RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 25-26, 2000

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 <http://www4.od.nih.gov/oba/documents1.htm>.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1] September 25-26, 2000

The Recombinant DNA Advisory Committee (RAC) was convened for its 79th meeting at 9:00 a.m. on September 25, 2000 at the National Institutes of Health (NIH), Building 31, Sixth Floor, Conference Room 10, 9000 Rockville Pike, Bethesda, MD 20892. Dr. Claudia A. Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on September 25 from 8:30 a.m. until 4:05 p.m. and on September 26 from 9:00 a.m. until 4:10 p.m. The following individuals participated in all or part of the meeting:

Committee Members

C. Estuardo Aguilar-Cordova, Harvard Gene Therapy Initiative
Dale G. Ando, Cell Genesys, Inc.
Xandra O. Breakefield, Massachusetts General Hospital
Louise T. Chow, University of Alabama, Birmingham
Theodore Friedmann, University of California, San Diego
Jon W. Gordon, Mount Sinai School of Medicine
Jay J. Greenblatt, National Cancer Institute, National Institutes of Health
Nancy M.P. King, University of North Carolina, Chapel Hill
Sue L. Levi-Pearl, Tourette's Syndrome Association, Inc.
M. Louise Markert, Duke University Medical Center
Claudia A. Mickelson, Massachusetts Institute of Technology

Ad Hoc Reviewers

Barry J. Byrne, Gene Therapy Center, University of Florida
David A. Dichek, University of California, San Francisco
Pedro R. Lowenstein, University of Manchester, United Kingdom
Anthony Rosenzweig, Massachusetts General Hospital
Richard O. Snyder, Harvard Medical School

Nonvoting/Agency Representatives

Jeffrey M. Cohen, Department of Health and Human Services
Philip Noguchi, Food and Drug Administration
Stephanie L. Simek, Food and Drug Administration

Executive Secretary

Amy P. Patterson, Office of the Director, National Institutes of Health

[11] 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.

Speakers

Michaele Christian, National Institutes of Health
Sonia I. Skarlatos, National Institutes of Health
David Strayer, Thomas Jefferson University

National Institutes of Health Staff Members

David Badman, NIDDK
Bobbi Bennett, OD
John T. Burklow, OCPL
John Scott Cairns, NIH
Sarah Carr, OD
Kelly Fennington, OD
Joe Gallelli, CC
Bob Jambou, OD
Sung-Chul Jung, NINDS
Richard Knazek, NCRR
Edward Korn, NCI
Tom Kresina, NIAAA
Kathy Lesh, OD
Mary Nuss, NIAID
Marina O'Reilly, OD
Pearl O'Rourke, OD
Gene Rosenthal, OD
Nava Sarvar, NIAID
Michael Sayre, CSR
Thomas Shih, OD
Lana Skirboll, OD
Sharon Thompson, OD
Chris Vargas, OD
Benjamin Wilfond, NHGRI

Members of the Public

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

I. Call to Order and Day One Opening Remarks/Dr. Mickelson

Dr. Mickelson, RAC Chair, called the meeting to order at 9:00 a.m. on September 25, 2000. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on August 22, 2000 (65 FR 51008). Issues to be discussed by the RAC at this meeting included reviews of four gene transfer protocols, discussion of issues for the December 2000 Safety Symposium on Cardiovascular Gene Transfer Research, update and report from the NIH Gene Transfer Data and Safety Assessment Working Group on serious adverse event (SAE) reporting, data management report, discussion of the scope and applicability of the *NIH Guidelines*, and an introduction to the proposed use of simian virus-40-based vectors in humans.

Dr. Mickelson introduced Dr. Jeffrey M. Cohen (Office for Human Research Protections), Dr. Barry J. Byrne (Gene Therapy Center, University of Florida), Dr. Pedro R. Lowenstein (University of Manchester, United Kingdom), and Dr. Sonia I. Skarlatos (National Heart, Lung, and Blood Institute [NHLBI]). Dr. Philip Noguchi, Food and Drug Administration (FDA), introduced Dr. Stephanie L. Simek, FDA's alternate liaison to the RAC.

Dr. Amy Patterson reviewed the NIH conflict-of-interest rules as a reminder to the RAC members of the importance of avoiding real and perceived conflicts of interest while serving on the RAC.

II. Minutes of the June 28-29, 2000 Meeting/Dr. Jon W. Gordon and Ms. Sue L. Levi-Pearl

Ms. Levi-Pearl indicated that the draft minutes of the June RAC meeting were accurate. The presence of a few typographical errors was noted.

Committee Motion 1

As moved by Dr. Gordon and seconded by Dr. M. Louise Markert, the RAC approved the June 28-29, 2000 minutes by a vote of 10 in favor, 0 opposed, and 0 abstentions.

III. Discussion of Human Gene Transfer Protocol #0007-407: *A Phase I, Double-Blind, Placebo-Controlled, Escalating-Dose, Multicenter Study of Ad2/Hypoxia-Inducible Factor (HIF)-1α/VP16 Gene Transfer Administration by Intramyocardial Injection During Coronary Artery Bypass Grafting (CABG) Surgery in Patients With Areas of Viable and Underperfused Myocardium Not Amenable to Bypass Grafting or Percutaneous Intervention*

Principal Investigator:	Todd K. Rosengart, M.D., Northwestern University Medical School
Sponsor:	Genzyme Corporation (represented by Ralph A. Kelly, M.D.)
RAC Reviewers:	Drs. Xandra O. Breakefield, Louise T. Chow, and Theodore Friedmann and Ms. Nancy M.P. King
Ad Hoc Reviewers:	Barry J. Byrne, M.D., Ph.D., Gene Therapy Center, University of Florida David A. Dichek, M.D., University of California, San Francisco (written review only) Pedro R. Lowenstein, M.D., Ph.D., University of Manchester, United Kingdom Anthony Rosenzweig, M.D., Massachusetts General Hospital (written review only)

Protocol Summary

The purpose of this clinical research study is to examine the safety and the potential ability of a new experimental study treatment for stimulating the growth of new blood vessels from existing blood vessels (angiogenesis). It is hoped that this treatment will improve the flow of blood within an area of the myocardium that is viable but not amenable to conventional CABG or percutaneous intervention in patients with coronary artery disease who are undergoing elective CABG surgery. This treatment, named Ad2/HIF-1α/VP16, is a new kind of gene transfer developed by the sponsor of this study, Genzyme Corporation. This investigational new drug (IND) transfers a gene into cells within the patient's

myocardium, which causes the cells to produce a modified form of a substance naturally produced by the body, HIF-1α.

The gene for HIF-1α/VP16 will be introduced into myocardial cells by using a type 2 adenovirus (Ad2), a common virus found in the human airway that, in its normal state, can reproduce and cause a cold. The vector (Ad2/HIF-1α/VP16) has been altered so that it cannot reproduce. The production of HIF-1α is a normal part of the patient's cellular response to low amounts of oxygen caused by reduced blood flow. When native HIF-1α or HIF-1α/VP16 enters these cells, it causes the cells to produce and release growth factors such as VEGF and other substances. These angiogenic growth factors have the ability to stimulate the growth of new blood vessels from existing blood vessels and, as a result, increase the flow of blood carrying oxygen to myocardial cells.

Genzyme has conducted efficacy, toxicity, and biodistribution studies in pigs and rats to support the proposed cardiac ischemia protocol. These studies are in addition to the intramuscular safety and toxicity studies conducted in support of Genzyme's critical limb ischemia clinical protocol (NIH Protocol #s 9907-327/328/329).

The proposed study will look at whether different doses of Ad2/HIF-1α/VP16 can be tolerated safely by direct injection into the heart muscle (intramyocardial injection) where the coronary artery is currently blocked and may not be helped by CABG or other common procedures used to clear arteries (e.g., angioplasty). The study design is a Phase I, randomized, double-blind, placebo-controlled, dose-escalation study. Twenty patients are anticipated to be enrolled, and five different doses of Ad2/HIF-1α/VP16 will be studied. The same five doses are also being studied and evaluated for safety in the Phase I critical limb ischemia clinical trial. The dose range was previously tested in animal studies and found to be safe for human testing. Four patients will be enrolled in each of five dosing groups, and one patient in each group will be randomized to receive placebo. A placebo group is included in the study because it may assist in providing an impartial evaluation of safety data by the investigator and by an independent Data and Safety Monitoring Board (DSMB) since adverse events (AE) may occur in participants whether or not they received the study drug. Each participant will receive a single dose of study drug or placebo in one administration.

Specific safety assessments have been included in the protocol to monitor for potential adverse experiences that could be related to the adenoviral vector in which the gene is placed, the HIF-1α gene contained in the adenoviral vector, the direct injection of the study drug or placebo into the heart muscle, or the progression of the patient's advanced coronary artery disease. These assessments are in addition to those routinely performed after elective CABG (e.g., laboratory testing for injury to the myocardium, electrocardiograms to monitor for abnormal heartbeats or rhythms, echocardiograms to look for extra fluid next to the heart or changes in the beating of the ventricular wall). This study also will include assessments to evaluate the extent of Ad2/HIF-1α/VP16-mediated new blood vessel growth to improve the flow of blood within the selected area of the myocardium and potential clinical outcomes relating to the ability of Ad2/HIF-1α/VP16 to increase blood flow in the heart and relieve chest pain (angina pectoris). Participant status will be monitored for 1 year after receiving Ad2/HIF-1α/VP16 or placebo during CABG.

Written Comments from Preliminary Review

Five RAC members recommended that the protocol warranted public review and discussion on the basis of several issues. Drs. Xandra O. Breakefield, Louise T. Chow, and Theodore Friedmann, Ms. Nancy

M.P. King, and four *ad hoc* reviewers submitted written reviews. The investigators responded to their comments in writing.

