DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
June 18-19, 1998

TABLE OF CONTENTS

XV. **Future Meeting Dates/Mickelson**

XVI. **Adjournment/Mickelson**

Acronyms for the June Minutes

The Recombinant DNA Advisory Committee (RAC) was convened for its seventy-first meeting at 9:00 a.m. on June 18, 1998, at the National Institutes of Health (NIH), Building 31, Conference Room 10, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Claudia Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on June 18 from 9:00 a.m. until 5:30 p.m., and June 19 from 8:30 until 4:00 p.m. The following were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Childrens Hospital
Dale G. Ando, Cell Genesys, Inc.
Jon W. Gordon, Mt. Sinai School of Medicine
Michael M.C. Lai, University of Southern California
M. Therese Lysaught, University of Dayton
Ruth Macklin, Albert Einstein College of Medicine
M. Louise Markert, Duke University Medical Center
Claudia A. Mickelson, Massachusetts Institute of Technology
Jon A. Wolff, University of Wisconsin Medical School

Executive Secretary:

Debra W. Knorr, National Institutes of Health
A committee roster is attached (Attachment I).

Non-Voting Representatives:

Daniel Jones, National Endowment for the Humanities
Melody Lin, Office of Protection from Research Risks
Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Lisa Carlton, NCI
Sarah Carr, OD
Greg Evans, NHGRI
Joseph Gallelli , OD
Christine Ireland, OD
Becky Lawson, OD
Rebecca Link, NHLBI
Mikel Miller, OD
Richard Morgan, NHGRI
Constance Noguchi, NIDDK
Brian O'Connell, NIDR

Gene Rosenthal, OD
Aiman Shalabi, NCI
Thomas Shih, OD
Minoru Tomizawa, NCI

Others:

Paul Aebersold, Food and Drug Administration
Jeff Akita, Genzyme Corporation
Victoria Allgood, GeneMedicine, Inc.
W. French Anderson, University of Southern California
Julie Andrews, Genetic Therapy, Inc.
Michael Ausborn, Genetic Therapy, Inc.
Kameron Balzer, Genentech, Inc.
Bridget Binko, Cell Genesys, Inc.
Amy Bosch, Targeted Genetics Corporation
Nell Boyce, New Scientist Magazine
Peter Burke, Deaconess Hospital
Jeff Carey, Genetic Therapy, Inc.
Francisco Castillo, Berlex Laboratories, Inc.
Sheila Connelly, Genetic Therapy, Inc.
Kenneth Culver, Codon Pharmaceuticals, Inc.
Lynn DellaPietra, University of Pennsylvania
Theodore Deweese, Johns Hopkins Institute
Julie Dorr, Newport School
Robert Engler, Collateral Therapeutics
Lara Frashure, Genetic Therapy, Inc.
Bruce Furie, Beth Israel Deaconess Medical Center
Angela Gallo, Genetic Therapy, Inc.
Donald Gay, Chiron Corporation
Debra Gessner, Collateral Therapeutics
Tina Grasso, GenVec, Inc.
John Grous, Calydon
Dan Henderson, Calydon
Joann Horowitz, Schering-Plough Research Institute
Tanya Houle, Genzyme Corporation
Dorothy Jessop, Public
Michael Kaleko, Genetic Therapy, Inc.
Steven Kradjian, Vical, Inc.
Toshi Kotani, Genetic Therapy, Inc.
Alexander Kuta, Genzyme Corporation
LaVonne Lang, Parke-Davis Pharmaceutical Research
Peter Larson, University of Pennsylvania
Denny Liggitt, University of Washington
Douglas Losordo, St. Elizabeth's Medical Center
Peter Loudon, Cantab Pharmaceuticals Research, Ltd.
Russette Lyons, Genetic Therapy, Inc.
James Markert, University of Alabama
Pran Marrott, Berlex Laboratories, Inc.
Cardinali Massimo, Food and Drug Administration

James McArthur, Cell Genesys, Inc.
Alan McClelland, Genetic Therapy, Inc.
Andra Miller, Food and Drug Administration
Austine Moulton, Food and Drug Administration
Patricia Murphy, Berlex Laboratories, Inc.
Patricia Novak, Collateral Therapeutics
Joanne O'Brian, Beth Israel Deaconess Medical Center
Janice Olson, Genzyme Corporation
Sheryl Osborne, NeuroVir, Inc.
Albert Owens, Johns Hopkins Institute
Amy Patterson, Food and Drug Administration
Rodney Pearlman, Megabios Corporation
Anne Pilaro, Food and Drug Administration
Joseph Posluszny, Berlex Laboratories, Inc.
Andrew Quon, American Medical Association
Blake Roessler, University of Michigan
David Roth, Collateral Therapeutics
David Roth, Beth Israel Deaconess Medical Center
Gabor Rubanyi, Berlex Laboratories, Inc.
Richard Selden, Transkaryotic Therapies, Inc.
Mercedes Serabian, Food and Drug Administration
Tomiko Shimada, Ambiance Awareness International, Inc.
Stephanie Simek, Food and Drug Administration
Jonathan Simons, Johns Hopkins Institute
Theodore Smith, Genetic Therapy, Inc.
Jurg Sommer, Calydon
Lorna Speid, GeneMedicine, Inc.
Jean Starr, Family Support Center
Kenichi Tamiya, Food and Drug Administration
Melissa Tice, Schering-Plough Research Institute
Douglas Treco, Transkaryotic Therapies, Inc.
Frank Tufaro, NeuroVir, Inc.
Thomas Valere, Wiley Europe
Patrick Walsh, University of Colorado
Scott Wheelwright, Calydon
Lisa White, The Blue Sheet
Ruth Wikberg-Leonardi, Collateral Therapeutics

Call to Order and Opening Remarks/Dr. Mickelson

Dr. Claudia A. Mickelson, Chair of the Recombinant DNA Advisory Committee (RAC), called the meeting to order at 9:05 a.m. on June 18, 1998. Notices of the meeting according to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* were published in the *Federal Register* on May 4, 1998, (63 FR 24712) and on May 26, 1998, (63 FR 28514).

Dr. Mickelson noted that an action under the *NIH Guidelines* was promulgated in the *Federal Register* of May 11, 1998, (63 FR 26018). This action provides an optional electronic submission format for the registration of human gene transfer protocols with the Office of Recombinant DNA Activities (ORDA).

Minutes of the March 10, 1998, Meeting

Reviewers: Aguilar-Cordova, Mickelson

Committee Motion 1

The RAC approved a motion made by Dr. Aguilar-Cordova and seconded by Dr. Markert to accept the minutes of the March 10, 1998, RAC meeting (with the incorporation of minor editorial changes) by a vote of 8 in favor, 0 opposed, and no abstentions.

Data Management Update
Summary: Greenblatt (presented by Aguilar-Cordova)

Protocol Registration

To date, a total of 244 human gene transfer protocols have been registered with ORDA including 30 gene marking protocols, 212 gene transfer protocols, and 2 non-therapeutic protocols. Therapeutic protocols include 23 for infectious diseases (all HIV-1 infection), 33 for monogenic diseases, 147 for cancer, and 9 for other diseases (rheumatoid arthritis, coronary and peripheral artery disease, arterial restenosis, and cubital tunnel syndrome).

Dr. Greenblatt noted that the following protocols are to be reviewed at the June meeting: 9802-232, 9802-235, 9802-236, 9802-237, 9802-238, 9804-244, and 9804-247. Complete submission materials have not been received for 9802-233 and 9802-234.

Since the March 10, 1998, RAC meeting, the following nine protocols have been recommended for sole Food and Drug Administration (FDA) review: 9801-227, 9801-229, 9801-230, 9802-231, 9802-239, 9803-240, 9803-241, 9803-242, and 9804-243.

9801-227
Lotze, Michael T.; University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; *IL-12 Gene Therapy Using Direct Injection of Tumors with Genetically Engineered Autologous Fibroblasts (A Phase II Study)*
NIH/ORDA Receipt Date: 1-2-98. Sole FDA Review Recommended by NIH/ORDA: 2-18-98

9801-229
Kadmon, Dov; Baylor College of Medicine, Houston, Texas; *Neoadjuvant Pre-radical Prostatectomy Gene Therapy (HSV-tk Gene Transduction Followed by Ganciclovir) in Patients with Poor Prognostic Indicators*
NIH/ORDA Receipt Date: 1-16-98. Sole FDA Review Recommended by NIH/ORDA: 2-13-98

9801-230
Cowan, Morton J. and Conant, Marcus A.; University of California, San Francisco, San Francisco, California; *Evaluation of the Safety and Effects of Ex Vivo Modification and Re-infusion of CD34+ Cells by an Antisense Construct Against HIV-1 in a Retroviral Vector* Sponsor: Enzo Therapeutics, Inc.
NIH/ORDA Receipt Date: 1-20-98. Sole FDA Review Recommended by NIH/ORDA: 3-26-98

9802-231
Malech, Harry L.; National Institutes of Health, Bethesda, Maryland; *Gene Therapy Approach for Chronic Granulomatous Disease*
NIH/ORDA Receipt Date: 2-2-98. Sole FDA Review Recommended by NIH/ORDA: 2-20-98

9802-239

Bergsland, Emily K.; University of California, San Francisco, San Francisco, California; *A Phase I/II Study of Hepatic Infusion of Autologous CC49-Zeta Gene-Modified T Cells in Patients with Hepatic Metastasis from Colorectal Cancer* Sponsor: Cell Genesys, Inc.
NIH/ORDA Receipt Date: 2-25-98. Sole FDA Review Recommended by NIH/ORDA: 3-17-98

9803-240
Rom, William N.; New York University School of Medicine, New York, New York; and Woo, Savio L.C.; Mount Sinai School of Medicine, New York, New York; Phase *I Trial of Adenoviral Vector Delivery of the Herpes Simplex Thymidine Kinase Gene by Intratumoral Injection Followed by Intravenous Ganciclovir in Patients with Advanced Non-Small Cell Lung Cancer*
NIH/ORDA Receipt Date: 3-3-98. Sole FDA Review Recommended by NIH/ORDA: 3-23-98

9803-241
Bensinger, William I.;University of Washington School of Medicine, Seattle, Washington; Parker, Pablo M.; City of Hope National Medical Center, Duarte, California; Henslee-Downey, Peggy J. and Abhyankar, Sunil; Richland Memorial Hospital, University of South Carolina, Columbia, South Carolina; Giralt, Sergio; University of Texas, MD Anderson Cancer Center, Houston, Texas; and Cornetta, Kenneth; Indiana University-Purdue University, Indianapolis, Indiana; *A Phase I/II Outpatient, Multicenter, Intrapatient, Multiple Dose Escalation Study of Herpes Simplex Virus Thymidine Kinase (HSV-TK) Transduced Mononuclear Cells in Subjects with Persistent or Relapsed Chronic Myleogenous Leukemia, Chronic Lymphocytic Leukemia, Multiple Myeloma, and Non-Hodgkin's Lymphoma after HLA-Matched Sibling Allogeneic Stem Cell Transplant* Sponsor: Chiron Corporation
NIH/ORDA Receipt Date: 3-27-98. Sole FDA Review Recommended by NIH/ORDA: 4-17-98

9803-242
Kipps, Thomas J., University of California, San Diego, San Diego, California; *A Phase I Study of CD 154 Gene-Transduced Leukemia Cells in Patients with Chronic Lymphocytic Leukemia*
NIH/ORDA Receipt Date: 3-30-98.

9804-243
Crystal, Ronald G., Cornell University Medical College, New York, New York; *Phase I Study of Direct Administration of a replication Deficient Adenovirus vector (Ad_{GV}VEGF121.10) Containing the VEGF121 cDNA to the Ischemic Lower Limb of Individuals with Peripheral Vascular Disease* Sponsor: GenVec, Inc.
NIH/ORDA Receipt Date: 4-10-98. Sole FDA Review Recommended by NIH/ORDA: 4-30-98

Protocol Amendments

Dr. Greenblatt noted that 12 protocol amendments were submitted to ORDA since the March 1998 RAC meeting. Seven amendments involved the addition of new principal investigators, clinical sites, or both. The other amendments were:

Two amendments to the protocol entitled: *High Dose Carboplatin and Etoposide Followed by Transplantation with Peripheral Blood Stem Cells Transduced with the Multiple Drug Resistance Gene in the Treatment of Germ Cell Tumors* (Protocol #9701-172): (1) Modification of the transduction procedure to reduce manipulation of the cells; the cytokine cocktail now includes granulocyte colony stimulating factor (G-CSF), stem cell factor, and megakaryocyte growth and development factor; (2) clarification of the G-CSF dosage.

The protocol entitled: *A Phase II Multi Center Open Label, Randomized Study to Evaluate Effectiveness and Safety of Two Treatment Regimens of Ad5CMV-p53 Administered by Intratumoral Injections in 78*

Patients with Recurrent Squamous Cell Carcinoma of the Head and Neck (Protocol #9709-214) is amended to include patients with needle inaccessible tumor of the head and neck region on a case-by-case basis. Providing non treatment of these lesions is not expected to affect negatively on the patient's ability to complete the study. The investigators amended the protocol to address a concern that a subset of typical patients with head and neck cancer was being excluded or withdrawn prematurely from the study.

The protocol entitled: *A Phase II Multi-Center Open Label, Study to Evaluate Effectiveness and Safety of Ad5CMV-p53 Administered by Intra-tumoral Injections in 39 Patients with Recurrent Squamous Cell Carcinoma of the Head and Neck* (Protocol #9712-226) is amended similarly to Protocol 9709-214 (see above).

The protocol entitled: *A Double Blind, Placebo-Controlled, Single Rising-Dose Study of the Safety and Tolerability of Formulated hIL-2 Plasmid in Patients with Squamous Cell Carcinoma of the Head and Neck* (Protocol #9705-190) is amended to modify the dosage for the third cohort from a single (0.6 mg) dose to multiple (0.6 mg) doses on Days 0, 3 and 7; then once per week for three weeks for a total of six doses.

Reports of Safety, Adverse Events, and Protocol Updates

Dr. Greenblatt noted that two amendments updating the status of the clinical studies (Protocols #9409-087 and #9209-026) and four safety reports were submitted to ORDA since the March 1998 RAC meeting:

The protocol entitled: *A Phase II Multi Center Open Label, Randomized Study to Evaluate Effectiveness and Safety of Two Treatment Regimens of Ad5CMV-p53 Administered by Intra-tumoral Injections in 78 Patients with Recurrent Squamous Cell Carcinoma of the Head and Neck* (Protocol #9709-214) reported two adverse events: (1) One patient experienced fever, chills, and vomiting following the first dose of Ad5CMV-p53. The fever and chills were considered to be related to the administration of AD5CMV-p53, while the vomiting was considered not to be related to the administration of AD5CMV-p53. (2) A follow-up report to an adverse event reported on January 30, 1998. the previously-reported patient experienced a second episode of bleeding from the oral cavity resulting in a decreased hematocrit (23-19 percent). The patient received two units of packed red cells and one unit of plasma. The patient underwent embolization of the right ligula facial artery trunk; the bleeding stopped.

The protocol entitled: *Phase I Study of E1A Gene Therapy for Patients with Metastatic Breast or Ovarian Cancer That Overexpresses Her2/neu* (Protocol #9512-137) reported a follow-up to an adverse event that occurred earlier. The event was reclassified as "possibly related" to the administration of a plasmid vector rather than "not related" to the study agent. The patient experienced nausea two days prior to the scheduled start of the second infusion of the third cycle of treatment. Nausea was resolved with medication and the patient received the second infusion of the third cycle. Three days later the patient returned to the hospital with protracted nausea, vomiting, and abdominal pain. The patient was hospitalized and recurred intravenous hydration, pain control, and nausea management; the patient was taken off the study. A computer tomography scan showed a large bowel obstruction and the patient underwent surgery to remove the bowel obstruction. Surgery indicated significant abdominal fibrosis. Because similar fibrosis was noted with other patients on the study, the event is now considered to be "possibly related" to the administration of the plasmid vector.

The protocol entitled: *Gene Therapy of Cystic Fibrosis Lung Disease Using E1 Deleted Adenovirus: a Phase I Trial* (Protocol #9212-035) reported that a patient experienced myalgia and flu-like symptoms within the first 24 hours of virus instillation. The maximum temperature was 101.2° F. Pulmonary function

test results were decreased. The patient experienced coughing with deep inspiration. Chest X-rays revealed a small infiltrate at the site of gene transfer. Three days following gene transfer a bronchoscopy was performed, after which the patient complained of continued occipital headache and right-side pleuritic chest pain, and the patient demonstrated inspiratory crackles over the right lower lung field. X-ray revealed extension of the infiltrate and some consolidation within the lateral basal segment of the right lower lung lobe. The patient no longer was running a fever, although still taking Tylenol. On the fourth day following vector administration, the patient continued to experience headache and mild nausea; consolidation within the lateral basal segment of the right lower lobe persisted. On Day 5 the lung infiltrate decreased and the nausea improved. The patient was discharged. Upon follow-up at Day 9, the infiltrate had resolved. In consultation with the FDA, the investigators treated the next patient at the same dosage.

Committee Motion 2

The RAC approved a motion made by Dr. Aguilar-Cordova and seconded by Dr. Macklin to accept the Data Management Report presented at the June 18-19, 1998, RAC meeting by a vote of 8 in favor, 0 opposed, and no abstentions.

Discussion on Gonadal Biodistribution of Gene Transfer Vectors/Mickelson

Dr. Mickelson provided an overview of the RAC's previous discussions on gonadal biodistribution of gene transfer vectors. At the December 15, 1997, RAC meeting, Drs. Steven Bauer and Anne Pilaro (FDA, Rockville, Maryland) reported the FDA's observation that multiple preclinical animal studies designed to assess vector biodistribution have demonstrated unexpected persistence of vector nucleic acid sequences in gonadal tissue. Presently, there is no information bearing upon the question of whether these sequences are intracellular or integrated. If intracellular, it is unknown whether these sequences are in gametes or somatic cells. Based on these limited data, the findings raise concern that administration of gene transfer vectors could lead to germ-line integration, a circumstance that would pose unknown risk to subjects participating in gene transfer clinical trials. Concurrently, the FDA indicated that sponsors are increasingly interested in gene transfer for less serious diseases, earlier intervention before manifestations of disease, and gene transfer for augmentation or enhancement purposes. Under the limits of confidentiality, the FDA could not discuss further specifics of the observations.

In an effort to gain additional data related to these observations, the RAC recommended that a letter should be sent to all principal investigators of clinical gene transfer protocols and to all Institutional Biosafety Committees (IBCs) registered with ORDA (more than 400) requesting any preclinical and clinical data related to this issue. ORDA received more than 80 responses to this request. Four responses indicated that vector sequences were detected in gonadal tissue in preclinical animal studies.

The four responses indicating that vector sequences were detected in either ovaries or testes in preclinical animal studies are summarized as follows: (1) Peter T. Scardino, M.D., Baylor College of Medicine, Houston, Texas, stated that they published a paper documenting their preclinical data (Timme, T. L., *et al.*, *Cancer Gene Therapy*, Volume 5, No. 1, 1998). In murine experiments with adenoviral vectors expressing the HSV-TK gene, 1 animal in 28 had evidence of vector DNA in testicular tissue by polymerase chain reaction (PCR) analysis. (2) Simon J. Hall, M.D., the Mount Sinai Medical Center, New York, New York, stated that in murine experiments, 1 animal in 14 had evidence of vector DNA in testes after injection of an adenovirus expressing HSV-TK into the prostate. No vector DNA sequences were observed in sperm aspirated from the epididymis. (3) Jeffrey Holt, M.D., Vanderbilt University, Nashville, Tennessee, stated that vector sequences were detected by PCR in ovaries and testes for up to four weeks following intraperitoneal and intraprostate injection of a retroviral vector in mice and rats. (4) Verma Fimbres, GenCell Division of Rhone-Poulenc Rorer, Inc., stated that they have studied the biodistribution

of adenovirus-p53 sequences. A weak signal for vector DNA was observed in the ovaries of nude mice following intratumoral administration. Vector DNA was detected in the ovaries on Day 3 (the signal was negative at Day 31). Vector DNA sequences were detected in ovaries of cotton rats following intraperitoneal administration, but not in the testes.

Four responses indicated that no vector sequences were detected in human gonadal tissue in follow-up studies as follows: (1) Genetic Therapy, Inc. (Gaithersburg, Maryland), indicated that no vector sequences were detected in the gonads in 45 autopsy samples obtained from 45 patients receiving retroviral vector administration. (2) Steven M. Albelda, M.D., (University of Pennsylvania, Philadelphia, Pennsylvania), stated that no evidence of gonadal distribution was observed in four testicular autopsy samples analyzed from patients with mesothelioma receiving the adenovirus vector expressing HSV-TK in pleural space. (3) Chiron Corporation (Emeryville, California) reported no evidence of inadvertent germ-line transfer in samples obtained from 118 human immunodeficiency virus (HIV) patients receiving intramuscular injection of a retrovirus encoding the HIV *rev* gene. (4) Introgen Therapeutics, Inc. (Houston, Texas) reported that no vector sequences were detected in the testes (autopsy tissue) following administration of an adenovirus vector expressing the p53 tumor suppressor gene in three lung cancer patients, and one patient with head and neck cancer. In the latter patient there was an initial positive finding in testes; however, this finding was subsequently found to be due to surface contamination of the samples during processing.

During its March 10, 1998, meeting the RAC recognized the need for improved detection methods and development of animal test systems to assess these observations further. At that meeting, the RAC recommended that a letter should be sent to the NIH Director advising that a Request for Applications (RFA) should be issued for the development of vector-specific animal test systems that will provide qualitative and quantitative assessment of potential germ-line integration for specific classes of gene transfer vectors.

A letter dated June 5, 1998, was forwarded from ORDA to Dr. Harold Varmus, the NIH Director with regard to the RAC recommendation to issue a RFA for animal biodistribution studies for gene transfer vectors. Dr. Mickelson stated that the RAC continues to encourage investigators to submit data related to vector biodistribution (both preclinical and clinical) to the RAC. Such data will greatly facilitate the research community's understanding of the issue and aid in evaluating the potential implication of such observations.

Other Comments

Dr. Robertson Parkman, *ad hoc* expert (Children's Hospital, Los Angeles, California) said that the first time the risk of inadvertent germ-line gene transfer was discussed by the RAC was when Protocol #9412-097 (A. Venook and R. Warren) was reviewed at the December 1994 RAC meeting. The RAC was concerned about hepatic artery infusion of an adenovirus-p53 vector for the treatment of hepatic metastasis of colon cancer. The RAC approved the protocol after consideration of the balance of risk/benefit ratio with regard to patients with advanced cancer. Dr. Parkman said that the risk of germ-line gene transfer is much greater for retroviruses particularly with respect to *in utero* gene transfer studies. He said that at the June 8-9, 1995, RAC meeting, Dr. Esmail Zanjani (University of Nevada, Reno, Nevada) presented data from sheep experiments suggesting that the presence of retroviral vector sequences in gonadal tissues may be due to contaminating lymphocytes rather than integration into germ cells. Dr. Parkman said that identification of vector sequences in the gonads should prompt further analysis to ascertain if the sequences are indeed present in the germ cells.

Dr. Mickelson proposed potential changes to Appendix M, *Points to Consider in the Design and*

Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider) of the *NIH Guidelines*. The potential changes would provide guidance to investigators regarding appropriate biodistribution studies for clinical protocols and issues that should be addressed in patient Informed Consent documents. Ms. Knorr noted that the proposed language is a first step toward amending the *NIH Guidelines*; the final language would be published in the *Federal Register* for public comments.

Dr. Gordon stated that the RAC will have to deal with new vectors in the future, e.g., amplicon herpesvirus or lentivirus vectors; the vectors used in previous protocols are based on adenoviruses or murine retroviruses. The recommended RFA is to find out what kind of animal models would be predictive for human applications. Before the biological consequences can be assessed in animal models, Dr. Gordon said that sperm samples of the subjects of gene transfer studies should be requested and tested for the presence of vector sequences by PCR analysis; techniques are available to separate sperm from contaminating lymphocytes for PCR analysis. Dr. Aguilar-Cordova had reservations about the value of such analysis; Dr. Lysaught agreed with Dr. Gordon that this type of analysis is the first step to address this difficult issue.

Dr. Noguchi said that he agreed with Dr. Gordon's assessment that the purported overall risk is low but there are no quantitative or qualitative data. The FDA's current perspective is that most patients treated so far have been sterile or have advanced disease. The FDA is concerned about patients with longer life expectancy who might receive gene transfer in the future.

Dr. Markert raised a concern about excluding fertile women from participation in gene transfer studies for fear of inadvertent gene transfer to the fetus. She noted that men are allowed to participate in the clinical trials by using barrier contraceptive usually for six weeks after receiving gene transfer. Dr. Noguchi responded that the FDA policy is to have equal participation in clinical trials regardless of gender unless there is an overriding medical concern.

Dr. Parkman asked whether there is any evidence of vector sequence integration into either ova or sperm except for direct injection of gene transfer vectors into gonads. Dr. W. French Anderson (USC, Los Angeles) said that to the best of his knowledge there is no evidence of any gene transfer to germ cells, including his own experiments of direct intravenous administration of retroviruses in monkeys. Dr. Noguchi said that absence of data is not data that shows absence; in most of the studies so far, the most sensitive and rigorous assays have not been applied to this question of germ-line integration. He said that the FDA cannot make a policy decision based on the current inadequate data.

