DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
June 9-10, 1994

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The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-eighth meeting at 9:00 a.m. on June 9, 1994, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Nancy L. Buc, Buc, Levitt, & Beardsley
Gary A. Chase, Georgetown University Medical Center
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, The Foundation of Human Genetics
David Ginsburg, University of Michigan
Robert Haselkorn, University of Chicago
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington
Robertson Parkman, Children's Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Gail S. Ross, Cornell University Medical Center
Bratin K. Saha, Emory University
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health

LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, Virginia Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).

Ad Hoc Consultant

Harold Ginsberg, National Institutes of Health/Columbia University

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

Niles Bernick, OD
R. Michael Blaese, NCI
Diane Bronzert, NCI
Sarah Carr, OD
Barry Goldspiel, CC
Toby Hecht, NCI
Christine Ireland, OD
Susan Jenks, NCI
Becky Ann Lawson, OD
Johnson Liu, NHLBI
Catherine McKeon, NIDDK
Koichi Miyamura, NHLBI
Joan Porter, OD
Muzaffar Quazilbash, NHLBI
Mallika Sekhar, NHLBI
Thomas Shih, OD
Bernard Talbot, NCRR
Chris Walsh, NHLBI
Debra Wilson, OD
Neal Young, NHLBI

Others:

Munir Abdullah, Glaxo, Inc.
Estuardo Aguilar, Baylor College of Medicine
W. French Anderson, University of Southern California
Mary Helen Binger, Hoffmann-LaRoche, Inc.
Bridget Binko, Cell Genesys, Inc.
Robert Boyd, Knight-Ridder, Inc.
Ronna Campbell, University of Pittsburgh
Jeff Carey, Genetic Therapy, Inc.

Rachel Carle, Genzyme Corporation
Barry Carter, Targeted Genetics Corporation
Jan Chappell, Genetic Therapy, Inc.
Yawen Chiang, Genetic Therapy, Inc.
Lori Clarke, Genetic Therapy, Inc.
Richard Cote, University of Southern California
David Curiel, University of Alabama
Karen Darcy, Magenta Corporation
Albert Deisseroth, MD Anderson Cancer Center
James Economou, University of California, Los Angeles
Chris Evans, University of Pittsburgh
Chuck Finn, Genetics Institute, Inc.
Olivera Finn, University of Pittsburgh
Terence Flotte, Johns Hopkins Hospital
Suzanne Forry-Schaudies, Genetic Therapy, Inc.
Ralph Freedman, MD Anderson Cancer Center
Joyce Frey, Food and Drug Adminstration
Lynn Frohnmayer, Mothers of Children with Fanconi Anemia
Morgan Gale, Hearings-on-the-line
Diane Gershon, Nature Magazine
Richard Giles, MD Anderson Cancer Center
Alan Goldhammer, Biotechnology Industry Organization
Richard Gregory, Canji, Inc.
Robert Grossman, Arrow International Marketing, Inc.
Elie Hanania, MD Anderson Cancer Center
Helen Heslop, St. Jude Childrens Research Hospital
Frankie Holmes, MD Anderson Cancer Center
David Holzman, BioWorld
Joseph Hughes, Quality Biotech
Edie Irvine, Genetic Therapy, Inc.
Jeffrey Isner, St. Elizabeths Medical Center
Bobbi Kamil, Cable in the Classroom
Michael Kanaley, The Novus Group
Karilyn Kelson, Mothers of Children with Fanconi Anemia
Martin Korbling, MD Anderson Cancer Center
Toshi Kotani, Genetic Therapy, Inc.
Steven Kradjian, Vical, Inc.
Gary Kurtzman, Avigen, Inc.
Al Kuta, Food and Drug Administration
Albert Lobuglio, University of Alabama
Michael Lotze, University of Pittsburgh
Bill Macaulay, University of Pittsburgh
Tamie Malaska, Targeted Genetics Corporation
Dan Maneval, Canji, Inc.
Tony Marcel, TMC Development
Eliot Marshall, Science Magazine
John Marshall, Magenta Corporation
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Andra Miller, Food and Drug Administration

Grant Mitchell, St. Justin Hospital
Micheal Nash, MD Anderson Cancer Center
Philip Noguchi, Food and Drug Administration
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Magenta Corporation
Robert Overell, Targeted Genetics Corporation
Andrea Pavirani, Transgene, Inc.
Michael Penn, Genetic Therapy, Inc.
Michael Pensiero, Genetic Therapy, Inc.
Jerri Perkins, Perkins & Perkins
Joan Personett, American College of Obstetricians and Gynecologists
Stephen Pijar, University of Maryland
Chris Platsoucas, Temple University School of Medicine
John Powderly, Georgetown Medical School
Raj Puri, Food and Drug Administration
Kathryn Pushkar, The Blue Sheet
Urban Ramstedt, Virus Research Institute
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
Paul Robbins, University of Pittsburgh
Jack Roth, MD Anderson Cancer Center
Leonard Schiff, Microbiological Associates, Inc.
Richard Scotland, Genzyme Corporation
Tomiko Shimada, Ambience Awareness International
John Shiver, Merck, Inc.
Robert Sobol, San Diego Regional Cancer Center
Ruth SoRelle, Houston Chronicle
Margi Stuart, Breast Cancer Action
Frank Sturtz, Progenitor, Inc.
Hideaki Tahara, University of Pittsburgh
Tom Tarlow, Chiron Corporation
Paul Tolstoshev, Genetic Therapy, Inc.
Matthew Tomaino, University of Pittsburgh
Karl Uhlendorf, The Blue Sheet
Cynthia Utley, GenVec
Dominick Vacante, Microbiological Associates, Inc.
Deborah Vaz, Virus Research Institute
Wanda de Vlaminck, Avigen, Inc.
Kenneth Walsh, St. Elizabeth Medical Center
Judi Weissinger, Applied Immune Sciences
Katharine Whartenby, Food and Drug Adminstration
Sharon Williams, Life Technologies, Inc.
Wei-Wei Zhang, MD Anderson Cancer Center
Laurence Zitvogel, University of Pittsburgh

I. CALL TO ORDER/DR. WALTERS

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting was published in the *Federal Register* on April 22, 1994 (59 FR 19200), and the proposed actions were published in the *Federal Register* on May 11, 1994 (59 FR 24618), as required by the*NIH Guidelines for*

Research Involving Recombinant DNA Molecules (NIH Guidelines). He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the proposal, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, and followed by the public at large.

New Members

Dr. Walters said that three new RAC members will participate in this meeting: (1) Gail S. Ross, Ph.D., Research Director, High-risk Infant Follow-up Program, New York Hospital, Perinatology Center, Cornell University, New York, New York; (2) Bratin K. Saha, Ph.D., Assistant Professor, Department of Pathology, Winship Cancer Center, Emory University, Atlanta, Georgia; and (3) David Ginsburg, M.D., Professor, Department of Internal Medicine and Human Genetics, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan.

Overview

Dr. Walters noted that 10 human gene transfer protocols will be reviewed at this meeting, 8 gene therapy protocols (6 cancer, 1 rheumatoid arthritis, and 1Fanconi anemia) and 2 gene marking studies (1 autologous bone marrow marking and 1 tumor infiltrating lymphocyte marking).

Discussion--Compensation for Research-Related Injuries

Dr. Walters summarized the meeting material regarding compensation for research-related injuries that was provided as a supplement to the RAC's previous discussions regarding provision of medical care to subjects who may be injured during the course of their participation in research.

Robert Levine's book entitled, *Ethics and Regulation of Clinical Research* , includes a discussion of the Yale University program on compensation for research-related injuries.
The University of Washington has a self-insured Human Subjects Compensation plan for research protocols that are not beneficial or therapeutic. Since 1979, the University of Washington plan has received 21 requests for payment resulting in a total outlay of $4,110.70. The most costly incident involved an elderly subject who broke her leg while participating in a fall prevention study. The injured subject required treatment costing $1,639.04. Most other requests averaged less than $600.

The Council for International Organization of Medical Sciences, Geneva, Switzerland, has published the *International Ethical Guidelines for Biomedical Research Involving Human Subjects* . These guidelines specify therapies that are provided free of charge for specific research-related injuries, and asserts the right of subjects to compensation in the event of disability or death.

On February 24, 1990, the U.S. Department of Defense (Army) issued regulations on the *Use of Volunteers as Subjects of Research.* According to these regulations, subjects who receive research-related injuries while in uniform receive compensation comparable to subjects who have been injured in combat. Civilian employees are covered under Worker's Compensation. Medical insurance and direct costs for contracting personnel are provided as part of the contract cost negotiated between the U.S. Department of Defense (Army) and the contractor.

Documents are included from the Royal College of Physicians and the Association of the British Pharmaceutical Industry that detail Great Britain's National Health System in which all medical costs

are covered for subjects injured during the course of their participation in research.

Dr. Zallen inquired about the current status of legislation relating to U.S. Health Care Reform and coverage for research-related injuries. Dr. Wivel explained that there are several health care proposals pending consideration at different Congressional committees and that specific language regarding compensation for research-related injuries has not been finalized in the legislation.

Temin Letter Regarding Potential for Recombination of Retroviral Vectors

Dr. Walters noted a letter dated December 2, 1993, from Dr. Howard M. Temin requesting that the RAC consider a specific safety issue regarding the potential for recombination of retroviral vectors. The letter states:

"I would like you to consider the following safety point relative to retrovirus vectors. Recombination, which could form replication-competent virus by recombination between the vector and packaging cell RNAs, only takes place upon infection of sensitive cells. Thus, even if there is no replication-competent virus in a vector inoculum, replication-competent virus could be formed upon infection. Does the testing now used for vector preparations used in patients adequately test for this possibility?"

Included in the meeting materials were comments by RAC members and representatives of industry responding to Dr. Temin's letter. Since this item was not included in the meeting agenda announced in the *Federal Register*, the discussion remained informal. Dr. Walters suggested that a working group should be established prior to the next RAC meeting to determine the RAC's plan of action with regard to this issue and invited suggestions for *ad hoc* consultants who could provide their expert comments on this issue.

Dr. Miller said that this issue is basically academic since current replication-competent retrovirus (RCR) assays adequately test for the recombination events alluded to by Dr. Temin. The standard S+L- marker rescue assays and the *Mus dunni* co-cultivation assay are sensitive enough to detect RCR both in the vector preparations and viruses generated following cell infection. Dr. Parkman concurred with Dr. Miller's statement. Dr. Post said that the question raised by Dr. Temin was not stated explicitly; and unfortunately, the question cannot be clarified since Dr. Temin is recently deceased. Dr. Post speculated that if the helper virus RNA is co-packaged with the vector RNA in virus particles, recombination might occur between these two RNA species upon infection of target cells to generate a novel retrovirus. Dr. Miller said that the current packaging cell lines are constructed with a split helper virus genome in order to reduce the probability of such a recombination event. Dr. Straus said that the question raised by a prominent scientist and Nobel laureate such as Dr. Temin could possibly be more profound that it initially appears. Perhaps, Dr. Temin was speculating about the possibility of generating RCR upon infection of sensitive cells *in vivo*, i.e., in the patient's body, as a result of recombination between the vector sequences and some endogenous elements within the target cells. Dr. Straus said that this issue should be carefully considered by the RAC to assure that current safeguards and testing procedures are adequate. Dr. Miller said that the current RCR testing procedures are designed to screen for such recombination events both before and after infection with the vector preparations. Dr. Philip Noguchi, Director of the Division of Cellular and Gene Therapies, Food and Drug Administration (FDA), agreed with Dr. Miller's statement that current testing procedures can adequately detect most occurrences of RCR; however, remote recombination events do occur during large-scale vector production. Dr. Noguchi encouraged the RAC to continue its discussion of this safety issue.

Update on *Accelerated Review Procedures*

Dr. Walters inquired about the status of the *Accelerated Review* procedures approved by the RAC at its March 1994 meeting. Dr. Wivel responded that these actions are included in the new incorporated version of the *NIH Guidelines*, which is in the final stage of approval by the NIH Director. The 1994 *NIH Guidelines* include new Appendices P and Q that address physical and biological containment for transgenic plants and animals in the greenhouse and animal facility settings. The environmental assessment for these new appendices has been recently approved.

Continuation of Discussion on Compensation for Research-Related Injuries

In regard to the RAC's earlier discussion regarding the provision of medical care to subjects injured during the course of their participation in research, Dr. Walters asked Dr. Smith to comment on Yale University's program for compensation for research-related injuries. Dr. Smith responded that Yale University provides coverage for all costs related to research-related injuries. Although he did not have data regarding the number and amounts of payments for claims under the program, he understands that the claims have not been overwhelming.

II. CHAIR REPORT ON MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that minor modifications were approved to the following human gene transfer protocols since the March 3-4, 1994, RAC meeting (Attachment II):

3/23/94	Protocol #9309-053	Investigators: Peter Cassileth/Eckhard Podack
3/23/94	Protocol #9206-023	Investigator: Cynthia Dunbar
3/23/94	Protocol #9206-025	Investigator: Cynthia Dunbar
4/11/94	Protocol #9212-034	Investigator: Ronald Crystal
4/13/94	Protocol #9303-041	Investigators: Robert Wilmott/Jeffrey Whitsett/Bruce Trapnell
6/01/94	Protocol #9209-020	Investigator: Robert Walker

III. MARCH 3-4, 1994, RAC MINUTES

The RAC approved a motion made by Dr. Chase and seconded by Dr. Doi to accept the March 3-4, 1994, RAC minutes with the inclusion of minor changes suggested by Dr. Chase by a vote of 15 in favor, 0 opposed, and no abstentions.

IV-A. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: PHASE I TRIAL OF A POLYNUCLEOTIDE AUGMENTED ANTITUMOR IMMUNIZATION TO HUMAN CARCINOEMBRYONIC ANTIGEN IN PATIENTS WITH METASTATIC COLORECTAL CANCER/DR. CURIEL*

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. David Curiel of the University of Alabama, Birmingham, Alabama. Dr. Haselkorn explained that the objective of this Phase I study is to determine whether intramuscular injection of plasmid DNA containing the cDNA

for carcinoembryonic antigen (CEA) induces an immune response (humoral or cellular) to CEA in patients with advanced colon carcinoma. CEA is an antigen that is present on the surface of embryonic cells in normal individuals. The immune response that recognizes CEA as a foreign antigen is deleted in early development. However, CEA frequently reappears in certain tumors such as colon carcinoma. The rationale of this study is to immunize cancer patients with the CEA antigen to overcome the immune tolerance and to induce immune response against cancer cells expressing the CEA antigen.

The materials submitted by Dr. Curiel reference an ongoing study conducted by Dr. Jeffrey Schlom of the National Cancer Institute (NCI), NIH. Dr. Schlom's study involves the administration of a recombinant vaccinia viral vector encoding CEA in order to elicit a CEA-specific immune response in cancer patients. In contrast, Dr. Curiel's study involves the administration of a plasmid DNA vector encoding CEA. Dr. Haselkorn asked the following: (1) How is an anti-CEA immune response expected if there is immune tolerance to the same antigen? (2) Since CEA has a molecular weight of 180,000 daltons and more than half of the mass is attributed to carbohydrate, are enzymes required to add the carbohydrate moiety to the CEA polypeptide? (3) Does antigen/antibody recognition require proper glycosylation of the CEA polypeptide? Dr. Haselkorn said that the original submission was poorly written; however, the investigators have clarified previous concerns in their written responses.