Dr. Breakefield's comments focused on the array of genes that will be turned on by HIF-1α/VP16 and the adenoviral vector; the potential toxicity of elevated inducible nitric oxide synthase (iNOS) levels; and the monitoring of lung function for vector accumulation and inflammation, which in rats occurred 90 days after myocardial administration of a high dose of the vector. She requested information about the clinical results of Genzyme Corporation's ongoing trial with patients with critical limb ischemia, especially information about which genes are turned on in muscle cells in response to HIF-1α/VP16.

Dr. Chow's comments discussed the paucity of knowledge about the role of HIF-1α; whether HIF-1α/VP16 induces expression of the same proteins as HIF-1 (erythropoietin, factor X, and iNOS); and inappropriate use of the words "treatment" and "therapy" in the informed consent document.

Dr. Friedmann's comments discussed whether safety, biodistribution, and gene expression studies have been conducted in nonhuman primates; the results of the biodistribution studies after intracardiac injection in the rat and whether any of these safety studies were repeated in the pig; which tests were used for systemic toxicity; how to interpret experimental results for participants in whom it is not possible to distinguish between benefits from the gene transfer and those from restored perfusion in other areas of the myocardium; and aspects of the placebo.

Ms. King's written review centered on consent form issues, including the description of adenovirus as a common cold, the risk of cardiac inflammation, use of terms such as "new study treatment" and "gene therapy treatment" in this Phase I trial, the need for clarification of the benefits section, and whether the risk of unwanted angiogenesis is theoretical or real.

Dr. Dichek's comments raised a number of specific questions, some dealing with the size of the study; the likelihood that no hypothesis regarding therapeutic angiogenesis could be formally tested in a study of this size; and the difficulty of differentiating between the effects of CABG and the gene transfer product and procedure.

Dr. Lowenstein's comments included questions about the titration of viral vector, the vector genomic structure, transcriptional control, immunogenicity, vector toxicity, preexisting circulating antibodies, and the longevity of expression. He also queried whether the investigators had taken steps to promote the performance of autopsies.

Dr. Anthony Rosenzweig, Massachusetts General Hospital, also prepared a written review, which included concerns about the choice of the target population; he expressed concern about the ethics of enrolling patients who have a reasonably good prognosis using standard medical options and the wisdom of subjecting them to the risks of Ad2/HIF-1α/VP16 gene transfer. Other concerns expressed by Dr. Rosenzweig included the fact that simultaneous CABG would likely cloud the ability of the investigators to deduce valuable lessons from this trial, the adverse effects of HIF-1α/VP16 (more detailed information was requested about how the chimeric transcription factor might alter transcription and what its downstream targets might be), and the potential adverse effects of intramyocardial adenoviral injection.

RAC Discussion

Dr. Kelly presented a summary of the protocol, the primary reviewers summarized their comments, RAC members posed additional questions, and the investigators responded to the comments and questions.

Like all the RAC reviewers, Dr. Friedmann expressed appreciation to Dr. Kelly and his colleagues for their thoughtful responses to comments and questions raised during the preliminary review and for the clear presentation at this meeting. Dr. Friedmann requested further information on the conditions under which it would be necessary to conduct extensive pharmacologic-toxicologic, biodistribution, and expression studies in the pig and in humans and to provide an explanation for why nonhuman primate studies would not be helpful to conduct prior to the initiation of this protocol.

Dr. Breakefield's comments centered on the fact that adenoviral vectors cause inflammatory and acute responses and that eventually most of the transduced cells in the heart would be rejected by the immune system; the difficulty in assessing vector toxicity because of the inflammation and healing, possibly leading to arrhythmias, which is also part of the disease's course; the potential toxicity of the novel protein HIF-1α/VP16; distinguishing between what was induced by the vector and the normal course of the disease in terms of the effect of induction of angiogenesis in adult tissues; and determining whether these relatively healthy participants constitute the most appropriate population for testing such a potent vector, given its additional risk.

Dr. Chow commented that the preclinical tests *in vitro* and in animal models seemed to have been carefully performed and that the wording in the consent document should avoid terms such as "treatment" and "therapy." She indicated that unless the RAC wanted to recommend nonhuman primate testing, she had no further comments or recommendations.

Ms. King focused on questions about the informed consent document, including how well risks are explained. She also provided a suggested rewrite of the wording on the potential benefits of the protocol and requested that a draft revised consent document be submitted to the RAC. She expressed disappointment that the Institutional Review Board (IRB) had not suggested these such changes during its review of the protocol.

Dr. Lowenstein offered several suggestions to the investigators: that they consider using stronger promoters that would allow substantial reduction of vector doses; look carefully at the immunogenicity of the HIF-1α/VP16 protein; and possibly enroll participants who do not have antibodies to adenoviruses, thereby potentially increasing transduction. He also requested that the investigators provide additional information about the bystander effect.

Dr. Byrne's comments reiterated Dr. Friedmann's concerns about the acute toxicities of the product, particularly those related to the timing of patient discharge. Dr. Byrne suggested that the patients be kept in the hospital until the first clinical observation point (1 week), rather than being released after 4 days. He questioned whether the increase in circulating VEGF observed in the rodent would be a realistic measure in humans. Dr. Byrne suggested that the imaging studies, which are used to assess outcome measures, be done in a centralized location to minimize intraobserver variability. He also expressed concern that the biodistribution data were obtained from rodents with beating hearts, whereas the patients will be on cardiopulmonary bypass at the time of virus administration.

Investigator Response

Dr. Kelly and colleagues responded to the reviewers' comments. He reported that the investigators have done extensive biodistribution and toxicologic studies and that, on the basis of their rat and porcine data,

the researchers felt confident in proceeding to human trials. Many histopathological examinations of hearts of treated rats and pigs have been done. Dr. Geoff Akita, Genzyme, added that the pig was selected for the bioactivity studies because it is considered the standard animal model for this type of research; nonhuman primates would not represent a significant improvement over the pig and the rat.

In response to a question from Dr. Mickelson, Dr. Noguchi pointed out that nonhuman primates may not be an appropriate model for two basic reasons. First, because primates are expensive to house, treat, and care for, conducting statistically valid time-course experiments, similar to those conducted in pigs or rats, may not be feasible. Second, primates may not be as sensitive as smaller animals to some of the vector's toxic effects and/or they may not have the relevant receptor. Dr. Noguchi indicated that the FDA will be exploring issues in preclinical pharmacology testing with its own advisory committee in the near future. Dr. Gordon suggested that a presentation from the Institute of Laboratory Animal Resources, part of the National Research Council, might be helpful in this regard.

Dr. Kelly explained the bystander effect as follows: When a gene is transferred into a cell, that cell becomes a "factory" for the genes; in this case, the HIF-1 that is made in the transduced cells then turns on a number of additional genes in that cell. Some of the products of these genes, such as peptide cytokines or angiopoietins, are transported out of the cells, and diffuse to have an effect on bystander cells some distance away from the transduced cell. The tranduced cells eventually are cleared by the immune system regardless of which apoptosis proteins are turned on or off. The biological effect is still hypothesized to occur due to the bystander effect in which the proangiogenic factors diffuse to receptors on ischemic tissue and induce blood vessel formation. In regard to vessel leakiness, in the critical limb ischemia trial, some edema was seen, but the response to the major protein that is induced (VEGF, which increases vascular permeability) is likely to be muted because of the robust upregulation of angiopoietins, which tend to decrease the amount of permeability that VEGF induces.

Dr. Kelly indicated that the researchers are concerned about vector toxicity. The participants would be at very high risk for developing ventricular arrhythmias because they have repolarization abnormalities resulting from underlying disease. Dr. Kelly agreed that the introduction of HIF-1α/VP16 could exacerbate heterogeneity, thus increasing the number of arrhythmias. He explained that theoretically the risk of arrhythmias should diminish if the gene transfer succeeds in bringing about an increase in vascular perfusion to the ischemic area. While the agent may increase the risk, if it has its intended effect, the risk should decrease. Dr. Kelly acknowledged that it may be difficult to predict whether the net effect will be beneficial or will exacerbate the patients' predisposition to arrhythmias. In the absence of gene transfer, this patient population experiences an 11 percent mortality rate, a significant proportion of which is arrhythmia related. The pig is known to be prone to ventricular arrhythmias in the absence of ischemia, so mortality tends to be high in this experimental model.

Dr. Richard J. Gregory, vice president for gene therapy at Genzyme, explained that the investigators were aware of the results referred to by Dr. Lowenstein regarding the strength of the mouse cytomegalovirus (CMV) promoter, but that they do not have direct experience with it. The investigators have a great deal of experience with the human CMV promoter, so they chose to move forward with a plasmid in an adenoviral construct that they understood well. Vector development is continuing at Genzyme, with second- and third-generation variations of the HIF-1 vector being developed, but at present, nothing is superior to the current HIF-1 vector. Regarding the antibody issue involving HIF-1, no reagents are currently available that will allow measurement of HIF-1 levels in humans; if a reagent is found, it will be incorporated into future studies.

With regard to the participation of patients who do not have preexisting antibodies, Dr. Gregory responded that there are few such patients. Genzyme has scanned human populations periodically to

determine antibody titres against Ad2 and found that almost everyone has a level of neutralizing antibody against it.

Dr. Kelly accepted Dr. Byrne's suggestion that participants remain in the hospital until after the first clinical assessment on day 7. He agreed that these patients have a higher mortality regardless of gene transfer, that their postoperative care is more complicated, and having them remain in the hospital for several extra days was a reasonable suggestion.

Dr. Aguilar-Cordova questioned the value of using a control of only one per cohort and how using a blind control would add value in evaluating the significance of SAEs. Dr. Kelly responded by explaining the extent to which the placebo effect takes place in angiogenesis trials—there is a large confounding effect on the positive side. Dr. Kelly explained that using one control per cohort has been successful in Genzyme's critical limb ischemia trial and that the degree of risk for saline injections is believed to be virtually zero. He acknowledged that control patients will have the burden of followup evaluations and exams. Dr. Kevin McEllin, director of clinical trials at Genzyme, stated that the investigators realize that the statistical comparisons cannot be done with such a small control number and that Genzyme plans to pool the placebo patients into one group, thus creating a group of five placebo patients which will be compared with the cohorts of three patients receiving each dose of the vector. This method will assist in the evaluation of SAEs and has been recommended by the investigators and the DSMB.