Dr. Wolff suggested investigating whether there is any evidence of germ-line transmission in patients who have natural adenovirus or HIV infection. Dr. Aguilar-Cordova said that since the 1960's investigators have looked into the possibility of retroviral DNA integration in HIV-infected individuals and no evidence of such integration has ever been reported. Dr. Wolff suggested that the issue should be investigated more rigorously; specifically, to determine whether there is any adenoviral DNA in germ cells of immunocompromised patients with high levels of viremia.

Dr. Mickelson said that an amendment to Appendix M of the *NIH Guidelines* emphasizing the need to submit data pertaining to gonadal biodistribution will build a body of information to address this vexing issue. Dr. Parkman said that requiring biodistribution studies in every protocol is an expensive proposition. He emphasized that the meaningful study should address the biological question of whether there is any DNA integration in reproductive tissues rather than just the presence of some vector sequences in gonads. Dr. Noguchi agreed that so far the question has not been addressed rigorously by most investigators.

Dr. Lai inquired how the FDA would handle the question if an investigator reports detection of vector sequences in gonads. Dr. Noguchi responded that the FDA conducts a risk/benefit type of analysis. It is less of an issue for patients who have advanced disease and who are sterile; the FDA needs definitive data in order to develop policy for cases in which patients do not have terminal disease and are sexually active. The FDA tries to work with the sponsor to give patients better information through the Informed Consent document including meaningful language regarding the possible implications of these findings.

Dr. Gordon noted that gene transfer vectors have to cross the blood-testes barrier in order to infect sperm and oocytes are protected from viral infection by a densely packed tissue barrier; however, no matter how minute the risk is, there must be solid scientific information to allay public fear. Dr. Noguchi said that the FDA is mainly concerned about policy decisions in response to reports of gonadal presence of vector DNA in animal studies.

Dr. Aguilar-Cordova noted that Dr. James Wilson's paper (in press) evaluated the potential of germ-line transmission after intravenous administration of recombinant adenovirus in the C3Hmouse. This study evaluated 814 offspring of animals administered with the vector; no evidence of germ-line transmission was detected. Dr. Aguilar-Cordova was concerned about the financial burden of testing patient sperm samples following vector administration.

Dr. Mickelson said that the FDA is asking for the RAC's opinion in terms of a policy decision for assessment of the germ-line risk. Dr. Noguchi said that the FDA would like the RAC to discuss publicly the issue of gonadal biodistribution so that if the risk is considered negligible, gene transfer studies can move forward confidently.

Dr. Ando suggested holding a Gene Therapy Policy Conference (GTPC) to address the issue of gonadal biodistribution.

Dr. Parkman outlined several questions to be addressed in the RFA or at a GTPC: What is the frequency of integration? Are there gene delivery systems that are more likely to integrate than others? Does the gonadal barrier break down more frequently in patients with specific types of disease? Do reproductive tissues have receptors for a particular type of virus? Are specific routes of administration more likely to cause integration than others? What are the biological consequences of integration? Is there a correlation between integration and the type of disease/disorder? For these studies, primates or dogs are more appropriate animal models than mice.

Dr. Noguchi said that efforts by the NIH to obtain increased understanding of this issue will greatly facilitate progress.

Dr. Mickelson said that until meaningful data exist relating to vector biodistribution, the RAC can recommend that appropriate information be disclosed in the Informed Consent document to assist patients in making decisions regarding participation in gene transfer clinical trials. Dr. Macklin said that the Informed Consent should include a description of the magnitude of the potential germ-line risk rather than just a low probability of such risk.

Dr. Parkman said that the biological consequence will most likely be disease-related. Dr. Gordon said that there are many scenarios that could potentially pose germ-line risks, e.g.,insertional mutagenesis. Some of the risks are application specific, e.g., prostate application could result in retrograde flow of vectors that bypass the blood-testes barrier.

Dr. Macklin stated that the Informed Consent document should provide the patients with meaningful information regarding germ-line risk, such as potential consequences of gonadal biodistribution, in order for the patient to make an informed decision.

Dr. Lai said that the first issue is to include relevant information in the Informed Consent document, and the second issue is to require all protocols to submit biodistribution data, including sperm. Dr. Markert said that sperm samples collected weeks and months following gene transfer would be more valuable than samples collected at the time of gene transfer (due to transient presence).

Dr. Parkman stated that inadvertent germ-line alteration is just a subset of the larger issue of germ-line gene transfer. He suggested focusing on the wider spectrum of the biology of intentional germ-line gene transfer; inadvertent alteration is just a toxicity question. Dr. Parkman said that the RAC previously invited Dr. James Neal from the University of Wisconsin to address the RAC regarding germ-line gene transfer. Germ-line alteration has the potential to pass on the genetic changes to the offspring. It could be done for the good intention of eradicating a bad gene from the human population. Dr. Parkman suggested that the RFA should focus on the larger issue of the biology of intentional germ-line gene transfer in animals, not simply on the toxicity of inadvertent germ-line alteration. Dr. Noguchi agreed that switching the emphasis from toxicity to biology is a very good idea; the issue will be appealing to academic investigators.

Dr. Mickelson suggested convening a RAC forum to discuss the issue of animal models for biodistribution.

Dr. Gordon said that inadvertent germ-line alteration is a different aspect of the issue of intentional germ-line gene transfer. Intentional germ-line gene transfer would employ a totally different strategy; one would never expose an embryo to adenovirus for germ-line correction. Therefore, both the biology and toxicology are valid issues.

Dr. Aguilar-Cordova agreed that the biology of germ-line gene transfer is important, but he said there is also a need to conduct toxicity studies separately. Dr. Gordon said that germ cell integration is a valid concern for integrating vectors such as lentiviruses. Dr. Lysaught noted that most investigators are not seriously considering the gonadal biodistribution issue in their protocols.

Dr. Mickelson asked that the RAC consider the proposed language as potential amendments to Appendix M. The potential changes would provide guidance to investigators regarding appropriate biodistribution studies for clinical protocols and issues that should be addressed in the Informed Consent document. Ms. Knorr asked for RAC volunteers to work on the proposed amendments for discussion at the September 1998 RAC meeting. Drs. Gordon, Ando, and Mickelson agreed to develop the proposed language further.

Appreciation of Retiring Members

At the end of the session, Dr. Mickelson noted that Drs. Lai, Lysaught, and Verma, along with Ms. Rothenberg, are completing their term of service as RAC members. She presented plaques and certificates to Drs. Lai and Lysaught, who were present at the meeting, in recognition of their service to the RAC. Ms. Knorr acknowledged the committed efforts made by all four retiring members and thanked them for their tireless dedication to the RAC.

Human Gene Transfer Protocol 9802-237 entitled: *Molecular Synovectomy by In Vivo Gene Transfer: A Phase I Trial*
PI: Blake Roessler, University of Michigan
Reviewers: Ando, Lai, Lysaught

Protocol Summary

Dr. Blake J. Roessler, University of Michigan Medical Center, Ann Arbor, Michigan, proposed conducting gene transfer experiments on eight patients (≥ 18 years of age) with rheumatoid arthritis (RA). pNGVL-TK is a plasmid expressing the HSV-TK gene under the control of a modified cytomegalovirus (CMV) promoter/enhancer. This protocol is a Phase I dose-escalation study of intra-articular administration of plasmid pNGVL-TK followed by systemic ganciclovir (GCV) in patients with active rheumatoid synovitis of the knees. This trial will study four doses of plasmid pNGVL-TK over a range of 1 ½ log doses (0.3 mg, 1.0 mg, 3.3 mg, and 10.0 mg). A constant dose of intravenous GCV (5 mg/kg twice daily for three days) will be used for each dose of plasmid pNGVL-TK tested. The investigator proposes to study two patients at each dose. The three major goals of this Phase I trial are to: (1) establish whether rheumatoid synoviocytes can be transfected *in vivo* using intra-articular administration of naked plasmid DNA, (2) establish the safety of the plasmid-based TK/GCV intra-articular administration, and (3) identify the biological effects specific to TK/GCV administration.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because it is the first proposed use of plasmid DNA in patients with chronic RA, a disease that is not life-threatening and for which alternative therapies exist.

Review – Dr. Ando

This is the first *in vivo* gene transfer for patients with systemic RA, an autoimmune disease. This disease is characterized by the autoimmune destruction of joint and other tissues. Immunologic characteristics include autoantibodies such as Rheumatoid Factor and nuclear antigens such as DNA. RA is a chronic relapsing disease that can be controlled with medication, but not cured. Aggressive treatment includes chemotherapy, anti-metabolites, immunosuppressive agents, and gold salts. The major clinical morbidity is destruction of joint tissues. Typical patients have multiple joint involvement, which generally includes fingers, wrists, elbows, shoulders, knees, ankles, and toes, in a symmetric pattern.

The current trial strategy is a regional therapy focused on the knees of patients with RA. Alternative therapies would include aggressive therapy for systemic disease, intra-articular steroids, and surgical synovectomy. HSV-TK/GCV treatment is essentially a regional genetic "synovectomy" predicated on the ability of this therapy to kill proliferating tissue. The effectiveness of this strategy, in theory, is extended by the ability of phosphorylated GCV metabolites to pass into neighboring cells and kill them with a "bystander" effect. Dr. Ando raised several questions and provided specific comments:

(1) Vector Biodistribution. The data presented suggest that DNA injected into the joint does not escape into the systemic circulation. Could this observation be due to differences in the sensitivity in the PCR assay used by the investigator? The intravenous study reported by the investigator shows markedly different tissue biodistribution compared with the literature. If it can be shown in humans that intra-joint DNA injection does not result in systemic exposure, then any germ-line risk or biodistribution risk would be minimal. Addition of this analysis to the study would be important to document this safety issue. Because plasmid DNA does not integrate and the gene transfer effect is transient, what level of vector biodistribution or germ-line transfer assessment should be recommended?

(2) Autoimmune disease. Because autoimmune disease is characterized by antibodies to self-antigens, should patients with anti-DNA antibodies be excluded? Has there been preclinical assessment of

anti-DNA antibody formation?

(3) Risk/benefit ratio. Alternative therapies exist for these patients. This fact should be discussed in the protocol and included in the Informed Consent document. The inclusion criteria require thatpatients cannot have received any disease-modifying therapy. What is the rationale for this particular entrance criterion?

(4) Efficiency of gene transfer. The level of gene transfer in preclinical studies is low and transient. The "bystander" effect may, however, increase the effect of gene transfer. The protocol has an excellent design for both invasive and non-invasive efforts to document gene transfer andsynovitis.

(5) TK immunogenicity The viral TK gene has been found to be immunogenic. Will patients be monitored for such an immune response? Data on TK immunogenicity will provide important information for the design of Phase II studies.

Dr. Ando stated that the investigator satisfactorily responded in writing to most of his questions.

Review – Dr. Lai

RA is a debilitating disease with no effective therapy. The currently available treatment, surgical synovectomy, is not effective in many cases. Thus, there is a strong need to develop alternative therapies RA is not a life-threatening disease; thus, the criteria for developing new therapies should have considered the availability of alternative therapies. This point should be clarified and made known to patients during the recruitment process. The investigator has provided preclinical studies that demonstrate the feasibility of this approach and its relative safety. However, the following questions should be clarified:

(1) DNA and lipid complexes were used in most of the published preclinical studies involving transfection of DNA into synoviocytes. Is there any reason why the investigator chose not to use lipid as a co-transfectant in this protocol? (2) Only 1 to 5 percent of the cells take up the DNA. Is there any evidence that the bystander effect is powerful enough to causecytotoxicity in most of the cells of the joint? (3) Is there any reason why GCV is administered intravenously rather than intra-articularly, so as to avoid any systemic side effects? (4) In the document,*Responses to Appendix M*, the investigator reasoned that other cell types are not likely to pick up the DNA, based on anatomical considerations. Have other cells actually been examined for the presence of plasmid DNA in preclinical studies?

Review – Dr. Lysaught

Dr. Lysaught provided a detailed written review previously to which the investigator responded in writing prior to the meeting. She noted that in a previous protocol reviewed by the RAC (Protocol #9406-074, Evans and Robbins), patients with RA received intra-articular injection of cells expressing the interleukin-1 receptor antagonist. The present study is the second protocol on RA registered withORDA.

The protocol is well written; the issues raised in Appendix M are clearly and specifically addressed. The investigators have examined gonadal tissue to address the issue of potential for germ-line transmission, a laudable positive step. Dr. Lysaught asked the investigator to provide an update on the longer term animal studies. How long were the animals followed? What was observed in terms of toxicity and efficacy? She asked the investigator to explain to the public the findings related to thehistopathological data of rabbits treated with gene transfer.

With regard to the Informed Consent document, Dr. Lysaught stated that except for one significant problem (described below), this Informed Consent is one of the best she has reviewed for the RAC. If the investigator agreed to revise the Informed Consent in response to her suggestions, she would recommend that this Informed Consent be utilized and disseminated to other investigators as a model Informed Consent document.

Dr. Lysaught would recommend the following changes to the Informed Consent document, all of which should be easy revisions to make. The first change, though easy to make, is conceptually significant. Throughout the Informed Consent the investigator uses the word "treatment." Given the experimental nature of this Phase I protocol, she recommended that this word be deleted from the document (it is used a number of times). Sentences could be easily reworded or the word "procedure" substituted. Related to this suggestion, the last paragraph on the first page of the Informed Consent does seem to overstate the therapeutic nature of this study. The purpose of the study is to assess transfection efficiency and safety; these goals are not mentioned in this section.

On the fourth page the Informed Consent document states: "Second, if you are a woman, if you are or may become pregnant, this research may involve unforeseeable risks to you, the embryo, or the fetus." Because pregnant women are excluded from this protocol, this phrase should be deleted.

Dr. Lysaught said the investigator explained the risks of the protocol well. The only item missing from the Informed Consent document, however, is a discussion of the risks of anaesthesia given during arthroscopy. This description should be added.

Investigator Response – Dr. Roessler

Dr. Roessler summarized the hypothesis of the intra-articular injection of plasmid DNA expressing HSV-TK gene followed by intravenous administration of GCV. The preclinical data suggests that this approach will be safe and will not be associated with significant toxicity or morbidity. The procedure may have beneficial clinical effects within the treated joint.

The entrance criteria of the protocol include: (1) Proven diagnosis of seropositive RA: Patients must have a diagnosis of RA as determined by the 1987 American College of Rheumatology criteria; (2) Age: 18 years or over; (3) Gender: Male or female; (4) Duration of symptomatic disease: Greater than six months but less than five years; (5) Severity of disease: Severe proliferative rheumatoid synovitis of the knee with a clinical indication for arthroscopic synovectomy. Evidence of synovial proliferation and rheumatoid synovitis in at least one compartment of either knee as determined by power Doppler ultrasonography.

Responding to the question of transduction efficiency, Dr. Roessler agreed that the efficiency of gene transfer by injecting naked plasmid DNA into the joint is low. The rationale for choosing this gene transfer procedure is based on consideration of the balance between efficient transduction and concerns about safety and biodistribution. Dr. Roessler presented data derived from rabbit experiments 24 hours after administration of a single dose of plasmid DNA. The transduction efficiency as compared to an adenovirus vector is relatively low; however, the plasmid DNA approach is preferable taking into account safety and biodistribution concerns.

Dr. Roessler presented biodistribution data. Conventional PCR analysis was used to assay tissue samples with an estimated sensitivity of detecting 10 copies of plasmid DNA per microgram of sample DNA. Twenty-four hours after a single intra-articular injection of plasmid DNA, no plasmid DNA sequences were found in spleen, lung, liver, kidney, gonadal tissue, or regional lymph nodes.

Dr. Roessler showed histopathological findings at 21 days following plasmid DNA administration to the joints followed by systemic GCV infusion. The data from the treated knee showed no overt damage to either cartilage or subchondral bone tissue.

Dr. Roessler presented data on longer-term rabbit studies. Albumin was injected into the knee joint to stimulate an arthritic response with repetitive dosing schedule to mimic chronic and progressive disease. One of the knee joints of each animal was treated with the TK plasmid. Specifically, no damage to cartilage or bone in the treated knee was observed compared with the untreated controls. The beneficial clinical effect was measured in terms of mean mediolateral knee diameter. During the entire course of the ongoing experiment (140 days) the treated knee showed sustained benefit compared to the untreated knee. Dr. Roessler noted one shortcoming of this model is that the experimentally induced RA will resolve by itself at the end of the experiment as opposed to the progressive nature of human RA.

Dr. Parkman inquired about the potential of the present gene transfer to provide sustained clinical benefit. Dr. Roessler responded that sustained benefit is obviously a desirable goal, but the interim goal of the present study is to compare TK gene transfer to the conventional medical therapy (intra-articular steroid treatment).

Dr. Lai asked if GCV alone will provide improvement. Dr. Roessler responded that such an experiment has not been performed; Dr. Roessler believed the joint improvement is due to combination of the TK plasmid and GCV.

Dr. Aguilar-Cordova said that in the TK/GCV cancer treatment paradigm, part of the *in vivo* "bystander" effect is due to immunological reaction to tumor cells stimulated by the TK/GCV regimen, i.e., stimulation of immune response against tumor associated antigen. He asked if there is any concern that similar immune stimulation will exacerbate RA, an autoimmune disease. Dr. Roessler responded that it is a legitimate concern about a pro-inflammatory response, but he noted that one of the hypotheses of RA is an inadequate immune response to eliminate the antigen that initiates the pathophysiological process. Dr. Roessler said that histological assessment of tissue removed from the joint treated by the TK plasmid will be performed to address the issue of inflammatory response.

Dr. Parkman inquired if patients with prior HSV infection (about 80 percent of adults) could develop an anti-HSV-TK immune response; HSV-TK expressed by the plasmid might be exported by the transduced cells and be presented as an antigen on the cell surface to induce an immune reaction. Dr. Roessler responded that he is collaborating with investigators at the University of Pennsylvania to develop antibody and cytotoxic T lymphocyte (CTL) assays to address this issue.

Dr. Lai asked if there are any data showing that injection of TK plasmid will induce anti-DNA antibodies. Dr. Roessler responded that most of these assays are for identifying human antibodies and are not applicable in the rabbit model.

Dr. Parkman asked if preimmunized rabbits will have beneficial results similar to the results in naive rabbits that receive the TK plasmid. Dr. Roessler said that this type of experiment has not been conducted.

Dr. Wolff asked if repeat administration of the TK-plasmid will produce sustained beneficial effects. Dr. Roessler said that the immediate goal is to compare the gene transfer procedure with the standard steroid treatment, which is not expected to have long term sustained benefit. The steroid treatments are repeated up to four times annually.

Dr. Ando said that a large number of safety studies have been performed related to DNA immunizations in both normal mice and autoimmune strains of mice. Administration of double-stranded circular *E. coli* DNA (similar to plasmid DNA) has not been shown to accelerate the lupus type autoimmune disease in these animals in contrast to single-stranded or open double-stranded DNA involved in lupus type disease. Therefore there is a large body of literature suggesting that the present approach will be safe. Dr. Roessler said that he has performed multiple doses of plasmid DNA experiments in primates and no anti-DNA antibodies have been detected. Dr. Parkman said that it is important to perform the experiment in animals preimmune to HSV to ascertain that TK gene transfer will not exacerbate the disease.

Dr. Markert noted that in the rabbit experiments, the serum chemistries of CO_2 values are lower than those of normal humans. Dr. Roessler said those are baseline values for rabbits.

Dr. Macklin noted that the $1,000 monetary compensation is relatively high and might constitute an undue inducement. She asked if the Institutional Review Board (IRB) raised any question regarding this amount. She said that the amount should be proportional to the risk and discomfort to undergo the procedure. Dr. Roessler responded that the money is to compensate for the additional arthroscopic procedure that patients are required to take in order to obtain tissue samples for analysis. Dr. Lysaught agreed that the compensation is reasonable; a similar rationale has been discussed by the RAC for compensating cystic fibrosis subjects undergoing bronchoscopy. Dr. Parkman noted that similar compensation has been discussed for the stereotactic injections of HSV-TK vector producer cells of brain tumor patients whose tumor cannot be surgically removed.

RAC Recommendations

At the close of the RAC discussion of this protocol, Dr. Lysaught noted that the Informed Consent document was well written and could serve as a model for other similar protocols. She recommended a few specific changes to the document such as eliminating the use of the word "treatment," which is misleading for a Phase I safety study.

At the conclusion of the discussion of this protocol, the RAC recommended that a letter summarizing the RAC discussion and containing Dr. Lysaught's recommendation be sent to the investigators and other concerned bodies.

Human Gene Transfer Protocol 9802-232 entitled: *Gene Therapy for Myocardial Angiogenesis*.
PI: Jeffrey Isner, Tufts University (Douglas Losordo representing Dr, Isner)
Reviewers: Wolff, Rothenberg (presented by Dr. Wolff)
Ad hoc: William Klaus, Duke University (presented by Dr. Wolff)

Protocol Summary

Dr. Jeffrey M. Isner, St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Massachusetts, proposed conducting gene transfer experiments on 30 patients (> 21 years of age) with a history of angina pectoris. phVEGF165 is a plasmid expressing the cDNA of the human 165 amino acid residue isoform of vascular endothelial growth factor (VEGF) under the transcriptional control of a CMV promoter. Eligible subjects must have stable exertional angina and areas of viable, but underperfused, myocardium who are not optimal candidates for surgical or percutaneous revascularization. Clinical responses will be evaluated by serial studies performed before and after gene transfer, including dobutamine stress "SPECT"-sestamibi myocardial perfusion, contrast stress echocardiography, exercise treadmill testing, and selective coronary arteriography. The protocol is a Phase I, single-site, dose-escalating, open-label study to determine the safety and bioactivity of direct intramyocardial gene

transfer of plasmid phVEGF165 in patients with symptomatic myocardial ischemia. A secondary objective is to determine the anatomic and physiologic extent of collateral artery development in patients receiving intramyocardial plasmid phVEGF165 gene transfer.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because it is the first proposal involving direct DNA injection into the heart.

Review - Dr. Wolff

The protocol proposes to inject a naked plasmid DNA expressing VEGF165 directly into the myocardium after surgical exposure. Based upon this group's results in limb ischemia (Protocol #9409-088), this approach is reasonable in the heart. Except for the surgical procedure, the protocol does not raise any increased risk over other protocols. The few potential risks are adequately addressed by the investigators in the Informed Consent document and in the protocol.

One concern involves the inclusion criteria. Given that this procedure is a surgical intervention (with the potential for morbidity), the inclusion criteria should be better indicated. Patients should have angina and objective evidence of myocardial ischemia despite "maximal medical therapy." This criterion is recommended for two reasons: (1) the risk vs. benefit consideration, and (2) to prevent changes in medical treatment that could affect the outcome. Maximal medical therapy could be specified (i.e., beta blockers, nitrates, and calcium channel blockers unless contraindicated) .

The endpoints appear to be reasonable. However, where is the validation of coronary angiographic indices of collateral blood flow? Dr. Wolff noted that his concerns were addressed by the investigators in their written response.

Review - Ms. Rothenberg (presented by Dr. Wolff)

Ms. Rothenberg stated in her written review that this protocol raises many of the same issues raised in the Crystal protocol (Protocol #9711-221) because they both involve the administration of an adenovirus vector to cardiac patients. Ms. Rothenberg focused on a few questions raised by the Informed Consent document, nothing that if the scientific reviewers believe that the science is sound, then these issues are relatively minor. Ms. Rothenberg's questions were: (1) Please clarifythe criteria for who will recruit patients into this study: Is it a cardiologist and/or a cardiac surgeon? Describe the patient enrollment process in more detail. (2) Justify that "open heart surgery" to get access to the heart to inject the VEGF gene is worth the risk. What are the risks of open heart surgery? (3) Do these patients really have no othe alternative therapies? The Informed Consent document is not clear about alternative therapies. Are medicines for angina considered as suboptimal treatment? (4) Clarify the risk of heart attack during the study. Is the risk due to the stress test, surgery, or the patient's condition? (5) There is no mention of consent or family discussion about autopsy in the Informed Consent document.

Review -Dr. Kraus (presented by Dr. Wolff)

In his written review, *ad hoc* expert, Dr. William Kraus, agreed with the general consensus of the RAC reviewers that once the RAC has considered these two protocols (#9802-232 and #9802-238) at the June meeting, that the RAC has effectively dealt with many of the possible variations for protocols dealing with gene transfer in the heart and that there will be little need to consider others in this amount of detail in the future. In association with the Crystal protocol (#9711-221) discussed at the December 1997 RAC

meeting, and these two being considered at this meeting, the RAC will have seen all the likely combinations of vectors and therapeutic genes. The one new issue that Dr. Kraus believes may be worthy of detailed consideration in future protocols, should they be presented, would be the use of other major classes of gene transfer vectors, such asadeno-associated virus or retroviruses. Many of the issues that were considered with the Crystal protocol resurface in these two and have been adequately addressed previously. Dr. Kraus' review outlined several issues worthy of consideration.