Review--Dr. Chase

Dr. Chase said that the protocol involves intramuscular injection of a plasmid DNA encoding the CEA gene, and the proposed treatment does not carry the risks associated with retrovirus vectors. He objected to the use of the term "vaccine." The term "vaccine" has traditionally been used to describe immunization for the prevention of disease, not treatment of preexisting disease. He objected to the Informed Consent document statement that patients will be responsible for any costs of medical treatment required as a result of research-related injury. Subjects should be informed that no benefit is expected from participation in this study. The Informed Consent document should be revised to reflect these concerns.

Review--Dr. Zallen

Dr. Zallen stated that the investigators have provided complete responses to Section I-D, Informed Consent, of the *Points to Consider*. She noted concern about the statement regarding payment for research-related injuries that medical treatment "is not provided free of charge." The use of passive voice in this statement makes it unclear who will be billed. Will the financial responsibility imposed by this statement constitute a barrier to the participation in this study? She inquired whether animal studies have been conducted to determine if the plasmid DNA spreads beyond the original site of injection.
Other Comments

Dr. Walters noted written comments submitted by Ms. Meyers stating her objection to the use of the term "vaccine" throughout the Informed Consent document and the protocol. Ms. Meyers expressed concern regarding patients' responsibility for costs of medical tests and research-related injuries. Subjects should not be excluded from participation in this study based on their inability to pay for such costs.

Ms. Buc commented that the statement in the Informed Consent document regarding compensation for research-related injuries does not exclude the patient's right to bring a lawsuit if such injuries resulted from negligence. Subjects have the right to choose to enter the protocol. Disclosure is the heart of Informed Consent; therefore, if the terms are clearly disclosed and agreed upon by the subject, the Informed Consent document is considered acceptable. Ms. Buc noted the inappropriateness of the RAC's continued discussions on these Informed Consent issues.

Dr. Smith asked whether there is the potential for toxicity resulting from high levels of CEA and anti-CEA antibodies in the blood. Have these toxicity issues been adequately addressed in animal experiments?

Dr. Parkman expressed concern about the immune response to CEA and immunoprotection of syngeneic CEA expressing tumors in the murine model. The RAC has always required that one consistent criterion for approving any human gene transfer protocol is to demonstrate immunoprotection against a preexisting tumor in an animal model. The preclinical studies submitted by the investigator has not adequately demonstrated immunoprotection. The antitumor effects observed in mice resulted from vaccine administration initiated 7 days after the CEA bearing colon carcinoma cells were transplanted. The antitumor effect should be demonstrated on tumors that have been established for a longer period such as 21 days or more. Another important concern is that the human CEA antigen was used in a murine model. The rationale for the proposed study is to break immune tolerance by immunization with the CEA antigen. The preclinical murine experiments in which immune responses were demonstrated against human CEA. Human CEA is a foreign antigen to mice and may induce an immune response by acting as a hapten, a co-stimulator; therefore, the preclinical experiments are irrelevant.

Dr. Haselkorn asked the investigators to elaborate on an experiment reported by investigators at the Memorial Sloan-Kettering Cancer Institute in which antibody responses to antigens associated with adenocarcinomas were induced by injection of human subjects with chemically treated bovine mucin. Dr. Doi inquired about the level of transgene expression, noting that an expression level of less than 1% is very low.

Dr. Parkman asked the investigators why immunization with plasmid DNA-CEA is expected to be superior to CEA expressed by the vaccinia vector currently under investigation by Dr. Schlom. He noted that Dr. Schlom's vaccinia vector protocol would not have been exempt from RAC review according to the RAC's revised definition of Footnote V-21, exempt vaccines. Dr. Wivel explained that Dr. Schlom's study was initiated before the RAC revised Footnote V-21.

Dr. Post said that the data obtained from the vaccinia-CEA trial should answer the question of whether human CEA can break tolerance and induce an immune response. The investigator has maintained that naked DNA injected into muscle is not integrated within the cell chromosomes, yet CEA has been shown to be persistently expressed for an extended period. Have sensitive experiments been performed to determine the exact percentage of DNA integration? Dr. Parkman asked whether the investigator has access to the data obtained from Dr. Schlom's vaccinia vector trial and whether the use of cytoxan in that trial is important in breaking the immune tolerance by eliminating suppressor cells.

Investigator Responses--Drs. Curiel and Lobuglio

In response to Dr. Haselkorn's concerns, Dr. Curiel stated that the muscle cells are capable of glycosylating the CEA appropriately and are recognized by a panel of specific antibodies. An immunological response is induced which is the ultimate functional test. Dr. Curiel explained that Dr. Schlom has demonstrated antitumor efficacy of vaccinia-CEA in mice with low tumor burdens.

In response to Dr. Zallen's question, Dr. Curiel said that the RAC has previously approved studies in which injected liposome DNA distributes systemically. Preliminary PCR assays indicate that as few as 5 DNA copies per mg of tissue are detectable one day following injection. DNA was detectable at the injection site, tongue, and gonads of animals. Seven days following injection, DNA was detected only in the tongue. This result suggests that if DNA localizes beyond the injection site, there is no persistent expression. In regard to transduction frequency, Dr. Curiel explained that the 1% frequency described in the protocol is based on published data and not derived from experiments conducted in his laboratory.

In response to Dr. Post's concerns, Dr.Curiel stated that consensus in the literature seems to be that the basis of persistence is not integration. Mixing experiments involving integrated and nonintegrated genes have been conducted by John Wolfe and published data demonstrates that a single integrated copy can be detected in 104 to 105 cells. Dr. Post stated that although he was unaware of the sensitivity data referred to by Dr. Curiel, a low level of DNA integration should be an acceptable risk.

Dr. Lobuglio of the University of Alabama explained that Dr. Schlom's Phase I vaccinia-CEA trial involves subjects with high tumor burden. There was no evidence of toxicity up to 107 virus particles per site. Immunologic response data is not yet available. Dr. Lobuglio stated that he has been contracted by Dr. Schlom's group to conduct a second vaccinia-CEA trial involving subjects with low tumor burden. This second trial will include cytoxan administration and establish evidence of immune response. The basis for the proposed plasmid DNA study is preliminary animal data demonstrating that CEA is immunogenic and can safely produce both immunoprotection and therapy.

Dr. Lobuglio explained that the monoclonal antibodies have been directed to the peptide portion of the molecule. The murine model demonstrated that intramuscular injection of this polynucleotide vaccine resulted in an immune response to the human CEA antigen. The presumption is that the human molecule contains the epitopes that are the target of the immune response.

The issue of breaking tolerance by immunizing an individual with a tolerant antigen is an overriding issue in tumor immunology. Data does not exist relating to the question ofCEA immunogenicity in humans. Published data indicate that an immune response to a fetal antigen (notCEA) has been observed in humans.

The term "vaccine" has been changed to "immunization" and "augmentation" throughout the protocol in response to concerns about vaccines inferring disease prevention. There is increasing evidence in the literature that post tumor immunization can produce a detectable immune response.

Dr. Lobuglio explained that Dr. Schlom administered vaccinia-CEA to mice 7 days following tumor cell implantation because tumor growth was rapid enough to become palpable and reach end stage in 28 days. Conversely, 10 to 14 days are required for gene expression by muscle cells*in vivo*. This time-frame between vaccine administration and tumor inoculation was a major constraint to testing this therapy.

In addressing the RAC's concern about compensation for research-related injury, Dr.Lobuglio stated that "all of the normal responsible issues are in place" regarding compensation for negligence. Only compensation for complications arising from the research are excluded. This exclusion will be clearly presented to all subjects.

In response to concerns about possible toxicity of circulating CEA and its immune complexes, Dr. Lobuglio said that no evidence of toxicity has been observed in subjects with circulating CEA receiving up to 1 gram of anti-CEA monoclonal antibody. One of the endpoints of the proposed study is to evaluate tissue injury resulting from an immune response to CEA. No such injuries have been observed in the vaccinia-CEA study. The proposed polynucleotide vaccine strategy is to overcome the diminished immune response to immunogens encoded by vaccinia which can occur in subjects previously exposed to vaccinia. In regard to Dr.Haselkorn's question about bovine mucin, Dr. Lobuglio stated that he was not knowledgeable about such data.

Other Comments

Dr. Parkman commented that the RAC has consistently held investigators to the standard that preclinical

evidence must be demonstrated on established tumors. The only data presented by the investigator involved a preimmunization model. Dr. Lobuglio agreed but stated that the proposed strategy was similar to that of the vaccinia trials. Dr. Post commented that a murine model may be irrelevant for the proposed study. Dr. Parkman noted cytokine trials in which animals have received both murine and human cDNA's. The investigator has based his hypothesis that this strategy will provide a better immune response than the vaccinia study without providing the necessary preclinical data. Dr. Lobuglio responded that an alternative, not necessarily better, method is proposed to achieve the desired outcome. The only way to evaluate the relative merits of the proposed study is to perform the human experiment. Dr. Parkman reiterated that preclinical evidence has not been provided demonstrating the efficacy of this treatment against metastases, the RAC's usual standard. If such studies are not technically possible, then the RAC should determine the next level of criteria.

Dr. Straus stated that the proposed direct DNA injection approach is inherently safer than the vaccinia approach; however, there is a lack of preclinical data to support this rationale. He recommended that the murine CEA gene should be used in a murine model. Dr. Miller said that although there is a lack of scientific evidence to support the proposal, the lack of data should be weighed against the potential risk from a recombinant DNA aspect. Since the proposed plasmid does not contain any viral elements, there is no danger of recombination. The RAC could reduce their standards for such innocuous trials. Dr. Chase agreed that the major purpose of this committee was protecting the public from hazards associated with the recombinant DNA aspects.

Dr. Straus recommended that the RAC should proceed cautiously in their deliberation of this new technology, i.e., direct DNA injection into muscle. Though probably a safe strategy, the proposal would be more acceptable with evidence that an immune response can be developed to murine CEA in a murine model. Dr. Smith noted that safety issues may vary depending on the life expectancy of the subject. Dr. Post commented that the treatments that the subjects have already received is far more toxic than the risks posed by the integration of a few DNA molecules.

Dr. Parkman reminded the committee that whatever action it takes on this protocol will have set a precedent. It is important that the RAC remain consistent so that future investigators have a clear understanding of the requirements and the committee maintains credibility. Dr. Walters asked Dr. Parkman to estimate the time that would be required to perform the relevant preclinical murine experiments. Dr. Parkman responded that 6-9 months would be an approximate speculation.

Dr. Lobuglio stated that he was not aware that the CEA gene had been cloned from a murine library. Each mouse strain has different immune responses; therefore, several different strains would have to be evaluated before drawing any conclusions. He noted that the murine model may not be totally predictive of the human experiment. A substantial amount of information exists on the use of this technology to produce immune responses that are efficacious against infectious diseases. Other investigators have demonstrated the antitumor potential of this model by mixing the splenocytes of immunized animals with tumor cells, and injecting them into naive animals. Total protection against tumor growth was observed *in vitro* at a ratio of 1:1, an amazingly high response.

Dr. Haselkorn said that he favors approval of this protocol. He asked the investigators to submit the immunological data derived from this study to the RAC as soon as possible. He said that it is unacceptable that data is unavailable from the vaccinia-CEA study after 20 patients have been entered onto the study.

Dr. Straus said that in absence of preclinical data involving the murine CEA gene or relevant data from animal tumor model, clinical data from the vaccinia-CEA trial would be an acceptable alternative. Dr.

Straus said that he cannot recommend approval of this protocol without reviewing these supporting data.

Dr. Saha asked whether the fact that different mouse strains respond differently to CEA is due to major histocompatibility complex (MHC) restriction. Dr. Lobuglio answered that lack of a consistent immune response is one reason that animal models will not be totally relevant to the human study.

Dr. Parkman agreed with Dr. Straus that the clinical data from the vaccinia-CEA study would be acceptable data to support this protocol. Dr. Haselkorn objected to inclusion of this contingency for approval since these data are from other laboratories and beyond the control of the present investigators. Dr. Haselkorn stated that even if the vaccinia-CEA study fails, the CEA-DNA injection might yield positive results. Ms. Buc agreed with Drs. Parkman and Straus' assertion that this protocol is not approvable without submission of adequate preclinical data.

Dr. Lobuglio explained that he is involved in the vaccinia-CEA study; however, the data is coded and the results are unknown. Dr. Miller maintained that it is illogical for the RAC to contingently approve a protocol based on data obtained from an experiment that was not reviewed by this committee. Dr. Wivel explained that the vaccinia-CEA experiment was considered exempt from RAC review based on the old definition of Footnote V-21 of the *NIH Guidelines*. The recently amended definition of Footnote V-21 would require RAC review of the vaccinia-CEA trial.

Dr. Curiel said that this trial will provide the data regarding the safety of polynucleotide vaccines which hold great promise for the treatment of many infectious diseases.

Dr. Smith asked whether the same preclinical data will be required if the investigators choose to treat patients with a resectable tumor rather than metastatic cancer. Dr. Lobuglio said that it is logical to begin this Phase I toxicity trial in subjects with advanced cancer.

Dr. Straus asked whether evidence of an immune response has been observed in the vaccinia-CEA study. Dr. Lobuglio responded that all of the subjects in the proposed study have advanced cancer; therefore, any immune response observed in the vaccinia-CEA trial is irrelevant for the proposed study. Dr. Straus said that polynucleotide vaccination is a new technology currently being developed for wide applications. Deliberation of the proposed protocol will set a precedent for the review of future protocols. Dr. Straus expressed concern about establishing a precedent for approval without adequate preclinical data.

Dr. Post asked whether an immune response to CEA in cancer patients is an adequate criterion for this study. Dr. Parkman said that a cellular immune response would be an adequate criterion rather than a humoral response alone. Dr. Lobuglio said that the vaccinia-CEA study involves immunological assays for humoral responses only.

Committee Motion

Dr. Haselkorn made a motion to approve the protocol. Dr. Zallen made a friendly amendment to revise the Informed Consent document incorporating the changes suggested by Drs. Haselkorn, Chase, and Zallen. The amendment was accepted by Dr. Haselkorn.

The RAC approved a motion made by Dr. Haselkorn and seconded by Dr. Miller to accept the protocol submitted by Dr. David Curiel of the University of Alabama, Birmingham, Alabama, by a vote of 10 in favor, 4 opposed, and no abstentions. RAC approval is contingent on the review and approval by the primary RAC reviewers of a revised Informed Consent document (as approved by the Institutional Review

Board) incorporating the changes suggested by Drs. Haselkorn, Chase, and Zallen.