Dr. Aguilar-Cordova asked whether the investigators will be following up any immunological consequences to the fusion protein (VP16). Dr. Akita responded that they do not have quality reagents to develop an ELISA test; they need an antigen to detect the antibodies made to VP16, and are trying to develop the required reagents.

Dr. Cohen commented that the consent form contains a good statement about the nature of a Phase I study, but it is buried in the middle of the form. He recommended that the statement be moved to the beginning of the form.

Dr. Gordon added a general comment cautioning that the RAC should avoid suggesting specific revisions to the protocols because the Committee should not assume responsibility for the way a protocol is written. Ms. King explained that the reason she provided specific language was to help investigators understand her points fully and because general statements can be misinterpreted. She indicated that she does not expect the investigators to use the suggested language verbatim.

Dr. Breakefield remphasized that the consent document needs to clearly inform patients that there is a significant unknown risk associated with this vector and that the vector could cause harm as well as possibly produce potential benefit.

Public Comments

No public comments were made.

RAC Recommendations

Dr. Mickelson summarized the following recommendations and observations:

- The rationale for using a placebo control with a single subject in a cohort of four subjects should be reevaluated in consultation with a biostatistician to assure the statistical power of the study design.

The informed consent document should be revised to include a statement about the odds and significance of being randomized to the control group.

- In order to ensure the consistency in the interpretation of imaging studies, such as SPECT nuclear imaging and magnetic resonance imagine (MRI), a centralized facility should be used to evaluate the outcome data.

- Because preclinical toxicity data indicated that toxicities can occur as late as seven days following vector administration, the research participants' hospital stays should be extended to seven days following vector administration to provide adequate monitoring of any adverse reactions.

- With regard to the informed consent document:

    - Since adenovirus can produce infections more severe than the common cold, the informed consent document should be revised to describe adenovirus along the lines of the following: *A common virus found in human airways and in its normal state can reproduce and cause a variety of respiratory infections, including mild cold-like symptoms, and more severe infections like bronchitis and pneumonia.*

    - In regard to the risk of cardiac inflammation, the relevant rat toxicology experiments should be described along the following lines: *In rat studies, microscopic evidence of minimal to mild inflammation in the heart muscle has been observed when the rats were given an injection of study agents that is ten times higher than the dose you will receive per injection.*

    - Throughout the consent form, potentially misleading terms such as "new study treatment" and "gene therapy" should be replaced with more neutral terms such as "study injections". "investigational study plan", and "gene transfer".

    - The benefits section should be clarified. Ms. King and Dr. Breakefield, RAC members, suggested that a statement along the following lines should be included:

        It is not possible to predict whether receiving the study injections will be of greater benefit to you than receiving CABG surgery alone, and it may increase your risk. Some preliminary results of other clinical studies using adenovirus gene transfer injections similar to Ad2/HIF-1/VP16 directly into the heart have shown that subjects tolerated the injections well, but the agent Ad2/HIF-1/VP16, has not been delived to human heart tissue previously. It is possible that in this study, the injections may show activity in creating new blood vessels in some subjects' hearts, which may reduce angina in some subjects. However, benefit to you is not the goal of this study. Instead, we want to see if the study injections are safe. We also want this study to provide a foundation for future studies of these injections, in hope of benefitting future patients with coronary artery disease.

    - To emphasize the experimental nature of this study, the following statement should be moved from the Alternatives section to the beginning of the informed consent document: *This is a phase I study, meaning it is primarily assessing safety and dose escalation, and it involves an experimental agent with which there is relatively little clinical experience.*

    - A statement regarding the possibility of harm to the research participants by the intervention should be included.

IV. RAC Discussion of Issues for the December 2000 Safety Symposium on Cardiovascular (CV) Gene Transfer Research/Dr. Friedmann

Dr. Friedmann described the recent work of RAC members with respect to the RAC's upcoming third safety symposium, which is scheduled to take place at the December 2000 RAC meeting. The goal of the symposium is to increase the understanding of the unique aspects of the field that require special safety considerations. Its purpose is to identify features related to the safety of CV studies, discuss issues related to selecting and ensuring the safety of research subjects, and examine the consent process specific to this field.

Potential specific topics for the symposium include monitoring, good clinical practice, product quality control, and entry criteria. The scope of the symposium is likely to include gene transfer to the heart and to peripheral blood vessels, how vectors are selected, vector features that are useful in the CV system, administration of vectors, patient selection, inclusion of placebo/control in study designs, and the nature of transgenes in the CV system.

The impetus for this safety symposium stems from the RAC receiving more interesting and complicated CV proposals. Thirty protocols have been registered with the Office of Biotechnology Activities (OBA) dealing with the heart and the peripheral vascular system. This field in general is moving rapidly. Protocols are likely to be submitted with greater frequency, and the special problems and difficulties would benefit from the information sharing that should occur at the conference. The conference will also benefit RAC members by broadening and deepening their knowledge of this area of research.

Dr. Friedmann listed the potential issues for the CV safety symposium, including the potential effects of transgene products on nascent tumors and tumor development, how to determine whether tumorigenicity might be a problem, the effect of growth factors and how to eliminate or reduce potential problems, selection and measurement of clinical end points, how to determine whether transgenes have had the intended effect, which technology should be used, and patient selection and the likelihood of response or interpretable outcomes.

Participants will include investigators, sponsors, cardiologists, basic researchers, ethicists, NIH and FDA staff members, and others. The symposium will be open to the public; use of the World Wide Web will enhance public access.

Dr. Friedmann asked RAC members to assist in sharply defining the goals and objectives of this safety symposium, suggest additional questions that would be suitable for such a symposium, and determine the type of input the RAC would like to have so that all RAC members will feel more comfortable when evaluating CV proposals.

RAC Discussion

Dr. Mickelson stated that the objective is not only to help the RAC assess protocols but also to help the field move forward, promote consensus on the nature of the important issues, and determine where the field ought to focus. Dr. Chow suggested that animal model systems should be added to the discussion; she agreed with Dr. Gordon's suggestion of using animal facility personnel. Ms. Levi-Pearl stated that patient advocacy organizations should be added to the list of participants.

Dr. Dale G. Ando recommended that overall focus be on the three basic vector models that will proceed to advanced trials, i.e., adenoviral E1/E3, E1/E4, and gutless vectors. Dr. Friedmann agreed that the symposium will not be able to cover all CV or all vector issues. The intent is to lay out the questions; rather than determine the current best candidate vector, the RAC should discuss the vector features and requirements that will be needed to move the field forward.

Dr. Aguilar-Cordova reminded the RAC that the focus of the conference should be on issues specific to CV disease. He also suggested that disseminating available data might be more fruitful than simply posing abstract questions.

Dr. Noguchi suggested that one session should feature the roles of the Federal Government, the public, and others in informing consumers. Dr. Friedmann agreed that the topic of various roles is so crucial that it might deserve an entire symposium. Dr. Mickelson added that some context should be drawn so that potential study participants are able to distinguish between the scientific unknown and the real risk of participating in the trial. Dr. Kelly agreed that this kind of feedback and discussion would be useful for Genzyme and other researchers and research companies.

Dr. Breakefield stated that a helpful topic would be how long inflammatory responses can be expected to continue.

Dr. Mickelson stated that fruitful public discussion will help other investigators and move the field forward. She suggested that additional ideas be e-mailed to the OBA for consideration.

V. Serious Adverse Event Reporting: Update and Report From the NIH Gene Transfer Data and Safety Assessment Working Group/Dr. Patterson and Michaele Christian, National Cancer Institute

Dr. Patterson introduced the topic of enhancing the assessment of toxicity and safety data in gene transfer clinical trials. Recent events in gene transfer studies have raised concerns and questions about the adequacy of Federal and local review and analysis of AEs, the scope of AEs reported to the NIH, and the best way to communicate safety information to the scientific, regulatory, oversight, and lay public communities. These recent events also have generated proposals for change from bioethicists, advocacy groups, the biotechnology industry, the scientific community, and the U.S. Congress. There is a wide spectrum of opinion about the optimal policy approach, ranging from those who believe in full access to those who believe data dissemination should be limited in order to prevent misperceptions about safety in this field.

The challenge for the NIH is to establish a data assessment system in human gene transfer research (GTR) that provides maximum protection to research participants and is feasible and useful to investigators, sponsors, and oversight bodies. The NIH has explored the range of concerns and opinions through RAC discussions and the deliberations of the Advisory Committee to the NIH Director (ACD) and its working group.

Following on those efforts, NIH established an internal ad hoc working group, composed of experts from a number of NIH Institutes and Centers (ICs) involved in GTR, and co-chaired by Dr. Michaele Christian, National Cancer Institute (NCI), and Dr. Patterson, to review existing and proposed mechanisms for systematic analyses of AE data. Dr. Christian presented the group's recommendations for an optimal system for monitoring safety in human gene transfer studies.

Dr. Christian reported that the group recommended the establishment of a standing RAC working group composed of scientists, clinicians, patient representatives, ethicists, one to two RAC members, NIH members, and an FDA liaison. The group would have expertise in basic and clinical GTR, biostatistics, ethics, and patient advocacy. Its functions would be to review all AE reports, analyze data for trends, identify those trends or single events that warrant public discussion and/or Federal action, and develop cumulative reports for presentation at the quarterly RAC meetings.

The primary responsibility for real-time response to safety concerns would continue to reside with the FDA and local oversight and review bodies; this working group would be a complementary and supplementary advisory group that would not replace real-time response. The operation of the working group would include rapid dissemination of results that could be released prior to RAC concurrence. The group would meet quarterly, in closed sessions prior to RAC meetings, and would be staffed by the OBA with dedicated staff members—medical officers, information specialists, and statisticians—to carefully assess these AEs.