Dr. Kraus stated that the present protocol proposes to use naked DNA as a vector for gene transfer of VEGF165. The protocol is well justified with supporting experimentation and documentation by the investigators in animal studies, and human work using similar techniques in peripheral artery disease (PAD). In general, gene transfer with naked DNA is much less worrisome that gene transfer with an adenovirus, as proposed in the Crystal protocol (#9711-221) in December 1997. There have been numerous preclinical studies using this technique (naked DNA gene transfer) with little evidence of adverse consequences. Use of the gene transfer by the investigators in the previous protocol involving PAD has been without incident. There is little reason to think that there will be any problems associated with this proposed study.

Dr. Kraus noted his concern related to the issue of patient selection. Choosing patients that "are not optimal candidates for surgical or percutaneous revascularization" begs the question of what criteria will actually be used to select subjects for this study. Does this criterion in reality mean "not candidates" or "candidates of last resort"? Dr. Kraus assumed that this protocol is not a compassionate use protocol. Why would a patient agree to participate in this study if surgical revascularization is a reasonable, even if suboptimal, option? Dr. Kraus noted that the proposed physiologic and imaging studies are justified, adding particular strength to the study. Such studies will likely yield very important information.

Investigator Response - Dr. Losordo

Regarding inclusion criteria, Dr. Losordo outlined the process by which patients are referred and then evaluated for participation in this trial. All patients are referred by their primary care physicians who have determined that the patients have failed all best possible medical treatments. The objective criteria include dobutamine stress "SPECT"-sestamibi myocardial perfusion, contrast stress echocardiography.

Regarding the use of coronary angiography as the measurement for the growth of new blood vessels, Dr. Losordo said that the use of this endpoint has been validated by autopsy studies and it is the best objective measurement to be used in this study. The angiography data will be corroborated by the data from improvement in echocardiography and sestamibi stress tests.

Responding to the risk of open heart surgery for gene delivery, Dr.Losordo stated that the surgery to be used is the left mini-thoracotomy, which has been performed in his institution for the past several years without mortality. The use of this procedure is justified because these patients are severely disabled without other treatment options; most patients who undergo this surgery are discharged from the hospital three days post-operatively.

Dr. Losordo responded to the question of why a patient would agree to this gene transfer procedure if surgery is available as an option. Dr. Losordo explained that patients included in this protocol do not have the option of surgery as determined by both their primary care physicians and by the eligibility review committee, which consists of physicians who are not participating in the protocol.

Dr. Losordo said that the protocol was initiated prior to RAC discussion. To date a total of eight patients have been treated under the protocol. Complete follow-up studies of three patients show that they have

registered clinical response in terms of reduction in symptoms and increase in their ability to perform physical activities; in two cases, the amount of nitrate medication needed to treat angina was reduced. The patients demonstrated clinical improvement in myocardial perfusion registered by sestamibi imaging and angiographic evidence of collateral new blood vessel growth.

Dr. Markert noted a discrepancy between data derived from Dr. Isner's previous protocol (#9409-088) involving PAD and animal studies regarding persistence of plasmid DNA in muscle, i.e., persistence in humans but not in animals. Dr. Losordo explained that, based on PCR analysis, the plasmid DNA present in humans is not integrated.

Dr. Wolff commented that it is good practice to have three independent physicians review the inclusion criteria for patients entering the study. He asked if the review committee also recommends the optimal medication for the patients during follow-up. Dr. Losordo responded that the primary care physicians are responsible for deciding on the optimal strategy for treating these patients (review committee may review the treatment, if needed).

Dr. Parkman asked about the volume to be injected to the heart. Dr. Losordo responded that a total of 8 cc is injected at four separate sites.

Dr. Lai inquired about transgene expression in patients receiving DNA injection. Dr. Losordo responded that in the peripheral blood vessel study an increase in plasma levels of VEGF was noted corresponding to the timing of gene transfer; no such data are available yet for this protocol.

Dr. Wolff noted the promising results so far, and he asked whether a control arm of injecting the empty plasmid is planned for a future study. Dr. Losordo responded that a control arm to evaluate the efficacy of the trial will be considered when a less invasive percutaneous system for catheter-based gene delivery is proposed in the near future; it is not justified to asked patients toparticipatein a control arm study using the more invasive surgical procedure. A crossover type of the study design will be used in the future control arm so that ultimately everybody would be treated with theVEGF plasmid after a sufficient waiting period.

Dr. Markert asked if plasmid DNA is detected in blood samples after DNA injection. Dr. Losordo said that they have not yet completed the data analysis.

Dr. Lai asked for clarification of what is meant by "patients are not optimal candidates for surgery". Dr. Losordo explained that they have struggled to come up with a proper wording for the inclusion criteria. It is intended to convey the meaning that the patients are not likely to benefit from any conventional revascularization interventions. The word "optimal" is chosen rather than "not candidate for surgery" because anybody can have an operation even with a minuscule chance being helped. Dr. Losordo said that bypass surgery is not an option for these patients.

Dr. Lai asked other members of the RAC to comment on whether the wording of the inclusion criteria should be clarified with regard to if the surgical intervention is an alternative therapy. Dr. Macklin noted that the primary care physician is the one to recommend participation, and if a patient refuses to accept the recommendation he or she will continue to be treated with medication. She noted that the statement in the Informed Consent document appears to be sufficient for the patients and their doctors to decide on participation. Dr. Lysaught noted a statement conveying such a message in the Informed Consent document, "My doctors have determined that I would not be best treated by a procedure such as an angioplasty (balloon dilation of my blood vessel), or coronary bypass surgery, therefore I may be eligible for participation in this research study." Dr. Parkman suggested an alternative statement that patients are

not expected to have an "acceptable outcome" if they undergo a procedure such as an angioplasty or coronary bypass surgery.

Dr. Macklin noted that the costs associated with treatment should be clarified in the Informed Consent document. The Informed Consent document currently states, "In the event that my participation in this trial results in a medical problem, treatment will be available at St. Elizabeth's Medical Center. Costs of any such treatment will not be reimbursed, nor will any other financial compensation be provided." Dr. Macklin said that costs related to short-term treatment of adverse events should be provided free of charge. Dr. Losordo explained that the language is part of the standard statement required by the Human Subjects Committee. Dr. Losordo stated that patients will not be liable for any costs associated with the protocol or any costs for treatment if complications occur as a result of their participation in the study. DrLosordo agreed to add an addendum to the standard statement advising potential subjects that any treatment for short-term adverse effects related to participation in this study will be provided free of charge.

Dr. Macklin asked if such a statement is in agreement with Federal regulations forIRBs. Dr. Lin responded that the Federal regulations require IRBs to address the issue of compensation to subjects injured as research subjects in the Informed Consent document; however, there is no requirement in the Federal regulations that the institution must compensate or provide treatment free of charge to patients; institutions can adopt individual policy. Dr. Macklin stated that, ethically, medical treatment related to participation in this study including treatment of adverse effects should be provided free of charge to the patients.

Dr. Mickelson noted her intention to abstain from this discussion of the protocol because she serves as a consultant to the St. Elizabeth's IBC.

Dr. Wolff said that there is a small chance that the gene transfer could induce antibodies againstVEGF, and he asked if anti-VEGF antibodies will be monitored in the follow-up study. Dr. Losordo responded that human VEGF is a natural protein and the likelihood of inducing such antibodies is small; he noted no indication of a physiologic reaction to repeat administration of VEGF plasmid in the patients treated in the peripheral blood vessel protocol. Dr. Wolff mentioned that there is a report of breaking immune tolerance when plasmid DNA expressing hepatitis B surface antigen was injected into the muscle of transgenic mice. Dr. Losordo noted that VEGF is a natural protein distinct from the foreign hepatitis antigen; nevertheless, he agreed to monitor anti-VEGF antibodies.

Dr. Lin asked why the RAC was discussing this protocol because it has IRB approval and has enrolled patients in the study. Dr. Mickelson explained that the RAC discussion is to serve the purpose of raising public awareness of novel gene transfer protocols, and in this case, to review different approaches to gene transfer for heart disease.

Dr. Markert asked if there are other examples, aside from the hepatitis antigen, that immune tolerance is broken by plasmid DNA injection. Dr. Parkman said if the post-translational processing such as glycosylation of the transgene product in muscle is different from the natural protein, such a protein might induce immune response. He cited an example of the factor VIII protein of hemophilia A. Dr. Parkman asked if the FDA has considered this aspect of the protocol. Dr. Noguchi responded that he needs to consult with the scientific reviewer of this specific protocol.

Dr. Wolff said that an immune response against a therapeutic gene product is more of a concern for the treatment of monogenic disorders for those patients who are born without the natural proteins. Dr. Noguchi noted that non-neutralizing antibodies have been observed in the patients receiving recombinant proteins, but not antibodies that would cause autoimmune disease. Dr. Ando explained that

immunogenicity is a standard clinical concern to be dealt with in all phases of clinical trials involving recombinant proteins. Dr. Lai said that all gene transfer protocols have a similar generic issue as to whether the transgene-derived proteins will be processed differently from the natural ones to induce an immune reaction.

RAC Recommendations/Comments

At the conclusion of the discussion, Dr. Mickelson summarized issues discussed in this protocol. Such issues include clarification of the inclusion criteria and recommendations regarding the Informed Consent document. The RAC made the following specific recommendations to be transmitted in a letter to the investigators and other concerned bodies: (1) The inclusion criteria should be modified such that enrollment should be limited to subjects who have angina and objective evidence of myocardial ischemia despite "maximal medical therapy" because of the potential risk of morbidity related to the surgical procedure. (2) The Informed Consent document should include a statement in the introduction about the importance of requesting an autopsy to assess the issue ofbiodistribution, and it should include a clear and understandable description of all surgical procedures. (3) The Informed Consent document should clearly articulate the subject's responsibility for any costs associated with this study. An addendum should be added advising potential subjects that any treatment for short-term adverse effects related to participation in this study will be provided free of charge. (4) The protocol should be amended to include follow-up studies to monitor for anti-VEGF antibodies.

Human Gene Transfer Protocol 9802-238 entitled: ***Phase I/Phase II Study of the Effects of Ascending Doses of Adenovirus Mediated Human FGF-4 Gene Transfer in Patients with Stable Exertional Angina***
PI: Joon Lee, University of Pittsburgh
Sponsor: Anthony Bourdakis, Berlex Laboratories, CA
Reviewers: Gordon (presented by Macklin), McIvor (presented by Macklin), Macklin
Ad hoc: William Kraus, Duke University (presented by Macklin)

Protocol Summary

Berlex Laboratories, Inc., Richmond, California, proposed to conduct amulticenter (up to 10 sites) gene transfer trial on a minimum of 48 and a maximum of 120 patients (30-75 years of age) with stable angina. The study involves intra-arterial administration of Ad5FGF-4 to the heart via a standard coronary angiography catheter and/or a guiding catheter and subselective catheter. Ad5FGF-4 is an E1A and E1B-deleted human adenovirus serotype 5 with a human fibroblast growth factor (FGF)-4 (hFGF-4) insert driven by a CMV promoter. The protocol is a Phase I/II, randomized, placebo-controlled, ascending-dose study in doses up to 10^{11} viral particles. Anti-ischemic effects will be evaluated by treadmill exercise test and by stress echocardiography at 4 and 12 weeks. Potential adverse effects due to FGF-4, the adenovirus vector, and the catheter will be evaluated. The objectives of the study are: (1) to evaluate safety and anti-ischemic effects of ascending doses of adenovirus mediated hFGF-4 gene transfer in patients with stable exertional angina, and (2) to select safe and effectivedose(s) for a subsequent study.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because it involves a new gene, hFGF-4, a new route of administration (via a catheter inserted into the coronary artery), and further discussion of the risk versus benefit considerations (less serious disease than previous cardiac trials).

Review - Dr. Gordon (presented by Dr. Macklin)

Dr. Gordon stated that this is a randomized, placebo-controlled protocol to instill an adenovirus vector carrying the gene encoding the angiogenesis factor, FGF-4, into the coronary arteries of patients with stable angina pectoris and one or two-vessel coronary disease. Patients with three-vessel disease will be accepted if one major proximal vessel has less than 70 percent stenosis. A minimum of 48 and a maximum of 120 patients will be studied. This is a multicenter study involving up to 10 centers.

The choice of FGF-4 as an angiogenesis factor was made on the basis of previous porcine studies, where instillation of this vector into coronary arteries improved left ventricular (LV) function and blood flow to an experimentally-induced ischemic region of the heart.

Escalating doses of adenovirus (up to a maximum of 10^{11} virus particles) will be instilled into coronary vessels. The placebo will consist of injection of vehicle alone. Patients will be followed in the hospital initially for 48 hours then overnight, and periodic follow-up visits will be made to assess cardiac function, primarily by stress EKG. This is a Phase I/II study with both safety and efficacy components.

Review of this protocol by the RAC was suggested by several members for the sake of completeness and comparison to similar protocols that have undergone RAC review. Some RAC members also were interested in the choice of angiogenesis genes as well as the novel methodology used for gene transfer. Some questions regarding the Informed Consent document were raised with the investigators during the interim period of submission to the RAC and its review. One RAC member was interested in the requirement that females be infertile, whereas males were encouraged to use barrier protection during the course of the study. One RAC member was interested in preliminary data in humans, perhaps available from similar protocols at other institutions. The following questions remain to be addressed: (1) What is the mechanism whereby this approach to gene transfer is presumed to be effective? If adenovirus fails under most circumstances to penetrate tissues beyond the vascular endothelium, where is the FGF-4 produced after coronary artery instillation? If the site of synthesis is the endothelium rather than the myocardium, how does it lead to improved cardiac perfusion? Are there differences in the structure of porcine versus human vascular endothelium that could weaken the porcine model as a predictor of results in the human? (2) The placebo consists of the vehicle alone rather than an empty vector. If the vector has a potential inflammatory effect and if inflammation can cause increased blood flow, would an empty vector be a better control? (3) Do the investigators plan to periodically test for the spread of virus to other organ systems following the gene insertion procedure? The investigators should discuss these plans, with particular reference to sperm samples. (4) Will post-mortem examinations be requested? (5) The preliminary evaluation of patients includes procedures such as mammograms and pap smears. Does the offer to conduct such tests provide an inducement to enroll in this study, and if so, does such inducement create any ethical concerns?

Review - Dr. McIvor (presented by Dr. Macklin)

In his written review, Dr. McIvor stated that his primary concern with the protocol pertains to the potential effectiveness of the study design in determining the safety and efficacy of administering Ad5FGF-4 into the coronary circulation.

The primary safety concern is the potential for an inflammatory response to the administered Ad5FGF-4 vector. Anticipated safety of the procedure is based on previous studies in the porcine model for ameroid ischemia, where histological studies indicate lack of an inflammatory response. Nevertheless, numerous studies from other laboratories show there is an acute inflammatory response in different organs and tissues following adenovirus mediated gene transfer. Indeed, quelling this response has become a major goal of the field. In the protocol, potential adverse responses to the Ad5FGF-4 vector will be assessed by

devising methods to assay for antibody formation against adenovirus, by clinical observation for adenovirus infection (fever, etc.), and by adverse effects on the liver and heart. These tests seem appropriately designed to track the status of the patient; however, will they adequately assess whether or not an inflammatory response has occurred and whether administration of the next higher dose of Ad5FGF-4 poses significant risk?

The effectiveness of the procedure will depend on efficient Ad5FGF-4 mediated delivery and expression of the FGF-4 gene. Unfortunately, the target organ in this case cannot be sampled to test directly for gene transfer and expression. Would it be reasonable to assay the patients plasma for elevated FGF-4 just after administration of the vector? This may be the only opportunity for testing the effectiveness of the procedure at the molecular level.

The protocol provides a detailed description of the tests that will be conducted to determine if Ad5FGF-4 administration has improved the condition of the patient, and the protocol has well-defined study endpoints (time to onset of angina and total duration on treadmill, ST segment depression ofECG, LV function). However, it was unclear what the quantitative difference is between normal individuals and affected patients. Is there variability within these groups? If so, what degree of quantitative improvement will be necessary to draw definitive conclusions with respect to the "normalization" of the patient's condition? Thus, an explanation of anticipated numerical results and their interpretation would be helpful in evaluating the potential efficacy of the protocol.

Review - Dr. Kraus (presented by Dr. Macklin)

Dr. Kraus stated that the present protocol proposes to study the effects of adenovirus-mediated gene transfer via coronary infusion into the ischemic regions of patients with stable angina. There are several issues with this protocol.

First, the criteria for selection of subjects with stable angina are not well defined. Do the criteria include patients with stable but severe angina not amenable to percutaneous angioplasty, or do the criteria include all comers with stable angina pectoris? This choice will make a difference in the number of patients needed to detect a benefit reliably, and a substantial placebo effect is possible if patients with fairly mild disease are used. The primary physiologic outcome, time to angina/ischemia on an exercise tolerance test, is fairly crude and may not be sensitive enough to detect significant differences. The lack of a sensitive outcome measure is likely the reason for the large number of proposed subjects (48-120). The investigators and sponsor might consider the use of more sensitive tests such as those proposed in the Isner study (Protocol #9802-232).

Review - Dr. Macklin

Dr. Macklin stated that her written review addressed ethical considerations involving recruitment of subjects, the risk/benefit ratio, confidentiality protections, and the Informed Consent document. (1) Recruitment of subjects. The protocol describes inclusion and exclusion criteria but does not describe the recruitment procedures. Dr. Macklin asked several questions: (a) How will potential subjects be identified, and by whom? (b) Who will approach them for their possible participation? (c) Who will have access to the records of these patients, other than the patients' personal physicians? (2) Risk/benefit ratio. The risk/benefit ratio in a study such as this one is very difficult to determine. Because the protocol is a Phase I/II study, and the first time the intervention is being attempted in humans, it is impossible to know whether there will be any benefit to the subjects who receive FGF-4. Subjects who receive a placebo will receive no direct benefit (except, perhaps, for the placebo effect). One RAC member noted during the period of assessment for review of this protocol: "Because escalating doses are contemplated the virus will

certainly be toxic at some point." Another RAC member expressed concern about toxicity in the context of the large number of patients to be enrolled, and especially considering the mildness of the disease (stable exertional angina). Another RAC member asked for a justification for the large number of patients enrolled in this Phase I study. Dr. Macklin asked several questions: (a) Would all of these patients be undergoing the stress echocardiogram if they were not in the study? (b) Would all of these patients be undergoing cardiac catheterization if they were not in the study? If the answer to (a) and/or (b) is "no", then the risk/benefit ratio of the entire study is open to question, apart from the matter of the possible toxicity of higher doses of FGF-4. (c) Because it is uncommon to include a placebo control group in a Phase I study, please justify the inclusion of the placebo groupfor all dose levels to be tested. (3) Confidentiality protections. The consent form includes the usual confidentiality protections. (4) Informed Consent document. (a) The Informed Consent is written at a high level of readability. Although most technical terms are explained, sentences are long and complex. Terms such as "experimentally induced condition," "inclusion criteria," "capillaries," "vascular," and "placebo" are not ordinary language expressions and require explanation or replacement by simpler terms. (b) The nature of the possible "toxic reaction" mentioned in item 5 under "Risks and Discomforts" should be explained.

Other Comments

Dr. Parkman said that the placebo group should allow crossover to the therapy group at a certain point of the trial. A second catheterization is valuable to assess the biologic endpoint of neovascularization.

Investigator Response - Dr. Engler

Dr. Engler is a cardiologist and a professor of medicine at the University of California, San Diego, California. He is also the Vice President and Medical Director of Collateral Therapeutics, Inc. (San Diego, CA), a partner of Berlex Laboratories. Dr. Engler is representing the 10 principal investigators and the sponsor of this protocol. Dr. Engler noted that the sponsor has responded in writing to most questions raised in written reviews. He used slide illustrations to respond to several major questions.

Regarding the type of target cells transduced by the adenoviral vector, Dr. Engler showed porcine data in which an adenovirus expressing the *Lac*Z gene was injected into the coronary artery. Cardiomyocytes as well as endothelial and interstitial cardiac cells were transduced.

Dr. Engler showed data from another pig study demonstrating thatFGF protein expression was detected in the left anterior descending and left circumflex artery bed following intra-arterial administration of the Ad5FGF-4 vector. Extensive analysis of a large number of organs demonstrated that FGF-4 protein was found only in the myocardium and not in any of the other organs examined.

Dr. Engler showed vector biodistribution data using primers specific for both thetransgene and adenoviral vector in the PCR analysis. Retina, liver, and skeletal muscle were completely negative following intra-coronary administration. In one animal left ventricle administration, there was evidence of adenovirus DNA in the testes at Day 5 but was negative at Weeks 4 and 12, indicating a transient presence of vector sequences.

Responding to the question regarding a possible inflammatory response, Dr. Engler stated that no inflammation in the heart has been observed following intra-coronary injection of adenoviruses expressing FGF-5, FGF-4, and VEGF into a large number of animals. He said that a similar lack of inflammation was observed in rabbits by other investigators. He speculated that the route and site of administration may be determinants of inflammatory reaction documented by published reports by multiple investigators.

Dr. Parkman noted that the supporting data demonstrating the lack of inflammatory response to the vector were derived from experiments involving nonimmune animals. He asked if any experiments have been conducted using preimmune animals, in which an immune response wouldbe stronger. Dr. Engler responded that they have not conducted anypreimmune animal experiments.

Dr. Parkman noted that adenovirus antibody status is not included either as an inclusion or exclusion criteria. Dr. Engler responded that he expects more than half of the participants will beseropositive, and that their antibody titers will be followed after vector injection. Dr. Engler noted that the porcine experiment showed no increase in antibody titer following intra-coronary administration of the vector; however a quick rise in antibody titer was observed following subcutaneous vector injection. Dr. Parkman said the latter is due to dendritic cell antigen presentation.

Dr. Parkman was concerned that persons with previous adenovirus infection as well as those with narrowed blood vessels may have a higher risk of developing an inflammatory response. Dr. Engler said that in human trials to date there is no specific evidence of marked immune response to the adenovirus other than those by local intra-organ injection of high dosage of the adenovirus vector. Dr. Parkman said that his concern relates specifically to the potential for a local cell-mediated immune response in patients with narrowed coronary arteries.

Dr. Engler said that the antibody titer will be measured by standard ELISA assay. The follow-up studies of plasma FGF-4 levels will be assessed at 1 hour, 1 day, 1 week, 2 weeks, and up to 12 weeks following vector administration; the schedule has been amended in the protocol after consultation with FDA officials.

Responding to the question of sample size, Dr. Engler said that the sample size was chosen based on the statistical considerations. The study is designed to detect a difference of 30 percent between patients receiving placebo versus those receiving the vector. There are 12 patients proposed for each cohort (9 receiving the active treatment patients vs. 3 receiving the placebo). The safety data regarding myocardial inflammation two weeks after vector administration will be thoroughly reviewed before moving on to the next cohort.

Dr. Aguilar-Cordova noted the sharp changes of serum FGF-4 levels observed in animal experiments. Based on these data, he suggested increasing the frequency of serum FGF-4 monitoring to a daily basis between Day 1 and Day 7.

In terms of the sample size, Dr. Aguilar-Cordova asked if three placebo patients per cohort adds to the significance of a Phase I study. Dr. Engler explained that the specific algorithm in the statistical consideration of the protocol will allow the detection of a 30 percent difference when the patient number is increased to a total of 120. Dr. Aguilar-Cordova was concerned with the small number of placebo patients in the low dose cohorts considering the invasive nature of vector administration. Dr. Engler noted that the number of placebo patients is cumulative as the trial moves up the dose cohorts. He said that the placebo group is important for safety assessment in this trial because the cardiac patients are prone to develop cardiac conditions, e.g., unstable angina, arrhythmia or even myocardial infarction; the placebo group will allow an assessment of whether these cardiac conditions are caused by the vector. Dr. Aguilar-Cordova inquired about the risk to cardiac patients from these procedures. Dr. Engler said that a diagnostic cardiac catheterization has a risk of about 0.1 percent for developing severe complications.

Dr. Parkman noted that the risk of cardiac catheterization is relatively low, and it is worthwhile to perform a second catheterization to obtain definitive information regarding the biologicalendpoints . Dr. Engler said

the sensitivity of coronary angiography to detect neovascularization is very small; the symptomatic endpoint relating to a patient's angina pectoris is the gold standard for approval of anti-anginal drugs. The proposed symptomatic endpoints include stress echocardiography and ST segment depression of EKG during exercise; these are the most sensitive tests to measure the functional endpoint of collateral angiogenesis.

Dr. Lysaught noted that safety and toxicity were not mentioned in the introduction of the Informed Consent document as the major purpose of the study. She suggested that words such as "therapy" and "treatment" should not be used. Dr. Lysaught stated that the use of placebo group in the Phase I study is not justified. Dr. Parkman said that the patients who sign up for the study will assume they have 75 percent chance of being in the treatment group. Dr. Engler explained that it is a judgment call regarding use the placebo group; it will provide useful safety information. Inclusion of the placebo group is the most efficient way to obtain preliminary information regarding safety and potential efficacy without exposing unnecessary number of subjects. Dr. Engler said that the control group is needed for obtaining any information regarding efficacy because there is a marked variability in exercise performance in patients with angina.