IV-B. ANNOUNCEMENT OF FDA SPONSORED MEETING

Dr. French Anderson of the University of Southern California, Los Angeles, California, announced that the FDA would be sponsoring an open meeting on "Production Issues for Human Gene Therapy" to be held immediately after the RAC meeting on June 9, 1994, in the adjacent conference room. The purpose of this meeting is to informally discuss issues surrounding the production of vectors for use in gene therapy protocols with FDA personnel. He said that this meeting would be the first of ongoing discussions between interested parties. This initial meeting is only to establish an agenda for future meetings. If there is sufficient interest, future meetings will be planned to coincide with RAC meetings.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: CLINICAL TRIAL TO ASSESS THE SAFETY, FEASIBILITY, AND EFFICACY OF TRANSFERRING A POTENTIALLY ANTI-ARTHRITIC CYTOKINE GENE TO HUMAN JOINTS WITH RHEUMATOID ARTHRITIS/DRS. EVANS AND ROBBINS*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Chris Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Parkman explained that there are two forms of interleukin-1 (IL-1), , and . IL-1 causes symptomatology associated with infectious or inflammatory diseases, and attracts granulocytes to the site of inflammation. The body also produces a natural IL-1 receptor antagonist protein (IRAP) which neutralizes the effect of IL-1 on its cellular receptors. So there is considerable research interest in attempts to relieve the IL-1 symptomatology either by systemic administration of IRAP to patients or by administration of gene modified cells producing IRAP. Three areas of clinical or preclinical studies have been performed involving IRAP. The first area is the treatment of sepsis caused by gram negative bacteria or endotoxin shock syndrome. The results of these human trials have not been optimistic. The second area is prevention of graft-versus-host (GVH) disease. Animal studies have yielded optimistic results involving bone marrow transplantation. The third area is the treatment of chronic inflammatory diseases such as rheumatoid arthritis; these studies have been met with varying degrees of success. Most IRAP studies have been conducted by industry, and the data has not been published yet in peer-reviewed journals.

The investigators have constructed an MFG-based retrovirus vector that encodes the genes for human IRAP and herpes simplex virus thymidine kinase (HSV-TK). The investigators propose to generate synovial fibroblasts from the knuckle joints of patients with rheumatoid arthritis who are scheduled to undergo surgery, transduce the fibroblasts with the IRAP vector, inject the transduced cells into the synovial space, and collect synovial fluid and joint material 1 week later to determine the presence and location of the transduced synovial fibroblasts and the level of IRAP in the joint fluid. This proposal is based upon the demonstrated efficacy of IRAP administration in either human clinical trials or preclinical animal studies.

The investigators indicated in their written response that they are not unswervingly committed to IRAP as the only antiarthritic agent in the human trial. Once the gene transfer techniques have been established, these methods can be employed to transfer genes other than IRAP. The proposed study is similar to a gene marking protocol in that a therapeutic outcome is not expected. He asked the investigators to clarify whether it is intended to be a marking protocol or a study with therapeutic intent since the criteria for approval will be different.

Dr. Parkman said that three outstanding issues remain: (1) Are the high titer vector producer cells described in the protocol currently available for the proposed study? (2) What is the transduction efficiency and the level of IRAP production in the proposed target cells? (3) If the present study is intended as a therapeutic protocol rather than a marking study,histopathologic data should be provided demonstrating the therapeutic effect, i.e., prevention of joint destruction in a rabbit model.

Review--Dr. Motulsky

Dr. Motulsky said that the proposed study is an innovative approach attempting to treat autoimmune disease in the affected joints of chronic arthritis patients. Current treatment is symptomatic and unsatisfactory. Although the IRAP gene is proposed for the current study, the investigators have reserved the option to use other cytokine genes if this procedure is successful. He asked the investigators to respond to the following questions: (1) What is the frequency of severe reactions? (2) What systemic or local reactions to IRAP have been observed that might cause patients to withdraw from the study? (3) What is the status of the experimental animal models described by Dr. Parkman? Preclinical data has been submitted in an antigen-induced arthritis rabbit model. The animals are presensitized to ovalbumin by intradermal injection and then given an intraarticular injection of ovalbumin in the knee joints to induce arthritis. What additional procedures will be required for this study that are not normally performed on patients with advanced knuckle joint arthritis? Dr. Motulsky recommended that a research clinical rheumatologist should be involved in this study to assist in patient evaluation and follow-up. The investigators agreed to include such an expert in this study. Dr.Motulsky recommended approval of this protocol if the requested information is provided.

Review--Dr. Zallen

Dr. Zallen explained that the investigators had already responded to several aspects of the protocol addressed in her written comments. She then raised the following additional questions: (1) Is the viral genetic material spread beyond the joint, specifically to gonadal tissue? This issue is of less concern in the proposed study since it involves only post-menopausal women; however, such information will have greater significance if this treatment is successful and patients of reproductive age will be involved in future studies. (2) The HSV-TK suicide gene has been incorporated in the vector construct as a safeguard. What are the undesirable side effects that will trigger the use of Ganciclovir (GCV) to eliminate the transduced cells? (3) Have the investigators demonstrated their capability of preparing human synoviocytes in a timely fashion for use in this experiment so that patients will not endure any greater risks including pain and discomfort while waiting for such treatment? What is the maximum time allowed for preparation of the gene altered cells? How would a subject be handled as far as the protocol was concerned if this timetable was not met? (4) The investigators' response indicates that a neutral person will be involved in the informed consent process, but the presence of such a person is not clearly indicated in the Informed Consent document. She stated that the changes suggested for the Informed Consent document have been incorporated, and the investigators have clarified that the costs of the research and any research-related injury will be covered.

Other Comments

Dr. Walters summarized the written comments submitted by Dr.Brinckerhoff. Dr. Brinckerhoff states that the synovial fibroblasts of young rabbit knee joints are easier to grow in tissue culture than the diseased tissues from knuckle joints of older subjects. There is a finite number of fibroblast doubling from cells obtained from human joints. Growing 1 x 107 synovial cells from tissue obtained from a single knuckle joint of an older patient with end-stage joint disease will be technically challenging and difficult to transduce. What is the transduction efficiency in the target cell population? Dr.Brinckerhoff commented

that it is unreasonable to evaluate efficacy based on data obtained from a 7-day experiment with fibroblasts obtained from a patient with chronic disease.

Dr. Walters summarized Ms. Meyers' written comments regarding the Informed Consent document. Several specific language changes were suggested to clearly describe the experimental nature of the treatment, recommendations regarding barrier contraception, and request for autopsy.

Dr. Parkman asked whether biochemical data is available regarding the chronic phase of the joint disease in the rabbit model. Dr. Straus was concerned that the IRAP gene introduced into the joints to interrupt the IL-1 mediated inflammatory reaction might diminish the joint's inherent anti-bacterial capacity in the event of a septic joint infection. This risk is more of a concern if the IRAP gene persists in surrounding tissues following removal of the joint. Individuals undergoing this type of surgery with severe rheumatoid arthritis are at particular risk for developing serious infection as a complication.

Dr. Miller raised several questions regarding the vector construct. The vector sequences provided by the investigators are those of the MFG backbone, not the insert. The proposed construct encodes genes for both IRAP and HSV-TK. Only the IRAP construct was used for the rabbit experiments. He suggested that the IRAP construct should be used for the human experiments in order to avoid possible complications due to immunogenicity of HSV-TK.

Dr. Parkman said that the HSV-TK gene is used for two purposes in this experiment: (1) to eliminate the transduced cells in the event of severe local reaction, and (2) as a surrogate marker to monitor transgene expression. Dr. Miller said that if the HSV-TK gene is to be used in the human study, the same vector construct should be tested in rabbit experiments. Dr. Miller asked the investigators to clarify whether the proposed MFG vector has the frame shift mutation that prevents viral *gag* gene expression or is it the original vector that expresses *gag*.

Dr. Walters remarked that the proposed study does not involve a life threatening disease. Dr. Parkman said that this protocol involves the first autoimmune disease study reviewed by the RAC.

Investigator Responses--Drs. Evans and Robbins

Dr. Evans said that the investigators are well aware that the proposed trial is for a chronic non-fatal disease; therefore, the safety issues are an overriding concern. For this reason, the HSV-TK gene was included as a means to eliminate the transduced cells (particularly for later trials that will last more than 1 week) in the event that the cells escape from the synovial space or cause an unexpected adverse effect. If the RAC considers inclusion of the HSV-TK gene unnecessary, the IRAP alone construct will be used for the human study. He noted that the IRAP construct was used for the preclinical rabbit experiments that demonstrated efficacy.

Dr. Evans presented expression data using the IRAP alone construct versus the IRAP/HSV-TK construct. A preliminary experiment with human synovial fibroblasts (passages 2 and 4) demonstrated between 20 and 50 ng of IRAP/1 x 106 cells/24 hours. This level of expression can be further boosted by multiple rounds of transduction. The current expression level exceeds the minimal level of 10 ng/1 x 106 cells/24 hours that resulted in a measurable response in rabbits. Dr. Parkman inquired about the amount required for maximum protection and whether the response is measured biochemically, i.e., glycosaminoglycan (GAG) release from the cartilaginous matrix (the presence of IRAP strongly protects the cartilage from breakdown). Dr. Evans responded that maximal protection is observed at 100 ng/1 x 106 cells/24 hours, and the response monitored is GAG production.

Dr. Evans presented additional data on GCV sensitivity of HSV-TK transduced cells. Responding to the question of immunogenicity of HSV-TK, he said that HSV-TK is expressed as a intracellular protein and should not be immunogenic. GCV administration is included as a precaution against toxicity from the treatment. GCV administration has been considered for every patient upon completion of the experiment. Dr. Straus was concerned about the rationale regarding the use of GCV, and he said that GCV can cause bone marrow toxicity, particularly in subjects who have bone marrow suppression. Dr. Bill Macaulay of the University of Pittsburgh (Dr. Evans' colleague) explained that at peak levels, GCV ranges between 4.5 and 10 g per ml and that 12.5 g per ml causes bone marrow toxicity. Dr. Evans commented that GCV use is not needed for the present short term experiment of 1 week.

Dr. Evans justified the use of IRAP based on its safety profile in human trials. There has been no toxicity or immunosuppression associated with the use of IRAP. Murine studies were conducted involving MFG-IRAP transduced hematopoietic stem cells that were transferred tosyngeneic irradiated host animals. No toxicity was observed in animals that expressed between 2 and 300 ng per ml of human IRAP for life. Upon subsequent challenge with human IL-1, a blocking response was observed. IRAP works well *in vivo* with rabbits and *in vitro* with mouse cells. Dr. Straus asked whether IRAP expression was sustained in the murine experiment. Is there a diminished response to pathogen challenge? Dr. Evans responded that these experiments have not been performed.

Responding to Dr. Miller's question about the MFG vector, Dr. Robbins said the proposed vector includes a frameshift mutation that blocks viral *gag* protein expression. The vector has previously been referred to as MFG-C, but is indicated as MFG in the present protocol.

Responding to Dr. Parkman's question regarding protection of the joint from tissue destruction, Dr. Evans explained that GAG release into the synovial fluid has been used to measure joint cartilage destruction. In the rabbit experiments, IRAP gene transduction blocked GAG release. Dr. Parkman agreed that these biochemical data reflect acute phase joint destruction, but a more relevant model is the effect of IRAP on the chronic phase of the disease. Dr. Evans said that the proposed 7-day study is not for the purpose of achieving clinical improvement, but to answer the question of whether the transduced IRAP gene is biologically active in an arthritic joint. Therefore, a more sensitive biochemical response has been chosen for this study. Dr. Evans said that most clinical studies on IRAP have been conducted by Synergen (Boulder, Colorado), and most of these data has not been published. The present study is independent of Synergen. Dr. Evans emphasized that the primary purpose of the study is to use the IRAP gene to develop a gene delivery system for treating joint diseases. If positive results are obtained, the same system can be adopted for future efficacy trials.

Committee Motion

Dr. Parkman made a motion to approve the protocol using the vector that does not contain the HSV-TK gene and submission of additional experiments on transduction efficiency. Dr. Evans concurred. Dr. Miller made a friendly amendment that safety data should be provided demonstrating no adverse effects of transduced cells outside the joint tissues. Dr. Parkman accepted the amendment.

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Zallen to accept the protocol submitted by Drs. C. H. Evans and Paul Robbins of the University of Pittsburgh, Pittsburgh, Pennsylvania, by a vote of 13 in favor, 0 opposed, and 1 abstention. RAC approval is contingent on the following: (1) the vector construct encoding the HSV-TK gene will not be used, (2) submission of data (a minimum of 4 reproducible experiments) demonstrating efficient transduction of human synovial cells with the vector that will be used in the human protocol, and (3) submission of safety data demonstrating that transduced synovial cells expressing IRAP do not traffic to other sites following intra-joint injection.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: USE OF A RETROVIRAL VECTOR TO STUDY THE TRAFFICKING PATTERNS OF PURIFIED OVARIAN TIL POPULATIONS USED IN INTRAPERITONEAL ADOPTIVE IMMUNOTHERAPY OF OVARIAN CANCER PATIENTS: A PILOT STUDY/DR. FREEDMAN*

Review--Dr. Dronamraju

Dr. Walters called on Dr. Dronamraju to present his primary review of the protocol submitted by Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas. This protocol is a resubmission of the proposal that was deferred by the RAC at its June 7-8, 1993, and March 3-4, 1994, meetings. The RAC deferred the protocol at its March 1994 meeting until the investigator returned to the full RAC with the following: (1) a modified protocol that includes a revised treatment schema, and (2) a revised Informed Consent document that describes the clinical procedures to be performed in language that is understandable to laypersons.

Dr. Dronamraju said that most of the previous questions raised by the reviewers have been answered by the investigator. The investigator has agreed to choose 1 month after infusion of the marked tumor infiltrating lymphocytes (TIL) as the time point for marker detection rather than 2 to 3 months as previously proposed. In response to Dr. Brinckerhoff's previous concerns, the investigator has clearly defined a successful outcome of gene marking. The specific uptake of markedTIL at the tumor sites will be supported when the mean numbers of marked cells in tumor tissue is significantly larger than the mean number of marked cells in the normal tissues. Dr. Dronamraju asked the investigator to clarify the meaning of their response to Dr. Brinckerhoff's review that "5% of the infected cells being marked, 9 evaluable patients will provide meaningful answer." In response to Dr. Brinckerhoff's written comments, the investigator has submitted polymerase chain reaction (PCR) assay data demonstrating his ability to detect 1 neomycin resistance (*neo*R) marked cell in 1 x 105 cells.

Dr. Dronamraju noted Ms. Meyers' written suggestion that the Informed Consent document should be revised such that patients will not be expected to pay for any research-related costs. The investigator indicated in his written response that the difficulties encountered previously in transducing CD8(+) cells have been overcome. Overall, Dr. Dronamraju said that all reviewers are in agreement that most questions have been answered satisfactorily.

Review--Dr. Brinckerhoff (presented by Dr. Dronamraju)
Dr. Brinckerhoff's written comments were presented by Dr. Dronamraju. Dr. Brinckerhoff states that the investigator has adequately responded to the issues previously raised by the RAC; therefore, approval of the protocol is recommended. Dr. Brinckerhoff recommended that for future protocols, investigators should present their protocol in a logical and thoughtful manner with supporting data and sufficient detail.

Review--Dr. Secundy (presented by Dr. Dronamraju)

Dr. Dronamraju summarized Dr. Secundy's written comments regarding the Informed Consent document. Dr. Secundy recommended that language should include the provision of medical care for any adverse effect that may possibly result from the treatment.

Other Comments

Dr. Zallen commented on the inconsistency of statements regarding medical costs in the Informed

Consent document. Item 11, which appears to be a standard statement required by the Institutional Review Board (IRB) of MD Anderson Cancer Center, stated that if the investigational agents become commercially available during the course of this study, the patients may be required to cover the cost of subsequent doses. The document states that costs related to medical care, including expensive drugs, tests, or procedures, shall be the patient's responsibility unless other funding sources contribute toward the costs. This statement is inconsistent with that in item 3, i.e., that there is no cost to the patients for the preparations of cells, viruses, or laboratory tests. Dr. Zallen asked the investigator to clarify this inconsistency. Dr. Walters added that this same concern was raised in Ms. Meyers' written comments.