RAC Discussion

In response to Dr. Friedmann's question about whether quarterly meetings of assessment group would be sufficient; Dr. Christian responded that if permanent staff members were assigned to support the activity, quarterly meetings should be sufficient. Permanent staff members would reside in the OBA and would receive and review AEs as they were reported. The group could meet more often than quarterly if an SAE required immediate action or if ongoing analyses suggested that more frequent meetings were warranted. This proposed group would conduct its analysis in closed session to protect patient privacy and present SAEs in a clinical context that would include analysis and interpretation, and the analysis and cumulative report would be released publicly at each quarterly RAC meetings.

Dr. Friedmann asked when the NIH Director is expected to act on this working group's recommendations. Dr. Patterson responded that a proposed action will be issued and sufficient time for public comment will be allowed (at least 30 days). The RAC will have to vote on the proposed action because it would amend the *NIH Guidelines*. Depending on the timing of release and depth and breadth of public comment, the RAC may vote either at a scheduled meeting or via public teleconference.

Dr. Gordon asked about the development of a database and whether it could help identify trends. Dr. Christian described the committee's discussions on this issue, including an attempt to promulgate consistent definitions and AE reporting mechanisms. She further explained that a database is being created to analyze the AE information, and that a database would be more robust and useful if it emanated from a common set of terms and definitions. Informatics support and staff (including database entry staff members) were among the additional staff recommended by the ad hoc group. Dr. Patterson and the OBA staff will be examining the current reporting system and will look at other systems to attempt to address the manner in which the AE data are submitted.
Dr. Aguilar-Cordova discussed the definition of SAEs that would be used. Dr. Christian pointed out that a recommendation was made to unify definitions with the FDA. Dr. Patterson added that the ad hoc group understood the desire to harmonize Federal reporting requirements as well as the expressed concerns that safety information be provided to the public in an analyzed format. She reiterated that proposed changes to NIH's reporting requirements must be approved by the NIH Director after additional public comments are gathered.

Dr. Markert asked for clarification about how the AE reports would be submitted to the OBA and the proposed committee. Dr. Christian responded that the AE reports would be submitted to the OBA as they

are now, but they would be analyzed by staff members dedicated to this proposed committee. The committee would then report their analyzed findings to the RAC.

Dr. Gordon expressed concern about the RAC receiving reports back from investigators to indicate whether and how the RAC's recommendations had been implemented. Dr. Mickelson responded that investigators are asked in writing to respond to the RAC recommendations. Dr. Patterson pointed out that one of the ACD's recommendations is that a mechanism be established for systematic follow-up by the investigators to the RAC recommendations. This recommendation was incorporated into the timing action and is currently being reviewed by the NIH Director.

Dr. Aguilar-Cordova asked how many additional OBA staff members would be needed to accomplish enhanced data assessment. Dr. Christian reiterated that the ad hoc group recommended the addition of two or three medical officers, a statistician, a data entry person, and an information specialist. These additional staff members were predicated on the current level of AEs received by the OBA. At the end of 2 years, the need to continue this heightened level of analysis will need to be reassessed. Dr. Aguilar-Cordova also asked about the function of patient representatives and ethicists on the assessment group, which will be carrying out a review of highly technical matters. Dr. Christian responded that ethicists and patient representatives would provide a unique perspective that would be valuable in creating context and interpreting the findings.

Public Comments

Ms. Janet Rose Christensen, Targeted Genetics Corporation, asked about SAE reporting and Phase IV review. She also suggested that members of the proposed working group/committee receive training on issues such as clinical development challenges and interactions with the FDA. Dr. Mickelson responded that the ACD Working Group recommended that reporting definitions and timing be harmonized with the FDA and that a single, electronic format for reporting SAEs be developed that would be acceptable to both the FDA and the RAC/OBA. Dr. Christian responded that the working group had no specific recommendation for Phase IV trials. Its primary focus is early research, when there is less known about toxicity, so data are being shared across trials and across vectors.

Mr. Paul Gelsinger, member of the public, expressed concern about the adequacy of resources to support the additional data assessment tasks. Dr. Skirboll responded that the OBA staff has more than doubled in the past year and that requirements for additional staff resources will be evaluated. She stated that NIH will implement what needs to be done with regard to the oversight of GTR and that tax dollars will be used efficiently and effectively.

Ms. Vera Hassner Sharav, Citizens for Responsible Care and Research, asked about changes in SAE reporting requirements and whether investigators would be held accountable. Dr. Christian responded that the proposed assessment group would be staffed with medical officers so that AE review would be timely and, if necessary, concerns could be communicated to the FDA or other entities. The assessment group would complement, not replace, FDA's regulatory reporting requirements and oversight procedures. Dr. Patterson added that there can be financial consequences for recombinant DNA researchers and their institutions if NIH requirements are not followed. In the near future, the NIH will establish a range of additional specific sanctions that will apply to investigators and institutions for types of noncompliance with the *NIH Guidelines*.

Additional Comments

Dr. Mickelson stated that additional discussion would take place at the December 2000 RAC meeting. Additional comments should be e-mailed to the OBA.

VI. Discussion of Human Gene Transfer Protocol #0006-406: *Erythropoietin (EPO) Administration in Hemodialysis Patients Using Vascular Grafts Lined With Transduced Smooth-Muscle Cells*

Principal Investigators:	William R.A. Osborne, Ph.D., University of Washington, Seattle Kimberly A. Muczynski, M.D., Ph.D., University of Washington, Seattle
Sponsor:	None
RAC Reviewers:	Drs. Ando and Markert and Ms. Levi-Pearl

Protocol Summary

The goal of this protocol is to test the safety of a gene transfer method for the sustained delivery of EPO to treat the anemia of patients with end-stage renal disease (ESRD). The anemia associated with ESRD is correctable by administration of recombinant EPO, a hormone that normally is synthesized in the kidney and regulates red cell production. The availability of EPO has reduced the need for transfusions and increased the quality of life for dialysis patients.

EPO is delivered intravenously or subcutaneously to all ESRD patients in the United States at a cost of more than $1 billion annually. The investigators have developed an experimental method for sustained delivery of EPO via smooth-muscle cells that are retrovirally transduced with the EPO gene. The EPO-expressing smooth-muscle cells are seeded into polytetrafluoroethylene (PTFE) vascular grafts, which, when ligated into the vascular system of a patient, provide a continuous source of EPO to the circulation. The method of EPO delivery proposed for this trial will involve no additional surgery for study participants other than that currently required to create their dialysis access grafts. PTFE vascular grafts are the standard grafts used for hemodialysis access in patients with ESRD.

The potential benefits of this method of EPO delivery include reduced cost of EPO therapy and maintenance of higher hematocrit levels.

Written Comments from Preliminary Review

This protocol was selected for review by seven RAC members because of its novelty in a number of areas, including the fact that it involves the use of both a device and a biologic and that a thorough revision of the consent document is needed. Dr. Ando, Ms. Levi-Pearl, and Dr. Markert submitted written reviews, to which the investigators responded in writing.

Dr. Ando's comments included the need to discuss, within the protocol and the consent form, the risk of replication-competent retrovirus (RCR) and the necessity of long-term followup; inclusion of a statement in the consent form about the use of a retroviral vector; and use of a dose-escalation, Phase I study design to evaluate the safety of this treatment.

Ms. Levi-Pearl's comments centered on the consent document. The submitted version of the consent document is not written for a lay reader. Ms. Levi-Pearl suggested a complete rewrite of the consent form, provided specific suggestions, and offered assistance in the rewriting process.

Dr. Markert's comments related to the stability of dose; continuation of participants' other medications (e.g., intravenous iron); participant safety issues such as continued monitoring; concerns about the clinical facility and Good Clinical Practices (GCP) plan; and two issues in the consent form—the need to mention the importance of autopsy in the event of death and information about removal of the PTFE graft. Dr. Markert also requested a copy of the audit for the laboratory facilities.

RAC Discussion

Drs. Osborne and Muczynski presented a summary of the protocol, the primary reviewers summarized their comments, RAC members posed additional questions, and the investigators responded to the comments and questions.

Dr. Markert began by noting that the principal investigator is not the dialysis center director, the optimal position for appropriate management and oversight of the study. Dr. Markert expressed concern that the investigators may not have adequate training in the conduct of clinical research and asked for information about the backgrounds of all key personnel. Regarding GCP issues, Dr. Markert reminded the investigators that an audit should be conducted prior to starting the study to ensure that everything is in place. Other concerns raised by Dr. Markert included the following:

- The consent document states that only the first dialysis treatment would occur at the University of Washington site, suggesting that the investigators plan to see the participants only twice–for the consent process and at the time of the first dialysis. This level of patient contact does not qualify as the "careful monitoring" warranted by the protocol. Given this level of care and follow-up, it also would be difficult to ensure appropriate attention to the documentation and reporting of AEs.

- The DSMB should review the possible changes in the patients' doses, decide whether patient enrollment should cease, and assist with data integrity and interpretation.

- A Good Laboratory Practices audit is necessary to ensure the quality of the laboratory procedures.

- With regard to the informed consent document, Dr. Markert suggested a number of specific and general changes. Information should be added about the continued need for intravenous (IV) iron and the possibility that supplemental EPO will be needed periodically. Information about the possible removal of the PTFE graft and its potential consequences should be condensed and placed in one place rather than scattered throughout the document. Discussion of the importance of autopsy should be added. In general, the content and organization of the document need to be revised. As currently written, the document is missing important information and consists of three large, but nonetheless incomplete, paragraphs. Subheadings should be added.