Dr. Markert agreed that the placebo group is a good idea. She noted that women who are fertile are excluded from the study while men can be included as long as they use barrier contraception for six weeks after vector administration. Dr. Engler responded that the concern for men is the potential germ-line transfer in the semen; requiring barrier contraception for the duration of the sperm cycle will assure no germ-line gene transfer. In women, the main concern is the effect of FGF-4 on the developing fetus. Administration of the vector to the heart could potentially produce circulating FGF-4. It is uncertain for how long the circulating FGF-4 will persist; therefore, the length of time the women are required to refrain from becoming pregnant is similarly uncertain. Dr. Engler noted that most of the women to participate in the trial will be post-menopausal; in the future, if the trial is promising and with accumulated data on serum FGF-4 levels, inclusion of women of childbearing potential will be considered. Dr. Markert was satisfied with the response.

Dr. Aguilar-Cordova said that fertile women may be included as long as they are cautioned to practice barrier contraception until their serum FGF-4 levels decrease. Dr. Lin said that the selection of the subjects in the clinical trial should be equitable. The NIH has a policy for inclusion of women in any NIH-funded clinical trials unless there is a compelling justification for precluding women. Dr. Noguchi said that it is a judgment call to determine whether the concern over an potential FGF-4 effect on the fetus is a compelling reason to exclude fertile women. He noted that women are not completely excluded from the study.

Dr. Lai asked why the vector DNA was detected in testes but not in liver or muscle in one animal when the adenovirus was administered to the left ventricle. Dr. Engler noted the route of administration is important, gonadal tissue is negative if the vector is given by the intra-coronary route. Dr. Engler explained that by the intra-coronary administration, over 99 percent of the vector was extracted from the blood circulation by the first pass through the heart. He calculated that the sensitivity of PCR analysis will not be able to detect any vector DNA outside the heart, consistent with the animal data.

Dr. Lai inquired about the results of the patients already treated in this protocol. Dr. Engler said that three patients have been studied and there have been no adverse reactions.

Dr. Ando said that it is commendable to use a placebo group in this study. He said that coronary bypass surgery itself is so painful that it tends to alleviate the anginal pain used as a study endpoint. To eliminate this placebo effect, the control placebo group is a good study design.

Dr. Macklin said that the use of a placebo group and inclusion of women can be topics for future RAC discussions of generic issues. She noted that reasonable people have different opinions regarding the use of a placebo group when the purpose of the study is to evaluate safety; she noted that the small size of the placebo group is debatable. Dr. Macklin noted that the RAC has no consensus on the use of placebo groups in gene transfer studies.

For background information regarding the issue of inclusion of women in clinical studies, Dr. Macklin noted a publication entitled: *Women in Clinical Research*, by the Institute of Medicine of the National Academy of Sciences. The book published four years ago was the result of a committee in which Dr. Macklin and Ms. Rothenberg participated. The main focus of the committee was to address the issue of enrolling women of childbearing potential and pregnant women in clinical trials.

Dr. Noguchi said that a placebo group for this protocol is a justified study design to assess safety issues; angiography has many uncertain adverse effects and a placebo group is useful to discern those effects. In terms of the issue of including women, the FDA agrees that women should be included in clinical studies.

Discussion Regarding the RAC Letter

Dr. Parkman asked a procedural question: because the protocol has already begun, what impact would it have if the RAC sent a letter to the FDA? Dr. Noguchi said that the FDA would consider any RAC recommendations. The FDA may put the protocol on hold if there is a compelling reason to alter or halt the trial; on the other hand, if there is just a difference in opinion, the FDA is prepared to allow the trial to go forward. Dr. Lin noted that the RAC letter will serve as a feedback to the IRB when it conducts the annual review of the protocol.

Dr. Lysaught stated that the introductory paragraph of the Informed Consent document should be modified to include a clear statement that the protocol is a safety study with no therapeutic intent.

Dr. Lai asked if there is a mechanism to obtain the ongoing data from the Crystal protocol (#9711-221) in order to facilitate the discussion of the present protocol. Dr. Mickelson said that there is no such mechanism. Dr. Aguilar-Cordova cautioned against using the data obtained from another protocol to make a judgment on the present protocol. Dr. Lai noted that most of the issues raised in this protocol were already discussed in the Crystal protocol. Dr. Mickelson said that the reason the protocol was voted for RAC discussion was that this discussion will complete the review of the cardiac protocols employing different gene delivery systems and different study designs for the treatment of the heart disease. Dr. Mickelson found the discussion very useful for the RAC and the public to understand this new approach to cardiovascular disease.

RAC Recommendations

Dr. Mickelson summarized the issues discussed with regard to this protocol, which would be included in the letter to the investigators: inflammatory response in preimmune animals, determination of antibodies elicited by adenovirus vector in patients, status and assessment of adenovirus antibody in the inclusion and exclusion criteria, increasing the frequency of assessing serum FGF-4 levels, reproductive and developmental consideration of vector administration, and the use of the placebo arm with small sample size. At the conclusion of the discussion, the RAC made the following specific recommendations, to be included in the letter to the investigators and other concerned bodies: (1) The RAC suggested that additional preclinical studies should be conducted using preimmune animals to assess any potential inflammatory response, because most patients will have had prior adenovirus infections. (2) Adenovirus antibody status is not included in the inclusion or exclusion criteria for this protocol, and should be

assessed.

(3) The frequency of assessing serum FGF-4 levels should be increased to include daily monitoring between Day 1 and Day 7. (4) The introductory paragraph of the Informed Consent document should be modified so that it clearly states that the protocol is a safety study with no therapeutic intent.

Human Gene Transfer Protocol 9802-235 entitled: *A Dose Escalating Phase I Study of the Treatment of Malignant Glioma with G207, a Genetically Engineered HSV-1*
PIs: James Markert, University of Alabama at Birmingham
Michael Medlock, Georgetown University, D.C.
Sponsor: Sheryl Osborne, NeuroVir, Inc., Canada
Reviewers: Gordon (presented by Ando), Verma (presented by Ando), Juengst (presented by Ando)
***Ad hoc:* Edward Wagner, University of California, Irvine (presented by Aguilar-Cordova)**

Protocol Summary

Dr. James Markert, University of Alabama at Birmingham, Birmingham, Alabama, and Dr. Michael Medlock, Georgetown University, Washington, D.C., proposed conducting gene transfer experiments on 24 patients (≥18 years of age) with malignant glioma. The vector, G207, was derived from the parental HSV-1(F) strain by deletions in both copies of the γ34.5 neurovirulence gene and a disabling insertion of the *E. coli LacZ* gene into the ICP6 region for use as an easily detectable marker, which allows for differentiation from HSV-1(F). The clinical strategy takes advantage of the virus' ability to infect and lyse cells. The first cohort will receive a single stereotactic injection of approximately 0.1 ml of G207 into a region of the tumor defined by magnetic resonance imaging (MRI). Additional cohorts will receive injections into multiple loci at doses ranging from 1×10^6 to 1×10^9 plaque forming units (pfu). The primary purpose of the study is to obtain safety information in a small number of individuals (three patients per group), with successive groups receiving escalating doses of G207 after appropriate intervals for evaluation of safety. As a secondary objective, patients will be followed serially by MRI for potential clinical response to G207.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because this study involves the first use of a replication-competent HSV vector, a modified human pathogen, for human gene transfer research; concerns related to potential toxicity to normal brain cells; and concerns about a potential immune response to the foreign proteins, (β-galactosidase and viral TK).

Dr. Mickelson noted that representatives from NeuroVir, Inc. made an informal presentation of the protocol at March 10, 1998, RAC meeting. She stated that Drs. Gordon, Verma, Juengst, and Wagner provided prior written reviews to which the sponsor responded in writing.

Review - Dr. Gordon (presented by Dr. Ando)

In his written review Dr. Gordon stated that this is a Phase I safety trial of G207, a replication-competent herpesvirus vector derived from HSV-1. This vector has both copies of the neurovirulence factor gene deleted, and has an insertion of the *LacZ* reporter gene into the ICP6 ribonucleotide reductase gene. The viral TK gene remains intact and offers a potential measure of safety by allowing retained sensitivity to acyclovir. In preclinical studies, this vector has been shown to effectively lyse, with minimal toxicity and no evidence of reactivation of latent herpes virus.

A maximum of 24 patients with glioblastoma multiforme which has proved refractory to standard treatment will be enrolled in this study. Patients will receive stereotaxic injections of escalating doses (ranging from 10^6 to 10^{10} pfu/ml). Patients will remain hospitalized and evaluated for toxic effects for 10 days following injection. Signs of toxicity to be monitored include death, stroke, hematoma requiring surgery, untreatable neurologic deterioration, unresponsive systemic infection, and disseminated HSV. Patients will be evaluated for signs of toxicity both clinically and by MRI. Vector shedding will be monitored in both saliva and blood. Patients will be evaluated 28 days, 3 months, 6 months, and 12 months after discharge. In the event of death, permission for postmortem examination will be sought.

Although efficacy of this therapy is not an objective of the study,MRI evaluation may provide incidental evidence regarding potential efficacy.

Dr. Gordon stated that this is a gene transfer protocol for an extremely serious disease in patients who have failed standard therapy. Given the desperate situation faced by these patients, the animal data available on G207, and the interest in exploring gene transfer as a new and potentially more powerful approach to treatment of malignant disease, this study is warranted. However, the following issues should be considered by the RAC: (1) This is a novel vector for clinical use. (2) This attenuated but replication-competent vector appears safe in animal studies but the potential hazards of its use, including neurovirulence and reactivation of latent herpes viruses, merits discussion. (3) This vector expresses the foreign bacterial *Lac*Z gene. This fact merits some discussion because of the potential for immune response. (4) The evaluation of toxicity merits discussion, because one parameter of toxicity is neurological deterioration, which may be the result of disease or treatment. (5) Should sperm samples be taken from male patients as part of screening for systemic dissemination of the vector?

Review - Dr. Verma (presented by Dr. Ando)

In his written review, Dr. Verma stated that glioblastomas are essentially incurable, particularly if there is a relapse after radiotherapy. Surgery may not be possible due to the location of the tumor so a relatively non-invasive procedure is the use of viruses to kill tumor cells.

Dr. Verma stated that overall this is a well-written proposal, and that considerable animal and *in vitro* data are provided. Fundamentally the main argument that the virus is notneurovirulent is a good one and as such may prevent its spread. However there are several issues that need to be addressed.

The first and foremost issue is recombination. There is a good possibility that a recombination event could occur between G207 and an endogenous herpes virus, and that this event may lead to amore virulent form. There is no mention in the protocol regarding testing the patient for herpes virus production. There is a good chance endogenous viruses might be activated, particularly because many of these patients would have been treated with radiation. This aspect is of considerable concern and needs an explanation.

The second concern is that although much of the animal testing was done with a virus titer of 10^7 pfu, the protocol involves administration of up to 10^9 virus particles. At such high titer there is no guarantee that infection of cells other than tumor cells may not occur. It would be useful to know the effect of injecting 10^9 virus particles into the cranium of the Aotus monkey.

Third, there is the concern about the potential for reactivation of latent virus. Overall most of the concerns lie with recombination with existing viruses, induction of endogenous viruses, and the ability to be

harbored as latent viruses. The good point of this proposal is that if there is any untoward effect, the herpesvirus can be destroyed by addition of GCV which is known to destroy the TK component of G207. Considering the fact that there are no other alternatives for glioblastoma, this protocol seems like a reasonable approach.

Dr. Verma noted that all of the investigators seem to be extremely competent and are capable of safely handling the vector. The Informed Consent document seems to be reasonable, because it clearly states that this is a safety study and that the patient may not receive any direct benefit. Furthermore the Informed Consent document states that this is the first use of a replication-competent herpesvirus, with the potential for unknown and untoward consequences.

In summary, Dr. Verma stated that this is a good proposal, but that there are concerns about recombination and latency which need to be dealt with before proceeding further.

Review - Dr. Juengst (presented by Dr. Ando)

In his written review, Dr. Juengst stated that this protocol was identified for discussion by the RAC because it involves the first use of a herpesvirus-derived vector in a human gene transfer protocol. The primary issues, therefore, will be questions of scientific design and clinical safety, which he is not qualified to address. Assuming that the protocol design is optimal and safe enough to merit pursuit, Dr. Juengst concentrated his comments on how the study is presented to potential subjects through the Informed Consent document. Overall, the investigators have done a good job describing the procedures and possible risks from the study, and the options of potential subjects with respect to participation. The description of the uncertainties surrounding any clinical benefit from the research is particularly clear. These descriptions have been reviewed and approved by the University of Alabama and the Georgetown University IRBs. Dr. Juengst's only concerns are minor: (1) The University of Alabama Informed Consent document should be clarified regarding the description of how the modified virus kills tumor cells. Is it the same process through which it normally causes cold sores (and herpes encephalitis), or is it a new capacity that has been engineered into the virus? The protocol suggests that the virulence is natural to HSV, and that it is only the ability of the virus to replicate that has been modified in the G207 construct; however, the Informed Consent document suggests that "the sponsor of the study has engineered the virus so that tumor cells will be killed when infected by this modified virus." The Georgetown University version of the Informed Consent document is much clearer on this point. He recommended that the University of Alabama should adopt the Georgetown University language under "the general plan of the research". (2) The Georgetown University Informed Consent document, however, is particularly difficult to follow by the use of the IRB's instructions to its authors as headings for the numbered sections. It is not clear if this is a record of the investigator's responses to a set of IRB questions, or the investigator's own description of the research to potential subjects, or a statement of the subject's understanding of the protocol.

Review - Dr. Wagner (presented by Dr. Aguilar-Cordova)

In his written review, Dr. Wagner noted that malignant gliomas are terrible, and there is no effective therapy. For this reason, heroic measures are warranted in an attempt to deal with them. The use of engineered HSV has been a method of great interest because of the virus's ability to infect neuronal tissue, its proclivity for establishing latent non-cytopathic infections in non-dividing neurons, and its high cytopathicity for dividing cells.

A major problem with the proposed study is that the virus has been engineered to reduce neurovirulence in order to enhance safety; however, in the process the virus may have lost full capacity to destroy the

target tissue. This problem was recently described in a paper co-authored by one of the developers of the test virus. This paper was not cited in the protocol (S. J.Advanil *et al*., *Gene Therapy* (1998) 5: 160-165). Because the protocol is a Phase 1 safety study, this is not a paramount consideration. The fact that the protocol is short on documentation throughout presents more of a problem. Dr. Wagner noted that any observations that might contra-indicate or add complications to the approach have not been included. A more defensible approach would be to cite the material and discuss why it is not applicable to the studies at hand. Dr. Aguilar-Cordova stated that this lapse is perhaps understandable, given the nature of the study and the way that such approaches are developed, but there are a couple of areas where Dr. Wagner has significant questions. (1) It is mentioned in passing that Dr. BernardRoizman's HSV vaccine was field tested in France about 15 years ago and found to be safe. This was taken as documentation of inherent safety in the use of HSV as a therapeutic tool. Unfortunately, there is no published formal documentation except for a number of anecdotal stories about this trial. An abstract was published in the 1992 ICAAC abstracts concerning the Pasteur-Mérieux Phase I trial with R7020. Cadoz *et al*. reported that all doses ($10^{2.2}$ to $10^{5.2}$ $TCID_{50}$) were tolerated in the seronegative subjects. Two doses were required to elicit neutralizing antibodies to HSV-1. Moderate local reactions with lymphadenopathies, fever and systemic reactions appeared after $10^{4.2}$ $TCID_{50}$ in HSV-1(+), a sufficient reason to stop trials in HSV1(+) recipients. While Richard Whitley and Bernard Roizman were listed as co-authors, they have disputed the severity of the reported findings. Dr. Wagner stated that these controversial observations should be stated in the protocol. (2) There is no mention of the fact that several laboratories have shown that removal of the (34.5 gene does not entirely abolish neurovirulence in some animal models. This is not surprising; no single gene in a virus with a genome as complex as HSV's can be expected to be the sole agent of any phenotype. Still, these findings may relate in some way to safety and it seems appropriate to include some discussion in the protocol. (3) There is a very minor point about which the IRBs of the hospitals involved do not seem very concerned. The protocol states that a single inoculation will be made, but at high doses in some projected studies the trial will involve multiple stereotactic injections of the tumor mass. The Informed Consent documents should be clarified with this regard.

These points aside, the protocol appears carefully constructed from the point of view of the medical techniques and patient safeguards to be employed, The engineered virus has usable control points which should add to its inherent safety, if not its efficacy. Dr. Wagner stated that If he were to suffer from this awful condition, he would give informed consent to participate in this protocol.

Other Comments

Dr. Aguilar-Cordova asked why a volume of 0.1 ml was chosen for the virus delivery?

Dr. Parkman inquired about the biological basis of selective lysis in brain tumor cells caused by G20 while normal neuronal cells are not affected.

Dr. Mickelson asked the investigators to elaborate on the function of the (34.5 neurovirulence gen

Dr. Louise Markert inquired as to the maximum volume of vector that can be safely injected into the bra

Dr. Ando inquired about the safety issues involved with using an replication-competent virus for cancer therapy.

Dr. Parkman asked how a herpesvirus vector, which is neurotropic , would be developed into a vehi the treatment of monogenic disorder affecting the central nervous system. Would a replication-competent virus be better than a replication-incompetent virus for this application? Dr. Aguilar-Cordova said that

replication-competent but attenuated virus is a better choice for cancer therapy.

Investigator Response - Ms. Osborne, Drs. Tufaro , James Markert , and Jeffrey Os

Ms. Osborne stated that Dr. Tufaro will address the question of the biological basis for specificity an issues of recombination, reactivation, and neuroattenuation . Dr. James Markert will address the clir issues of injection volume, toxicity, and disease progression. Dr. Jeffrey Ostrove will address the issue biodistribution of G20

Dr. Tufaro explained the biological basis of specificity. Two genetic changes have been made in G207 order to attenuate it and still allow it to replicate in brain tumor cells. The normal neuronal cells can be infected but the deletion of the γ34.5 neurovirulence gene makes the virus incapable of furthe perpetuating and spreading in neuronal cells. The γ34.5 neurovirulence gene encodes a protein tha reduces the antiviral effects that cells normally turn on in response to virus infection. Normally after infection of a cell, the protein synthesis machinery of the cells is shut down due to phosphorylation of eukaryotic elongation factor. The γ34.5 neurovirulence gene product temporarily suspends tha phosphorylation to allow the virus to propagate in the cell. HSV deleted γ34.5 gene is attenuated and it does not cause disease in animal models, e.g., mouse and Aotus monkey. The second inactivati of the ribonucleotide reductase gene provides an additional safety feature of discrimination of dividi non-dividing cells.

Dr. Parkman asked how neurovirulence relates to differentiation of malignant vs. nonmalignant cells? D Tufaro explained that glioblastoma cells are not derived from neurons. When a glioblastoma ce infected with G207, the virus replicates efficiently and spreads to the surrounding tumor cells. When the virus encounters neurons at the edge of the tumor mass, the neuronal cells get infected but the virus has an eclipse in neuron.

Dr. Lai said he had difficulty visualizing how the virus establishes target cell specificity. Dr. Tufaro explained that the virus is capable of infecting a variety of cells, but that the virus is targeted to the tumor by direct stereotactic injection into the tumor mass. Dr. Lai asked in terms of the wild type HSV wha of cells are infected when the virus is injected to the brain. Dr. Tufaro explained that both glial cells neuronal cells are infected, and if the virus is in the ventricle, ependymal cells are infected as well. H can also track along the neuronal pathways to the peripheral nervous system. When the attenuated G207 is used, the virus infects cells at the needle track but does not spread away from the track. When a high dose of G207 (10^7 virus particles) was injected into the mouse brain, it caused no toxicity. Dr. Tufaro sa that wild type HSV can infect most cell types and stimulate them to divide and finally cause cell lysis in neurons does the virus persist and cause latent infection.

Dr. James Markert said that wild type HSV can cause a hemorrhagic encephalitis which will kill all c the brain. G207 is deleted in γ34.5 and the ribonucleotide reductase genes and cannot replicate in r cells, but it can replicate in tumor cells or dividing cells because these cells provide *in trans* the missing gene functions of G207. When G207 is injected into mouse brain, it does not cause any clinical effects. Dr. Lai asked what type of cells in which the G207 replicates in the brain? Dr. James Markert responde that there are only low levels of virus replication in oligodendrocytes and glial cells. In glioma c however, G207 replicates efficiently and causes cell lysis

Dr. Aguilar-Cordova noted that different values of LD_{50} of HSV were reported in the literature. Dr. Jame Markert explained that there is a lot of difference in neurovirulence between different strains of HS in many studies from different laboratories.

Dr. Lai asked if the released virus will harm normal tissue after killing the glioma cells by G207 infection. Dr. Tufaro responded that they have studied the toxicity of G207 in the very sensitive Aotus monkey model. After injection of a very high dose of G207 intracranially, no toxicity was noted even though the vector DNA was detected by PCR analysis; it is essentially an eclipsed genome that does not harm normal tissue. There is no known natural passage for the virus to spread from the brain to other peripheral tissue. Dr. Parkman asked if the residual virus can be eliminated with a systemic administration of GCV. Dr. Tufaro said it is not part of the protocol, but if there is any indication of encephalitis, GCV administration could be an option.

Dr. Wolff asked what is the mitotic index of the tumor cells. Dr. James Markert responded that at any given time point the dividing tumor cell population is approximately 10 percent. Dr. Wolff noted that the efficacy of the gene transfer will depend on the balance of how fast the cells are dividing and how fast the host system is eliminating the virus. Dr. James Markert said the clinical outcome will be evaluated from the data to be gathered from the study. He noted that at the high dosage cohorts, multiple injections (up to 10 sites) are used to facilitate spreading of the virus to the entire tumor mass.

Dr. Mickelson asked how much damage would be expected to the normal tissue surrounding the tumor by infection of a large amount of virus amplified by its replication within tumor cells. Dr. James Markert responded that he expects little damage to normal tissue based on his observation of no pathology at the site of injection with a high dosage (10^9 pfu) of G207 in Aotus monkeys. Dr. Mickelson asked what is virus to tumor cell ratio in the proposed study. Dr. James Markert explained that the protocol calls for an area of tumor of at least 1 cm in size, i.e., 10^{11} cells. It would be approximately one virus particle per 10 cells at the highest dosage.

Dr. Parkman was concerned about potential inflammatory response in the closed space of the brain after injection of G207, especially in patients preimmune to the virus. Dr. James Markert noted that brain edema is a severe toxicity in mouse experiments because the brain space of the mouse is very small. For brain tumor patients he anticipates less of a concern because most patients are on steroids (immunosuppressive medication) and the glioma patients usually have profound immune dysfunction due to secretion of immunosuppressive cytokines, e.g., tumor growth factor-β (TGF-β) by tumor cells. In the Informed Consent document, the patients are advised about the potential for developing brain edema and if needed, higher dosage of steroid will be used or brain debulking surgery performed to relieve the symptoms. Dr. Parkman asked if the patients will be pre-screened with an *in vitro* blastogenic assay to assess the patients for the likelihood of reaction to the vector. Dr. James Markert responded that in brain tumor patients a stronger immune response against tumor cells is beneficial. If there is any brain edema caused by inflammatory response, there are routine medical measures to deal with this complication.

Dr. Wolff asked about the vector biodistribution studies using a vector expressing the *LacZ* marker protein. Dr. Ostrove explained that in mouse and Aotus monkey experiments a low level of β-galactosidase was detected at the needle track one day after intracranial injection of the virus, an indication that the virus had not spread to normal brain cells; however, the viral DNA could be detected by PCR analysis up to 2 months following virus injection. Dr. Wolff noted that the lack of β-galactosidase expression is unexpected. He expressed concern about a possible immune reaction against normal neurons with persistent virus infection.

Dr. Lai asked if infectious virus can be recovered from the tumor cells in tumor-bearing mice injected with G207. Dr. James Markert said that infectious virus could be recovered at 14 days; however long term data are unavailable. Dr. Lai was concerned about possible long-term toxicity due to the persistence of infectious virus in residual tumor cells. Dr. Tufaro said that data published in 1994 demonstrated no persistent infection or inflammation in rats out to two years following administration of a HSV virus simi

to G207. These animals demonstrated complete cure of tumors.

Dr. Lai asked if there is any evidence of reactivation. Dr. Tufaro responded that in animals previousl infected with wild type HSV in the brain, virus reactivation was not observed after a second injection wi G207. In the clinical protocol, virus reactivation will be studied in patients treated with G207.

Dr. Louise Markert applauded the inclusion of a provision in the University of Alabama Informed Conse document for obtaining a Durable Power of Attorney in the event that a patients decision-making ability is impaired during participation in the study. Ms. Osborne said the sponsor has used this Informed Consent as a model for other sites, but it is institutional policy to have this specific language included in their Informed Consent document.

Dr. Macklin noted that the University of Alabama Informed Consent document states that the study sponsor will reimburse subjects for costs associated with medical treatment for an injury resulting from participation in the study. Ms. Osborne said that the sponsor will cover the costs even though the university will not. Dr. Macklin noted that such a statement of sponsor reimbursement is missing from the Georgetown University Informed Consent document. Ms. Osborne said she does not know why Georgetown University does not require such a statement.