Dr. Parkman asked whether the ongoing TIL trials would be discontinued if this marking study demonstrates no selectivity or specificity of the TIL cells in tumor versus normal tissue.

Investigator Response--Dr. Freedman

Responding to Dr. Dronamraju's question regarding 5% marking of infected cells, Dr. Freedman said 5% refers to the dilution effect. The TIL cultures are expanded in 2 bioreactors at a transduction rate of 10%; therefore, each bioreactor will have 5% of the transduced cells. Regarding the cost of medical care, subjects will not be required to pay for such costs; however, the MD Anderson IRB insists that the statement in item 11 be included. Dr. Zallen said that inclusion of two inconsistent statements in the Informed Consent document is unacceptable. The costs and risks that are unique should be stated explicitly for each protocol rather than inclusion of a general statement in all Informed Consent documents. Dr. Freedman responded that he will request that the IRB address this issue. Dr. Parkman commented that there are no unique risks associated with this marking protocol that do not apply to the ongoing TIL trial except for the *neo*R marker gene. Dr. Chase agreed with Dr. Zallen's concern about the contradictory statements in the Informed Consent document. Dr. Walters suggested inclusion of the following statement in item 11: "Please see item 3 for costs that will not be charged to you in this study." Ms. Buc said that the statement as written in item 11 is uninformative as to covered costs and is inconsistent with the statement in item 3.

Responding to Dr. Parkman's question regarding the possibility of a negative outcome of this study, i.e., non-specific trafficking of TIL to tumor, Dr. Freedman stated that the TIL trial will continue regardless of the outcome of the marking study. Dr. Parkman said that conducting a study that will not impact the future course of investigation is inconsistent with the strategy of conducting clinical research. Dr. Chris Platsoucas of Temple University (a co-investigator) explained that mechanisms other than TIL cell trafficking, e.g., enhanced cytokine production by TIL may contribute to the antitumor effect; therefore, the TIL trial should continue regardless of the outcome of the marking study.

Dr. Post inquired whether this protocol would have been considered exempt under the *Accelerated Review* process. Dr. Wivel responded that this protocol is a resubmission of a protocol that was deferred by the RAC; therefore, the *Accelerated Review* guidelines are not applicable.

Committee Motion

Dr. Dronamraju made a motion to approve the protocol with a request to resolve the discrepancy regarding items 3 and 11 of the Informed Consent document as previously suggested by Dr. Zallen.

The RAC approved a motion made by Dr. Dronamraju and seconded by Dr. Post to accept the protocol submitted by Dr. Ralph Freedman of MD Anderson Cancer Center, Houston, Texas, by a vote of 13 in favor, 1 opposed, and no abstentions.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: USE OF DOUBLE MARKING WITH RETROVIRAL VECTORS TO DETERMINE THE RATE OF RECONSTITUTION OF UNTREATED AND CYTOKINE EXPANDED CD34(+) SELECTED MARROW CELLS IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION/DRS. HESLOP, BRENNER AND KRANCE*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Childrens Research Hospital, Memphis, Tennessee. Dr. Miller explained that autologous bone marrow transplantation (ABMT) is often used in pediatric patients to allow the administration of high dose chemotherapy. Bleeding and infection are risks often associated with ABMT. To improve ABMT methods, the marrow is often pre-incubated with growth factors that increase the rate of engraftment. If engraftment can be significantly enhanced, smaller amounts of marrow may be harvested in the future. The investigators propose to determine whether the addition of growth factors enhances engraftment and persistence of the engrafted cells. Two portions of marrow will be marked with *neo*R using two distinguishable retrovirus vectors, LNL6 and G1Na. One portion of the cells will be treated with growth factors and the other will remain untreated. Both portions of cells will be reinfused at the time of transplant. The time and persistence of engraftment will be determined.

Dr. Miller said that the proposed protocol is similar to other marking protocols previously approved for the same laboratory. The investigators are competent and experienced. He recommended approval of this protocol. He noted that the Institutional Biosafety Committee (IBC) recommended Biosafety Level (BL) 2 physical containment for the retroviral vectors, but BL1 is sufficient according to the *NIH Guidelines*.

Review--Dr. Chase

Dr. Chase recommended that a statistician should be consulted for data analysis and accurate determination of the growth pattern differences between treated and untreated cells across a heterogeneous pool of subjects.

Review--Ms. Buc

Ms. Buc commented on minor inconsistencies in the Informed Consent document as follows: (1) All items in the "statement of understanding" are not included in the Informed Consent document, and (2) the statement "I know my or my child's records will not be given to anyone outside the hospital unless I agree" is not necessarily true as these records could be subpoenaed. She commended the investigators for writing such an understandable Informed Consent document.

Dr. Walters commented that this Informed Consent document could serve as a model for other investigators. He noted Ms. Meyers' written comments suggesting that recommendations for long-term follow-up and a request for autopsy should be included in the Informed Consent document.

Ms. Buc said that the proposed study promises to definitively answer important questions regarding bone marrow engraftment and holds the promise for improving current therapy methods.

Investigator Response--Dr. Heslop

Dr. Heslop agreed to incorporate minor changes in the Informed Consent document suggested by Ms.

Buc.

Dr. Miller noted the IRB's provisional approval of the protocol pending RAC review. Are there other issues raised by the IRB that have not been addressed? Dr. Heslop answered that there are no outstanding issues; provisional approval refers to informing the IRB of any changes in the protocol required by the RAC. Dr. Post commented that this protocol would have qualified for the *Accelerated Review* process.

Committee Motion

The RAC approved a motion made by Dr. Miller and seconded by Ms. Buc to accept the protocol submitted by Drs. Helen Heslop, Malcolm Brenner, and Robert Krance of St. Jude Childrens Research Hospital, Memphis, Tennessee, by a vote of 14 in favor, 0 opposed, and no abstentions.

VIII. WORKING GROUP ON DATA MANAGEMENT--SEMI-ANNUAL DATA MANAGEMENT REPORT/DR. SMITH

Dr. Smith summarized the semi-annual data reports submitted by the PIs of NIH-approved human gene transfer protocols. A total of 219 patients have been entered on NIH-approved human gene transfer studies (68 subjects since the last reporting period). Of the 72 protocols recommended for approval by the RAC, 63 have been approved by the NIH Director, 39 are considered active, 10 are considered closed, 21 are pending FDA approval, and 9 are pending NIH Director approval (due to failure of the investigators to meet the RAC's stipulation requirements for approval).

A new column has been added to the Semi-Annual Data Management Report entitled, "Biological Efficacy." This column is applicable only to gene therapy protocols. The term "published" is entered in this column for protocols in which demonstration of biological efficacy has been published in a peer-reviewed journal.

The "*In Vivo* Evidence of Gene Transfer" column has been modified to include additional information regarding gene expression. For the gene marking studies, information will be given to indicate detection of marked cells in the recipients. For gene therapy protocols, information will be collected regarding detection of the transgene in recipients' cells and expression of the gene product. Such information is irrelevant for vaccine protocols.

Dr. Smith made the following recommendations regarding future semi-annual data reports: (1) demonstration of the virus or gene product in a site other than the target should be reported as an "adverse effect", (2) investigators should indicate the number of subjects who have undergone autopsy and provide relevant data, and (3) a letter should be sent to investigators who have not enrolled any subject onto the protocol for several years inquiring whether the study should be considered "closed."

Dr. Chase asked about the compliance rate for data reporting. Ms. Debra Wilson of the NIH Office of Recombinant DNA Activities (ORDA) said that the response rate is over 90%. Dr. Ross asked if information regarding the number of eligible patients identified versus the number of subjects entered. Dr. Smith said that with one exception (Dr. Deisseroth's study) such information is not requested or available. Dr. Smith pointed out one discrepancy regarding patient enrollment in the protocol by Dr. Galpin, et. al. (Protocol #9306-048). This protocol was submitted for RAC review on a voluntary basis since NIH funding was not involved. The protocol has accrued 16 patients while the RAC approved a maximum of 15 subjects. No explanation has been provided for over-accrual. Drs. Parkman and Straus said that Viagene, Inc., should be notified since it is the sponsoring company and bears partial responsibility for data reporting. Ms. Buc said that if the protocol is not obligated to have RAC review under the *NIH Guidelines*;

therefore, data reporting should be collected on a voluntary basis. Dr. Post concurred with Ms. Buc's statement. Dr. Zallen said that the investigators have benefitted from the RAC review and approval process; therefore, data reporting should be required. Dr. Chase said that future studies submitted on a voluntary submission should be approved with the stipulation that data reporting is mandatory. Ms. Wilson stated that the results obtained from this trial, i.e., immunological assays, may have bearing on future protocols submitted by the same sponsoring company. Dr. Noguchi agreed with the statement made by Ms. Wilson. Dr. Noguchi stated that the FDA encourages companies to voluntarily submit their protocols for RAC review, otherwise, public review by FDA committees would be required.

Ms. Sheryl Osborne of Viagene, Inc., responded that the immunological data from Dr. Galpin's trial is contained in a coded format; therefore, the results are not yet available. Ms. Osborne stated that some of the subjects enrolled in this trial are on the placebo arm; perhaps, accounting for the discrepancy in patient number.

Dr. Smith pointed out an adverse event report dated March 31, 1994, submitted by Dr. Oldfield for a subject entered on the HSV-TK/GCV glioblastoma study (Protocol #9206-019). A hemorrhagic complication occurred as a result of multiple intratumoral injections (44). The adverse event was not directly related to gene transfer. Dr. Walters said the RAC will follow up on this event. The other significant adverse effect was observed in Dr. Crystal's cystic fibrosis (CF) study (Protocol #9212-034). Dr. Wivel said that one subject developed pneumonitis related to the high dose of adenovirus vector. The investigators have reduced the vector dose.

Summarized below are the categories of human gene transfer protocols that have been approved by the RAC to date:

	T	M	Total (T + M)
RAC Approved	50	22	72
NIH Director Approved	41	22	63
Categories of RAC-Approved Protocols			
Cancer	32	4	36
Cystic Fibrosis	7	0	7
SCID/ADA	1	0	1
Acute Hepatic Failure	0	1	1
Familial Hypercholesterolemia	1	0	1
Gaucher Disease	3	0	3
Bone Marrow Marking/Cancer	0	15	15
HIV(+)	5	2	7
Alpha-1-Antitrypsin Deficiency/Acute Lung Injury	1	0	1

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: USE OF SAFETY-MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF BREAST CANCER: A PILOT TRIAL/DRS. DEISSEROTH, HORTOBAGYI, CHAMPLIN, AND HOLMES*

Review--Dr. Brinckerhoff (presented by Dr. Dronamraju)

Dr. Walters called on Dr. Dronamraju to present the primary written review of Dr.Brinckerhoff for the protocol submitted by Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas. Dr. Brinckerhoff stated that this study is based on Protocol #9306-044, previously approved by the RAC for the treatment of ovarian cancer. This proposal differs in three aspects: (1) patient eligibility is limited to breast cancer not ovarian cancer; (2) peripheral blood mononuclear cells will be transduced with the gene for multidrug resistance type-1 (MDR-1), not autologous bone marrow cells; and (3) the peripheral blood cells will undergo immunohistochemical analysis to rule out the presence of contaminating breast cancer cells.

A retrovirus vector encoding the MDR-1 gene will be used to transduce autologous peripheral blood hematopoietic stem cells. Prior to transduction, these stem cells will be cryopreserved while the patient undergoes chemotherapy. Following chemotherapy, 50% of the CD34(+) cells will be thawed, transduced with the MDR-1 vector, and infused into the patient. The MDR-1 marked cells will be monitored by means of a methylcellulose late progenitor colony culture system and a PCR assay for the MDR-1 gene. The acquisition of chemotherapy resistance by stem cells will be monitored using culture assays by drug selection. These studies will evaluate whether the introduction of the MDR-1 gene into peripheral blood CD34(+) cells confers chemotherapy resistance; therefore, allowing for a more intensive Taxol regimen. Ultimately, increased doses of chemotherapeutic agents may lead to improved prognosis for breast cancer patients.

Dr. Brinckerhoff stated that this proposal is a logical extension of Dr. Deisseroth's ongoing ovarian cancer protocol (Protocol #9306-044). Although this protocol may have qualified for the *Minor Modification* process, a change in the tumor target raises a number of important questions that should be addressed; therefore, approval as a minor modification is not appropriate. The investigators proposed transduction of peripheral blood mononuclear cells rather than bone marrow cells in order to reduce the probability of transducing contaminating cancer cells in the marrow. What is the probability of tumor cell contamination? In their written response, the investigators stated that the immunohistochemical assay is capable of detecting 1 contaminating cancer cell in 100,000 normal cells. The investigators provided published data on assay sensitivity. Dr. Deisseroth was asked to expand on the ability of his laboratory to perform these assays at the same level of sensitivity. Dr. Brinckerhoff asked the investigators to respond to the following questions during his oral presentation: (1) Is the transduction rate of peripheral blood mononuclear cells as efficient as that of bone marrow cells? (2) Will increased doses of Taxol exceed the capacity of the MDR-1 transduced cells to confer resistance? Dr. Brinckerhoff stated that if satisfactory responses are provided to all of these concerns, approval of the protocol is recommended.

Review--Dr. Dronamraju

Dr. Dronamraju expressed concern about the Informed Consent document statement, "costs related to my medical care including expensive drugs, tests or procedures... required by this clinical research study shall be my responsibility." This statement poses ethical and moral dilemmas and will preclude participation if the patient's financial status changes during the course of their participation in the study. Dr. Dronamraju inquired about the transduction efficiency. In the investigator's written response, they stated that the transduction rate for peripheral blood cells is as efficient as bone marrow cells. The investigators anticipate that approximately 50% of the subjects entered on the study will complete the protocol. Patients will be followed systematically for 5 years.

Review--Ms. Meyers (Presented by Dr. Dronamraju)

Dr. Dronamraju summarized the written comments submitted by Ms. Meyers on the Informed Consent document. Ms. Meyers suggested the following: (1) patient follow-up should be for life rather than for 5

years, (2) the vector manufacturer should be added as a party who may access patients' medical records, and (3) patients who are poor or uninsured should not be excluded from participation in the protocol. In the investigators' written response, they stated that MD Anderson Cancer Center will provide free care to uninsured residents of Texas.

Other Comments

Dr. Parkman asked about patient accrual in the ovarian cancer protocol (Protocol #9306-044). Dr. Smith asked if there is an exclusion criterion based on a defined number of contaminating breast cancer cells in the marrow.

Investigator Response--Dr. Deisseroth

Dr. Deisseroth explained that patients entered on this study will have Stage III/IV breast cancer and failed initial therapy but have not received Taxol. Subjects will have negative bone scans and negative marrow involvement as demonstrated by microscopic and immunohistochemical methods. The immunohistochemical assays are sufficiently sensitive to detect one contaminating cancer cell in 1 x 106 normal cells. The combination of patient eligibility criteria and assay sensitivity will ensure that the MDR-1 chemoprotection gene will not inadvertently transduce tumor cells. Prior to the collection of peripheral blood cells, subjects will receive a dose of cyclophosphamide to reduce the number of circulating cancer cells. CD34(+) selection of peripheral blood cells will further preclude the possibility of contaminating cancer cells since the latter are CD34(-). The proposed strategy of this study is that introduction of the MDR-1 chemoprotection gene into CD34(+) peripheral blood cells will allow delivery of high dose Taxol post-ABMT to eradicate breast cancer cells.