Dr. Ando raised some of the issues related to replication-competent retrovirus (RCR), product manufacturing, and dosing. Regarding RCR, FDA guidelines with respect to long-term followup should be referenced in the protocol and followed. Even with *ex vivo* research, RCR can be generated, and the FDA guidelines specify that no RCR should be present in the gene product. Dr. Ando suggested that the investigators contact the National Gene Vector Laboratories (NGVL) to discuss whether testing the supernatant will eliminate completely any risk of RCR in the transduced cell product which cannot be tested directly. Regarding manufacturing, good manufacturing practices (GMP) require that controls for the reagents, procedures, production records, testing, and quality assurance are in place during every step

of the manufacturing process. However, the protocol does not describe procedures consistent with GMP. Dr. Ando also asked why the protocol is not designed as a dose-escalation study, which, given that there is an accepted treatment for the disorder, he believes would result in more useful safety information.

Ms. Levi-Pearl focused on the informed consent document and recommended a complete rewrite. Her main concerns were the document's lack of emphasis on the experimental nature of the trial. She suggested that text be separated into more paragraphs and that the following sections and section headings be added: the purpose of the study, the potential risks and benefits, procedural specifics, reasons for investigator cessation of subject participation, the implications and risks of the subject withdrawing from the experiment, privacy and issues of confidentiality, and costs (those that are covered by the clinical trial and others that are the responsibility of the subject or his or her insurer). She provided the investigators with a list of other items that are missing (e.g., alternatives to participation) or in need of elaboration.

Dr. Mickelson reiterated concerns about whether the facility's infrastructure is adequate to provide comprehensive oversight of the complex procedures involved in this experimental biologic-device protocol.

Continued Discussion and Investigator Response

Dr. Jay J. Greenblatt stated that this protocol is inadequate as written. An extensive background section is needed as well as sections on pharmaceuticals and AE reporting. He urged the investigators to take advantage of the many model protocols available from various NIH ICs, including the National Cancer Institute. Dr. Greenblatt asked whether the investigators had performed any studies in nephrectomized animals, given that the animal models did not appear to simulate the clinical situation. Dr. Osborne responded that causing kidney ablation in a large animal (e.g., a sheep) requires the animal to be dialyzed to produce the research conditions; he reiterated that the small-animal data were adequate to show that EPO delivery could be achieved in a large animal.

Ms. King questioned whether there was a more direct way to address the problem of the cost of EPO and the effects of Medicare reimbursement policy. She asked how the experimental method would improve on the current method of administering EPO. The investigators explained that establishing a more stable level of hematocrit may be possible with gene transfer.

Dr. Mickelson asked why the investigators chose a one-dose treatment rather than dose escalation. Dr. Osborne responded that a dose-escalation study would not be unreasonable. However, the one-dose approach was selected in order to provide participants with a potentially therapeutic dose.

Dr. Noguchi suggested that the investigators consult with some of the researchers who have conducted earlier trials.
Dr. Aguilar-Cordova asked about whether the grafts would have a consistent number of cells and separation of cells. Dr. Osborne responded that they have been able to achieve consistency of seeding, pumping, and measuring of secreted EPO.

Dr. Gordon commented that this type of protocol should be of special interest to the RAC because it is an example of cell therapy, which has many long-term potential advantages, such as stable transduction of cells.

Dr. Osborne addressed some of the RAC reviewers' concerns. He indicated that the requirements for GMP and the documentation of AEs will be carried out by the trained clinical research center staff and experienced members of the gene transfer research core at the University of Washington. The core staff

members will advise the investigators and will supply additional personnel to fulfill reporting and other requirements. The PG13 packaging system has a good record for producing replication-incompetent virus. RCR should be detectable during cell culture prior to seeding of the graft and the NGVL will document that the virus is RCR-free. Dr. Muczynski agreed that the consent form could be revised on the basis of RAC reviewers' suggestions.

Public Comments

No public comments were made.

RAC Recommendations

Dr. Mickelson summarized the RAC recommendations and observations.

- The infrastructure needed for comprehensive oversight of the development of a medical device for gene transfer is inadequate at this academic site. Documentation of the protocol's adherence to good clinical practices, good manufacturing practices, and quality assurance and control of vector development and production should be improved. A standard operating procedure for adverse event reporting should be placed before proceeding with the clinical trial.

- The use of model protocols available to assist investigators with the development of clinical protocols should be considered. National Cancer Institute (NCI) template protocols are available at the NCI-Cancer Therapy Evaluation website. (ctep.info.nih.gov/Templates). Dr. Jay Greenblatt, RAC member, offered to help in this regard.

- To improve comprehension by the research participants, the informed consent document should be revised by shortening the paragraphs and including sub-headings. Since this is the first time that this experiment is to be performed on humans, such terms as "experimental study/research" and "preliminary investigation" should be used throughout the document. Ms. Sue Levi-Pearl, RAC member, offered to help with revision of the informed consent document.

- Because this study is intended to be a phase I dose escalation safety study, the doses used in the protocol should not begin at the therapeutic levels. The initial dose level should be lowered in order to enhance research participant safety.

VII. Data Management/Dr. Greenblatt

Dr. Greenblatt reported that 10 new protocols were submitted to the OBA since the last reporting period; 6 were exempted from review by the RAC; and 4 were reviewed at this RAC meeting. This brings the total number of protocols submitted to OBA to 409 GTR protocols. The breakdown of the 409 is as follows:

370	gene transfer
37	cell marking
2	normal volunteers
249	cancer
50	monogenic diseases (e.g., cystic fibrosis)

38 other diseases (e.g., coronary artery disease and peripheral artery disease)
33 infectious diseases (e.g., HIV)

Amendments and Updates and Adverse Events

Since June 1, 2000, 70 amendments and updates have been submitted to the OBA, almost all of which included changes in eligibility criteria, new study sites, increased participant enrollment, or protocol changes requested by the FDA. A total of 617 AEs were reported during the period June 1 to September 1, 2000. Of those 617, 15 percent were unexpected and considered possibly associated with the study agent. Dr. Greenblatt noted many events occurred months or, in some instances, years before the reporting date, indicating that many investigators are still in the process of submitting overdue reports in order to fulfill reporting requirements.

Dr. Greenblatt expressed concern about being the sole RAC reviewer of the AE reports and support for the establishment of an assessment committee that would have the time and expertise to conduct a thorough evaluation. He also recommended the use of a standard reporting format.

RAC Discussion

A discussion ensued regarding new NIH policies for enhanced clinical trial monitoring. The new policy requires grantees to outline a plan for data monitoring in all phases of clinical research. The policy requires improved data monitoring but DSMBs are not mandated for early-phase clinical trials.
Dr. Markert questioned whether gene transfer studies would be required to establish a DSMB because the policy seems to require DSMBs for studies of vulnerable populations or high risk or novel research.
Dr. Patterson responded that the NIH is requiring clinical trial monitoring plans to be in place for Phase I and Phase II trials; Phase III studies are already required to form DSMBs. NIH ICs have the discretion to institute additional requirements for their own grantees. Dr. Skarlatos described NHLBI's requirements.

Drs. Aguilar-Cordova and Breakefield suggested that at a future meeting the RAC discuss the merits of a more consistent approach across all the NIH components and consider whether to provide advice to the NIH Director in this regard.

Public Comment

Dr. Shirley Clift, Cell Genesys, commented on the public's perception of the RAC's authority and influence and the importance of regulatory clarity.

VIII. Day One Closing Remarks/Dr. Mickelson

Dr. Mickelson thanked the participants and adjourned the first day of the September 2000 RAC meeting at 4:05 p.m. on September 25, 2000.

IX. Day Two Opening Remarks/Dr. Mickelson

Dr. Mickelson opened the second day of the September 2000 RAC meeting at 9:00 a.m. on September 26, 2000.

X. **Scope and Applicability of the *NIH Guidelines*/Dr. Mickelson**

Dr. Mickelson led a brief discussion of the scope of the *NIH Guidelines*. The definitions related to recombinant DNA and gene transfer have not changed since 1990, when Appendix M was added. Given

rapid developments in recombinant technologies, a group was formed a year ago to examine the scope of the *NIH Guidelines* and to explore whether new areas of research should be added.

Dr. Gordon suggested that one of the goals of the effort should be to make the language of the Guidelines more general so that it does not become outdated as rapidly. For example, the reference to "sequence-specific oligonucleotides" should be changed to "oligonucleotides." Dr. Aguilar-Cordova cautioned that the language should not be so general that it would encompass unintended areas.

Dr. Mickelson stated that the current *NIH Guidelines* is essentially silent on many of the newest technologies in both plant and animal genome modification. For instance, perhaps nuclear transfer techniques in animal research should be included. Dr. Mickelson indicated that human cloning research would not be included because policy in this area is determined in other venues at NIH.

Dr. Cohen suggested that general definitions should be included in the *NIH Guidelines*, with reference to a list, separately published, that could be changed when needed. This method is used by the Office for Human Research Protections (OHRP) and by the FDA. Dr. Mickelson agreed that this method would be useful.

Ms. King suggested that the language in Appendix M regarding "patient" and "treatment" should be changed to, for example, "research subject" and "gene transfer." Dr. Patterson agreed systematic changes emphasizing the experimental nature of gene transfer research would be beneficial.

Dr. Mickelson requested that the working group continue to explore the scope issues and, if possible, be prepared at the next RAC meeting to make recommendations about how the *NIH Guidelines* should be revised. The group's specific objective is to develop a proposed action for discussion at its December 2000 meeting. Dr. Mickelson requested volunteers for the working group. Drs. Aguilar-Cordova, Ando, Friedmann, and Greenblatt and Ms. King volunteered to serve on this working group. Dr. Mickelson agreed to continue as chair of the group. She indicated that the group will look at several models, take RAC comments into account, and request review from the other RAC members via e-mail before the next RAC meeting.