Dr. Lai asked how many protocols using replication-competent viruses have been registered with ORDA Dr. Shih responded that the present herpesvirus protocol and the adenovirus protocol (#9802-236) to b discussed in the next day's session of the RAC were the only two protocols using replication-competent viruses for gene transfer research that were registered with ORDA . Dr. Noguchi noted there is anothe adenovirus protocol using an E1B-deletion mutant of adenovirus (Onyx-015) for cancer therapy that has been conducted outside the scope of the *NIH Guidelin*for human gene transfer. Dr. Lai stated that the use of a replication-competent virus in the present protocol raised several novel issues for RAC discussion.

Dr. Lai was concerned about the target cell specificity of G207. Dr. Tufaro explained that G207 is conditional replication mutant similar to Onyx-015 (a hundred-fold preferential replication in tumor cells with p53 mutations versus cells expressing wild type p53). The same magnitude of specificity to tumor cells (approximately a hundred-fold) can be demonstrated with G207 in dividing vs. non-dividing cells or in neuronal vs. tumor cells. Dr. Lai asked about the biological basis for conditional replication. Dr. Tufaro responded that the mechanism for the biological basis of the specificity is not completely understood. He emphasized that lack of toxicity in Aotus monkeys at a dose of 10^7 plaque forming units (pfu) indicates wide discrimination between tumor cells and normal cells.

Dr. Mickelson asked how the investigators are going to explain to patients about the specific toxicity of G207 to their tumor. Dr. James Markert emphasized that the biological basis of specificity is no completely understood; however, patients will be told that there is a relative degree of sparing of normal brain tissue and that the virus seems to divide preferentially in tumor tissue. The virus has safety features built into it; should there be any evidence of viral encephalitis, G207 can be eliminated by subsequent systemic ganciclovir or acyclovir administration. In addition, the virus is temperature-sensitive and if patient develops a fever greater than 39.5^{o} C, virus replication will be inhibited.

Dr. Noguchi stated that the FDA has benefitted from the 's discussion of this herpesvirus protoco especially the discussion of the latency of herpesvirus infection. He noted that neurovirulence i common concern for attenuated viruses; he cited live polio vaccine virus as an example for which the biological mechanism is also not completely understood.

Dr. Aguilar-Cordova asked how many patients have been treated in this study. Dr. James Markert responded that six patients have been treated and no adverse events were observed related to the agent.

Dr. Louise Markert asked what are the investigat thoughts about the RAC sending a letter regarding the RA's discussion. Dr. James Markert said that the letter should clearly state that it is for informat purposes and not to cause unnecessary alarm to the Institutional Review Board if there are no serious concerns over the protocol. Dr. Noguchi said that the FDA favors more disclosure and the letter is a good mechanism for relaying the important isues that have been discussed. Dr. Parkman noted that th investigators responded satisfactorily to most of the questions raised by the RAC, and that the letter will convey to the investigators the consensus of the RA's discussion

Dr. Mickelson summarized the major RAC discussion points that will be included in the letter to the investigators and other parties: (1) the biological basis of selective tumor cell lysis caused by injection G207; G207 shows greatly enhanced replication in dividing cells compared withresting or non-dividin cell lines. The sponsor explained that the specificity for brain tumor cells was mainly due to deletion of both copies of the ¥34.5 neurovirulence gene. The mechanism of action of ¥34.5 gene is not well understood. A disabling insertion of the *E. coli LacZ* gene into the ICP6 (viral ribonucleotide reducta region provides an easily detectable marker, which allows for differentiation from the parental laboratory strain, HSV-1(F). Safety studies in the Aotus monkey demonstrated no toxicity. The precise mechanis for tumor cell specificity is not completely understood. (2) The potential for an inflammatory response to G207 when injected into the closed space of the brain. (3) The immune status of the patients to HSV , (the potential for virus reactivation, and (5) the potential antigenicity of vector expression of the foreig bacterial *LacZ* gene. The RAC endorsed the inclusion of a provision in the Informed Consent document for obtaining a Durable Power of Attorney in the event that a patients decision-making ability is impaired during participation in the study. Dr. Mickelson noted that the sponsor and investigators have adequately responded to all of the questions raised by the RAC.

RAC Recommendations/Comments

Based on the discussions, the RAC was satisfied with the responses provided by the investigators and sponsor; therefore, there were no specific recommendations.

RAC Forum for Replication-Competent Viruses

Dr. Lai suggested a formal discussion on the generic issue of replication-competent viruses. Dr. Mickelson stated it is useful to have a forum to discuss the development of different classes of vectors tha are replication-competent. Dr. Noguchi suggested that such a forum should include vaccine developments that employ live attenuated viruses.

Germ-line Gene Therapy

Dr. Louise Markert noted that a recent conference on the subject of germ-line gene transfer was held a the University of California Los Angeles (UCLA) on March 20, 1998. She raised the issue of whether the *NIH Guidelin* should be amended so that the RAC could begin discussion of the important scientific and ethical issues surrounding germ-line gene transfer. Currently, the *NIH Guidelines* state: "[the] RAC will not at present entertain proposals for germ-line alterations but will consider proposals involving somatic cell gene transfer. The purpose of somatic cell gene transfer is to treat an individual patient, e.g., by inserting a properly functioning gene into the subject's somatic cells. Germ-line alteration involves a specific attempt to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring." Dr. Mickelson noted that some o

the safety issues including the potential for germ-line alteration will be addressed at the Gene Therapy Policy Conference on *in uter* gene transfer in December 1998. Dr. Aguilar-Cordova noted that the possible toxicity of germ-line alteration related to *in uter* gene transfer is a separate issue from intentional germ-line gene transfer. The latter is a much broader issue.

Dr. Parkman stated that the RAC could initiate a process by which *ad hoc* experts are invited to "educate" both the committee and the public on the scientific, safety, and ethical implications of germ-line research. However, he cautioned against revising the *NIH Guidelin* regarding this issue, as revised language could given the unintended impression that the RAC and the NIH endorse the submission of clinical tria

For the public record, Dr. French Anderson (USC) stated that the RAC should not change the wording the *NIH Guidelin* nor initiate a discussion of entertaining germ-line gene transfer proposals. Public discussion of this issue could be misinterpreted as an endorsement to proceed with such trials. Dr. Anderson said that several prominent scientists including Dr. James Watson (Cold Spring Harbor Laboratories, New York) at the March 1998 UCLA meeting suggested that the RAC consider germ-line gene transfer. Dr. Mickelson stated that she has consulted with Dr. John Fletcher, a participant of the UCLA conference, about the meeting. Although there was a policy recommendation for the RAC to change the *NIH Guidelin* in this regard, this recommendation did not represent the consensus of the group, but rather, represented personal opinions of a few participants.

Dr. Lysaught commented that the RAC should not give the appearance of being proactive regarding it "entertainment" of germ-line gene transfer protocols; the public reaction will be quite different from the opinion of some scientists.

Dr. Noguchi stated that the FDA welcomes public discussion of the issue before such protocols are submitted, noting that "we [the FDA] cannot ban anything." He encouraged the RAC to establish an appropriate mechanism to conduct public discussion of the issue without the appearance of endorsement.

Dr. Aguilar-Cordova stated that the RAC is the appropriate forum to discuss the implications of germ-line gene transfer research. He suggested RAC discussion of the issue, but not necessarily a change to the *NIH Guidelin* Dr. Parkman agreed.

Dr. Macklin said that the way in which the RAC approaches this issue is important. The RAC should invite speakers with balanced viewpoints and discuss the issue in an objective atmosphere; she noted criticisms being raised against the National Bioethics Advisory Commission on its discussion of the human cloning issue.

Dr. Anderson noted that the RAC is an ideal place to address the issue of germ-line gene transfer to discuss the science of germ-line gene transfer; however, the discussion should not result in changes to the NIH Guidelines or a reversal of the 's moratorium on "entertaining" germ-line gene transfer clinical trials. In response to Dr. Noguchi's concern regarding possible submissions of germ-line protocols to the FDA in the future, Dr. Anderson suggested that any Investigational New Drug (IND) applicatio submitted to the FDA involving germ-line gene transfer should be deferred to a public Federal advisory committee and the RAC. Dr. Noguchi said that both the FDA and the RAC should be prepared for such a scenario well in advance of a formal submission to the FDA. The RAC is the ideal forum for having a societal debate of the germ-line gene transfer issue. Ms. Knorr suggested if the RAC decides to condu discussion on this issue, a carefully crafted *Federal Register* notice outlining the specific objectives of such a discussion would clearly articulate the RA's intention so as not to be misconstrued to indicate a change in RAC policy. Dr. Lysaught said that the RAC discussion should not be limited to scientifi issues and should cover the social and ethical issues as well.

Dr. Anderson announced that he and Dr. Esmail Zanjani intend to submit two prototypical protocols *uter*gene transfer at the September RAC meeting. These protocols involve: (1)*ex vivo* autologous feta hematopoietic stem cell transplantation for " thalassemia , and (2) direct fetal intraperitoneal injec noted that the issue of inadvertent germ-line gene gransfer may be raised as a potential effect of one o both of these pre-protocols. Ms. Knorr noted that RACdiscussion of these protocols would most like handled in a format different from fully developed protocols. She anticipated that such discussion would be considered a "first step" in initiating an ongoing public dialogue of the science, safety, and ethics of in utero gene transfe

In conclusion, the RAC endorsed Dr. Parkman's recommendation to invite numerous ad hoc experts as part of an ongoing educational process and in accordance with its mandate to ensure public awareness of the scientific, safety, and ethics issues related to germ-line gene transfer. However, the RAC agreed that ***such discussions should not be viewed as an endorsement of germ-line gene transfer*** and that the intent of this process should be clearly articulated to the public, perhaps as a statement published in the *Federal Register*.

Human Gene Transfer Protocol 9804-244 entitled: *A Phase I Study Using Direct Combination DNA Injections for the Immunotherapy of Metastatic Melano*
PI: Patrick Walsh, University of Colorado
Reviewers: Markert , Mackl
Ad hoc: **Robertson Parkman, Children's Hospital of Los Angeles**

Protocol Summary

Dr. Patrick Walsh, University of Colorado Health Sciences Center, Denver, Colorado, proposed conducting gene transfer experiments on 18 patients ($8 years of age) with metastatic melanoma. Th therapeutic DNA/liposome formulation, C192, contains equal weights of purified plasmid pMB287 expressing the human Interleukin-2 (IL-2) cDNA and plasmid pMB288 expressing the superantige staphylococcus enterotoxin B (SEB). Both of the gene inserts are expressed under the control of a promoter. This protocol is a Phase I study to evaluate the safety of C192 in subjects with metastatic melanoma. Three subjects will receive one of six escalating doses (10, 100, 250, 500, 1,000, and 2,000 µg) of C192. Subjects will receive direct injection of the plasmid DNA coding for IL-2 and SEB int cutaneous melanoma metastasis. Subjects will also be monitored for any potential clinical effect at th site of injection and local or distant metastasis.

The Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because this study represents the first use in a clinical trial of the gene encoding SEB

Review - Dr. Marker

Dr. Markert stated that the goal of this research protocol is to investigate safety and to identify an toxicities associated with injection of plasmid DNA encoding IL-2 and SEB . The genes are injected a cationic liposome/DNA complexes. The investigators have chosen patients with metastatic melanom who have a very poor long-term survival rate. The study will also examine gene expression in tumor tissues and look for plasmid DNA in the circulation after injection. The investigators will examine anti-tumor immune responses and characterize the clinical responses.

Background

SEB is a classic superantigen in that it binds to both major histocompatibility complex (MHC) cla molecules and to the β chain of the T cell receptor (TCR). All T cells carry a TCR . Approximately percent of TCRs are composed of α and a β chain. There are approximately25 families of β chains based on the sequence of the variable region of this chain (Vβ). A given superantigen binds to a limite set of Vβ families. Binding of a superantigen to a TCR results in activation of that T cell and generat cytotoxic activity. Systemic administration of superantigens leads to toxicity secondary to massive T activation and cytokine release. The rationale for this protocol is that expression of a superantigen by tumor cell will result in locally high concentrations of superantigen and thus activation of T cells in tha area only which will kill the tumor cells (in part via release of tumor necrosis factor).

To develop stronger immune responses to tumors, the investigators are using co-stimulating molecules. IL-2 provides a strong second signal to T cells to prevent them from undergoing apoptosis. In animal models the use of IL-2 transfected tumor cells leads to local anti-tumor responses but does not genera immunity to challenge with tumor later. Superantigens are another type of stimulating molecules whic can activate up to 30 percent of T cells. With both signals together, the superantigen should signa through the T cell receptor and IL-2 would then function as a second signal. It is hoped that some specific anti-tumor activity may be elicited in the T cells.

Animal studies have found the combination of IL-2 and superantigen to be more effective tha superantigen alone, so the combination is being used in this protocol. The investigators report that som tumor models in mouse models regress completely with this therapy. Of relevance to this study, the investigators have studied pet dogs with spontaneous melanoma. Seven of 11 dogs treated with the DNA therapy achieved partial or complete remissions.

Gene Vector

The SEB gene is the focus of the review. Plasmid pMB288, which expresses SEB , is based on a co laboratory plasmid, pUC . It contains the hCMV -IE promoter/enhancer, a chimeric intron splice site from human β globin gene, the splice site from a human immunoglobulin gene intron , and the SV early polyadenylation site. A number of issues regarding gene expression and vector distribution hav been addressed in preclinical animal studies. Antigen expression has been found in 10-20 percent of injected tumors. CTL activity has been elicited in mice treated with this construct. There are approxima 9×10^{12} copies of plasmid per 50 µl of solution. Although much of the plasmid may be lost in the injection, copy numbers per cell after injection may be as high as 10^3/cell. The plasmid remains extrachromosoma The expression is maximal at 48 hours and is much decreased to undetectable levels by 72-96 hours. Injections in animals have shown transient inflammation of the injection site by the empty vector or the gene-containing vector. No vector has been detected in the gonads at two or seven days after the final injection in mice.

Study design

Three patients will be treated with each of six doses of liposome complexed plasmid DNA. Injections w occur every other week for a total of 6 treatments during 12 weeks. Up to three tumor nodules will be injected. In addition, if possible, the nearest draining lymph node will be injected. The initial dose is 5 µg of each plasmid. The dose will escalate to 1000 µg of each plasmid.

Measurements

SEB production will be assessed using polyclonal SEB antisera and immunocytochemistry o biopsy sections. Gene transfer into tissues will be assessed by PCR of plasmid DNA. Revers transcriptase (RT)-PCR will assess gene expression. CTL and T cell proliferative assays wi conducted on peripheral blood mononuclear cells (PBMC) to see if circulating T cells developed that ar reactive to the tumor. Monoclonal antibody analysis of TCRβ composition of T cells infiltrating tumor will be done to see if the Vβ families expressed are consistent with SEB stimulation. A variety of measures be recorded to document effect of treatment on tumor size, progression, and time to death. Semen samples are not being examined.

Critique

This is a novel approach to stimulate immune responses to tumors. The use of superantigens , althoug new, is similar to other approaches in that the investigators hope to activate T cells to kill tumor cells with this approach. As a Phase I study, the investigators present their research goals in a clear fashion. The concerns of the RAC with respect to the use of a new gene are adequately covered in the proposal.

One weakness of this proposal is the lack of detailed animal data regarding the development in PBMC MHC -restricted CTL or proliferative responses to the tumor cells. It is unclear if these tests are a developed in the investigator's laboratory. In addition, with the small numbers of patients in this study, the investigators have not discussed whether it is even possible to see any increase in CTL activity given t standard deviations in response expected. This weakness, however, is in only one set of the aims of this study, and should not detract from the overall quality of the project.

A second weakness of the proposal is lack of a control arm in which empty vector (instead of the vector containing SEB) is administered. This issue may have been addressed in animal studies. Given the co of production of clinical grade vector, it may not be feasible to do this control in humans.

Review - Dr. Macklin

Dr. Macklin addressed the following ethical considerations in the protocol: recruitment of subjects, risk/benefit ratio, confidentiality protections, Informed Consent document, and other ethical considerations.

Recruitment of Subjects

The protocol does not provide details of the recruitment process. However, the response to Appendix M-II-C-3 of the *NIH Guidelin*says that "public notices of the clinical trial will be displayed in clinics throughout the Denver area." The protocol states that "the referring physician, the attending physician, and surgical oncology or the relevant clinical department, the patient, and family members will make a joint decision regarding the appropriate treatment with conventional therapy." Dr. Macklin raised several questions. (1) To whom are the public notices addressed --patients who are prospective subjects or their physicians? (2) Are the "referring physicians" those caring for the patients with melanoma? (3) Who will actually approach the patients to ask whether they are interested in participating in the trial?

Risk/Benefit Ratio

The risk/benefit ratio is favorable, as the disease is fatal for which no completely effective alternative therapies exist. No other experimental therapy has proven to have a significant effect on the natural progression of the disease; other experimental therapies have led to significant morbidity and even mortality. Possible beneficial effects of this intervention are that treated tumor nodules may regress

completely and patients may develop systemic tumor immunity that leads to regression of other, non-treated tumor nodules. A possible adverse effect is the development of significant inflammation in or near the treated tumor nodule(s). If significant inflammation develops in or near tumor nodules, th experimental procedure will be suspended and treatment for the inflammation will be considered. Other side effects described in the Informed Consent document may cause considerable discomfort, but do not appear to be life-threatening.

Confidentiality Protections

Steps to protect confidentiality are not described in the protocol; however, the Informed Consent document promises the usual confidentiality protections.

Informed Consent Document

The Informed Consent document is generally clear and written at a reasonably acceptable level of comprehension. However terms such as "surgically excised" or "excision" should be replaced by "surgically removed." A couple of statements in the Informed Consent require elaboration or clarification. (1) "In mice with melanoma, this drug can treat melanoma if it is used in the manner to be tested in this trial for safety." What does it mean to say the drug "can treat melanoma"? The statement could be interpreted to mean "successfully treat melanoma," and might be rephrased to avoid that implication. (2) "…the injections may stimulate immunity against some of your tumor cells.." What is the net effect of this stimulation of immunity?

Other Ethical Considerations

(1) The Informed Consent document mentions HIV testing prior to enrollment. Will pre- and post-test counseling be provided?

(2) The Informed Consent document states that if subjects are injured by the research they will have to pay for any needed care. However, it is customary for researchers conducting a clinical trial to provide treatment for short-term medical care and immediate treatment for injury without cost to the research subjects. Could such medical care be provided in this study in case of injury? If it cannot, the statement that subjects will have to pay for medical care in case of injury should be placed in a separate paragraph in the Informed Consent, under a separate heading. The statement must not be embedded in a paragraph under the heading "Steps for minimizing risks."

(3) Other information in this same paragraph should also not be placed under the heading "Steps for minimizing risks." The information about financial value from any permanent cell lines developed also belongs in a separate paragraph. The information that describes confidentiality protection can be deleted, as it appears in the paragraph regarding confidentiality at the bottom of the page.

Review - Dr. Parkman

Dr. Parkman said that the animal model analogous to the human tumor for the preclinical studies should be an animal that already has a tumor burden. Dr. Parkman noted that most of the studies were performed with primary genetically modified B16 cells. What was the impact of the injection of the transduced B1 cells into animals already bearing non- transduced B16 cells? Usually one likes to see a immunotherapeutic benefit in an established tumor model because it is the preclinical model for the proposed clinical studies, rather than just a change in the immunogenicity of the transduced ce

Dr. Parkman noted that most of the preclinical studies involved combination of the superantigen SEB granulocyte-macrophage colony stimulating factor (GM- CSF) and contained very little data o combination of SEB with IL-2 as proposed in the clinical protoco

Dr. Parkman asked the investigators to explain how SEB would function as a co-stimulating molecule i the immunotherapy strategy. Is the mechanism analogous to the other well studied co-stimulating molecule B7 (CD80)? What is the normal immunological response to SEB in skin testin

Investigator Response - Dr. Walsh

In response to Dr. Markert's question of assays for immune response, Dr. Walsh said that autologou tumor cell lines will be established from pretreatment biopsy specimens for the CTL assays. The succe rate of establishing the cell lines is only about 80 percent; therefore, peripheral blood mononuclear cells will be collected and frozen at the beginning and at several time points after trial initiation in order to do the CTL assays if the autologous tumor cells are successfully established. β skewing will be assessed to see whether particular subsets of T cells are being expanded or deleted. Dr. Walsh said that his IRB suggested for safety reason to include only patients who are serologically positive for antibodie to SEB because those patients have been found to be less susceptible to the toxic shock syndrom induced by SEB . Dr. Walsh said that when humans are exposed to the superantigen , the first reacti local Vβ skewing and the superantigen can actually anergize the patients so that the stimula lymphocytes will undergo apoptosis and are eliminated. Therefore, the peripheral blood normal TCRβ repertoire will be assessed in order to determine if there is any Vβ skewing after exposure to the superantigen

Dr. Markert noted that the exact time course of Vβ changes is not known and collection of only two peripheral blood samples may not be adequate to detect the changes. Dr. Walsh agreed to do more frequent sample collections.

Dr. Parkman noted that the proposed CTL assays only assess the precursor cells because the T cells stimulated and expanded *in vitro* before the assay. Dr. Parkman said that the CTL assay should includ assessment of both the circulating CTL as well as the precursor CTL in the blood because the m presence of precursor cells does not necessarily indicate there are circulating mature T cells. Dr. Walsh responded that he would perform both assays for precursor and existing T cells. He explained that the analysis of TCRβ repertoire will allow determining the broad population of cells that are capable of reacting with the superantigen , and by doing this at various time points one should be able to determin the fate of those cells. Dr. Parkman said that in addition to the precursor assay, it would be better to see whether there is any circulating CTL by performing an assay against chromium-labeled melanoma cell without *in vitro* expansion. Dr. Walsh agreed to perform the assay suggested by Dr. Parkman. Dr. Parkman noted that a success rate of 80 percentin establishing autologous tumor cell lines is g enough to perform the CTL assays, and that there should be adequate amount of blood available fro adult patients for this type of assay.

Dr. Markert asked why only one biopsy is planned for the study. Dr. Walsh responded that multipl biopsies would remove most of the tumor mass and hence any positive effect would not be able to be determined.

Dr. Parkman said that melanoma sometimes responds to an antigen such as Bacille Calmette -Gue (BCG); therefore, it is important to assess the anti-tumor effect of distant metastasis. Dr. Parkman said inclusion criteria should include patients with at least two cutaneous lesions so that effects on distan metastasis can be studied in all patients. Dr. Walsh responded that the primary goal of the Phase I study

is to assess toxicity not efficacy, and it is too limiting in a Phase I study to include only those patients who have at least two lesions. Inclusion of patients with metastatic melanoma can be considered in the futu Phase II study. The major endpoint for this study is the immune response.

Dr. Aguilar-Cordova asked if the stimulation by a superantigen will increase the risk of tumor metastasi Dr. Walsh explained that the function of the superantigen is not similar to the co-stimulating molecule such as B7 (CD80). The major points of the present study are the massive stimulation with a superantigen , and in the presence of the second IL-2 signal to eliminate the possibility of anergy . whole point of immunotherapy with the superantigen is to break the tolerance to tumor associate antigens. Dr. Walsh said that there is no evidence of any tumor growth stimulation in animals treated with superantigens

Dr. Ando asked if different ratios of SEB to IL-2 in the combination therapy might produce a differen outcome? Dr. Walsh responded that there are no data examining the effects of varying the ratio, but the combination of SEB and GM- CSF works better than either factor al

Dr. Aguilar-Cordova asked if any TCR β skewing has been observed in animal studies. Dr. Walsh responded that there is no evidence of any elimination of a particular Vβ subpopulation of the peripheral blood in the animal studies. The Vβ skewing assay is to see whether there is any change in the relative ratios of the various TCRβ subpopulations over different time points after SEB administratio

Regarding the biological basis of SEB immunotherapy, Dr. Walsh referred to a publication by Steven W Dow *et al.*, *In Vivo* Tumor Transfection with Superantigen plus Cytokine Genes Induces Tu Regression and Prolongs Survival in Dogs with Malignant Melanoma, *J. Clin . Invest. 1*2406-2424, 1998. He explained that when both genes are administered to the animal, both protein products are secreted to the microenvironment of the transduced cells. An inflammatory response ensues includin generation of CD4+ and CD8+ T cells. The second stimulating IL-2 signal prevents anergy of stimulate lymphocytes and it may provide a signal for natural killer (NK) cells as well. In addition, there may b nonspecific NK cells stimulated by cytokine releas

Dr. Wolff asked about the transduction efficiency. Dr. Walsh explained that in*in vivo* experiments of intra- tumoral injection the tumor cells immediately adjacent to the needle track are positive for express of a reporter gene. The number of cells transduced depend on the number of injections to the tumor m approximately 1 percent of cells are transduced

Dr. Lai asked about the potential toxicity of SEB secreted after transduction. Dr. Walsh responded tha there has been no evidence of systemic toxicity in the dog experiment because the amount of SEB produced is very small and localized. Similar lack of systemic toxicity was observed in the more sensitive rabbit experiments performed by other investigators.