Dr. Deisseroth stated that the 10% transduction efficiency observed in CD34(+) peripheral blood cells is equivalent to the transduction efficiency of CD34(+) bone marrow cells. Responding to the question of possible cancer cell contamination, he responded that the immunohistochemical assay used to screen patients is sensitive enough to easily identify patients who have cancer cells circulating in their peripheral blood. The bone marrow will be assayed for contaminating cancer cells. Since bone marrow has a contamination rate 1,000 times higher than that of peripheral blood mononuclear cells, a negative assay demonstrated on bone marrow cells reduces the probability of peripheral blood contamination to less that 1 in 1 x 108 cells. CD34 selection should further reduce this possibility to less than 1 in 1 x 109 to 1 x 1010 cells. Since a maximum of 2 x 107 cells will be transduced, the chance of transducing the MDR-1 gene into a single cancer cell is extremely small. Any possible risk to patients is insignificant compared to the potential for benefit. He expects that 20 patients entered on the study should yield 10evaluable patients, i.e., participation through completion of the study.

Dr. Deisseroth responded to questions regarding the Informed Consent document. Patients will be monitored for life. MD Anderson Cancer Center will provide free medical care to uninsured patients who are residents of Texas. For those patients who have insurance, financial counselling will be provided to ensure that there is sufficient insurance coverage before admission to the protocol. Research-related costs will not be charged to patients; these costs are provided by research grants. Dr. Chase expressed concern about contradictory statements in the Informed Consent document regarding disclosure of financial obligations. Dr. Deisseroth responded that the Informed Consent document will be revised to clarify this issue and submitted to the IRB.

Dr. Holmes commented that the IRB has formed a subcommittee specifically to address the issue on providing mechanisms for payment of medical costs that are not considered as routine patient care. Dr. Chase applauded this effort by the MD Anderson IRB and said that letters should be sent to otherIRBs to

urge similar action. Dr. Wivel noted that such an action is beyond the purview of the RAC.

Dr. Deisseroth said that no patients have been entered on the ovarian cancer protocol because FDA approval is pending.

Dr. Richard Cote of the University of Southern California (co-investigator) explained the immunohistochemical assay to detect contaminating cancer cells in peripheral blood and bone marrow samples. The test employs a mixture of monoclonal antibodies directed to cell surfaceglycoproteins and cytoskeletal elements of cells of epithelial origin including breast and ovarian carcinoma cells. He described spiking experiments involving a mixture of breast cancer and normal bone marrow or peripheral blood CD34(+) cells. At varying concentrations, between 2 and 5 tumor cells were detectable among 1 x 106 normal cells. The presence of breast cancer cells in bone marrow is biologically relevant since patients are more likely to have recurrence at this site following chemotherapy. Dr.Deisseroth reiterated that screening patients for bone marrow metastases is a major safety feature.

Dr. Deisseroth described murine experiments in which CD34(+) cells were transduced with MDR-1. These mice were resistant to Taxol at a maximum dose of 30 mg/kg body weight. This dose is equivalent to the highest dose proposed for the human study; therefore, the same degree of safety is expected for th human protocol. In response to Ms. Meyers' written comments, DrDeisseroth said that patients will undergo life-long follow-up. There is no manufacturer involved in the vector production; the vectors will be produced in the investigators' laboratory.

Dr. Anderson commented on his personal experience regarding negotiation of medical coverage with the local IRB. IRB negotiation involves the IRB as well as the hospital administrators, board of trustees, and legal counsel. Ms. Buc responded that extensive negotiation is required only for deciding who is responsible for such costs. The issue of Informed Consent document clarification merely involves an amendment. Such amendments are easily dealt with at theIRB level. Dr. Deisseroth noted that most patients entered on this study have failed numerous other treatments. The "spirit" of the Informed Consent document is disclosure, i.e., possible unforeseen complications not directly related to gene therapy. Medical care will be provided to subjects independent of their economic status.

Committee Motion

The RAC approved a motion made by Dr. Dronamraju and seconded by Dr. Parkman to accept the protocol submitted by Drs. Albert Deisseroth, Gabriel Hortobagyi, Richard Champlin, and Frankie Holmes of MD Anderson Cancer Center, Houston, Texas, by a vote of 13 in favor, 0 opposed, and no abstentions.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: RETROVIRAL MEDIATED GENE TRANSFER OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C GENE TO HEMATOPOIETIC PROGENITORS OF GROUP C PATIENTS/DRS. LIU AND YOUNG*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Johnson M. Liu and Neal S. Young of NIH, Bethesda, Maryland. This protocol is a resubmission of the proposal that was deferred by the RAC at its December 2-3, 1993, meeting. The RAC deferred the protocol until the investigators returned to the full RAC with the following: (1) murine data demonstrating *in vivo* expression of the Fanconi anemia complementation group C (FACC) gene and safety data accumulated over a period of 4 months demonstrating that theFACC-transduced cells do not result in any untoward effects;

(2) data as cited in Dr. Cynthia Dunbar's semi-annual data report (Protocol #9206-025) regarding the possibility that "stem cell factor could favor the growth of leukemic versus normal progenitors during *ex vivo* culture periods"; and (3) revision to both the protocol and Informed Consent document to modify the eligibility criteria regarding the necessity for bone marrow examination following each reinfusion.

Dr. Parkman said that this protocol is a meritorious study. Preclinical studies demonstrate that *in vitro* transduction of the FACC gene into lymphoblast cells of Fanconi anemia patients results in the normalization of sensitivity to mitomycin C treatment and a reduction in the number of induced chromosomal breaks. Two outstanding questions remained following the previous RAC review: (1) What are the long-term effects (6 months) of the FACC transduced gene in murine bone marrow cells? and (2) Does the presence of granulocyte colony stimulating factor (G-CSF) during the transduction procedure promote the outgrowth of preleukemic cells? Dr. Cynthia Dunbar (Protocol #9206-025) previously reported that G-CSF provided a growth advantage to chronic myelogenous leukemia (CML) cells. In response to these questions, the investigators concluded: (1) published literature demonstrates that the administration of G-CSF to Fanconi anemia patients does not increase the probability of developing myeloid malignancy. (2) Data published by Dr. Cynthia Dunbar indicates that although the presence of the transmembrane form of the stem cell growth factor promotes a selective growth advantage to CML cells, the soluble form of this factor has no deleterious effect. The proposed transduction procedure includes the soluble form of the stem cell growth factor rather than the transmembrane form; therefore, selective growth advantage is not a safety concern. The investigators have provided long-term toxicology data derived from murine experiments demonstrating that no lymphoproliferative abnormality has been observed for a period of 6 months. Dr. Parkman stated that the RAC's previous concerns have been satisfactorily answered by the investigators; therefore, approval of the protocol is recommended.

Review--Dr. Smith

Dr. Smith agreed that most of the RAC's concerns have been addressed by the investigators. The question of stem cell factor in the transduction procedure has been addressed. The data demonstrated that this factor's effect is specific to CML cells whereas normal progenitors are not as sensitive. Although one may disagree as to the relative merits of various components of the growth factor cocktail to be used in the transduction procedure, the investigators have now provided sufficient reassurance to allow the proposed transduction procedure. The investigators have provided adequate data in response to toxicology questions relating to the long-term effect in an animal model. He recommended approval of the protocol.

Other Comments
Dr. Post asked the following questions: (1) Was the transduced FACC gene continuously expressed during the course of 6 month murine experiments? (2) Was the FACC gene expressed in cell colonies growing out of the transduced human CD34(+) peripheral blood cells?

Investigator Response--Dr. Liu

Dr. Liu responded to several issues regarding quantitation of FACC RNA. FACC RNA was detected by the reverse transcriptase polymerase chain reaction (RT-PCR) assay of murine peripheral blood cells. Northern Blot analysis demonstrated low level FACC expression that was detectable at the end of the 6 month murine experiment. DNA PCR was used to demonstrate the presence of the transgene in human bone marrow cells. A functional assay was employed to determine improved colony growth. Data was presented on 3 Fanconi anemia cell lines representing 3 different mutations of the FACC gene. Normalization of mitomycin sensitivity was demonstrated by transduction of FACC gene into the cell line containing a stop codon mutation of the FACC gene. The corrected Fanconi CD34(+) cells had a growth

advantage over the defective cells, a fact that would favor engraftment of the transduced cells. Data was presented demonstrating transgene expression of transduced lymphoblast cell lines as well as cells of Fanconi anemia patients. The investigators looked at this sensitivity to mitomycin C in terms of chromosomal aberration and found that after gene correction, creation of cytogenetic abnormalities was reduced to normal. Data indicates that FACC is not an oncogene but plays a role in DNA repair. Fanconi anemia cells have an unusually prolonged G2 cell cycle phase. Introduction of the FACC gene into these cells diminishes the chance that cells with unrepaired DNA will enter into mitosis.

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Smith to accept the protocol submitted by Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, by a vote of 13 in favor, 0 opposed, and no abstentions.

XI. AMENDMENT TO PART I-D OF THE *POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA INTO THE GENOME OF ONE OR MORE HUMAN SUBJECTS (POINTS TO CONSIDER) OF THE NIH GUIDELINES REGARDING INFORMED CONSENT/DR. ZALLEN*

Dr. Zallen provided background information about the efforts of the Working Group on Informed Consent regarding revision of Part I-D, Informed Consent, of the *Points to Consider*. At the December 2-3, 1993, RAC meeting, Dr. Gary Ellis, Director of the NIH Office for Protection from Research Risks (OPRR), suggested that many of the RAC's concerns regarding informed consent could be addressed by amending the *Points to Consider*. At the March 3-4, 1994, RAC meeting, the working group presented two draft versions of revised Part I-D of the *Points to Consider* for RAC consideration. Following a lengthy discussion, the RAC recommended that elements of the two draft documents should be incorporated into a single document for review and approval by the RAC. Following a working group telephone conference, a revised document was developed.

Part I-D-1 of the revised document requests information regarding the informed consent process. Part I-D-2 describes issues that should be addressed in the Informed Consent document. Part I-D-2-a describes general requirements for research involving human subjects. Part I-D-2-b describes special requirements specific to gene transfer research, i.e., use of barrier contraception, long-term follow-up, request for autopsy, and protection from the media.

Dr. Motulsky commented on Part I-D-2-a-(5) which includes verbal descriptions of quantitative information relating to risk. The proposed language is inconsistent with the common usage of the terms *rare*, *uncommon*, *common*, and *frequent.* The proposed document defines *rare* as < 0.1%; *uncommon* as < 1%; *common* as 1 to 10%; and *frequent* as > 10%. Dr. Motulsky explained that the more common usage of these terms is as follows: *rare* as < 0.5%; *uncommon* as 0.5 to 2%; *occasional* as 2 to 15%; *common* as 15 to 35%, and *frequent* as > 35%. Dr. Parkman expressed concern about assigning numerical values to verbal descriptors since most risks, i.e., adverse events, cannot be precisely quantitated due to the extremely small number of patients who have been entered onto gene transfer trials to date. MsBuc said that the use of verbal descriptors is acceptable if used to disclose the investigators' quantitative estimate of risks. Dr. Smith agreed with Dr. Motulsky's quantitative definition. Dr. Chase said that quantitative information can be determined from an appropriate probability model; therefore, the verbal descriptors are acceptable as defined. Dr. Walters asked the RAC whether the proposed terms should be associated with a numerical value. Drs. Smith and Miller emphasized that quantitative definition are preferable. Ms. Buc said that investigators retain the option not to include these terms in their Informed Consent documents.

Dr. Zallen said that Ms. Lori Andrews (a RAC member who was not present) suggested revision of the autopsy section to indicate that permission for autopsy will be requested but it is not a requirement.

Regarding Part I-D-2-b-(1) on barrier contraception, Ms. Buc said that it is inappropriate to exclude pregnant women from participation in gene transfer studies since it assumes that a woman's right is subservient to that of the fetus. Dr. Zallen said that this exclusion is to protect the gene therapy research from being associated with any untoward effect such as a malformed newborn even if it is not directly due to gene transfer. Dr. Walters commented that this exclusion will prevent inadvertent gene transfer to the fetus. Dr. Parkman added that the recommended duration of barrier contraception should be specified. Dr. Chase said that this exclusion is a balance between the females right to treatment versus the desire to protect the nascent field of gene transfer research. Dr. Motulsky concurred with Dr. Chase's comments.

Regarding the statement, "In the consent form, subjects should be informed about...any costs for medical treatment...as a *direct* result of research-related injury", Dr. Parkman said that the word *direct* is problematic based on variable interpretation and recommended that the term *direct* be deleted. Ms. Buc suggested that the phrase, "In the consent form" is redundant and should be deleted.

Dr. Walters suggested that the RAC empower Dr. Zallen and the ORDA staff to incorporate the suggested editorial changes.

Ms. Buc reiterated her concern about excluding women from participation in Phase I/II gene therapy trials since such studies are often the last hope of benefit for terminally ill patients. Dr. Chase said that the issue is the right of pregnant women to a treatment versus a collective right to safeguard a new form of medical experimentation. Both men and women are urged to practice barrier contraception with no reference to gender. Dr. Parkman suggested that the statement, "female subjects should be informed..." should be eliminated to avoid gender discrimination. Dr. Miller noted that most investigators would be reluctant to perform such a novel treatment on pregnant women. Ms. Buc stated that due to the controversial nature of this issue, the RAC should not take a position on the exclusion of pregnant women. Drs. Ross and Smith said that participants should be informed of the possible risk rather than outright exclusion. Dr. Parkman asked whether the FDA has a policy on this issue. Dr. Noguchi said that the FDA would not dismiss such a protocol outright unless there was clear scientific reason as to why women of child bearing age or pregnant women should not be included in the protocol. Dr. Parkman concluded that the investigators should: (1) describe whether pregnant women will be included in the study, and if not, provide an explanation for their exclusion, (2) describe possible risks to the fetus, and (3) include specific recommendations regarding contraception. Dr. Parkman suggested a new title for Part I-D-2-b-(1) entitled: "Reproductive Considerations." Dr. Noguchi said that Dr. Parkman's recommendations are consistent with FDA policy.

Committee Motion 1

The RAC approved a motion made by Dr. Chase and seconded by Dr. Haselkorn to accept the proposed amendments to Part I-D, Informed Consent, of the *Points to Consider* by a vote of 13 in favor, 0 opposed, and no abstentions. These amendments were approved with the exception of Parts I-D-2-b-(1) (Barrier Contraception) and I-D-2-b-(3) (Request for Autopsy), deletion of the words "in the Informed Consent document" where appropriate, and the inclusion of minor editorial changes.

As to the use of verbal descriptors for possible risks, Dr. Chase suggested that if these descriptors are used to convey quantitative information, such terms should be thoroughly explained to the patients.

Committee Motion 2

The RAC approved a motion made by Dr. Chase and seconded by Dr. Motulsky to amend Part I-D-2-a-(5) (Possible Risks, Discomforts and Side Effects) by a vote of 13 in favor, 0 opposed, and no abstentions. The following sentence will be added to the current language:

"If verbal descriptors (e.g.,*rare*, *uncommon*, or *frequent*) are used to express quantitative information regarding risk, these terms should be explained."