XI. Discussion of Human Gene Transfer Protocol #0001-372: *A Phase IA, Single-Dose, Dose-Escalation Study of MiniAdFVIII Vector in Patients With Severe Hemophilia A*

Principal Investigators:	Gilbert White II, M.D., University of North Carolina, Chapel Hill Arthur R. Thompson, M.D., Ph.D., University of Washington, Seattle
Sponsors:	GenStar Therapeutics (represented by Wei-Wei Zhang, M.D., Ph.D.) and UroGen Corporation
RAC Reviewers:	Drs. Breakefield, Friedmann, Juengst, and Mickelson
Ad Hoc Reviewers:	Pedro R. Lowenstein, M.D., Ph.D., University of Manchester, United Kingdom Richard O. Snyder, Ph.D., Harvard Medical School

Protocol Summary

The primary purpose of this clinical investigation is to assess the safety of a novel adenoviral ("minimal" or "gutless") vector termed "miniAdFVIII." The secondary purpose is to determine whether gene transfer

can be used to produce circulating, functional levels of FVIII, a protein that aids in blood clotting and is deficient in patients with severe hemophilia A. Patients who have hemophilia A are currently treated with clotting factors, but most patients receive treatment only for bleeding events and not to prevent bleeding.

Preclinical studies performed at several independent laboratories indicate that gutless adenoviral vectors have improved safety and efficacy profiles compared with earlier generation adenoviral vectors. In the miniAdFVIII, most of the viral DNA has been removed and replaced with the FVIII gene, a normal gene designed to assist the liver in making FVIII. The miniAdFVIII is designed so that it does not multiply and disseminate to the rest of the body. This approach has been tested previously in laboratory animals, suggesting that administration of miniAdFVIII could produce circulating, functional levels of FVIII in the blood. The study will help determine whether miniAdFVIII can transfer the FVIII gene to produce the FVIII clotting factor in patients with severe hemophilia A.

Written Comments from Preliminary Review

This protocol was selected for review by four RAC members because it represents the first clinical application of the helper-dependent adenoviral vector system in an *in vivo* gene transfer study and because of unresolved safety and scientific issues. Ms. King reported that she has abstained from participating in the review of the protocol because she is affiliated with the clinical site, the University of North Carolina. Drs. Breakefield, Friedmann, Juengst, and Mickelson submitted written reviews, as did *ad hoc* reviewers Drs. Lowenstein and Snyder, to which the investigators responded in writing.

Dr. Breakefield's written review centered on the toxicity that would result from administering large doses intravenously. She requested further information about the level and nature of the helper virus present in the vector stocks. Other specific issues in Dr. Breakefield's comments included quantification of vector stocks, vector dosing, participants' liver status, presence of several antibody and inhibitor levels in participants prior to vector administration, and genotyping of all participants.

Dr. Friedmann's comments discussed characterization of the starting material, *in vivo* tissue distribution and duration of transgene expression, host immune response, and the use of this class of vector for treatment of a chronic disorder.

Dr. Juengst's comments centered on the need for more animal data on the biodistribution of the vector and the extent of the vector's ability to target the liver exclusively. Dr. Juengst asked whether the canine biodistribution data were relevant to this trial and whether nonhuman primates should be tested for transgene expression outside the liver. Dr. Juengst also questioned the comprehensiveness of the informed consent in both the document and in discussions that occur during the consent process.

Dr. Mickelson's comments centered on the expectations of vector targeting and distribution in humans, use of a transient vector that would require repeat administrations in a patient population with a chronic genetic disease, potential vector immunogenicity and toxicity and triggering of anti-Factor VIII (FVIII) antibodies in recipients, and the potential effect of preexisting adenoviral antibodies on possible efficacy.

Dr. Lowenstein's comments discussed titration of viral vector and ancillary vector, vector genomic structure, transcriptional control, immunogenicity, vector toxicity and dose, and injection of virus into the liver.

Dr. Snyder's written review included questions about empty virions contaminating the miniAdFVIII preparation, the production cell line, determination of the miniAdFVIII titer, risks associated in expression of FVIII from sites other than the liver, and establishment of baseline levels of FVIII protein

and activity prior to vector administration. In regard to the possibility that research participants develop inhibitors, or antibodies against FVIII, Dr. Snyder questioned whether overexpression or expression from tissues other than liver would result in generation of inhibitors due to problems in FVIII protein processing and, if this occurred, who would pay the cost of treating affected participants.

RAC Discussion

Drs. White, Thompson, and Zhang presented a summary of the protocol, the primary reviewers summarized their comments, RAC members posed additional questions, and the investigators responded to the comments and questions.

Dr. Friedmann thanked the investigators for responding in writing and in their presentation to many of his initial questions. He said that he remained concerned about the use of a less precise assay that would be incapable of adequately characterizing the starting material both chemically and in terms of purity. Dr. Friedmann asked to see some evidence of physical targeting—where the particles are going, what cells in other tissues are transduced, and the duration of expression in other tissues.

Dr. Breakefield discussed the possibility that participants would generate FVIII antibody inhibitors. She supported the investigators' approach to targeting the liver by using a liver-specific promoter that seems capable of decreasing immunogenicity, but suggested that further testing to confirm tissue specificity was needed. She asked about coverage of the treatment costs for participants who develop inhibitors. Dr. Breakefield also expressed concern that the titer method be capable of detecting empty particles since they can contribute to toxicity and interfere with therapeutic efficacy.

In his absence, Dr. Mickelson summarized Dr. Juengst comments. Dr. Juengst raised questions about the relevance of the canine biodistribution data to the different viral constructs proposed, the value of conducting additional nonhuman primate research to test for the transgene expression outside the liver and its potential effects, the addition of a short statement in the consent document about the relevance of hepatitis C and HIV testing to the protocol, and inconsistencies in the two consent documents with regard to the use of term "gene therapy."

Dr. Mickelson stated that while the protocol is a potentially valuable development in gene transfer vector technology, several key points had not been addressed by the preclinical studies. Because of the vector's inherent immunogenicity, an immune response might be triggered in the participants. She noted that the inclusion of a strong albumen promoter might offset the lack of a targeting benefit in the vector. She also suggested that it would be important to find out whether antibodies arise when the appropriate transgene is used in the appropriate species (e.g., murine FVIII used in a mouse) and when there are repeat administrations.

Dr. Snyder raised additional questions about the animal model (hemophilic dogs were transduced with the proposed vector) and the extent of the researchers' experience with the model and how it would compare to a nonhuman primate model.

Dr. Lowenstein stated that the development of inhibitor antibodies of FVIII needs to be analyzed more carefully. He echoed Dr. Mickelson's concern that the albumen promoter's ability to reduce the immunogenicity be tested, and he agreed with Dr. Breakefield's request that the virus be tested in human hepatocytes. Dr. Lowenstein stated that readministration should be a major positive aspect for the use of gutless vectors and that the protocol is important in exploring that possibility.

Continued Discussion and Investigator Response

Dr. Mickelson asked why the platelet counts dropped at the highest doses of miniAd vector. Dr. White responded that the drop is likely a result of the adenovirus, that the drop is short-lived, and that there appears to be a brisk bone marrow response to it.

Dr. Lowenstein stated that the protocol is important because it is the first application of the gutless adenoviral vectors in a clinical trial and has enormous potential in the development of gene therapy. He also commented that these vectors have a number of advantages, some of which have not been explored by the researchers. For example, the literature indicates that these vectors are much more persistent even in preimmunized animals. Dr. Lowenstein suggested that the researchers should use real-time polymerase chain reaction (PCR) to detect the amount of vector genome in the vector preparation, ancillary (helper) vector, and any replication-competent genome. He also suggested that the researchers provide the exact vector particle number using electron microscopy counting. Dr. White agreed to use PCR and to determine total viral particles so that a direct comparison can be made between ancillary adenovirus and total viral particles.

Dr. Friedmann expressed concern about the potential for overexpression of FVIII and its consequences, biodistribution, and immune responses at other sites. Dr. Thompson responded that while some recent epidemiologic data indicate that elevations of FVIII (twice the normal level) can slightly increase the risk of thrombosis, FVIII levels more than tenfold above normal can be tolerated without acute adverse events.

In response to Dr. Friedmann's question about how the investigators will monitor the participants' responses to the vector and to the transgene, Dr. Zhang stated that antibody development will be monitored weekly.

Dr. Markert suggested that the investigators look at the proliferative response of some of the participants after gene transfer to determine whether the route of administration might be causing a suppression of T-cell proliferation by the product.

In response to Dr. Breakefield's concern regarding reimbursement of the treatment costs for participants who develop inhibitors, Dr. Sobol, GenStar Therapeutics, stated that the sponsor will pay for the cost of any injury to the participants, including development of inhibitors.

In response to several concerns about the possible effect of inflammation with FVIII vector administration, Dr. White responded that it will be monitored closely as part of the protocol. The inflammation produced by the vector is considerably less than that produced by other generations of adenoviral vectors.

Dr. Zhang agreed that the proposed vector could be tried on primary cultures of human hepatocytes, as suggested by Dr. Lowenstein. Dr. Lowenstein commented that this testing could determine whether the promoters work the same way in gutless vectors as they do in replication-competent adenoviral vectors or first-generation vectors.

Ms. Levi-Pearl requested that the OBA let the RAC know when the protocol is initiated and how it progresses.

Dr. Ando suggested that, for vectors like this one with potential liver toxicity, the investigators should review data on interleukin-6 (IL-6) in preclinical models and in humans, since IL-6 is an acute-phase reactant and a harbinger of liver injury. It would be useful to accumulate data from preclinical models

and human trials to determine whether there is any predictive value in dose escalation for safety. Dr. White indicated that the investigators would be sure to include measurements of prothrombin time, partial thromboplastin time, and IL-6 in the clinical protocol.

Dr. Breakefield urged the investigators to characterize the vector stocks more thoroughly before they are used clinically and to conduct animal model tests to determine whether the promoter does reduce the risk of inhibitor development. Dr. Friedmann agreed that these were important concerns and added that he would like to see a more thorough biodistribution expression study performed.

Dr. Ann Pilaro, FDA, stated that although the agency had concerns about the potential for the development of inhibitors, she did not believe that further animal studies of inhibitor development using species specific FVIII (i.e., canine FVIII in canines), which could take several years to develop and conduct, would provide important new information and that FDA would not require further animal studies prior to authorizing the trial to proceed. To address the safety concern regarding inhibitor development, however, the protocol includes a stopping rule in the event that one participant develops inhibitors.