Dr. Parkman said that most animal studies in mice or dogs were performed with the combination of GM- CSF and SEB rather than IL-2 and SEB . Dr. Walsh said their recent data suggest the comb SEB with IL-2 is more effective than with GM- CSF . However, he did not bring the IL-2 data with him he would provide the data if requested. Dr. Parkman said that such data should be included in the protocol. Dr. Walsh agreed.

Dr. Aguilar-Cordova asked what percentage of melanoma cells are MHC Class 2 positive, and whethe SEB needs a second signal for its action. Dr. Walsh responded that GM- CSF is to turn on the anti presenting cells. SEB is cable of inducing immune response by itself and IL-2 is to provide the secon signal to prevent anergy and to generate a more vigorous response. Dr. Parkman asked for th

quantitative difference between SEB combination with either IL-2 or GM- CSF especially for anti-tu effect on distant metastasis. Dr. Ando said that the data included in the protocol suggested better local response in combination with IL-2 than with GM- CSF but no data on metastatic tumors were inclu

Dr. Markert said that the superantigen may turn on the cells carrying a part-ul ceptor and release massive amount of cytokines. The dendritic cells will present the tumor antigens from the dying cells ar induce CTL that are not necessary specific to th with which the superantigen interacts. Dr. Wals agreed that the major function of the superantigen is to break the tolerance as opposed to generatin specific responses and allowing the immune response to become effective against melanoma.

Dr. Aguilar-Cordova asked Dr. Walsh to explain the difference between his study and that of Dr. Gary Nabe's study involving HLA-B7 immunotherapy (Protocol #9202-013). Dr. Walsh said that the HLA-B7 protocol is to elicit allogeneic immune response against tumors while the superantigen is a nonspec stimulation of immunity by breaking tolerance. The latter approach is more broadly applicable. Dr. Aguilar-Cordova noted that toxicity seen in the HLA-B7 protocol was minor and he expects it should be the same for the present protocol.

Dr. Markert asked if any skewing of cells occurred in the dog study. Dr. Walsh responded that skewing was detected only in the local area around the tumors; no skewing of cells was evident in the peripheral blood. Dr. Markert noted that the observation is consistent with the lack of systemic toxicit

Dr. Parkman suggested that an assay using TCR antibodies could be used to block the CTL respon in order to determine if the response correlates with a specific V family. Dr. Walsh agreed to include the blocking assay in his protocol.

At the conclusion of the RAC discussion of this protocol, Dr. Mickelson summarized the discussions and noted that the investigators responded in writing prior to the meeting to most questions raised in written reviews. The additional discussion points include assessment of circulating T cell repertoire (precursors as well as existing CTLs), the preclinical data using combination of SEB with GM- CSF or IL-2, in of patients with distant metastasis, thebiology of action involving SEB , and clarification of the Infor Consent document regarding both the patient's financial responsibility for adverse events and the use of autologous tumor cell lines. She noted that Dr. Parkman suggested a T antibody blocking experiment.

RAC Recommendations

The RAC made the following specific recommendations, to be included in the letter to the investigators and other concerned bodies (1) The RAC recommended that the Informed Consent document should be modified for the purpose of clarifying: (a) the use of autologous tumor cell lines, and (b) the patien financial responsibility for any costs associated with treatment of potential adverse events. The investigator agreed to clarify these issues in the Informed Consent document. (2) The RAC recommended that the protocol should be amended to include evaluation of subjects' circulating T cell repertoire prior to treatment and the TCR repertoire in the prestudy tumor biopsies. In addition, more frequent pbl after the treatment should be studied for TCR repertoire (not just week 6 and 12). As the investigator have pointed out, it is unknown what effects there will be of treatment with SEB on circulating T repertoire in the human.

Compensation for Injured Research Subjects

Dr. Mickelson noted that in response to the RA's earlier discussion on the issue of compensation for

costs arising from research-related injuries, Dr. Melody Lin, Office for Protection from Research Risks (OPRR), distributed an article on this subject published in the *Journal of American Medical Association* on June 17, 1998 (Vol. 279, No. 23, page 1854). Dr. Parkman stated that the RAC discussed this issue previously. At that time, the RAC submitted a position paper to former NIH Director, Dr. Bernadine Hea recommending the indemnification of subjects injured in the course of gene transfer research. Furthermore, this recommendation was made when there was a debate in Congress on national health care reform.

Dr. Parkman noted that at his institution, patients who enter clinical trials at the NIH -sponsored Genera Clinical Research Center (GCRC) are covered; any untoward financial consequences from their bein subjects for clinical research are totally reimbursable. Dr. Markert said that as a GCRC Director of institution she is not aware of such resources being earmarked for this particular purpose.

Dr. Macklin noted that the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research issued a recommendation that the issue of compensation for injury of research subjects be studied and dealt with in a systematic and definitive way. The report was released in 1982, and no follow-up has occurred on this issue. Dr. Macklin said that one should make a distinction between financial compensation and providing direct medical treatment free of charge to patients. Many institutions provide short-term necessary care free of costs to the research subjects. As a point of clarification, Dr. Parkman said his institution provides medical treatment rather than reimbursement for medical costs related to the injuries. Dr. Macklin noted that most patients are entered into the trials in a hospital setting; the hospital staff and facility are available for the care of injured subjects. Dr. Markert s that proper guidance on this issue may be provided in Appendix M of the *NIH Guidelin*

Dr. Gordon noted that most patients entered into human gene transfer protocols already have advanced disease; it is frequently difficult to make a distinction between injuries that might haveresulted from gen transfer procedures and injuries that resulted from natural progression of the disease. Dr. Mickelson agreed that the issue is complex and it requires future discussion.

Human Gene Transfer Protocol 9802-236 entitled: *A Phase I Study of the Intraprostatic Injection of CN706, a Prostate-Specific Antigen Gene-Regulated Cytolytic Adenovirus in Patients wit Locally Recurrent Cancer Following Definitive Radiotherapy*.
PI: Jonathan Simons, Johns Hopkins University
Sponsor: Dan Henderson, Calydon , In
Reviewers: Ando, Aguilar-Cordova, Juengst (presented by And

Protocol Summary

Dr. Jonathan W. Simons, Johns Hopkins University School of Medicine, Baltimore, Maryland, proposed conducting gene transfer experiments on up to 30 patients (≥18 years of age) with recurrent or persistent carcinoma of the prostate. CN706 is an attenuated, replication-competent adenovirus that has been genetically modified by inserting the 2.2 kb fragment containing the promoter and enhancer elements of the cloned prostate specific antigen (PSA) gene to a region upstream of the E1A gene of the virus. Th gene-modified adenovirus replicates preferentially in human PSA -producing prostate cell

Preclinical studies of CN706 demonstrate the generation of an oncolytic infection in PSA -producing and xenograft tumors. Five cohorts of three subjects will receive two courses (Days 1 and 4) of CN706 one of five dose levels. The total dose, in viral particles, will depend upon volumetric assessment of the prostate. Doses will range from 1×10^{10} to 1×10^{12} particles per 3-5 cc of prostate volume. Subjects will

receive up to 10 injections of CN706 using a transperineal approach. The primary objective of this stud is to determine the maximum tolerated dose (MTD) of CN706 when administered by local injection int the prostate. Secondary objectives include evaluation of antitumor activity, time to disease progression systemic bioavailability and distribution, and monitoring of the immune response.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol based on the following: (1) this protocol represents the first study submitted to the RAC for the use of a replication-competent adenovirus, (2) the protocol involves the use of a prostate-specific promoter to target gene expression, and (3) the vector will be administered to a site anatomically close to germ cells.

Review - Dr. Ando

Dr. Ando stated that adenovirus type 5 (Ad5) has been used for vaccine studies of army recruits and several significant efforts have been made to use replication-competent adenovirus for the treatment of cancer. Recently an E1B-deleted mutant, Onyx-015, has been used for the treatment of pancreas and head and neck cancers. These studies (outside the RAC purview of human gene transfer research) are ongoing and widely discussed in gene transfer meetings. Various strategies are being used to attenuate or to provide specificity of the replication-competent adenoviruses, i.e , conditional replication in p5 mutant cells of Onyx-015 and specific replication in PSA expressing cells of CN70

Dr. Ando stated that the focal points for RAC discussion are: the issue of specific expression of CN706 in the prostate, decreased systemic replication of adenovirus, and nonspecific toxicity of adenovirus.

This protocol is a Phase I study of CN706, a prostate specific replication-competent adenovirus for the regional treatment of locally advanced prostate cancer that has relapsed after radiation therapy. Alternative therapy consists of palliative androgen blockade, and no curative therapy exists. The novel feature of this protocol is that the adenovirus has been engineered so that expression of E1A is driven by the PSA promoter. *In vitro* studies showed differential proliferation in prostate cell lines compared with non-prostate cell lines. *In vivo* efficacy studies showed ablation of prostate tumors in a xenograft tumo model and induction of prostatitis in normal animal

Studies showed toxicity to liver and abdominal adhesions. Vector biodistribution shows adenoviral DN in all tissues except the brain at Day 5; DNA was cleared completely by Day 30, including germ-line cells. Culturable virus in urine could only be detected on Day

Manufacturing is by standard methodology. Testing for replication-competent adenovirus is not applicable because the virus is replication-competent. Nested PCR with good sensitivity has been developed to lo for wild type adenovirus contamination or recombinations

In terms of clinical trial, the Phase I study is an intrapatient dose escalation of direct injection of CN706 prostate cancer patients who have elevated PSA levels after radiation therapy. The clinical rationale ar objectives of the study are sound. The protocol is well designed for the evaluation of safety, gene transfer, and potential therapeutic effect.

Dr. Ando raised several specific questions. Are the *in vitro* and *in vivo* effects of the PSA promote affected by testosterone levels? Can the investigators clarify the *in vitro* results comparing replication of CN706 and CN702 (a related virus lacking the PSA promoter) in prostate and non-prostate cell lines? I there a maximal tolerated dose of CN706 in rodents? Has there been a comparison of lethal doses of

CN706 and CN702 in mice? What differences, if any, have been observed between CN706 and CN702 in the toxicology studies? Do these studies support the added safety value of tissue-specific expression? What rationale was used for the maximal dose? What is the function of the control CN702 in " LNCaP xenograft model with respect to tumor shrinkage and toxicity? Because hepatitis has been seen an adenovirus is trophic to the liver *in vivo*, would the investigators consider excluding patients with known viral hepatitis or a history of liver disease even if the liver function tests are within three-fold of normal value? Adenoviral dissemination has been seen for up to five days. Will the virus be monitored in the patients? Is there any impact of the virus on the environment or on healthcare workers? Is the strategy for treatment for local control or to take advantage of the transient dissemination for systemic treatment of metastatic diseas

Dr. Ando said that the investigators adequately responded to most of his questions in the written response.

Review - Dr. Juengst (presented by Dr. And

Dr. Juengst stated that this protocol was identified for discussion by the RAC because it involves the us of a replication-competent viral vector in tissue that is relatively close to the anatomical site of germ-cell production. The CN706 vector has been modified to replicate selectively in cellsproducing PSA . E though some replication apparently occurs in other cells, there is no evidence to date of germ-line infection by viral vectors derived from adenoviruses in general or CN706 in particular, and the investigators have included both sperm analysis and post-mortem autopsy efforts to assess any unanticipated infection of the germ cells. It is not clear to Dr. Juengst what else the investigators could to minimize or prepare for this risk. However, Dr. Juengst would defer to his scientific colleagues, if furt precautions are warranted.

The Informed Consent document developed for this study does a good job of describing the protocol in lay terms. However, the "Benefits" section does seem to convey a "therapeutic misconception" about the purpose of the study. It is described throughout the protocol as a traditional Phase I study, and introduced in the Informed Consent correctly as a "study of the side effects" of CN706 injection. However, under "benefits", the investigators cite their "hope" that "this treatment with CN706 could slow down or stop your prostate cancer and reduce your blood PSA level." The investigators acknowledge that "no prediction can be made on how well CN706 will work," but this statement suggests (unwarranted) confidence that it will work to some extent. A clear statement that this study is not intended as a "treatment" for the patient is still required.

Review - Dr. Aguilar-Cordova

Dr. Aguilar-Cordova echoed most of the questions raised by Dr. Ando. He asked if preclinical studies have been conducted in preimmunized animals because most patients are expected to have antibodies adenoviruses. He noted that CN706 is a E3-deleted virus and thus more immunogenic. Have the investigators considered using a virus with intact E3 genes? The virus particle units are used as dose levels and a quantitative relation of viral potency should be described. The nested PCR assay fo detection of wild type virus with intact E1A could be complicated by the presence of E1A sequences in the 293 cells used for vector production. Is there any Southern blot analysis or differential PCR amplification to differentiate the vector vs. wild type virus in " LNCaP " cell studies? Dr. Aguilar-Cordov asked the investigators to explain the rationale of vector delivery to the prostate by the transperineal approach.

Other Comments

Dr. Parkman asked a question about threshold dose in naive vs. preimmunized hosts. He noted that th dose/response curve is the result of a balance between virus-induced cell lysis vs. elimination of infecte cells mediated by CTL responses. In a preimmunized host, which already has cellular immunity would expect the need to transduce a higher proportion of tumor cells in order to be effective, because transduced cells will be eliminated by CTL . Dr. Parkman asked how much of the dose/response cur shifted in the naive vs. preimmunized host

Dr. Markert noted a discrepancy on the length of time (one week in the Informed Consent document vs two weeks in the protocol) that the patients are required to keep the Foley urinary bladder catheter in place.

Dr. Aguilar-Cordova asked the investigators to elaborate on the effectiveness of CN706 vs. CN702 in preclinical studies. In the cotton rat experiments, the maximum dosage is 5×10^{10} virus particles and no MTD is found. In the mouse experiments, $_{50}$ was found to be 2 to 5×10^{11} virus particles per mouse. These are all PSA negative animals, and there should be no difference between CN706 vs. CN70

Dr. Macklin noted an ambiguous statement in the Informed Consent document regarding medical care if the subjects are hurt by participating in the study: "If you think you have been hurt by being in the study, or not treated fairly, you should call the Joint Committee on Clinical Investigation at (410)-955-3008, or the Johns Hopkins Bayview Medical Center Institutional Review Board for Human Research (410)-550-185 to receive help or advice, including help finding medical care if needed. The Johns Hopkins University, The Johns Hopkins Hospital, the Johns Hopkins Bayview Medical Center, Calydon , or the Fede government do not have any program to pay you if you are hurt or have other bad effects which are not the fault of the study doctors." Dr. Macklin said that the statement needs to be clarified with regard to whether the patients will be cared for free of charge, and what is the policy if the bad effects are the fault of the study doctors. How does a patient determine if the bad effects are not the fault of the study doctors? Dr. Macklin noted that the statement appears to be a standard statement of institutional policy, but it needs to be clarified for the patients.

Investigator Response - Drs. Simons and DeWees

Dr. Simons stated that Dr. DeWeese will describe the anatomic specificity of vector injection to th prostate.

Dr. Simons used slides to present his data and responses to RAC questions. He said the replication-attenuated adenoviruses are interesting anti-cancer therapeutics that make use of the molecular knowledge to target either the specific molecular defects of cancer cells (e.g., p53 mutation) or a specific site of cancer (e.g., prostate). A virus such as Onyx-015 is not applicable to prostate cancer because only one in four prostate cancer patients have p53 mutations whereas more than 95 percent have elevated levels of PSA expression. Significant antitumor activities were observed for CN706 in xenograft model of prostate cancer. There are only nine prostate cancer cell lines available for *in vitro* study; therefore, this restricts some of the analyses on specificity of CN706.

Dr. Simons said that CN706 was developed at Calydon , Inc., by Dr. Dan Henderson and his colleagues CN706 employs the PSA promoter/enhancer to target oncolytic infection to PSA -producing pro cancer cells. The PSA promoter/enhancer contains androgen-response elements that are stimulated b the presence of normal testosterone in cancer patients. In " LNCaP " cells with a virus carrying a reporte gene, gene expression increased at least 50 to 100-fold in the presence of androgen. Although the virus is capable of killing tumor cells in the absence of androgen, patients are intentionally chosen to have

normal androgen levels in order to have a better antitumor effec

Dr. Simons presented the data from the xenograft model published by Ron Rodrigue *et al.*, (*Cancer Research 57:* 2559-2563, 1997). One can eradicate the xenograft tumor by CN706 administration. *In vitro* studies with prostate cancer cell lines showed selective preferential replication of CN706 over the control virus, CN702. Dr. Simons stressed that CN706 does not have an infinite therapeutic specificity. The proposed protocol is to study the specificity in humans.

Dr. Simons introduced Dr. DeWeese to address the issue of anatomic specificity. The virus is to b administered to the prostate by the same procedure used in brachytherapy to deliver radioactive "seed The anatomic specificity of vector delivery will complement the molecular specificity of the vector construct.

Dr. DeWeese said that administration of CN706 is based on a widely used technology for brachythera based on the precise anatomic definition and localization of the prostate gland in relation to the surrounding structures. Dr. DeWeese said that the technique is better than ultrasound guided transre delivery and will enable homogeneous distribution of the virus. A standard highly specific three-dimensional planning computer program is available to guide the delivery. Homogeneous delivery of the vector is important for the treatment of multifocal prostate cancer. Dr. DeWeese showed a sli illustrate the delivery instrument that allows a preplanned delivery procedure with an anatomic specificity complementing the biologic specificity of CN706.

Dr. Aguilar-Cordova said that in brachytherapy a computer program has been developed to calculate th distribution of radiation, and he asked how the program can be used to calculate virus distribution. Dr. Simons showed data from the mouse model that simulates virus delivery to the patients.

Responding to the question of the ratio of virus particle to infectious unit, Dr. Simons said the ratio is 10 to 1.

Dr. Parkman said the nude mouse experiments were performed on immunodeficient animals; there is r immune response to reject virus infected cells, and the situation is different from the patients whose immune systems are intact. Dr. Simons responded that the xenograft model of a PSA -expressing tu in athymic mice is the only model available for the preclinical study. It takes about 100 to 1,000 viru particles in a volume of 25 µl to eliminate a tumor of 1 cm^3 in size.

Dr. Wolff asked what evidence exists that CN706 is expressing the E1A gene in a PSA -specific manne as would be expected from the PSA promoter/enhancer elements. Dr. Simons responded that there ar no data directly addressing the question of E1A expression in early infection; the data are on virus titers, an indicator of late phase virus replication cycle.

Responding to the question of retaining E3 gene in the construct, Dr. Simons said that if the present study turns out to be promising such an improvement in vector design will be considered for future study.

Regarding the question of preimmunized animal study, Dr. Simons said such experiments are in progre and there are no data yet to address the issue of whether the dose/response curves would be different.

Dr. Simons stated that the Informed Consent document will be changed to state that a Foley catheter is to be in place for 14 days as stated in the protocol.

Dr. Aguilar-Cordova commented on Dr. Simons' responses to his questions. He said that the virus with intact E3 may prove to be more efficacious that the E3 CN706. Dr. Aguilar-Cordova noted that the present virus injection technique is more invasive than transrectal injection, and it needs to have a better anima study to justify its use in patients. He suggested using the PSA independent CN702 virus in a synger mouse model to investigate the vector delivery technique.

Dr. Aguilar-Cordova asked if the differential virus titers seen in *in vitro* studies regarding CN706 vs. CN702 are due to a difference in the time course of infection; at the end of the experiment, the titers for both viruses may approach the same value. Dr. Simons responded by showing data on thetime course virus infection; a differential of 10-fold difference is maintained during the entire time course of infection.

Responding to the question of transrectal vs. transperineal injection, Dr. DeWeese explained tha cancer is a multifocal disease and the transperineal route is a preferred approach to ens homogeneous distribution of the vector.

Dr. Lai noted that although *in vitro* data show preferential replication of CN706 in PSA positive cells experiments in cotton rats indicate that the virus is distributed to many organs including the brain, heart, kidney, and liver. In addition, CN706 causes prostatitis and peritonitis in cotton rats. Dr. Lai asked if th virus is similarly distributed to all these organs after administration by injection to the prostate of patients. As a point of clarification, Dr. Simons said cotton rats, which are permissive hosts for virus replication, were used to assess the question of E3 gene leakage. The Copenhagen rat experiment was actually to assess the inflammatory effects of intraprostatic delivery. There was dose-limiting prostatitis either injection with CN706 or CN702. In humans, there are data related to Onyx-015 indicating the spread of the virus to many organs. Dr. Lai said in the data presented for the cotton rat model, there is vector DNA detected by PCR analysis in many different organs. He asked about the virus titers in these organs a compared with that in prostate, i.e., whether CN706 replication is specific for the prostate. Dr. Simons responded that there is no PSA in cotton rats or Copenhagen rats and one should not expect to see su specificity of virus replication. Human studies with CN706 will provide data regarding whether the virus replicates specifically in the prostate.

Dr. Lai was concerned about widespread inflammation caused by CN706. Dr. Noguchi explained that virus replication *per se* does not cause inflammation; it is the virus coat proteins in high dosage that is toxic by an unknown mechanism. Dr. Lai asked if the toxicity will be higher if the virus does replicate to a high titer in a particular organ? Dr. Simons responded theoretically, yes. In the clinical trial reported for Onyx-015, there was viremia and fever in the treated patients; this could be managed with medication These symptoms are expected for the present trial. Dr. Noguchi noted that the toxicity may be related to the dosage and the trial will show whether there is a therapeutic window that will have antineoplastic effect with manageable systemic toxicity; unfortunately, there is no animal model that can test both the replicative and inflammatory components of toxicity of these adenoviruses because the human virus do not replicate well in animals. Dr. Simons said that there is a stopping rule in the protocol that if toxicity is encountered, the trial will go no further.

Dr. Aguilar-Cordova noted that the cotton rat is a good animal model for respiratory tract application of adenoviruses, but may not be the best model for transperineal application. Dr. Aguilar-Cordova said th the best toxicity data are those related to the human trial of Onyx-015 and live adenovirus vaccine trials in Army recruits. Dr. Wolff noted that the Army vaccine trial is by oral administration. Dr. Simons agreed that a careful human study will address the toxicity issue.

Dr. Lai was still concerned about the spread of CN706 in animal experiments that caused peritonitis and prostatitis ; in humans there is a potential that the virus is capable of causing toxicity in tissue without

He noted that the protocol is the first time that a replication-competent adenovirus is to be administered via an unnatural route of infection in humans. Dr. Noguchi said that the Onyx-015 trial was initiated about four years ago without RAC review because it used a natural adenovirus deletion mutant. The data obtained so far indicate oncolytic activity and someinflammation at the injection site in head and n tumors. Dr. Noguchi said that CN706 is different from Onyx-015 but short of human trial it is difficult to predict toxicity based on any available animal models.

Dr. Aguilar-Cordova noted the injection site is very close to germ cells. He asked if there are any data to address the issue of viral DNA integration in germ cells. Dr. Simons said that the investigators and their IRB have seriously considered this issue. There is a plan to obtain seminal plasma from patients befor and after treatment and to preserve the samples for future use if a proper technology is developed to assess the viral DNA integration issue. At present, there are no data indicating spermatocyte gen transfer in humans; Dr. Simons said that they are using the clinical trial to collect data prospectively in an attempt to address this issue.

Dr. Gordon noted that it is a daunting task to assess if any vector DNA detected in a sperm sample by PCR analysis is indeed in the sperm cell proper and is integrated into the host cell genome. In addition only a few sperm cells might be positive and the vast majority of sperm cells will not fertilize an egg. Dr. Gordon said in his study with mice, at least a hundred-fold excess of adenovirus particles is needed to infect a sperm cell.

Dr. Gordon shared the same toxicity concern raised by Dr. Lai that the virus can cause toxicity independent of PSA expression as suggested by the rat experiments. Dr. Noguchi said that the huma trial will start with a much lower dosage to assess the potential maximum tolerated dose.

Dr. Wolff asked what is the difference between human cells and murine cells in supporting replication o $E1A^-$ adenoviruses. Dr. Aguilar-Cordova responded that murine cells are not as efficient as human cell in supporting replication of the human adenoviruses, wild type, or $E1A^-$. But in extrapolating the toxicity data from animals to humans, the body size difference is also a factor.

Dr. Parkman noted that all anti-cancer drugs have toxicity, and the question is what is the level of toxicity that is unacceptable. The trial will start from a low dose to determine the tolerable dose level. Dr. Noguchi noted that gene transfer in general has been remarkable for a lack of toxicity as compared with chemotherapeutic compounds.

Dr. Aguilar-Cordova said that adenovirus replication is mostly extrachromosomal and the chance of DN integration is low. But for the sake of scientific inquiry, he asked if any*in vitro* experiments in permissive cells have been performed to determine the incidence of integration. Dr. Simons responded that he does not have any data on CN706.

Dr. Parkman recommended that the Informed Consent document should request permission to archive gonadal tissue as part of the surgical management of disease. Archived tissue will be valuable fo assessing any potential viral DNA integration in gonadal cells. Dr. Simons agreed to the suggestio

Dr. Aguilar-Cordova asked if the patients would accept orchiectomy after gene transfer. Dr. Simon responded that if the patients agree to such a procedure, the removed testicles would be valuable samples for future study.

