules is a concern. To the extent possible, safety endpoints and stopping rules should be identified and discussed in the protocol.

- Since clinical effects of the experimental agent at lower doses is not expected, the rationale for inclusion of placebo control groups at these dose levels is inadequate and should be reevaluated.

- For subjects who are eligible to participate but may nonetheless be found to have a proximal coronary occlusion or stenosis > 70%, consideration should be given to altering the protocol defined distribution of the experimental agent between the right and left coronary arteries to lower the risk of reflux or the experimental agent into the aorta.

- In order to ascertain whether there have been any local effects of the gene transfer, it may be helpful to obtain a myocardial biopsy in subjects during the cardiac catheterization performed four weeks after the delivery of the transgene. As such, consideration should be given to adding this procedure to the protocol.

- The exclusion of women of childbearing potential should be reconsidered for those women who agree to use a medically acceptable form of birth control (e.g., oral contraceptives, levonorgestrel implant, and medroxyprogesterone acetate injection).

- The language in the informed consent document is too complex and should be simplified.

- If a myocardial biopsy is added to the protocol, the informed consent document should be modified to specify the increased risks. This is particularly important if the placebo groups are maintained because the risk/benefit ratio would be different for subjects in the placebo groups.

- The informed consent document should clarify that a coronary angiogram will be performed as part of the protocol and that it would also be performed when clinically indicated.

- The informed consent document should include the theoretical risk of hypotension resulting from the infusion of nitroprusside as well as the measures that would be taken if this occurs.

G. Committee Motion 7

It was moved by Dr. Simari and seconded by Dr. Lo that the above recommendations be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 11 in favor, 0 opposed, 1 abstention, and 0 recusals

XVII. Closing Remarks and Adjournment/Dr. Wara

Dr. Wara thanked the participants and adjourned the meeting at 2:35 p.m. on March 11, 2004.

_____

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: _____

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Attachment II
Public Attendees

Jim Ackland, Biologics
Ronald D. Alvarez, University of Alabama, Birmingham
Zoe Arvanitakis, Rush University Medical Center
David Bartlett, University of Pittsburgh
Raymond T. Bartus, Ceregene
David A. Bennett, Rush University Medical Center
Kathie M. Bishop, Ceregene
Mike Blaese, Oragenics, Inc.
Phillip Boucher, FDA
Andrew P. Byrnes, FDA/CBER
Joy L. Cavagnaro, Access Bio
Sunil Chada, Introgen Therapeutics, Inc.
Cheauyun Chen, FDA/CBER
Eric Chojnicki, Oragenics, Inc.
Shirley M. Clift, Cell Genesys
Charles Conrad, MD Anderson
Margaret Crowley, Eberlin Reporting Services
David T. Curiel, University of Alabama, Birmingham
Juan Fueyo, MD Anderson
Stephanie Haas, University of Florida
H. Kirk Hammond, University of California, San Diego
Ray Harris, SAIC
Joanne S. Hawana, F-D-C Reports, Inc.
Jeffrey D. Hillman, Oragenics
Ying Huang, FDA
Don Jantzen, Pfizer Consumer Healthcare
John V. Kelsey, FDA/CDER
Frederick Lang, MD Anderson
Susan Leibenhaut, FDA
Russette M. Lyons, Biotechnology Consultant
Elanchezhiyan Manickan, FDA
Maritza McIntryre, FDA/CBER
Andra E. Miller, Biologics
Jeffrey M. Ostrove, Ceregene
Joanna Peterkin, Ceregene
Cynthia Rask, FDA
Michael J. Rosenberg, Health Decisions
Sheryl Ruppel, SAIC-Frederick, Inc.
Jude Samulski, University of North Carolina
Donna Savage, Capital Consulting Corporation
Jean Schoen, Eberlin Reporting Services
Michele M. Schoonmaker, Congressional Research Service
Ana Schwartz, FDA/CBER
Tatiana Seregina, NewLink Genetics.
Mento A, Soponis, Oragenics
Earl Stibitz, FDA
Constance E. Stone, University of Florida
Elizabeth Sutkowski, FDA/CBER
Mark H. Tuszynski, University of California, San Diego
Dominick A. Vacante, Ceregene
Erin D. Williams, Congressional Research Service
Peter K. Working, Cell Genesys

Bob Zahradnik, Oragenics, Inc.
Yongje Zhou, FDA

Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
ACH	acetylcholine
AC_{VI}	adenylyl cyclase type VI
AD	Alzheimer's disease
Ad5	adenovirus-5
ADA-deficient SCID	adenosine-deaminase-deficient severe combined immunodeficiency
AE	adverse event
cAMP	cyclic adenosine monophosphate
CD	cytosine deaminase
CF	cystic fibrosis
ChEI	cholinesterase inhibitor
CHF	congestive heart failure
CNS	central nervous system
CRAD	conditionally replicative adenovirus
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
FACA	Federal Advisory Committee Act
FDA	U.S. Food and Drug Administration
FOIA	Freedom of Information Act
GAD	glutamic acid decarboxylase
HIV	human immunodeficiency virus
IBC	institutional biosafety committee
IND	investigational new drug
IRB	institutional review board
MTD	maximum tolerable dose
NBM	nucleus basalis of Meynert
NGF	nerve growth factor
NGVL	National Gene Vector Laboratories
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	NIH Office of Biotechnology Activities
OD	Office of the Director, National Institutes of Health
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RAID	Rapid Access Intervention Development
RGD	arginine-glycine-aspartic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SAE	serious adverse event
S. mutans	*Streptococcus mutans*
SR	somatostatin receptor
vvDD-CDSR	double-deleted vaccinia virus plus CD/SMR