Public Comments

Two individuals from the public offered comments on this protocol. Mr. Patrick Collins, National Hemophilia Foundation (NHF), stated that the NHF has been meeting with GenStar Therapeutics to assist in enhancing the consent document and other elements of the protocol. Roland Herzog, Children's Hospital of Philadelphia, reported that researchers at GTI/Novartis have prepared a paper (in press) on a study of adenoviral vector expressing canine FVIII from the albumen promoter. The results show that when introduced to hemophilia A dogs, inhibitors developed against canine FVIII.

RAC Recommendations

Dr. Mickelson summarized the following issues to be addressed in the RAC's letter:

- Gene transfer and potential toxicity may be affected by the presence of ancillary vector, replication competent virus, and defective vector (empty virus-like particles) in the mini-Ad vector preparations. Therefore, quantitative and qualitative assays should be developed for titration of vector, ancillary vector, defective vector, and recombinant virus as in the following suggestions:
- real time PCR, viral particle quantification by electron microscopy, and/or ELISA for virion particles
- The titers of vector and possible contaminants should be normalized by using the same techniques; and the development of a measure of mini-Ad vector titer by using cells infected with saturating levels of ancillary adenovirus should be considered.

- The biodistribution of the vector and transgene expression should be evaluated in appropriate animal models to address concerns about potential for tissue damage and the effect of transgene expression in tissues other than the liver. The liver specificity of the human albumin promoter should be confirmed by comparing expression in primary human hepatocytes to other human primary cell types in vitro as well as in animal studies in vivo.

- Since it may contribute to the induction of inhibitor, the levels of Factor VIII protein contamination in the vector preparations should be determined by ELISA.

- NIH OBA should be informed of any adverse effects related to the development of inhibitor to FVIII in research participants receiving miniAdFVIII vector.

- A statement should be included in the informed consent document describing the need to first evaluate the safety profile of the miniAdFVIII vector in participants who do not have other medical conditions including infections by human immunodeficiency virus (HIV) and hepatitis C virus (HCV). The University of Washington informed consent document should be modified to refer to 'gene transfer" rather than "gene therapy/treatment." The University of North Carolina informed consent document's explanation of gene transfer should be clarified along the following lines: *Gene transfer uses DNA to produce proteins in the body that may have therapeutic benefit. These gene transfer methods are experimental.*

Discussion ensued about whether the RAC should specify that recommendations should be incorporated before the clinical trial moves forward. Some RAC members were satisfied that the recommendations could be implemented simultaneously with starting the trial.

XII. Discussion of Human Gene Transfer Protocol #0006-404: *A Multicenter, Double-Blind, Placebo-Controlled, Phase II Study of Aerosolized AAV-CF in Cystic Fibrosis (CF) Patients With Mild Lung Disease*

Principal Investigators:	Richard B. Moss, M.D., Stanford University School of Medicine Bonnie Ramsey, M.D., University of Washington, Seattle
Sponsor:	Targeted Genetics Corporation (represented by Thomas C. Reynolds, M.D., Ph.D.)
RAC Reviewers:	Drs. Aguilar-Cordova and Breakefield and Ms. King
Ad Hoc Reviewer:	Dr. Snyder

Protocol Summary

CF, the most common inherited disease in North America, is caused by the mutation of a gene known as cystic fibrosis transmembrane conductance regulator (CFTR). Normal functioning of this gene is required for the movement of water and salt across airway cells. CF causes abnormal mucus build-up in the lungs, which leads to serious lung disease.

Gene transfer attempts to replace the missing CFTR gene. Clinical studies so far have involved vectors with transient effects, which mean that they may not be ideal candidates for the development of a treatment. Targeted Genetics Corporation has developed a different type of vector, called tgAAV-CF, which is based on an adeno-associated virus (AAV). Many people have been infected by naturally occurring AAV which does not cause disease. AAV is able to maintain its DNA for long periods in the cells that it enters. Tests of AAV vectors carrying the CFTR gene have shown it to be biologically active in cells *in vitro* and in animals. Sixty previous study participants have received the vector without serious side effects.

Complications of CF include ongoing lung infection, inflammation, and destruction. This study proposes to administer three doses of tgAAV-CF in repeat administrations to the lungs of participants with CF. Measurements will be taken to determine whether the vector is present and active in the lung.

Participants older than 12 years will receive either active drug or placebo via inhalation. Bronchoscopies will be performed on only the first two cohorts to measure vector activity in the lung; age restriction on these two cohorts is 15 years of age or older. Results from this trial will be used to design end points for future studies.

Written Comments from Preliminary Review

This protocol was selected for review by four RAC members because of issues related to the protocol design, including the age of participants at enrollment, inclusion of a control group, and questions about the protocol's claims of potential benefit from using AAV to deliver genes to appropriate lung cells.

Dr. Aguilar-Cordova's comments discussed the use of a placebo control, the questionable expectation of benefit when no expression was detected in previous study participants, potential adverse effects of the use of bronchoscopy, lack of data on expression *in vivo* and neutralizing antibody responses, and the need for a clearer statement in the informed consent document regarding participants' odds of receiving placebo and that all participants are to undergo bronchoscopy.

Dr. Breakefield's comments focused on the unknown risk of multiple doses of AAV vectors to the lungs of CF patients and the possibility that the risk may be greater to younger participants because it may accelerate the course of the disease. Dr. Breakefield also raised questions about the comprehensibility of the informed consent document to participants 12 to 17 years of age. She requested information about whether AAV delivered via nonpulmonary routes causes inflammation and clarification regarding various specifics of the nonhuman primate study.

Ms. King's comments focused on the potential for direct benefit and how that potential is worded in the consent form as well as the enrollment of minors, especially 12- to 14-year-olds.
Dr. Snyder's comments dealt with the timing of dose administration related to a potential humoral response; whether the vector will be distributed to the gut and, if so, whether side effects are anticipated if transduction of the gut cells occurs; requiring participants to suspend antibiotic use before baseline screening; whether the proposed dose (at only three times higher than the Phase I trial) could result in improved lung function; and the parameters that would constitute success and justify progressing to Phase III.

RAC Discussion

Drs. Moss, Ramsey, and Reynolds presented a summary of the protocol, the primary reviewers summarized their comments, RAC members posed additional questions, and the investigators responded to the comments and questions.

Dr. Breakefield noted that this is the first time multiple dosing will be used with this vector, which has the potential to increase inflammation to the lung. She suggested that there is no need to include younger patients to establish whether inflammation is increased. She suggested confining at least the first trial to subjects 18-years or older to establish whether multiple dosing increases inflammation, especially since only normal animals have been administered multiple doses. CF patients already have inflammation and therefore could be more susceptible to developing a toxic response. In terms of potential benefit, Dr. Breakefield stated that in the first trial involving 60 participants, there was no indication of benefit and no evidence of gene expression in the lungs.

Ms. King echoed Dr. Breakefield's concerns, stating that the RAC's question is *when*, not *if*, it is appropriate to move into a pediatric population. Ms. King stated that the potential benefits were

overstated in the consent document. She commended the investigators for using consent monitors in the protocol, because they will be helpful to this trial and because consent monitoring will need to be put in place for many future trials.

Dr. Snyder thanked the investigators for their thoughtful responses to his questions and then posed several additional questions. He requested comment on the discrepancy between conflicting sets of data regarding the observation of neutralizing antibodies and their interference with repeated administration of the vector. Dr. Snyder asked the investigators to comment on the need for increasing gene expression from the vector and how that relates to increasing the dose to achieve an efficacious end point.

Dr. Aguilar-Cordova questioned the use of a placebo control because of its low statistical power, the use of bronchoscopy for placebo controls who have no possibility of benefit, and the expectation of potential benefit. Dr. Aguilar-Cordova stated that the informed consent document should contain a clear statement telling participants their odds of being in the placebo group.

Investigator Response

In response to Dr. Snyder's concerns, Dr. Reynolds explained that this study did look at neutralizing antibody responses. In participants receiving vector at the highest dose ($1x10^{13}$), none manifested neutralizing antibodies in the bronchial wash fluid. With regard to measuring efficacious end points, Dr. Reynolds indicated that in the previous studies expression was not detected in the lung possibly because bronchoscopy reaches down only to the fourth-generation bronchi and not to the deep lung and branched airways to detect gene expression there.

With respect to the placebo group, Dr. Reynolds explained that the first eight (six active and two placebo) participants will undergo a bronchoscopy at the end of the study; those participants would be age 15 and older. Having placebo participants will assist, among other things, in dealing with the fact that most of the CF trials have shown a considerable false-positive rate in molecular measures whether by AAV, adenoviral, or liposomal delivery. The control group, although quite small (N=2 in this first arm of the trial), will allow verification of efficacious results, if they occur, in the six active study participants. Dr. Ramsey explained that the investigators hope that induced sputum will replace bronchoscopy as the measure of what is occurring in the central airway; however, so far they have been unable to validate induced sputum as an outcome measure; therefore, bronchoscopy is the only way to sample the central airway directly.

In response to several RAC members' questions about using participants who are age 18 and older for the first multidosing exposure to check for inflammatory response, Dr. Reynolds responded that developing a drug for CF must be done safely but also expeditiously. The placebo participants represent the investigators' response to the need to add appropriate statistical power to the trial to examine efficacy. Dr. Reynolds explained that people with CF who survive to adulthood are not the same as children with the disease and that demonstrating efficacy is more difficult in adults; investigators are maximizing the chance to see therapeutic benefit by proposing to involve younger participants. He indicated that the investigators believed the RAC would be comfortable with including participants as young as 15 in the first group because of approval of a prior trial in which participants the same age were involved. In addition, the investigators had received approval from the local IRBs and the FDA and are working with the Cystic Fibrosis Foundation, which routinely deals with CF patients in trials. In her presentation, Dr. Ramsey discussed the need to move CF trials into the pediatric population. CF is a progressive disease with the greatest decline in lung function occurring in the pediatric population. By adulthood, fixed structural defects have developed that are irreversible even if gene transfer of CFTR is successful. Also other CF studies have shown that younger patients have an increased response to therapeutics.