Dr. Aguilar-Cordova asked if there is any viral shedding through the urine. Dr. Simons said that his IBC

also was concerned about the same issue. A urine sample will be archived for furtherstudy , and he wil not be surprised to find short-term virus shedding in the urine after vector administration. A nested PCR assay has been developed to analyze any recombinant wild type adenovirus in the urine.

Dr. Lai noted that the Copenhagen rat study shows toxicity at a dose 50 to 500-fold of the initial human dose, and he asked if there is any toxicity at a dose comparable to the initial clinical dose. Dr. Simons said that the toxicology study was conducted with consultation with FDA officials to determine the toxicity at the maximum dose that can be given to the animals; toxicity at the lower dose levels was not determined. The starting dose in terms of patient safety is well within the established range for a Phase I trial.

Dr. Mickelson summarized the major discussion points. She noted that most of the questions raised in the written review were satisfactorily responded to by the investigators in writing. The RAC discussed the observed dose/response curve resulting from the balance of virus-induced cell lysis and elimination o infected cells mediated by CTL responses. The RAC discussed whether this balance could be affected adenovirus preimmunization . The RAC inquired about the replication specificity of CN706, noting that a high doses CN706 induces widespread pathology in Copenhagen rats. The RAC discussed the issue of whether a therapeutic window exists that will produce a favorable therapeutic index in the prostate. The investigator presented additional data demonstrating that the specificity of CN706 oncolytic activity i based on vector design and anatomic delivery to the prostate. The RAC discussed the potential for CN706 biodistribution to gonadal cells, noting the cotton rat and Copenhagen rat biodistribution

RAC Recommendations

At the close of its discussion of the protocol, the RAC made the following specific recommendations to be included in the letter to investigators and other concerned bodies: the Informed Consent document should be modified to: (1) include information explaining the importance of conducting an autopsy in the event of death. This notification that an autopsy will be requested should include a clear explanation regarding the need to obtain gonadal tissue for assessment of virus DNA integration into germ cells; (2) reques permission to archive gonadal tissue as part of the surgical management of disease. Archived tissue w be valuable for assessing potential viral DNA integration into gonadal cells. Although the presen technology is not yet fully developed to assess whether integration occurs in germ cells or adventitious cells of sperm samples, it is anticipated that such assays will be available in the near future; (3) clarify the issue of compensation for subjects who could potentially be injured as a result of their participation in the study and any address financial responsibility for medical costs.

Dr. Macklin agreed to provide samples for Informed Consent document if asked by the investigators.

Functions of the RAC

At the conclusion of the discussion of Protocol #9802-237 on June 18, 1998, the RAC stated its intent to send a letter to the investigator(s), with copies to the IRBs , IBCs , OPRR , and the FDA, follow discussion of a protocol. The letters will inform relevant parties of the RAC discussion, including remaining issues that should be addressed and areas of concern. The letters will also address positive aspects of the protocol and the Informed Consent document.

Additional Comments

Dr. Parkman asked what is the product of RAC deliberation under the current practice of RAC review. Dr. Mickelson said the letter to the investigators is to outline in broader terms the issues that were raised in

the review and responded to by the investigators, and to convey RAC recommendations or concerns, if any. The letter is to be addressed to the investigators with copies sent to the IRB , IBC, OPRR , and FDA. Responding to Dr. Aguilar-Cordova's question on how the letter will be generated, Ms. Knorr stat that the RAC should strive to have a consensus of the major recommendations and ORDA will draft th letter in consultation with RAC reviewers.

Dr. Parkman said that at the December 1996 RAC meeting a motion was approved with regard to the issue of a feedback mechanism of RAC discussion. The RAC recommendation would be forwarded to the FDA, and the FDA would inform the RAC with any resolution of the issues upon interaction with the investigators. Ms. Knorr noted that the issue of the follow-up mechanism will be deliberated by th Appendix M Subcommittee with regard to amendments to Appendix M of the *NIH Guidelin*

Dr. Aguilar-Cordova noted that the letter should be for informational purposes; it is entirely up to the investigators, the FDA, IRB , and IBC to decide on how they will deal with RAC recommendations. Ms Knorr said the RAC should resolve the question of whether the letter is simply for informational purpos or whether there should be a follow-up mechanism in place to see if specific RAC recommendations have been implemented. Dr. Markert said that she favored having the RAC forward the letter to the investiga with no reporting requirements back to the RAC.

Dr. Lysaught noted that the feedback of how its recommendations are resolved will help the RAC dea with similar issues in subsequent protocol submissions. Dr. Mickelson asked if feedback from the FDA is feasible in terms of its confidentiality statute. Dr. Noguchi said that the FDA would be pleased to respond to RAC letters. Dr. Parkman agreed that the feedback from the investigators or the FDA will help the RAC to know whether RAC concerns are resolvable.

Dr. Gordon said that if the purpose of the letter is simply for information, he would rather not send any letter to the investigators, IBC, or IRB . Dr. Lysaught said that she favors sending the letter but mandating a response. Dr. Markert favored sending the letter, but an immediate response should not b required because some data may take time to acquire. Dr. Parkman said that the data on preclinical studies such as immune response in preimmunized animals should be available to the RAC upo request. Dr. Macklin agreed with Dr. Gordon's assessment not to send a letter regarding every protocol the RAC reviewed; a letter is only appropriate when there is significant concern by the RAC. Dr. Lysaugh noted that a standard letter to the principal investigator of every reviewed protocol would help to mitigate any misunderstanding relating to political issues concerning certain kinds of protocols. Dr. Lai agreed with Dr. Lysaught about sending a letter to show appreciation to an investigator who has taken time t participate in a RAC discussion.

Dr. Ando asked for the viewpoint of Dr. Roessler , the investigator of Protocol #9802-237. Dr. Roessl responded that he believes that IRBs and IBCs would welcome the feedback from the RAC, but he not think that the RAC should mandate any response. Dr. Parkman agreed that sending letters about all reviewed protocols would eliminate the contentious issue of why a particular protocol was chosen for review; in his view, the IRB and IBC will benefit from RAC expertise because often the local bodies onl give provisional approval pending Federal review, ifany . Ms. Knorr added that the letters could prov educational information to IRBs and IBCs . Dr. Ando said that the minutes of RAC deliberation are a useful to the local bodies. Dr. Gordon agreed that the minutes are useful.

A motion was made by Dr. Ando and seconded by Dr. Gordon to send a letter and a portion of the "draft" minutes pertaining to the discussion of a particular protocol to the investigators with copies to the IBC, IRB , OPRR , and the

Considering the time required to prepared draft minutes (one-two months), Dr. Aguilar-Cordova made a friendly amendment that the letter be sent out as soon as possible after the RAC meeting and the draft minutes to follow, if requested.

Dr. Mickelson noted that the letter could mention the following areas of RAC review: safety, study design, and ethics or informed consent issues. Dr. Gordon was concerned about the amount of work involved in drafting detailed letters when many protocols are reviewed at a single RAC meeting. Dr. Parkman noted that ORDA should be able to handle the task without too much additional burden to RAC reviewer

Committee Motion 3

A motion was made by Dr. Ando and seconded by Dr. Gordon to send a letter to the investigator(s) wit copies to the IBC, IRB , the FDA, and OPRR after RAC public review of a novel human gene trans protocol. The letter will inform relevant parties of the RAC discussion, including remaining issues that should be addressed and areas of concern, and highlight positive aspects of the protocol. The letter will be useful to IBC consideration of future gene transfer clinical trials and will assist the IRB during annua review of the study. The motion passed by a vote of 6 in favor, 1 opposed, and 1 abstention.

Continued RAC Discussion on June 19, 1998

Dr. Mickelson asked Ms. Knorr to provide background on previous RAC discussions on the issue of fut functions of the RAC. Ms. Knorr stated that during the March 1998 RAC meeting, Dr. Lana Skirboll Associate Director for Science Policy) recommended that the RAC begin development of a vision statement that would articulate its roles and responsibilities in response to the changes implemented October 31. 1997. regarding NIH approval authority of human gene transfer protocols. Ms. Knorr as the RAC to conduct a preliminary discussion of the major points to be included in the vision statement to be drafted by ORDA for a formal discussion at the September RAC meeting. Once the RAC develope the plan, this statement could be published in the *Federal Register* for public comment.

Dr. Gordon said he has given a lot of thought to the future role of the RAC. From his understanding, the intention of the NIH Director, as transmitted by Dr. Skirboll , is to focus on emerging gene trans technology and controversial ethical issues of human gene transfer research, e.g., germ-line gene transfer, rather than reviewing each gene transfer protocol in detail. Dr. Gordon suggested a way to get out of the current pattern of reviewing each protocol in the absence of approval authority. Some types of protocols may be reviewed with a less rigorous level of scrutiny, e.g., adenovirus administration into the coronary artery which is similar to the myocardial application previously reviewed by the RAC. These types of protocols may be reviewed by a few primary RAC reviewers in detail and reported back to the full RAC withouthaving to have the investigators come to a RAC meeting. In terms of Dr. Mark idea of accepting protocol submission before approval by a local IBC and IRB , Dr. Gordon foresees a advantage to this submission procedure but a proper safeguard needs to be worked out so that the RAC will not be inundated by helping the investigators to work out all the fine points of their protocols before submission to their IBC and IRB . Dr. Gordon stated that close communication with the FDA is importar the new role of the RAC, because the FDA is relying on the RAC to bring up controversial issues for public discussion.

Dr. Noguchi noted that the FDA has a mechanism to review the study design and safety of protocols and those issues need not be dealt with by the RAC. For identifying significant issues for RAC discussion, Dr. Noguchi suggested having a subcommittee of the RAC responsible for tracking issues, which could consult with the FDA to recommend topics for RAC discussion.

Dr. Mickelson noted from the protocols discussed at this RAC meeting that there is ambiguity in the definition of what is considered to be a "novel" protocol and that there is some degree of uncertainty regarding what is to be accomplished by public RAC discussion.

Dr. Parkman stated from his experience as a reviewer that a protocol tends to be reviewed less rigorously if it has received FDA approval. He suggested an idea for the RAC to consider novel "prototype" protocols that are still under development; the RAC may work with the investigators to address the outstanding issues before final presentation to review committees. In terms of RAC discussion of overarching issues involving a group of protocols, the RAC should be able to discuss these issues within a shorter time frame than that requiring *Federal Register* notice for public discussion. Ms. Knorr explained that a *Federal Register* notice is required only for announcement of the meeting and the RAC may discuss the overarching issue within that framework.

Dr. Markert said she found that having the investigators and sponsors come to the RAC discussion is v helpful. She said that the problem of RAC review of a protocol after the fact of FDA approval could be obviated by not requiring prior IBC and IRB approval for submission to ORDA . Her recommendation accept protocols prior to IBC and IRB approval will afford the RAC the opportunity to discuss the protoc at an earlier time point. Dr. Markert said that the current modified procedure of RAC discussion of nove issue without holding up initiation of a protocol partly resolves this dilemma of the submission time frame. She said that the feedback to the IBC and IRB contained in the RAC letters serves the educationa purpose of RAC discussion. Dr. Mickelson agreed that the investigators appreciate the feedback from RAC discussion.

Dr. Ando said it is useful to review a novel protocol by focusing on new issues and to distill the essence of RAC discussion in order to benefit future studies related to it. As an example, he mentioned the discussion of the adenovirus prostate protocol (#9802-236) that uses a tissue specific promoter/enhancer for transgene expression. The issue of how to validate tissue specific expression in animal studies is a important issue for future protocols using the same approach.

Dr. Mickelson said that RAC review of individual protocols was useful, both for the identification of specific research design issues and for public awareness that will ensure continued progress in the field. She noted that issues relating to Informed Consent documents were the most difficult to identify without protocol review. One problem, however, is that only those protocols undergoing full review have the benefit of Informed Consent document review. She suggested that the RAC explore mechanisms by which all Informed Consent documents are scrutinized, which would optimize patient understanding of all aspects of the research process and procedures.

Dr. Macklin noted that the RAC needs to clarify the criteria used to decide which protocols are "novel" and need to be reviewed at regularly scheduled public meetings. Part of the disagreement among RAC members in terms of the definition is that novelty is a completely separate issue from the degree of potential risk. Should the RAC review a protocol that is novel but poses little risk? Dr. Macklin noted from the RAC discussion of protocols, issues of risk and benefit received more deliberation that novelty of the gene transfer procedure *per se*.

Dr. Gordon responded that he is not surprised that different RAC members assess a given protocol differently in terms of the need for RAC review because RAC members have different expertise. It is useful for the RAC to decide on what kinds of protocols need to be reviewed. Dr. Gordon pointed out one area in which the RAC can contribute to the field of gene transfer in general is to establish standards for Phase I and Phase II clinical trials, including issues related to study design. For Phase II studies, appropriate control arms are essential to assess efficacy.

Dr. Noguchi stated that some issues in protocol design and clinical outcome are controversial and that public discussion of such issues is useful to advance the field of gene transfer research; however, he cautioned that the RAC should not supersede the FDA's regulatory authority of gene transfer protocols.

Dr. Gordon noted an example of a clinical trial involving adenovirus-p53 in patients with hepatocellular carcinoma. Twelve patients were to be studied; only five of those patients were expected to have the p53 mutations. Dr. Gordon said he found the study design with such a small patient number to assess any endpoint of efficacy to be very unsatisfactory; the Informed Consent should clearly inform the patients to the fact that the study is a toxicity study without any expectation of efficacy.

Dr. Markert noted an example from the discussion of Protocol #9802-238 that placebo arm of a Phase study is a controversial issue and that the RAC did not reach a consensus.

Dr. Macklin was concerned about the fairness of selecting certain protocols for RAC discussion based simply on "novelty." The investigators of protocols selected for RAC review underwent an added burden and at the same time received the expert consultation from the RAC, while others were unfairly excluded from both the burden and benefit.

Dr. Lai noted that historically the RAC stressed safety over scientific merit in its previous approval of human gene transfer protocols.

Ms. Knorr suggested that the RAC can use th*NIH Guidelin*to provide guidance for research design that is equitably available to all investigators.

Dr. Noguchi said that the public benefits significantly from RAC discussion, and that very few sponsors view this process as a waste of time. He said several issues were worth discussion by the full RAC, and he suggested that some issues are more worthy than others, e.g., safety concerns and partial correction issues surrounding *in uter*research and germ-line research.

Dr. Aguilar-Cordova said the RAC is an educational and deliberation body, and that members should review the RAC statement of purpose to resolve questions about the function of the RAC. He noted that the RAC is not a Study Section that reviews proposals for NIH funding; review ofscientific merit of s designs is not the top priority of the RAC. Nor it is a matter of fairness in deciding which protocols should be reviewed if it serves an educational purpose.

Dr. Macklin said that the criterion of "novelty" that merits RAC discussion should be clarified, and that RAC discussion should focus on the novel features of the protocols.

Dr. Markert noted that the RAC stated the rationale when selecting a protocol for review. She said th RAC discussion has sharpened a number of issues that were not anticipated in the initial written review but that were important, e.g., breaking of tolerance in the superantigen protocol (#9804-244). The RA discussion should focus on new and novel aspects of gene transfer research. Dr. Gordon said the RAC should not be bound to review only certain aspects of a protocol; if there is a flaw in the study design it should be pointed out and the Informed Consent document should not leave patients with a false sense of efficacy. Dr. Markert said that she frequently offers specific comments that ORDA forwards to investigators; a great deal of dialogue occurs without full RAC review of a protocol. Dr. Gordon said that the RAC comments should be responded to by the investigators.

Dr. Macklin noted that her review of protocols is not confined to the novel aspects, e.g., the ethical aspects

of the clinical trial. Dr. Macklin suggested a general policy statement that all Informed Consent documents should not make any implication of any treatment potential for the Phase I/II studies. Dr. Gordon agreed, and said that the RAC should be able to inform the investigators about its concerns over a specific Informed Consent document.

Ms. Knorr noted that the RAC can incorporate the recommendation on generic issues in the prope section of Appendix M of the *NIH Guidelin*, e.g., Section M-III for informed consent issues. She asked the RAC to reconsider the present process for identifying a protocol for RAC review, i.e., the number of votes need to recommend review and whether there should be categories of RAC discussion other than "review "or "no review."

Dr. Aguilar-Cordova asked why the Informed Consent documents for Phase I gene transfer trials should be different from Phase I trials of other investigational new drugs? He said if a protocol is selected for full RAC discussion, the review should not be limited to just the novelty aspects. Dr. Macklin said that from her experience of serving on several IRBs , there is no other type of Phase I studies that promises bene and treatment to the subjects. She said that gene "therapy" is a terminology that leads to misunderstanding.

Dr. Gordon suggested a review category in which a RAC subcommittee reviews the protocol in detail and reports back to the full RAC without necessarily asking the investigators to participate at a RAC meeting. Such a review can be a limited review of particular issues.

Dr. Lai said one unique aspect of gene transfer protocols is their enormous publicity; Phase I studies are frequently being construed as potential therapies. Any patients who are willing to participate in the trials feel that they will be helped by the protocol. He asked if OPRR reviews any Informed Consent docume Dr. Lin responded that OPRR reviews only Informed Consent documents prepared by small institution that do not have their own IRB ; OPRR relies on IRBs to review most protocols including Inf Consent documents.

Human Gene Transfer Protocol 9804-247 entitled: *A Phase I Safety and Dose Escalation Trial of Autologous Transfected Human Fibroblasts Producing Human Factor VIII in Patients with Se Hemophilia A*
PI: David Roth, Harvard Medical School
Sponsor: Kurt Gunter, Transkaryotic Therapies, Inc.,
Reviewers: Verma (presented by Mickelson), Mickels
***Ad hocs*Robertson Parkman, Children's Hospital of Los Angeles and Haig Kazazian , Univer of Pennsylvania (presented by Mickelson)**

Protocol Summary

Dr. David R. Roth, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, Massachusetts, proposed conducting a gene transfer experiment on nine subjects (≥ 13 years of age) with severe hemophilia A. A plasmid expressing human factor VIII (hFVIII) will be used to transfect autolo fibroblasts. The cloned DNA encodes a hFVIII gene in which the B domain has been deleted (B hFVIII). The modified hFVIII gene has been cloned into a pBR322-based plas

The plasmid contains sequences designed to maximize the expression of BDD hFVIII in hu fibroblasts, as well as a selectable neo^R gene. Dermal fibroblasts will be isolated by biopsy and expanded in culture. Expanded fibroblasts will be transfected by electroporation with the plas encoding hFVIII and implanted via laparoscopy. Three subjects will be entered into each cohort; eac

cohort represents escalating doses between 1 x 10^8 and 4 x 10^8 fibroblasts. The objective of this study is to investigate the safety of non-virally transfected autologous human fibroblasts producing hFVIII implanted within the peritoneum of hemophilia A patients.

Rationale for Full RAC Discussion

The RAC recommended full public discussion of this protocol because it represents a new disease, a new gene (hFVIII), and a new delivery method (transfected fibroblasts into the omen

Dr. Mickelson noted that the sponsor requested that certain portions of the responses to reviewers' comments be held confidential. Dr. Mickelson stated that holding part or all of a protocol confidential goes counter to the goal and intent of RAC public discussion; a closed session, however, may be convened if requested by the sponsor. She asked the RAC to consider only those portions of the protocol that are in the public domain in the open session. All primary reviewers signed certifications regarding confidentiality of information.

Dr. Ando said that he abstained from review of the protocol due to conflict of interest. Dr. Mickelson noted that Dr. Verm's review will not be presented due to a possible conflict of interest

Review - Dr. Mickelson

Dr. Mickelson emphasized that she is very glad to see a gene transfer clinical protocol dealing with hemophilia A (factor VIII deficiency). This disorder affects a relatively large number of people, second only to cystic fibrosis. Given the large number of people affected and the fact that there is a wide therapeutic margin, this disorder has always seemed a reasonable target for gene- basedtreatment . There is a goo chance that even marginally efficient gene delivery/expression systems will help this patient population.

The clinical protocol and the Informed Consent document were well written. The time and effort put into answering Appendix M, *Points to Consider,* was evident. The questions outlined below should not be interpreted as criticisms but as an attempt to clarify points or issues that might be of interest to the public, patients and other researchers.

Even though this is a Phase I study, portions of the follow-up regime seem aimed at looking at duration and efficacy of expression of the injected factor VIII expressing cells. Dr. Mickelson agreed with this approach, although the study population is very small. It would be a shame not to attempt to obtain some preliminary expression and duration data from the unique study group generated by this procedure. However, Dr. Mickelson would hope that the investigators will be conservative in their interpretation of the results and realize the weakness that the small population size places on the data.

Dr. Mickelson raised several specific questions: (1) She would like to see a description in the responses to Appendix M that mentions that a Phase I clinical trial utilizing fibroblasts transfected with the huma growth hormone (hGH) gene was in progress. She is not sure this protocol has been registered wit ORDA . If it has not, why not? The hGH protocol employs recombinant DNA in humans and should seen by the RAC and be part of the ORDA database. (2) Patient Study Group. Shoul one criterion for inclusion in the study group be factor VIII genotype and not just a combined phenotype of hemophilia? Will the study participants' factor VIII gene be characterized? Is that information known? Is factor VIII therapy given to overcome bleeding disorders due to traits other than protein absence such as a factor VII protein with altered von Willebrand factor binding? (3) Transfected Cells. The number of transfect proposed for implantation seems low given the amount of protein that must be produced in the human recipient to reach potentially therapeutic levels. Dr. Mickelson would appreciate some information on how

the range of cell numbers to be tested was reached (whether the range to be tested exceeds what may be the highest cell numbers required). What protein production rate is the minimum required for a transfecte clone to be expanded for use in the trial? What clonal protein production rate is required to reach minimally effective therapeutic level in humans? A quick estimate would seem to indicate that a very high factor VIII production rate or more than $4x10^8$ cells might be needed. Once a high producing clone has been selected for use, is any information about the site of factor VIII integration sought, such as asking whether integration has occurred in known tumor suppressor genes? Whether multiple vector copies are inserted in tandem or scattered throughout the genome? (4) Immune Reaction. Factor VIII gene-knockout mice have been generated. Wouldn't studies using this transfected autologous fibroblast system in mice be the most relevant preclinical model rather than rabbit fibroblasts in nude mice? Were studies parallel to those outlined in this protocol conducted in factor VIII gene-knockout mice? If not, Dr. Mickelson would think it quite important because the other clinical trial cited by the investigators utilizes a gene product (hHG) that is already present in the recipient and thus not "new or foreign" to the host immun system. One objective of this gene transfer system is the long-term persistence of the transfected cells the experiment works, these patients will be exposed to the "new" protein for a much longer time than for treatment of a "bleeding episode". It would seem that the longer these cells persist and express the insert the more likely that antibodies to factor VIII will arise. What will or can be done for patients who develop antibodies during the course of the study? Is subsequent immune suppression or tolerization contemplated? (5) The Informed Consent document. The Informed Consent document should include additional information in Section D4,*Risks and Discomfort*, discussing what may happen if a participant develops antibodies to factor VIII as a result of this procedure. Dr. Mickelson would presume that steps would be taken to lessen the response as this response would be detrimental to future effectiveness of the normal factor VIII replacement treatment. These risks should be outlined as well.

Review - Dr. Kazazian (presented by Dr. Parkma

Dr. Kazazian stated that the strategy of transkaryotic therapy refers to administration of autolo fibroblasts that have been transfected with a desired gene of interest into the body in order to effec sustained delivery of the desired gene product. The investigators propose to apply this approach to factor VIII delivery to correct "severe" hemophilia A. Although this proposal appears to be a rational and promising strategy for gene transfer, he said it would be unthinkable to pursue Phase I clinical trials based on the preliminary findings described.

The only animal model tested for factor VIII delivery to the circulation from transplanted fibroblasts is an immunodeficient mouse model. A higher order animal system which more closely mirrors humans, suc as dogs or non-human primates, should be tested to determine whether findings in a small animal model can be extrapolated to a larger animal system.

The mice used for the preliminary studies were immunodeficient . No studies using immunocompete animals were described. The impact that an immune response can have on the efficacy of this approach is potentially large. Inhibitors (antibodies) to the circulating factor VIII may compromise its function and bioavailability. Even though the patients selected have not yet developed inhibitors to factor VIII, they may do so with this new mode of delivery. Similarly, a cell-mediated immune response may compromise levels and duration of factor VIII expression. Furthermore, chronic inflammation at the site of fibroblast transplantation could have detrimental effects on the host organism.

The duration of expression that would be achieved in humans cannot be extrapolated from the data described. First, a cell-mediated immune response may lead to complete loss of expression over even a short period of time. The model system used to test this approach is insufficient to address this issue. How human fibroblasts would fare in transkaryotic therapy for factor VIII replacement has not been addresse

Reference to studies in human subjects who have received human growth hormone-expressing fibroblasts is made to suggest that this approach can give rise to prolonged expression in the absence of an immune response. However, this model is irrelevant to the proposed studies because human growth hormone is a "self" antigen in all the subjects tested. Assuming that the patients to be recruited for this proposed Phase I clinical trial are severe hemophiliacs who express no factor VIII, the factor VIII generated by transplanted fibroblasts would likely be seen as "non-self" by the immune system and trigger an immune response.