Further discussion ensued regarding proposed language for Part I-D-2-b-(1) regarding contraception. Dr. Parkman summarized the conclusions as follows: (1) the subject will be informed concerning the possible risks of the experiment to the fetus, (2) the subject will be informed concerning the necessity for barrier contraception during the active phase of the experiment, (3) the investigator will define the duration of the active phase of the experiment, and (4) the inclusion/exclusion of pregnant women in the experiment should be addressed. The working group will make minor editorial changes as necessary.
Committee Motion 3

The RAC approved a motion made by Dr. Parkman and seconded by Ms. Buc to amend the title of Part I-D-2-b-(1) by a vote of 13 in favor, 0 opposed, and no abstentions. Part I-D-2-b-(1) will be entitled *Reproductive Considerations*.

XII. DELETION OF APPENDIX L, *RELEASE INTO THE ENVIRONMENT OF CERTAIN PLANTS* , **OF THE** *NIH GUIDELINES/DR. WIVEL*

In a letter dated April 29, 1994, Dr. Nelson A. Wivel, Executive Secretary, RAC, requested that Appendix L, *Release into the Environment of Certain Plants*, be deleted from the *NIH Guidelines*. Dr. Wivel stated that Appendix L should be deleted based on the following: (1) Section I of the *NIH Guidelines* allows experiments to proceed that are reviewed and approved by another Federal agency that has jurisdiction for review and approval without the necessity for NIH review or approval (except for experiments involving human subjects) (52 FR 31849); (2) the RAC has not reviewed any deliberate release experiments involving recombinant DNA since 1984; (3) at its May 30-31, 1991, meeting, the RAC recommended deletion of Section III-A-2 of the *NIH Guidelines* regarding environmental release; and (4) experiments involving deliberate release into the environment are currently reviewed within the framework of existing Federal regulations, i.e., the Environmental Protection Agency and the United States Department of Agriculture.

The RAC approved a motion made by Ms. Buc and seconded by Dr. Chase to delete Appendix L from the *NIH Guidelines* by a vote of 13 in favor, 0 opposed, and no abstentions.

Appendix L will be deleted as follows:

"APPENDIX L. RELEASE INTO THE ENVIRONMENT OF CERTAIN PLANTS

"Appendix L-I. General Information

"Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Working Group, and specific approval by the NIH Director. Such experiments also require the approval of the IBC before initiation. Information on specific experiments which have been approved will be available in ORDA and will be listed in Appendix L-III when the Guidelines are republished.

"Experiments which do not meet the specifications of Appendix L-II fall under Section III-A and require RAC review and NIH and IBC approval before initiation.

"Appendix L-II. Criteria Allowing Review by the RAC Plant Working Group Without the Requirement for Full RAC Review.

"Approval may be granted by ORDA in consultation with the Plant Working Group without the requirement for full RAC review (IBC review is also necessary) for growing plants containing recombinant DNA in the field under the following conditions:

"Appendix L-II-A. The plant species is a cultivated crop of a genus that has no species known to be a noxious weed.

"Appendix L-II-B. The introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants.

"Appendix L-II-C. The vector consists of DNA: (i) From exempt host-vector systems (see Appendix C); (ii) from plants of the same or closely related species; (iii) from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants; (iv) from plant pathogens only if sequences resulting in production of disease symptoms have been deleted; or (v) chimeric vectors constructed from sequences defined in (i) to (iv) above. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests.

"Appendix L-II-D. Plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review by the IBC. Copies must also be submitted to the Plant Working Group of the RAC."

XIII-A. AMENDMENT TO PART VI OF THE *POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING EXPEDITED REVIEW OF SINGLE PATIENT HUMAN GENE TRANSFER PROTOCOLS/DR. WIVEL*

In a letter dated April 29, 1994, Dr. Nelson A. Wivel, Executive Secretary, RAC, requested that Item #4 of Part VI, *Expedited Review of Single Human Gene Transfer Patients*, of the *Points to Consider*, should be amended to clarify submission requirements for *Expedited Review*.

The *Procedures to be Followed for Expedited Review* currently reads:

"4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols.

The proposed amendment reads:

"4. Regardless of the method of review, the *Points to Consider* must be the standard of review for all gene transfer protocols; therefore, submission of the *Points to Consider* is required."

The RAC approved a motion made by Ms. Buc and seconded by Dr. Chase to amend Part VI of the *Points to Consider* regarding expedited review of single patient human gene transfer protocols by a vote of 13 in favor, 0 opposed, and no abstentions.

XIII-B. CHAIR REMARKS

Dr. Walters noted that there is an expanding volume of information that is currently published regarding human gene therapy. He noted two new journals in this field, i.e., *Gene Therapy* and *Cancer Gene Therapy*, in addition to *Human Gene Therapy* which is now published monthly. Dr. Parkman remarked that NIH has expanded its funding of research programs involving human gene therapy. One such example comes from the National Heart, Lung, and Blood Institute which is requesting gene therapy proposals for the treatment of heart and cardiovascular diseases. The National Institute of Child Health and Human Development has recently requested applications for prenatal gene therapy research proposals for the treatment of degenerative central nervous system diseases.

XIV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I TESTING OF GENETICALLY ENGINEERED INTERLEUKIN-7 MELANOMA VACCINES/DRS. ECONOMOU, GLASPY, AND McBRIDE*

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California. This proposal is an open label, Phase I clinical trial to evaluate the safety and immunological effects of administering IL-7 producing allogeneic and autologous melanoma cells to patients with metastatic melanoma. Three groups of 5 patients will receive 1 x 107 irradiated, unmodified autologous tumor cells mixed with increasing numbers of IL-7 transduced allogeneic melanoma cells (M24 cell line). The number of transduced cells will be adjusted to produce increasing doses of IL-7 between 10 and 1,000 ng/24 hours. The vaccine will be administered as 3 biweekly subcutaneous inoculations. The study design will allow a dose-escalation evaluation of local and systemic toxicity. A final group of 10 patients will receive 3 biweekly inoculations of their autologous melanoma cells transduced with an IL-7 retrovirus vector. Antitumor immunity will be measured by tumor cell skin tests, tumor biopsies, generation of specific antibodies for autologous tumor and M24 cells, and generations of cytotoxic T lymphocyte (CTL) precursors.

Dr. Motulsky noted that this is the first proposal involving the IL-7 transgene in human subjects. The investigators have submitted both *in vivo* and *in vitro* experiments demonstrating an antitumor effect against melanoma cells. Data indicates stable cytokine expression and induction of CTL in mixed lymphocyte tumor cell cultures. The investigators are currently conducting similar studies using the IL-2 transgene; therefore, they possess sufficient expertise to conduct the study. The retrovirus vector will be produced and tested by Targeted Genetics, Inc., Seattle, Washington.

Review--Dr. Doi

Dr. Doi asked the following questions: (1) Has the IL-7 transduced M24 cell bank been certified by the FDA? (2) Have rodent and monkey experiments been conducted to determine IL-7 toxicity? (3) The doses of IL-7 to be administered to patients are 10, 100, and 1,000 ng/24 hr. Since the maximum obtainable

level of IL-7 production is 10 ng IL-7/1 x 106 cells/24 hr, 1 x 108 transduced M24 cells would have to be administered to achieve the highest dose in addition to the autologous cells. Is this large number of cells feasible? (4) Have high dose IL-7 producer cells been successfully cloned? (5) Should the HSV-TK gene be deleted from the vector construct to prevent possible complications? (6) Is quantitative data available demonstrating the superiority of IL-7 over IL-2, IL-3, IL-6, and tumor necrosis factor?

Review--Ms. Meyers (presented by Dr. Walters)

Ms. Meyers raised three issues of concern in her written comments: (1) the use of the term "vaccine" throughout the protocol and the Informed Consent document, (2) recommendations for barrier contraception only for females and not for males, and (3) the legitimacy of approving simultaneous studies involving multiple cytokines.

Other Comments

Dr. Smith asked if the proposed dose of irradiation sufficiently prohibits proliferation yet maintains adequate IL-7 production.

Dr. Parkman asked the following questions: (1) What are the results of the previously RAC-approved melanoma protocol involving IL-2? (2) Is there any data derived from preclinical animal experiments demonstrating an antitumor effect against preexisting tumor? Such a model would be more clinically relevant than this 10 day preimmunization model.

Dr. Zallen asked how the investigators would avoid conflict of interest in eliciting informed consent from subjects for whom they provide care. Is there a match between the human leukocyte antigen (HLA) locus of the subject and the proposed cell line?

Drs. Dronamraju and Chase asked how the investigators arrived at the proposed number of 5 patients per dose escalation group. Dr. Parkman asked the investigators to justify their choice of IL-7 over IL-3 since the data indicates that IL-3 is more effective in the preimmunization model. Dr. Smith inquired whether the same subjects will be targeted as the ongoing IL-2 trial (Protocol #9309-058). If the same individuals are targeted, how will decisions be made regarding assignment of subjects to individual protocols?

Investigator Responses--Drs. Economou and Overell

Dr. Economou stated that Dr. Robert Overell of Targeted Genetics Corporation, Seattle, Washington, would respond to questions regarding cell bank certification, toxicology studies, and inclusion of the HSV-TK gene. In response to Dr. Doi's question regarding the autologous arm of the study, Dr. Economou said that a success rate of 90% has been achieved in establishing primary cultures from melanoma patients. Transduction of these cells results in a level of IL-7 production between 10 and 50 ng IL-7/1 x 106 cells/24 hours. There will be no attempt to clone high producing autologous cells. 1,000 ng IL-7/1 x 107 cells/24 hours will be the upper limit for IL-7 production. Bulk transduced cells will be selected for hygromycin resistance and are expected to produce IL-7 within the range of between 10 and 50 ng/24 hours. In the allogeneic arm of the protocol, doses of 10, 100, and 1,000 ng IL-7/24 hours will be achieved. The immunological response will be determined and compared to the autologous tumor cell data in which IL-7 production is variable.

Regarding Ms. Meyers' concerns, Dr. Economou stated that both male and female patients would be counselled with regard to contraception. Premenopausal women must have had a negative pregnancy test prior to entering the study.

Responding to Dr. Smith's question, Dr. Economou said that data on the effects of irradiation on cytokine production has been published in the journal *Blood* (reprint was appended in the submission). The M24 cell line transduced with the IL-7/HyTK retroviral vector has stable IL-7 production over 7 days after 10,000 cGy irradiation. Dr. McBride stated that 10,000cGy is more than adequate to inhibit growth of these tumor cells. Dr. Smith asked if similar data is available for the primaryautologous tumor cells and if these cells are clonogenic following irradiation? Dr. Economou responded that stable IL-7 production was maintained for greater than 7 days butclonogenic assays were not performed. M24 was selected because this cell line has been administered to hundreds of patients in polyvalent allogeneic vaccine trials at the University of California, Los Angeles (UCLA). For these previous vaccine studies, 5,000 cGy irradiation was used and no evidence of tumor growth was observed at this dose.

In regard to the results of the IL-2 melanoma study, DrEconomou explained that the IL-2 trials were delayed due to a change in the vector supplier. Therefore, both the IL-2 and IL-7 protocols will be conducted in parallel which would allow for comparisons between the two studies. Both studies are similar in design.

Dr. Economou explained that the animal studies are difficult to perform using a preexisting tumor model because these tumors are anaplastic and grow so rapidly that the animals die within 3 weeks. There is insufficient time to generate systemic tumor specific immunity to control the tumor growth. Perhaps the model system could be modified to allow a longer preexisting period and reducing the inoculating tumor dose; however, such a modified system would not reflect the clinical situation. He stressed that the human study is the only real test of this treatment in which safety and immunity will be determined.

Dr. Parkman stated that the lack of adequate preclinical data is a major weakness in this proposal. The previously approved IL-2 study was based on substantial preclinical data in an animal model that demonstrated an effect onmetastatic tumors. IL-7 is a new cytokine and the scientific basis for this study is clearly inadequate. IL-3 is superior to IL-7 inpreimmunization model experiments. What is the rationale for choosing IL-7? Dr. Economou answered that both IL-3 and IL-7 demonstrate the most significant cytokine effect in animal models. IL-3 was not chosen for this study because its biology is not well understood. IL-7 is more of a T cell specific cytokine. CD4 and CD8 cells are largely the cells that are attracted to tumor sites. Dr.Economou explained that his laboratory has been studying the effect of IL-7 transduced cells on melanoma for several years. Tumor infiltrating lymphocytes isolated from IL-7 transduced tumors demonstrate tumor specificcytotoxicity. Dr. Parkman suggested the possibility of using the B16 melanoma model since B16 was the preexisting tumor model used for the IL-2 preclinical studies.

Dr. Overell questioned whether the RAC has maintained consistency in its expectations regarding preclinical data. Dr. Parkman said that the RAC has consistently required demonstration of anantitumor effect in a preexisting tumor model. Clear rationale for therapeutic benefit should be demonstrated in preclinical studies even for a Phase I toxicity study. Dr. Post stated that it may be preferable to examine the data as a whole and determine if there is clear rationale for the proposal rather than defining specific experiments. Dr. Parkman expressed concern that IL-7 has never been administered to humans; therefore, toxicity is unknown. Dr. Chase stated that there is no statistical rationale for the proposed study. What conclusions can be drawn from a study involving 5 patients in each experimental group?

Dr. Walters asked Dr. Noguchi to comment on this issue. Dr. Noguchi responded that the 219 patients entered in RAC approved trials to date do not provide a sufficient base by which to address common toxicity, i.e., 1 in 100 subjects. The primary goal of a Phase I study is to estimate toxicity. Toxicity in drug trials is usually not observed until the Phase II study. He said that 5 subjects per group is preferable to 3, particularly for rare genetic diseases.

Dr. Economou explained that he has consulted with Dr. William Cumberland, Professor of Biostatistics at UCLA School of Public Health, regarding statistical analysis and the size of the treatment groups. Because of the number of variables in the proposed study, the number of subjects to be entered in each group is statistically difficult to determine. It is the opinion of the investigators that 5 subjects would yield useful data regarding toxicity and immunological responses.

Responding to Dr. Smith's question on patient selection, Dr. Economou explained that metastatic melanoma patients who have failed conventional treatment are referred to UCLA and are eligible for several protocols that are being conducted simultaneously. Usually subjects are triaged with at least 2 cycles of chemotherapy. Subjects with subcutaneous or regional disease are then offered the option of entering one of the adoptive immunotherapy trials. Subjects that are not entered on an adoptive immunotherapy study are offered other systemic trials. The vaccine trials are offered as a possible third level of treatment. Entry on one research protocol does not preclude entry into another study. Some subjects can be entered onto more than one study in a sequential fashion. Dr.Haselkorn recommended that a patient advocate should be assigned to assist subjects in understanding the studies and making informed decisions.

Dr. Chase stated that the choice of the number of patients based on budget and duration of a protocol is a reasonable one if there is no other statistical criteria. Entering subjects sequentially on studies could lead to unrecognized consequences that could cloud results. Dr. Economou responded that melanoma patients usually demonstrate very clear responses to beneficial treatment. Given sufficient time between entry on individual protocols it is possible to determine the effectiveness of each treatment. The major objectives of the present study are toxicity and immunological endpoints which are easily discernible.

Dr. Parkman asked about the specific criteria for entering patients on either the IL-7 or the IL-2 protocol. Dr. Economou responded that subjects will be assigned in a random fashion. Dr. Chase commented that randomization of patient entry is an acceptable procedure.