Since this is a disease that strikes children, Dr. Cohen expressed his belief that including participants as young as 15 in this clinical trial is within ethical bounds; children with CF are relatively sophisticated patients because they have been spent much time in the medical system. He reminded the investigators that participating as a research subject in this trial may constitute a health risk that is above minimal levels, in large part because of the use of bronchoscopy to measure vector activity. The current consent form should be rewritten in simpler language; children and parents need to know that participants may receive the placebo.

Dr. Gordon requested that the investigators provide to him via e-mail an explanation of the rationale for applying gene transfer to CF, given the large surface area of the lungs that must be treated.

Dr. Snyder followed up Dr. Reynolds' statement that the third dose in nonhuman primates produced a low level of neutralizing antibodies by clarifying that the third AAV dose the subjects receive is their fourth exposure to AAV (the initial, pretrial infection plus three doses during the trial). As a result, it is possible that the level of neutralizing antibodies may be of concern.

Public Comments

Cynthia Dunafon, a University of Chicago CF patient, participated in summer 1999 in a Johns Hopkins Phase I trial using AAV placed in her nose and lungs. She described the research and the dynamic process of consent, reiterating that consent is an active process with the informed consent document as the centerpiece.

RAC Recommendations

- Given the unknown and potential risks associated with multiple dosing of AAV vectors to the lungs of patients with cystic fibrosis (CF), and the possibility that these risks may be greater to younger, as compared to older, patients, enrollment of participants under 18 years of age may not be appropriate because the vector may accelerate the progression of their disease. Therefore, the inclusion of research participants less than 18 years of age should be re-evaluated.

- If the enrollment of participants under the age of 18 is proceeded with, the assent form of the informed consent document should be revised to ensure its comprehensibility to adolescents (age 15-18).

- In the event that patients under age 15 are enrolled, please provide to OBA a blinded summary of the safety data from the participants age 15 and above. In addition the assent form may need further revision to ensure its comprehensibility for participants age 12-15.

- The use of a placebo-control arm should be reconsidered. Although the investigator and sponsor agreed to increase the number of research participants in the control arm to enhance statistical power of the study (from 18 participants in active drug arm and 6 in control arm to 18 participants in both active and control arms), the RAC remains concerned about the scientific rationale for and the ethical issues associated with the use of a placebo-control arm, especially in view of the risk-benefit factor to CF patients undergoing bronchoscopies. At least, consideration should be given to delaying its use until after the first eight study participants, who are to undergo bronchoscopies, have been treated.

Dr. Mickelson stated that, as with all RAC recommendations, RAC members will be asked to review the draft language before the recommendations are finalized and sent to the investigators and sponsors.

XIII. An Introduction to the Proposed Use of Simian Virus-40 (SV-40)-Based Vectors in Humans/Dr. David S. Strayer, Thomas Jefferson University

Dr. Strayer introduced the RAC to the system of gene delivery vectors based on simian virus-40 (SV-40). Dr. Strayer offered a preliminary exposition of a clinical application of these vectors to human gene delivery, with a formal proposal to be made at a later date. A group of investigators will propose to apply this system to bone marrow progenitor-cell gene delivery in patients undergoing bone marrow transplantation for AIDS-related lymphoma. The investigators will attempt to deliver as a transgene a single-chain Fv antibody directed against HIV type 1 integrase.

In the SV40 derived vector, both the T antigen genes and all the capsid genes are deleted. These functions are supplied in *trans* by packaging in COS-7 cells. Transgenes of up to 5 kb can be inserted into the vector. The vector can transduce most cell types because the SV40 receptor is believed to be MHC class I which is present on almost all nucleated cells. Transduction levels are increased by multiple exposures to the vector. The vector integrates in a random manner not only in respect to the cellular genome, but also the vector which could result in disruption of the transgene.

In vivo studies in mice have shown up to 95% transduction of liver with transgene expression detected out to 14 months. After 7 or 8 inoculations of vector, no neutralizing antibodies against SV40 were detected. Studies of *ex vivo* transduction of $CD34^{+}$ cells with the anti-integrase vector done in SCID mice and baboons showed transgene expression and no evidence of hematologic or chemical toxicity. Although all of the parameters for the clinical study are not fully in place and plans for vector production are not complete, these aspects of the project were outlined briefly. Dr. Strayer also outlined the investigators' ongoing concerns and the approaches being applied to resolve these issues.

In addition to introducing this system to the RAC, Dr. Strayer sought input on behalf of the other investigators regarding scientific questions about the application of the recombinant SV-40 gene delivery system in humans and safety considerations that should be addressed.

RAC Questions and Comments

Dr. Ando asked for an explanation of the manufacturing process, which Dr. Strayer described. The SV-40 genome is cloned into a plasmid, and the viral genome is excised from the plasmid, purified, recircularized, and then transfected into Cercopithecus monkey (COS) cells. SV-40 produces a lytic infection in these cells. After 3 or 4 days, researchers lyse the COS cells and then use that lysate to infect COS cells to amplify the virus. There are three or four passages between the transfection stage and the recovery of approximately $1x10^{13}$ to $1x10^{14}$ infectious units of virus. For laboratory use, a stock of virus is established and then amplified when needed. For human studies, the virus is generated from plasmid each time it is needed to minimize the possibility of contamination or impurities in the production process. Dr. Aguilar-Cordova suggested that making a master viral vector stock might be preferable to retransfecting every time since this procedure has worked effectively for the similar manufacturing of adenovirus.

In response to a question from Dr. Mickelson about the detection of replication-competent contaminants using plaque assay, Dr. Strayer indicated that researchers believe that it would be more efficacious to look for reacquisition of T-antigen, which would have to be present for the virus to be replication competent.

Dr. Snyder asked about the possible side effects of potential random breakage of the vector. Dr. Strayer responded by identifying three possible side effects: 1) Some integrations would interrupt expression of the transgene; 2) random integration into a critical gene disrupting cell function; 3) integration causing

activation of an undesired gene. Based on laboratory studies conducted thus far, there is no reason to expect that SV-40 integration will have results that are any different from those experienced with retroviral vectors.

Dr. Aguilar-Cordova queried whether basic distribution studies had been conducted in animals using various routes of delivery and whether the potential for germ-line transduction had been examined. Dr. Strayer explained that the first study is proposed to be *ex vivo* gene therapy rather than *in vivo* delivery. By intravenous or intraperitoneal inoculation, virus distribution was detected in the rat liver, spleen, kidneys, skin at injection site, and brain, but detailed studies of germ-line transmission have not been conducted. *Ex vivo* transduction and reimplantation have been done in nonhuman primates, but direct inoculation into nonhuman primates has not been performed.

XIV. Chair's Closing Remarks/Dr. Mickelson

Dr. Mickelson outlined a number of issues that will be discussed in further detail at the December RAC meeting: the final timing action; the proposed action on serious adverse event reporting and the data assessment board; and further development of modifications to the scope of the *NIH Guidelines*. The Safety Symposium on Cardiovascular Gene Transfer Research will be held during the December meeting.

XV. Adjournment/Dr. Mickelson

Dr. Mickelson adjourned the meeting at 4:10 p.m. on September 26, 2000.

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

_____
Amy P. Patterson, M.D.
Executive Secretary

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

Date:

_____
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Chair

Attachment I
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Attachment II

Attendees

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Valder Arruda, Children's Hospital of Philadelphia
Ann Besignano, Capital Consulting Corporation
Mike Bond, Capital Consulting Corporation
Quinn Bryan, student (Eli Lilly)
Jeffrey W. Carey, GenVec
Joy A. Cavagnaro, Access Bio
Yung-Nien Chang, Virxsys
Jan Chappell, DirectGene
Margaret Charette, Genzyme
Janet Rose Christensen, Targeted Genetics
Shirley Clift, Cell Genesys
Laura R. Coleman, Eli Lilly
Patrick Collins, National Hemophilia Foundation
John Cutt, Novartis
Cynthia Dunafon, cystic fibrosis patient
Thomas Eggerman, Food and Drug Administration
Laura Emig, Genzyme
Celia Farber, *Talk Magazine*
Frederick A. Fletcher, Amgen
Jim Foss, Stellar Systems
Jeffrey L. Fox, science writer, editor
Jeffrey Friedman, Collateral Therapeutics
Paul Gelsinger, citizen
Ishtaq Ghafour, *The Blue Sheet*
Robyn Goldman, Capital Consulting Corporation
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J. Fraser Wright, Avigen
Wei-Wei Zhang, GenStar Therapeutics
Kathryn Zoon, Food and Drug Administration

Attachment III

Abbreviations and Acronyms

AAV	adeno-associated virus
ACD	Advisory Committee to the Director
Ad2	type 2 adenovirus
AE	adverse event
CABG	coronary artery bypass grafting
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CMV	cytomegalovirus
COS	Cercopithecus monkey
CV	cardiovascular
DSMB	data safety monitoring board
EPO	erythropoietin
ESRD	end-stage renal disease
FDA	U.S. Food and Drug Administration
FVIII	Factor VIII
GCP	good clinical practices
GMP	good manufacturing practices
GTR	gene transfer research
HIF	hypoxia-inducible factor
HIV	human immunodeficiency virus
ICs	Institutes and Centers (of the NIH)
IL-6	interleukin-6
IND	investigational new drug
iNOS	inducible nitric oxide synthase
IRB	institutional review board
IV	intravenous
NGVL	National Gene Vector Lab
NHF	National Hemophilia Foundation
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities (formerly ORDA, Office of Recombinant DNA Activities)
OHRP	Office of Human Research Protection (formerly OPRR)
PCR	polymerase chain reaction
PTFE	polytetrafluoroethylene
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
SAE	serious adverse event
SV-40	simian virus-40
VEGF	vascular endothelial growth factor