It is not clear whether the patients to be recruited for this trial will be characterized with regard to (1) the nature of their mutation, (2) whether any endogenous non-functional factor VIII protein is synthesized, and (3) the severity of their disease. These parameters could have a significant impact upon whether an immune response will be generated and limit levels and duration of factor VIII expression. Clearly, any recruited patients for this study will need to be characterized for their molecular defect.

In summary, the Phase I study proposed should not be carried out at this time. "Good science" would require that the study first be carried out in factor VIII-deficient mice, or, if that is not technically feasible, in factor VIII-deficient dogs.

Review - Dr. Parkman

Dr. Parkman said his first question is the appropriateness of the preclinical animal model in terms of how long the transgene expression is expected to persist. He questioned whether the immunodeficient r with human cells are appropriate models for the human study. The second question is whether the fibroblasts obtained from older patients are able to expand from a single fibroblast clone to the number of cells (10^8) proposed for administration to patients. The third question is whether the immunogenicity o factor VIII produced by fibroblasts is the same as the natural protein.

The inclusion criteria include patients who have received prior treatment with factor VIII replacement therapy. Dr. Parkman said it should be clarified whether the prior treatment was with a recombinant protein or a natural substance. The inclusion criteria also require that patients should not have inhibitors to factor VIII. Dr. Parkman stated that it is better that the inclusion criteria directly measured inhibitors or stated that inhibitor levels would be determined. The issue of an inhibitor antibody development against the recombinant factor VIII is an important one. It would be better that the patients do not have detectable inhibitors prior to the gene transfer study. Dr. Parkman asked whether the investigators can show any difference in the immune response of animals (mice or rabbits) to the recombinant protein as compared to the wild type human factor VIII.

Other Comments

Dr. Mickelson noted that the protocol is well written and it addresses a monogenic disease affecting a relatively large number of people. The investigators have responded to most of the questions in writing without any restrictions on the confidentiality of the response.

Investigator Response - Drs. Selden, Roth, and Trec

Dr. Selden said that he is the founder of Transkaryotic Therapies, Inc. (TKT), and he will address fo public the basic issues that were raised by the reviewers, and detailed specific questions will be answered in a closed session. He explained that TKT has maintained a low profile to develop gen transfer in order not to create false hope and unrealistic expectation for the patients. He was concerned

that anything discussed in a public forum will have an adverse effect on their ability to protect their technology in terms of patents. Dr. Selden responded to the following major questions raised by reviewers:

(1) Do the nonclinical studies performed by the sponsor TKT support the proposed Phase I study? I VIII deficient mice and dogs are available. Would studies using transfected autologous fibroblasts in animals be the most relevant nonclinical mode

Dr. Selden stated that in assessing the safety of transkaryotic therapy, the investigators have focused extensive testing and characterization of the product intended for administration to patients, i.e., transfected clonal human fibroblasts producing human proteins. To assess thenonclinical efficac long-term toxicity of transkaryotic therapy, the investigators have utilized human and rabbit fibroblast producing human proteins.

The use of mouse or canine cells in autologous models for the treatment of factor VIII deficiency ha limited use for assessing the safety and efficacy of a human gene transfer product. In the gene-knockout mouse model for hemophilia A, the implantation of clonal mouse fibroblasts stably transfected with mouse factor VIII coding sequence has major drawbacks. First, mouse fibroblasts commonly undergo spontaneous neoplastic transformation in culture, typically following approximately 10-15 cumulativ population doublings (CPD), essentially ensuring transformation and immortalization of the cloned cells The efficacy data generated in such a system would not be representative of normal human (non-immortalized) cells in patients. Second, the mouse factor VIII protein expressed would be recognized as foreign in the gene-knockout mouse model, and an immune response and production of anti-mouse factor VIII antibodies would be expected. Furthermore, the safety of human cells expressing a human protein would not be evaluated in this system. Delivery into mice of human factor VIII by transfection w the human coding sequence would not be useful as the human protein would also be recognized as foreign, and anti-human factor VIII antibodies would be produced.

Similarly, the use of VIII-deficient dogs presents problems in developing a suitable model for testing the safety and efficacy of a human gene transfer treatment. One problem is that the human protein would induce an immune response in dogs. In addition, the canine protein may also induce an immune response in severely deficient animals (unlike the clinical protocol, where the patients are selected based on their lack of inhibitor development). The use of human cells in dogs is also problematic. Perhaps most importantly, the use of stably transfected clonal canine fibroblasts expressing canine factor VIII doe test the safety or efficacy of the product under development for use in humans.

For these reasons, the investigators have focused their efforts on systems that allow the long-term delivery of human factor VIII by stably transfected normal clonal cells. Both human and rabbit fibrob implanted into immunodeficient mice meet this objective. Human fibroblasts are remarkably resistant t spontaneous transformation in culture; there has never been a documented report of spontaneous transformation of a human fibroblast in culture. In their own work, examining thousands of independent clonal cell strains, the investigators have never observed a transformation event in a transfected non- transfected human clonal strain. Although human cells can survive for extended periods af implantation into immunocompromised mice, the investigators have demonstrated that rabbit ski fibroblasts show generally greater survival while also maintaining the *in vitro* and *in vivo* growth properties of normal, non-transformed cells. The improved survival of such xenogeneic implants allow human factor VIII to be delivered at physiologically relevant levels over extended periods. Long-term delivery of human proteins is essential for evaluating the toxicologic effects of chronic human factor VII delivery in an animal model.

In summary, other animal model systems of an animal or human factor VIII protein expressed by autologous animal cells could certainly be devised and tested. However, these models do not specifica test the safety or efficacy of the product intended for administration into patients.

(2) Will patients develop antibodies (inhibitors) or other immune responses to factor VIII transkaryotic therapy? What have the sponsor and investigator done to minimize the risk ofantibody development? I patient were to develop antibodies, how would the patient be medically managed?

Dr. Selden stated that it is unlikely that patients will develop a clinically significant immune response to factor VIII transkaryotic therapy. Their extensive analysis of factor VIII produced by clonal autolo human fibroblasts demonstrates that the protein has the structure and function expected for factor VIII isolated from normal human plasma. Therefore, the investigators believe that hFVIII transkaryotic th will carry the same risk for antibody development as conventional factor VIII replacement therapy.

Nevertheless, the investigators have taken precautions to minimize the risk of development of antibodies to factor VIII. Patients who are enrolled in the study will have had lifelong exposure to factor VIII replacement products without development of neutralizing anti-factor VIII antibodies (inhibitors). Clinical experience predicts that most patients who are destined to develop inhibitors do so within the first few years of replacement therapy, while contemporary studies using high purity factor VIII (antibody purified or recombinant) demonstrate inhibitor development much earlier, after a median exposure time of nine days. Because all patients eligible for entrance into this protocol must be negative for inhibitors after years of exogenous factor VIII exposure, this cohort of patients represents a group selected for very low likelihood of inhibitor formation. A positive clinical response of the patient to conventional factor VIII therapy and the absence of circulating inhibitor antibodies in a patient with a significant clinical exposure to exogenous human factor VIII are the most useful predictors of the likelihood of generating inhibiting antibodies. The investigators believe that a careful review of these baseline data should provide adequate protection to patients participating in the trial.

If, despite their best predictions, inhibitor formation occurs, standard approaches to treat patients will be undertaken. Often inhibitors are transient and can easily be overcome by using higher doses of factor VIII. Alternatively, porcine-derived factor VIII may be successfully employed. Bypassing agents, including prothrombin complex concentrates and recombinant factor VIIa , can also be utilized. Recombinant VIIa is particularly promising and Dr. Roth, the principal investigator, has significant clinical experienc with the use of recombinant factor VIIa for patients who have developed to inhibitors to factor VIII. Last immune tolerance protocols have been developed and can be very effective.

If conservative medical therapies are unsuccessful in achieving a long term reversal of inhibitors, surgical resection of the implanted fibroblasts will be undertaken. Only after all reasonable and standard medical approaches were exhausted would the investigators recommend that the cells be surgically resected Their best efforts at surgical resection may not succeed in removing all of the implanted cells (as is the case with essentially any surgical resection) although it is anticipated that most would be removed. Complete or significant reduction in cell number would probably be adequate to reverse the untoward effect.

All patients will be fully informed before entrance into the study that the experimental nature of this endeavor leaves the investigators unable to anticipate every possible risk and outcome and, in particular, that they cannot guarantee that every cell can be surgically resected to reverse the experimen

(3) Can the required number of cells be obtained from a single stably transfected cell? Will the cell become tumorigenic durin *in vitro* growth?

Dr. Selden stated that in developmental studies and in their Phase I clinical study of hGH transkaryot therapy, the investigators have consistently been able to expand stable transfected clonal skin fibro through a number of generations sufficient to produce in excess of 10^9 cells using the procedures described in this proposal. The investigators have studied the *in vitro* properties of fibroblasts derived from human donors of a variety of ages, including fibroblasts from subjects as old as 80 years. In these experiments the investigators have not observed a significant correlation between *in vitro* fibroblast growth properties and the age of the subject donating fibroblasts.

The manufacturing process involves the parallel growth of multiple autologous factor VIII-expressin fibroblast clones; an optimal clone is ultimately selected for patient implantation based on TKT standar operating procedures, in-process testing, and lot release testing, that include, among other things, tests fo tumorigenicity , sterility, and endotoxin . The transfected autologous fibroblasts are exte characterized for tumorigenicity and growth properties prior to administration to patients. Autologous will not be administered to patients which do not meet the FDA-reviewed lot release specifications for *in vitro* BDD hFVIII expression and other safety paramet

In contrast to the problems associated with tumorigenicity testing of heterogeneous cell population resulting from multiple different gene integration events (as occurs in almost all viral gene transfer systems in clinical use today) in which the ability to detect a rare tumorigenic subpopulation is difficult tumorigenicity testing of clonal cell lines is an extremely sensitive method for detection of cellu transformation because all cells share the same properties. This notion is supported by their work demonstrating that human fibroblasts are remarkably resistant to spontaneous transformation in culture; there has never been a documented report of spontaneous transformation of a human fibroblast in culture In thousands of clonal strains studied *in vivo* and *in vitro* experiments performed to support the development of TK's nonviral gene transfer technology, and thro*in vitro* lot release testing of clonal cell strains for the hGH Phase I clinical study, TKT has never observed a tumorigenic human fib clone. Finally, the investigators have demonstrated lifetime expression of BDD hFVIII (as well as l result never achieved in any other gene transfer system.

Other Comments

Dr. Markert asked what cell type normally produces factor VIII. Dr. Selden responded that factor VIII i normally produced by liver hepatocytes . Dr. Markert asked if factor VIII produced by the transfe fibroblasts will induce T cell as well as B cell immune responses because the wild type factor VIII is different from any mutant form made in patient's cells. She was concerned that transfected fibroblast placed in the omentum are not easily removable, and she asked why it is necessary to place those cell in the omentum . Dr. Selden responded that they do not believe there will be either a B- or a T-cell imm response; the fibroblasts may be removed from the omentum if necessary. Dr. Markert said that pla cells in muscle will allow easy access for biopsy to determine the outcome of cell implantation. Dr. Selden explained that the major purpose of the study is to investigate the safety issue mentioned by Dr. Markert

Dr. Markert noted that transfected fibroblasts are grown in a medium containing penicillin, and she a if patients allergic to penicillin will be excluded from the study. Dr. Selden responded that the improved tissue culture medium does not contain penicillin.

With respect to the Informed Consent document, Dr. Markert said that the statement regarding the surg procedure to remove the transplanted cells from the omentum should be clarified. Dr. Roth responded the Informed Consent has been amended to state clearly that while it is not the intention of the protocol to remove those cells, if there is an adverse indication an attempt would be made to remove those cells. It is

specifically stated that the investigators cannot guarantee that every individual cell could be removed.

Dr. Aguilar-Cordova asked to clarify why rabbit fibroblasts in nude mice are a better model than gene-knockout mice or factor VIII deficient dogs. He asked as a safety issue whether the patients receiving the factor VIII producing fibroblasts will be refractory to the standard replacement treatment if an immune response is developed. Dr. Treco responded that the goal of the study is to assess the produc producing a human protein in a human cell. He noted that human cells do not show reasonable survival in the immunodeficient test mice to allow long term testing of factor VIII exposure, and rabbit cells are better choice. The human factor VIII produced in rabbit cells is properly glycosylated . Dr. Roth said tha factor VIII made by transfected human cells has biochemical and functional characteristics similar to fa VIII used in replacement therapy; in his experience, long term exposure to such a product poses little risk to the development of an inhibitor.

Dr. Markert noted that factor VIII produced by a different cell type, i.e., fibroblasts vs. hepatocytes , r processed to peptides differently to induce a T cell response. Dr. Treco responded that fibroblasts are "professional" antigen presenting cells, and in many animal studies no such T-cell response has been demonstrated by expressing a variety of proteins in fibroblasts; he noted that fibroblasts lack the accessory molecules such as B7 and MHC Class 2 T-cell receptor to be efficient antigen presenting ce

Dr. Aguilar-Cordova noted that fibroblasts can use the MHC Class 1 pathway to present the antigen an induce an immune response. Dr. Treco responded that such an immune response is unlikely as shown immunocompetent mouse studies. He said if the CTL response does occur it will eliminate the tra cells and cause the failure of transkaryotic therapy but will not prevent the patients from receivin standard replacement treatment.

Dr. Parkman stated that the protocol should be amended to include a stopping rule so that the trial will be terminated if two or more subjects out of the nine proposed patients develop either a CTL or antibod response to human factor VIII. The stopping rule will allow termination of the study if toxicity is encountered; however, he considered the likelihood of such a toxicity to be small. Dr. Roth agreed to the recommendation.

Dr. Aguilar-Cordova asked if such a statement about the immune response is included in the Informed Consent document. Dr. Roth responded that toxicity is specifically stated.

Dr. Parkman asked whether all patients will be genotyped in terms of factor VIII gene mutations. Dr. Trec responded that not all patients will be genotyped; any patients who developed an inhibitor would be genotyped. Dr. Treco noted that based on extensive review of the hemophilia A mutant database, onl five genotypes with point mutations have more than a 50 percent chance of developing inhibitors. Deletion or insertion mutations in fact have even less chance of developing inhibitors. Only about 50 patients in the total database of 586 patients have some correlation of genotype to inhibitor phenotype. He noted that only patients who do not develop inhibitors to thereplacement therapy will be included in th study in order to minimize the risk. Dr. Treco concluded that genotyping is not a predictive test fo entrance to the study.

Dr. Mickelson inquired why the canine model is not appropriate to the protocol. Dr. Treco responded th they have made progress in improving the technique of growing the canine fibroblasts in tissue culture and are optimistic for using this model for future preclinical studies. Although they are committed to development of a canine model, the data on rabbit fibroblasts are sufficient to support the safety trial proposed in this protocol. Dr. Mickelson noted that canine hemophilia A model is useful to study the issue of immune response to factor VIII because there is no background production of endogenous factor VIII.

Dr. Selden noted that the canine model is more useful for assessing long term delivery of factor VIII than it is for assessing the immune response. Toxicity related to immune response has to be evaluated in human hemophilia A patients.

Dr. Selden also noted that genotyping has little predictive value with respect to inhibitor development in the patients. Contrary to what has been believed before, patients with factor VIII gene deletions have recently been found to have less risk of developing inhibitors. However, for patients who develop any inhibitors, their genotypes will be determined in order to build a knowledge base to see whether the genotype may yield some predictive value.

Dr. Gordon inquired how long the transfected fibroblasts will persist in the omentum after th implantation. Dr. Selden explained that fibroblasts are a type of cell with a low turnover rate and a long lifetime. In animal experiments, the implanted fibroblasts continue to produce the recombinant protein over a long period of time; however, the human data can only be obtained from the human trials.

Dr. Gordon asked how expression of the transgene can be assessed if the patients are concurrentl receiving replacement therapy with the same protein. Dr. Selden responded that factor VIII levels in patients with be measured after the implantation for up to two years to determine if the fibroblasts produce any additional factor VIII. Dr. Selden emphasized that the protocol is a safety study and such an efficacy endpoint is not its primary goal.

Dr. Mickelson asked if the fibroblasts can secret proteins into the circulating blood. Dr. Selden explained that fibroblasts secret a lot of proteins into the interstitial space and some of those proteins enter the circulation.

Dr. Richard Morgan (NIH National Center for Human Genome Research) inquired about th bioavailability of factor VIII injected subcutaneously. Dr. Selden responded that bioavailability of subcutaneous implantation is not favorable; for this reason the gene-modified cells were implanted in the omentum . The availability data will be obtained from the study. Dr. Morgan asked whether the patient have to bear the expensive cost of inhibitor therapy if they develop any inhibitors to factor VIII. Dr. Roth responded that the patients will not be liable for those costs.

Dr. Macklin noted that the monetary compensation of $2,500 offered to the participants is higher than that offered in most clinical trials. Dr. Roth said his IRB agreed to this level of compensation. The Informe Consent document was amended to state that if a patient withdraws prematurely from the study, he or she will be paid on a prorated basis. Dr. Macklin inquired the basis for calculating the amount of monetary compensation. Dr. Roth responded that there is no givenstandard to determine the amount but h considers $2,500 to be fair to compensate the subjects without the appearance of coercion.

Dr. Parkman said he had one question that was based on the proprietary information submitted by the sponsor, and he asked if he should raise that question in a closed session. Dr. Anderson objected to holding a closed session and reminded the RAC and study sponsor that one of the committee's primary purposes is public accountability. He said that holding any discussion closed to the public would be contrary to the purpose and tradition of the RAC. He noted that in the entire history of RAC review of human gene transfer protocols there was only one brief closed session when proprietary information regarding the exact formulation of a DNA/liposome complex protocol was discussed. Dr. Parkman added that another occasion was when patient confidentiality was involved in a single-patient gene transfer protocol by Dr. Ivor Royston. Dr. Anderson noted that the sponsor and investigators of the present prot have demonstrated a laudable spirit of cooperation with RAC review, and so far most questions have been satisfactorily responded to in public. He suggested continuing the discussion in the public forum.

Dr. Mickelson agreed with Dr. Anderson that there may not be a need to go into a closed session. She asked the sponsor and Dr. Parkman to discuss Dr. Parkman's question privately to see whether his question can be raised and responded to in public. Dr. Selden explained that the reason he previously requested a closed session was to present some detailed proprietary technical data to the RAC. He agreed that he might be able to respond to Dr. Parkman's question in public.

While waiting for a private conference between Drs. Parkman and Selden regarding the question based on the proprietary submission material, Dr. Anderson asked if there is any rule pertaining to proprietary restriction. Dr. Noguchi said the FDA's confidential information includes patient identity, financial information, and trade secrets related to product manufacturing.

After examining Dr. Parkman's question, Dr. Selden agreed to respond to Dr. Parkman's question in public.

Dr. Parkman noted that in the human growth hormone study, the transduced fibroblasts were give subcutaneously and were removed to assess the inflammatory response. He asked why not implant the transduced cells subcutaneously in the present protocol? It would be much easier to access the site fo biopsy and to remove the implanted cells.

Dr. Selden said he is pleased to answer this question in an open session to assure public confidence that they are proceeding with the clinical trial with the patients' best interest in mind. In response to Dr. Parkman's question, Dr. Selden explained that the subcutaneous route is not a good way to deliver factor VIII; there is no need to propose a trial that will not be useful for future therapeutic purposes. He noted tha there was no local inflammation induced by implanting growth hormone producing fibroblasts under the skin. With respect to the question of why not implant the cells under the skin, Dr. Noguchi noted that presentation of antigens by dendritic cells in the subcutaneous tissue may pose more risk of toxicit related to immune response in the factor VIII protocol.

Public Comments

Ms. Julie Dorr (Kensington, MD), a mother of a hemophiliac child and a patient advocate, asked several questions. (1) Granted this study is a Phase I safety trial; however, presuming the trial goeswell , what i the eventual expectation in terms of percentage of correction for people with hemophilia? Dr. Selden responded that optimism should be tempered by the fact that he started this project in 1978 and the clinical trial is just beginning to start now. The goal is to have complete phenotypic correction and the phenotypic result can be achieved even with only a 25 percent of normal factor VIII expression level. (2) Ms. Dorr asked if the cells to be infused into the patients will be derived from the punch biopsy. Dr. Selden responded yes. The cells to be reinfused back to the patients are autologous fibroblasts expanded the biopsy specimen in tissue culture. (3) Ms. Dorr asked whether the patient population is only those affiliated with his medical center or from a broader group. Dr. Roth responded that most of the patients wil be those who are affiliated with the Center for Hemophilia Care and Thrombotic diseases at the Bet Israel Deaconess Medical Center, but that an attempt will be made to include patients throughout Massachusetts and the New England area. (4) Ms. Dorr asked if there are any racial/ethnic barriers to the eligibility criteria. Dr. Roth responded there are no racial or ethnic stratification criteria. (5) Ms. Dorr asked if any of the proprietary information contained in the ORDA submission package will be available to th participants of the study. Dr. Selden responded that they would try to make as much information available to the public as possible. (6) Ms. Dorr asked what is the time lapse between the completion of the safety study and a subsequent trial to evaluate efficacy. Dr. Selden responded that if there is no safety problem, the next trial can be started in about a year; if there is any safety problem, the investigators have to go

back to basic research before attempting another clinical trial.

As a point of clarification Dr. Mickelson noted that most of the confidential information contained in the ORDA submission package is not proprietary *per se*; it is the sponsor's feeling that proper responses to those questions may require presentation of proprietary technical data.

Dr. Michael Kaleko (Genetic Therapy, Inc., Gaithersburg, MD) asked from a research perspective whether the clinical trial should be attempted without adequate preclinical studies performed on the available mouse and dog models. He noted that hemophilia A is a nonfatal disease. Dr. Parkman explained that the gene-knockout mouse model is not a perfect model for the human disease. Most hemophiliacs have circulating nonfunctional factor VIII; there is more of a chance in developing an immunologic reaction to the transgene product in a gene-knockout mouse that has no backgroun expression of the protein. Dr. Kaleko noted that the hemophiliac dog model is a natural disease that is relevant model to the human study. Responding to Dr. Kaleko's comments, Dr. Noguchi noted that from the FDA perspective the serious life-threatening diseases include a wide variety of "nonfatal" diseases. It is not the seriousness of the disease *per se* that is the real factor in deciding on a clinical trial; it is how much scientific information is expected to be gained from the proposed clinical study.

Mr. Steven Kradjian (Vical Inc., San Diego, CA) said that he appreciated Dr. Anderson's effort in keeping the entire RAC discussion of the protocol in a public session. He noted that his company has always embraced the philosophy of open discussion of human gene transfer clinical trials. The questions coming out of today's discussion highlight the value of public discussion that builds public confidence. He said it is a sunny day in the history of the RAC and human gene transfer research.

Dr. Gordon suggested putting an inducible toxin gene into the transduced fibroblasts to facilitat elimination of the transplanted cells if needed. Dr. Selden said that such an option was considered and rejected due to additional safety concerns associated with the suicide gene. Dr. Selden would consider the option if a better technology is developed in the future. Dr. Noguchi agreed with Dr.Selden that it i riskier in the face of current technology to include an inducible toxin gene in the vector construct.

Dr. Macklin noted that the Informed Consent document is clear, lucid, complete, and is written in a language understandable by the patients.

Dr. Parkman asked the biologic reason for not implanting the transduced cells subcutaneously. Dr Selden explained that factor VIII is a large molecule and it is not readily bioavailable when administere under the skin.

Dr. Markert asked if there is any risk of immunological response to neomycin phosphotransferase . Selden noted no significant adverse effects have been observed in many clinical trials involving the *ne*^{R} gene in gene marking studies.

Dr. Mickelson summarized the specific issues discussed by the RAC. Specific issues discussed by the RAC included the biology of fibroblasts transduced with hFVIII . The RAC asked if the transduced would produce and process the hFVIII protein differently from the natural protein and the possibility o mediating CTL and antibody responses. The RAC discussed the use of appropriate preclinical anima models. The investigators stated that they are beginning to develop a canine model for hemophilia A-factor VIII, which is a more appropriate model than the factor VIII gene knock-out murine model. Th RAC asked whether genotype analysis of patients would be valuable for the study. The investigators responded that such genotyping has limited value for this clinical study. The Informed Consent document has been amended to include discussion of the surgical removal of transplanted fibroblasts, if necessary.

The RAC noted the relatively high monetary compensation for participation in the trial.

RAC Recommendations

At the conclusion of its discussion of this protocol, the RAC made the following specific recommendation to be included in a letter to the investigator and concerned bodies: the protocol should be amended to include a stopping rule so that the trial will be terminated if two or more subjects develop either a CTL antibody response to hFVIII

Future Meeting Dates

The next RAC meeting will be September 24-25, 1998, at the NIH , Building 31C, Conference Room 10 Bethesda, Maryland. The next NIH GTPC will be on the topic *in vivo* gene transfer, and has been rescheduled for December 7-8, 1998, in Bethesda, Maryland. Previously, the date for this GTPC wa September 24, 1998.

Adjournment

Dr. Mickelson adjourned the meeting at 3:15 p.m. on June 19, 1998.

Debra W. Knor
Executive Secretary

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

Date: 09/24/98

Claudia A. Mickelson, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health