In response to Dr. Haselkorn's suggestion regarding assigning a patient advocate, Dr. Economou stated that neither he nor Dr. Glaspy are of the opinion that a neutral person is necessary to discuss informed consent with patients. The research is secondary to patient care and all subjects receive comprehensive information about all research protocols for which they are eligible. Subjects are frequently referred to studies other than the adoptive immunotherapy trials.

In response to Dr. Zallen's concerns, Dr. Economou explained that although patients are HLA typed, an HLA match is unnecessary because the M24 cell line is used for cytokine production and not antigen presentation. Dr. Overell explained that M24 has been used in a number of clinical trials at UCLA. This cell line is part of a biologics master file that Targeted Genetics, Inc., will be filing with the FDA. Validation tests are outlined in the submission materials. With regard to toxicology studies, experiments are currently being conducted in monkeys and mice. In the monkey model, animals receive recombinant IL-7. In the murine experiment, animals receive multiple administration of irradiated transduced tumor cells. Both of the ongoing animal models will be evaluated for evidence of toxicity. Dr. Parkman asked whether there is data documenting IL-7 induced abnormalities in transgenic mice, e.g., vitiligo (development of white patches in skin), or other autoimmune phenomena as a result of responses to the melanin within these cells. If such an observation would occur, toxicities should be disclosed in the Informed Consent document.

Dr. Overell responded to Dr. Parkman's question on the rationale for the starting dose of 10ng IL-7/24 hours. This dose is based on data derived from murine experiments that included doses between 10 and

100 ng/24 hours. Dr. Parkman expressed concern about whether themurine data is an adequate basis for the rationale. The norm for other cytokine studies is to determine doses by extrapolation of data from human systemic toxicity studies. Dr. Post inquired if the monkey andmurine toxicity studies have been completed. Dr. Overell said that these studies have recently been initiated. The monkey study involves doses 100 times over the dose proposed for the human trial. Themurine studies will be used to test for autoimmune disease. Dr. Parkman commented that these toxicity data are needed for writing a scientifically based Informed Consent document. Dr. Smith added that it will be difficult to approve the protocol without these toxicity data. Dr. Parkman stressed that this protocol is the first application of IL-7 to human subjects, and the present proposal is not supported by adequate preclinical data. Dr. Noguchi commented that he would agree with Dr. Parkman that FDA would not approve a trial without extensive negotiation regarding the study design and data from toxicology experiments. It is entirely appropriate for the RAC to raise these concerns.

Dr. Motulsky said that considering the lack of toxicology studies, he would recommend deferral of the protocol until the investigators return to the RAC with these data. Dr. Miller asked for clarification regarding the toxicology data that would be required. Dr. Parkman inquired whether IL-4 was given to humans systemically before approval of the IL-4 gene therapy protocol. Dr. MichaelLotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, stated that IL-4 was administered systemically in combination with IL-2 to 100 patients before the IL-4 gene therapy trial was proposed, but extensive toxicology data in animal models was not required by the RAC. Dr. Parkman commented that this clinical scenario is different in which no human IL-7 data is available. Dr. Overell said that negotiations have already occurred with the FDA regarding the kind of toxicology data that will be required. He asked whether the data required by the FDA would be satisfactory for the RAC. Dr.Wivel commented that the FDA required data will serve the purpose. Dr. Parkman noted that a justification for the starting dose is necessary and the result related to scleroderma. Dr. Walters concluded that the toxicology study design negotiated with FDA will be acceptable to the RAC. Dr. Parkman said that due to the serious deficiency of preclinical data, it would require a new submission for the future RAC review, not just provisions of toxicology data.

Committee Motion

Dr. Motulsky made a motion to defer the protocol. Dr. Parkman made a friendly amendment that efficacy data must be provided from preexisting tumor models when the protocol is resubmitted. DrMotulsky did not accept the friendly amendment.

The RAC approved a motion was made by Dr. Motulsky and seconded by Dr. Doi to defer the protocol submitted by Drs. Economou, Glaspy, and McBride of the University of California, Los Angeles, California, by a vote of 12 in favor, 0 opposed, and 2 abstentions. The RAC deferred the protocol based on insufficient toxicology studies and failure to demonstrate biological efficacy. Dr. Parkman recommended that data demonstrating biological efficacy in a preexisting murine tumor model be provided when the protocol is resubmitted. Dr. Motulsky said that he would not require such data. Dr. Walters noted that Dr. Miller abstained from voting due to his collaboration with Targeted Genetics, Inc..

Chair Note

Dr. Walters welcomed Dr. David Ginsberg of the University of Michigan as a new member of the RAC and Dr. Harold Ginsberg as an *ad hoc* consultant for the adenovirus protocol submitted by Dr. Jack Roth of MD Anderson Cancer Center, Houston, Texas.

XV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: CLINICAL PROTOCOL FOR MODIFICATION OF TUMOR*

SUPPRESSOR GENE EXPRESSION AND INDUCTION OF APOPTOSIS IN NON-SMALL CELL LUNG CANCER (NSCLC) WITH AN ADENOVIRUS VECTOR EXPRESSING WILDTYPE p53 AND CISPLATIN/DR. ROTH

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. Jack A. Roth of M.D. Anderson Cancer Center, Houston, Texas. Dr.Haselkorn explained that this protocol involves the *in vivo* transduction of wildtype p53, a tumor suppressor gene, into lung cancer cells to inhibit cell division, i.e., prevent tumor growth. The p53 protein forms complexes with normal transcriptional factors that control the expression of other genes that promote cell division through the activation of DNA synthesis. Cells do not divide in the presence of functional wildtype p53. In the absence of wildtype p53, cells divide in an uncontrolled manner. A substantial number of NSCLC have been shown to have the defective p53 gene. The investigator proposes to replace the defective gene with the corrected p53 gene to inhibit tumor cell division. Murine and human lung cells have been transplanted into nude mice and this strategy has been demonstrated to be functionally effective.

This human protocol involves the delivery of the wildtype human p53 gene to lung tumors. The delivery process involves modifying an adenovirus such that early genes required for early transcription and DNA replication are replaced with a human wild type p53 gene. The adenovirus vector, Ad5CMV-p53, will be injected into NSCLC patients.

The investigators have demonstrated that the addition of cisplatin at the time of transduction by the adenovirus-p53 construct induces apoptosis (programmed cell death). Cisplatin alone has been shown to reduce growth of tumor cells in culture. The p53 construct by itself effects a 28% reduction in the cell growth. However, the combination of cisplatin and the p53 adenovirus construct have a synergistic effect. This phenomenon forms the scientific basis of the human protocol.

Dr. Haselkorn raised the following questions: (1) What is the scientific explanation for the synergistic effect of cisplatin and p53? (2) Since this protocol requires injection into a defined tumor, why is the tumor not surgically removed? He said that most of the previous concerns raised in the primary written review have been adequately addressed by the investigators.

Review--Dr. Straus (presented by Dr. Haselkorn)

Dr. Haselkorn summarized Dr. Straus' written comments. Dr. Straus stated that the combined treatment of the p53 gene and cisplatin was a potentially exciting Phase I study. The investigators propose 2 study groups with up to 21 patients in each group. One group will receive direct injection of the adenovirus construct directly into the tumor mass. The second group will have the adenovirus construct injected into the pleural space (for patients with a pleural disease). Dr. Straus suggested that subjects should receive either the adenovirus vector or cisplatin alone as a preliminary toxicity study prior to administering the combination. The current 2 week window between administration of the adenovirus alone and its use in combination with cisplatin may not be adequate to assess toxicity and the potential for adenovirus replication and shedding. Immunosuppression caused by cisplatin may augment virus shedding. He suggested that a background rate for virus shedding should be determined, and the treatment groups and total number of subjects should be reconsidered with this aspect in mind.

Dr. Straus suggested that the Informed Consent document should be revised with the following modifications: (1) the statement that the adenovirus vector "cannot cause disease" should be deleted, (2) the incomplete sentences should be corrected, and (3) the ambiguous statements regarding medical

costs should be revised such that these issues are clearly disclosed. Dr. Straus concluded that approval is recommended contingent on incorporation of the suggested revisions.

Review--Mr. Capron (presented by Dr. Haselkorn)

Dr. Haselkorn stated that Mr. Capron provided a very detailed written analysis of the protocol. Mr. Capron states that the preclinical studies sufficiently justify the human trial. The injection of the recombinant adenovirus does not pose an unacceptable risk to other individuals who may come in contact with these subjects. Mr. Capron raised several questions about the number of patients entered on the study and the statistical design. He recommended several changes in the Informed Consent document for clarification.

Review--Ms. Meyers (presented by Dr. Haselkorn)

Dr. Haselkorn presented Ms. Meyers' written concern about the Informed Consent documents regarding medical costs and life-long follow-up. Ms. Meyers questioned whether 21 patients are justified for this Phase I trial.

Review--Dr. H. Ginsberg (Ad hoc)

Dr. H. Ginsberg commented on the biology of the adenovirus construct. One advantage of the proposed construct is that the inserted p53 gene replaces the E1b viral gene, thus preventing the complex formation between p53 and the 55 kilodalton E1b protein. He noted; however, that the cell transduction at a high multiplicity of infection may augment the expression of early genes. This vector is not a totally defective virus. He expressed concern that the pathogenicity of the virus has not been considered. Although viral DNA expression is required for the pathological response, the vector can produce severe pathogenic effects in cotton rats, mice, and non-human primates. Pathogenicity must be assessed prior to administering this construct to patients with pulmonary disease. One advantage of this vector over previous adenovirus vectors used in CF protocols is that the early region 3 (E3) has been retained that helps protect the cells from cytopathic effects.
In regard to the issue of potential pathogenicity, Dr. H. Ginsberg explained that some animal models do not demonstrate clinical signs of adenovirus infection similar to humans; therefore, the choice of an animal model is important. The cotton rat is the model of choice because clear pathogenic effects of adenovirus infection are evident. Adenovirus pathogenic effects do not require viral replication. If large doses of adenovirus vector are administered, expression of early genes can produce such effects in the absence of viral replication. Pathogenicity is first observed in the alveoli followed by peribronchial and perivascular infiltration. Epithelial cells are not affected; therefore, epithelial cell brushings are inadequate to test for pathogenicity. There should be histologic examination of the tissue. Because the vector does not replicate, large doses of the vector, i.e., between 1 x 109 and 1 x 1010 plaque forming units (PFU), would be required to determine pathology in the human lung.

Other Comments

Dr. Doi noted the adverse event reported by Dr. Ronald Crystal (Protocol #9212-034) with regard to adenovirus vector administration. Dr. H. Ginsberg said that the major pathological response is the result of early cellular immunity, predominantly CTL responses. Deletion of E1a does not prevent early gene expression, a fact that will limit multiple inoculations of adenovirus vectors. Dr. Haselkorn commented that the adverse event reported for Dr. Crystal's CF study was related to target cell destruction resulting from an antibody response to late viral proteins. Since this proposal involves the destruction of tumor cells, such an effect could be beneficial. Dr. Haselkorn asked the investigators to elaborate on this concern. Drs. Miller, Parkman, and Haselkorn asked Dr. Roth to address the phenomenon of the "bystander effect."

Dr. Miller asked about the dose of virus proposed for the human study and whether assays have been performed to determine the presence of helper virus. Is sequence data available for the junction points of p53 and the vector backbone? How will the virus be injected into the tumor mass?

Dr. Smith asked how this protocol relates to Dr. Roth's previous NSCLC/retrovirus vector protocol in terms of patient entry. He asked Dr. H. Ginsberg how far the adenovirus vector might be expected to traffic outside the immediate injection area. Dr. H. Ginsberg responded that escape of the vector outside the injection area is unlikely because the transgene will only react within the cell.

Dr. Parkman asked about the percentage of transduced cells necessary to observe the "bystander effect." Has the "bystander effect" been observed with the adenovirus vector as with the retrovirus vector? What percentage of cells is expected to be transduced using this vector *in vivo* or *in situ*?

Dr. Post noted that the protocol calls for the administration of the adenoviral vector alone followed by a combination of the vector and cisplatin. Will the preliminary administration of the vector alone result in an immune response against the second injection? In regard to intrapleural administration, where is the virus expected to target?
Dr. Saha raised a concern that p53 gene mutations could possibly convert the tumor suppressor gene into an oncogene. What is the likelihood of such an occurrence, i.e., p53 undergoes somatic mutation?

Dr. Haselkorn explained the bystander effect is a phenomenon where more cells are killed than are actually infected or transduced by the vectors. One model for this postulates that a diffusible substance is released by dying cells that causes apoptosis of surrounding cells. Dr. Parkman explained that apoptosis is a programmed cell death triggered by either a natural or pharmacological substance.

Dr. H. Ginsberg cautioned against assaying for adenovirus in the supernatant of the transduced cells since infected cells will not be lysed immediately and released into the supernatant. More than 90% of adenovirus is associated with the cell pellets.

Investigator Response--Dr. Roth

Dr. Roth hypothesized about the mechanism for the combined effect of p53 and cisplatin. The function of wildtype p53 is to cause a delay in cell cycle progression that allows for the repair of damaged DNA. If p53 is nonfunctional, the cell will continue to divide and DNA damage will accumulate. Introduction of wildtype p53 into cells damaged by irradiation induces apoptosis (programmed cell death). A similar mechanism might be operative as a result of excessive cisplatin DNA damage. Cisplatin is a DNA crosslinking agent.

In response to the reviewers comments regarding the Informed Consent document, Dr. Roth explained that suggestions have been incorporated to the extent allowed by the IRB. Paragraphs 10 and 11 regarding the compensation for research-related injuries and medical costs including expensive tests or procedures cannot be changed or removed at this time. He stated that he will work with the other MD Anderson investigators and the IRB on this issue.

Dr. Roth explained that resectable tumors will be removed surgically. However, due to invasion of vascular and bronchial structures, not all tumors confined to a local region are surgically resectable. These non-resectable areas, as well as recurrences and isolated metastasis, can be injected by a computed tomography (CT) guided needle. Tumors are usually soft and easily injected. Dr. Roth presented a slide demonstrating the spread of the injected material throughout the tumor mass as

indicated by the *lacZ* marker. Distribution of the vector to the peripheral blood circulation is limited.

Dr. Roth addressed the safety features of the proposed vector construct. The cytomegalovirus (CMV) promoter, which expresses p53, is susceptible to inactivation. Therefore, p53 expression is transient, and the p53 protein is no longer detectable 15 days following transduction. If there is any mutation in the p53 gene, the mutant protein would be expressed only for a short time.

Dr. Saha asked if the investigator is capable of detecting a virus with the mutant p53 gene. Dr. Roth presented data from a spiking experiment in which a transforming Abelson leukemia virus was mixed with 1 x 1010 adeno-p53 virus particles. Foci formation was not observed upon infection of NIH3T3 cells. Dr. Miller commented that Abelson virus is a retrovirus, not an adenovirus.

Dr. Roth presented diffusion experiments using the *lacZ* marker gene. Following a single injection, the vector was distributed to over 60% of the tumor cells as estimated by a planimetric measurement. With the bystander effect of the tumor killing activity of the transduced cells, most cells in the tumor mass will be treated. He presented data from the orthotopic lung cancer model to verify that this is a highly reproducible system. By flushing the tumor sites with the adeno-p53 vector, no evidence of tumor at the p53 treated site was observed after 30 days. Sequential injections were used in the murine model. The first injection of the adeno-p53 construct reduced the growth of the 30-day established tumor. The second injection with the combination of p53 and cisplatin completely inhibited tumor growth and induced apoptosis. These *in vitro* and *in vivo* experiments have been accepted for publication in the *Journal of the National Cancer Institute*.

Patient eligibility will be limited to subjects with local or regionally advanced NSCLC. The intrapleural administration arm of the study involves subjects with malignant pleural effusion who are not eligible for surgery, radiation, or other local treatment. For intrapleural administration, the adenovirus vector will be delivered through a chest tube. Dr. Post asked if adenovirus vectors have been previously delivered to the pleural cavity in any human trial. Dr. Roth answered that human subjects have not received adenovirus vectors intrapleurally to date. Dr. Post explained that unlike intratumoral injection, normal cells in the pleural cavity will be transduced by the adenovirus construct. Dr. Post asked if biopsies of eligible subjects will be screened by PCR for the p53 gene mutation. Dr. Roth responded that demonstration of this mutation is included in the eligibility criteria.

Dr. Roth explained that the rationale for injecting larger tumors and including pleural disease is that a more concentrated adenovirus vector preparation can be obtained than with retrovirus vectors.

Dr. Parkman expressed concern that the transduction of normal cells within the pleural cavity might result in pleuritis or pleurisy. Dr. Post asked if the wildtype p53 gene might render normal cells in the pleural cavity more susceptible to cisplatin. Dr. Parkman asked if mouse or monkey experiments have been performed to determine toxicity of the adeno-p53 construct when injected into the pleural cavity following cisplatin treatment. Dr. Roth responded that these experiments have not been performed. Dr. Parkman said that although the majority of the cells lining the pleural cavity are nondividing, the lining is continuously renewed by the dividing stem cells underneath. Destruction of these stem cells may result in serious pleural disease.

Dr. Roth presented PCR data in response to concerns about wildtype replication-competent adenovirus contamination of the vector preparations. One wildtype virus particle is detectable among 1 x 109 vector particles. The patient dose will range between 1 x 1010 and 1 x 1011 particles; therefore, a maximum of 100 wildtype virus might be expected. One order of magnitude higher (1,000 wildtype virus particles) is required to cause human disease. Dr. Miller commented that a plaque assay is a more biologically

relevant assay than PCR.

Dr. Wei-Wei Zhang of MD Anderson Cancer Center (co-investigator) explained that thewildtype helper virus has been assayed by two methods: (1)PCR of the E1a gene, and (2) DNA labeling of HeLa cells infected with the virus. Helper virus was not detected in a background of 1 x 109 vector particles.

Dr. Roth presented slides demonstrating p53 expression with no effect on bronchial epithelial cell proliferation at 7 days. Dr. Haselkorn asked if the experiment has been performed in combination with cisplatin. Dr. Roth responded that thecisplatin experiment has not been performed. Dr. Parkman suggested that the best model for the effect of the combination therapy would be to administer these agents to the pleural cavity of cotton rats.

Dr. Roth presented histological data from Balb/c mice experiments involving intrabronchial administration of adenovirus constructs. At the highest virus dose (1 x 1010PFU), slight evidence of perivascular infiltration was observed with no evidence of pneumonia. Dr. Roth quoted a letter from Dr. James Wilson of University of Pennsylvania stating that no pathogenicity was observed in 8 subjects receiving 5 x 107 PFU of their adenovirus constructs which have both E1 and E3 deletions and are more pathogenic than the proposed construct in which the E3 gene has been retained. Dr. Roth stated that all research-related costs of the trial are underwritten by a sponsored research agreement.

Dr. Roth addressed the possible mechanism of the "bystander effect." Upon transduction with the adeno-p53 vector, molecules are secreted from thetransduced cells that affect killing of the nontranduced cells. These protein molecules have a molecular weight in the range between 30 and 100 kilodaltons. These molecules may be released from cells undergoing apoptosis. Dr. H. Ginsberg commented that the E3 gene has been reported to inhibit apoptosis.

Dr. Roth noted that there is no shortage of eligible patients for this study. Eligible patients will be screened through a conference mechanism. Based on these discussions, subjects can make informed decisions regarding protocol participation.

Responding to concerns about sequential administration of the vector, Dr. Roth said thatcisplatin could be administered with the first dose of the adeno-p53 vector.

Dr. Parkman asked whether the clinical trial could be separated for the two groups of patients, those with local lesions and those with intrapleural lesions. Additional toxicology experiments should be conducted prior to approval of the intrapleural arm of the study. Dr. Roth agreed that the two arms of the study could be separated. Dr. Parkman favored approval only for the local disease arm of the study.

Dr. Zallen confirmed that as with other MD Anderson protocols, problems remained with Section 11 of the Informed Consent document that requires patients to pay for costs related to medical care. Dr. Roth reiterated his willingness to work on this issue with the IRB. Dr. Parkman suggested that this issue could be clarified by inclusion of a statement that patients will not be responsible for any research-related costs; however, they may be required to cover some of the costs for treatment.

Drs. Parkman and H. Ginsberg suggested cotton rats should be used for the toxicology studies since they are the most sensitive to adenovirus. However, if the investigators still insist on using mice, the C57/Black strain is preferable.

Dr. Miller raised a concern about spreading of the adenovirus vector carrying a mutated p53 gene with oncogenic potential to cells outside the injected areas or to other individuals. The safety data presented

did not rule out the generation of mutant virus in the vector preparations. Dr. Roth disagreed with Dr. Miller's interpretation stating that cells transduced for 2 weeks should not have any oncogenic potential. Dr. Smith suggested that subjects should be monitored for virus shedding. Dr. Miller suggested and Dr. Roth concurred that patient's sputum will be collected and assayed for the presence of adenovirus on 293 cells expressing the viral E1a and E1b genes.

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Haselkorn to accept the protocol submitted by Dr. Jack A. Roth of M.D. Anderson Cancer Center, Houston, Texas, by a vote of 11 in favor, 1 opposed, and 1 abstention. Approval of the protocol is contingent on the review and approval of the following by the primary RAC reviewers: (1) Intra-pleural administration of the adenovirus vector will be eliminated from the protocol; therefore, a revised protocol and Informed Consent document are required; and (2) The protocol will be revised to include patient sputum titration assays of the adenovirus on 293 cells (for both the wildtype and vector adenoviruses) to be conducted until virus is no longer detectable (patients will be isolated for a period of 1 week).

The RAC deferred the intrapleural administration portion of the protocol until the investigator returns to the full RAC with a revised protocol that includes toxicity data demonstrating the effect of the adenovirus vector and cisplatin in an appropriate animal model. The RAC *strongly* recommended the cotton rat as the most appropriate model.

XVI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: INJECTION OF GLIOBLASTOMA PATIENTS WITH TUMOR CELLS GENETICALLY MODIFIED TO SECRETE INTERLEUKIN-2 (IL-2): A PHASE I STUDY/DRS. SOBOL AND ROYSTON*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Robert Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California. This protocol is a resubmission of a protocol that was disapproved by the RAC at its December 2-3, 1993, meeting. The majority of the RAC members concluded that the preclinical data derived from a single patient protocol was inadequate to justify the proposal.

This revised protocol involves subcutaneous injection of irradiated tumor cells secreting IL-2 in an attempt to stimulate antitumor immunity in patients with glioblastoma. Both autologous and allogeneic human leukocyte antigen A2 (HLA-A2) positive tumor cells will be used. Preclinical animal data and data from the single patient entered on the previous study indicate the possible efficacy of this approach. Since their previous submission, the investigators have documented the dose of irradiation required for complete tumor cell killing and that this dose allows continued IL-2 production for at least 2 weeks. The protocol is well written and has been approved by both the local IRB and the IBC. Potential danger to patients or other individuals from a recombinant DNA standpoint is low. Dr. Miller recommended approval of the protocol.
Review--Dr. Straus (presented by Dr. Wivel)

Dr. Wivel summarized Dr. Straus' written comments. Dr. Straus stated that the protocol is not fundamentally changed from the prior submission. This resubmission addresses most of the concerns raised by the RAC members during the previous review. However, the investigators have failed to address the issue of the necessity for the two study arms, i.e., autologous and allogeneic tumor cells. The

use of allogeneic cells is not scientifically validated; therefore, the investigators have agreed to delete the allogeneic arm of the study in their written response to the primary review. Dr. Straus recommended approval of the protocol based on deletion of the allogeneic tumor cell arm of the study.

Review--Ms. Meyers (presented by Dr. Walters)

Dr. Walters summarized the written comments submitted by Ms. Meyers. Ms. Meyers questioned the use of a cell line derived from the deceased glioblastoma patient. However, the investigators have since agreed to delete the allogeneic tumor cell arm of the study. Ms. Meyers expressed concern about the use of immunotherapy for the treatment of brain tumors. The assumption that intracranial tumors can be cured by immunotherapy is based on an article published in 1983. Adoptive immunotherapy has not led to any major therapeutic breakthroughs for cancer treatment.

Investigator Response--Dr. Sobol

Dr. Sobol stated that based on Dr. Straus' and Ms. Meyers' comments, the trial will only involve autologous cells. IL-2 transduced cells will be compared to nontransduced cells. Dr. Sobol agreed to the reviewer's suggestion that both transduced and nontransduced cells should receive identical doses of irradiation. The highest dose of irradiation will be used that does not interfere with IL-2 production.

Committee Motion

The RAC approved a motion made by Dr. Miller and seconded by Dr. Post to accept the protocol (with deletion of the allogeneic cell study arm) submitted by Drs. RobertSobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, by a vote of 13 in favor, 0 opposed, and no abstentions.

XVII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: IL-12 GENE THERAPY USING DIRECT INJECTION OF TUMOR WITH GENETICALLY ENGINEERED AUTOLOGOUS FIBROBLASTS/DR. LOTZE*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Miller noted that the investigator did no meet the deadline for submission of data; therefore, the review process has been impeded. At the time of submission, the entire vector sequence was not included, the vector was misnamed, preclinical data on IL-12 production by transduced cells was submitted late, and the Southern blot data was incomplete. Discussion ensued regarding the appropriateness of reviewing this protocol given the timely delay in submission of relevant data. A decision was made to review the protocol despite these delays. The RAC members noted their dissatisfaction with the manner in which the investigator handled the submission.

Subjects will receive intratumoral injections of irradiated autologous fibroblasts that have been transduced with the gene for IL-12. The investigator hypothesized that local production of IL-12 may promote tumor destruction. The RAC has previously recommended approval of studies involving other cytokine genes with this strategy. Dr. Miller raised several issues: (1) Is the retroviral vector construct, TFG-IL-12-neo, stable and suitable for use in humans? Since IL-12 has 2 subunits, the proposed vector expresses both subunits and *neo*R which will be used as a selectable marker. Expression of these 3 genes is accomplished using 2 identical ribosome entry sites (IRES) that allow translation of all 3 gene products from a single mRNA species. The feasibility of this approach should be documented, i.e.,

adequate levels of IL-12 production and lack of rearrangement during transduction. Southern blot analysis of vector DNA from transduced cells should be provided to demonstrate the lack of rearrangement. (2) Why did the investigators propose a different vector for the human study than was used for the preclinical experiments. (3) What is the rationale for initiating the IL-12 trial when the results of the IL-4 trial have not been submitted? (4) The animal data indicates that only intermediate doses of IL-12 are effective in tumor reduction, high doses of IL-12 have little antitumor effect. Does the IL-12 dose have to be carefully titrated in humans to obtain a response? (5) IL-12 toxicity studies have not been submitted for human subjects or large primates; therefore, can possible adverse effects to humans be predicted for the proposed doses of IL-12?

Review--Dr. Parkman

Dr. Parkman stated that there are 3 critical issues that should be addressed for any protocol: (1) the vector, (2) the preclinical data, and (3) the toxicity data.

In regard to preclinical studies, data was submitted only for a preimmunization animal model. Animals were preimmunized with IL-12 transduced cells. Data was not submitted from a preexisting tumor model until yesterday. These data demonstrated IL-12 transduced cells resulted in regression of contralateral tumors. Systemic IL-12 toxicity data demonstrated tumor regression 28 days following inoculation of the primary tumors. The proposed starting dose for the human protocol is 10 ng IL-12/24 hours. This dose is 1/70th the dose used for the murine systemic administration studies. In primates, the maximum tolerated dose of IL-12 is as high as 1 mg/kg body weight. Dr. Parkman said the safety margin represented by these recent studies is more than adequate. However, the RAC's concerns about the nature of the vector remain to be resolved.

Review--Ms. Meyers (presented by Dr. Walters)

Dr. Walters summarized the written comments submitted by Ms. Meyers. Ms. Meyers noted that the types of cancer proposed for treatment and the number of patients are not clearly stated in the protocol. Will the maximum number of subjects entered on the study be 18 or 43? Ms. Meyers suggested that Informed Consent issues, i.e., costs for non-negligent injuries, alternative therapies, life-long follow-up, contraception, and autopsy should be clearly described.

Other Comments

Dr. Parkman inquired about the exact vector that will be used for the human study. He agreed with Dr. Miller's comments that the issue of vector rearrangement should be addressed. Dr. Chase inquired about the rationale for testing a broad range of interleukin cytokines. Does the adoptive transfer of cytokine-producing cells hold promise for the treatment of cancer? Discussion ensued about the use of cytokines for the treatment of cancer. The consensus of the RAC was that this area of research may be beneficial based on preclinical data; therefore, human studies are of value.

Dr. Zallen agreed with the comments submitted by Ms. Meyers noting that the description of the murine virus should be revised such that it is more understandable to laypersons.

Investigator Response--Dr. Lotze

Dr. Lotze explained that the terms TFG-IL-12 and TFG-IL-12-Neo are used interchangeably. The vector is derived from the MFG vector that was made by Dr. Richard Mulligan of the Whitehead Institute, Cambridge, Massachusetts. MFG was designed for a single gene insert, whereas, DFG is for a double

gene insert. The vector construct encodes the genes for the p35 and p40 subunits of IL-12.TFG refers to the DFG construct with the addition of *neo*R.

With regard to Dr. Miller's question regarding the vector structure, Dr.Lotze explained that *neo*R was placed between the *p35* and *p40* subunit genes to prevent splicing out of *neo*R following selection of transduced cells in G418. Southern blot analysis confirms the structure of the vector DNA in the transduced cells. This construct has two identical 600 base pair IRES elements for internal initiation of protein synthesis. There was concern that homologous recombination of these elements would splice out the middle portion of the insert sequences; therefore,*neo*R was inserted between these 2 elements to prevent splicing. Autologous fibroblasts will be transduced with the TFG vector, selected in G418, tested for IL-12 production, and injected into the patient. Southern blot analysis of a fibroblast cell line transduced with TFG demonstrated the appropriate 4 kb band, not a 2 kb band. These data indicate that there is no evidence of a splice event.

Dr. Lotze explained that the IL-12 cytokine was chosen based on promising preclinical data. Data suggests that IL-12 is involved in early immune response and rejection of subcutaneous tumors. The interleukins are part of a complex immunological process in which significant knowledge is being gained. He hypothesized that positive results from both the IL-12 and IL-4 human trials could lead to a combination therapy of these 2 cytokines. IL-12 has been extensively tested in primates and has been demonstrated effective at doses much lower than those that are toxic, many orders of magnitude below toxic levels observed in primates.

The *lacZ* mobilization assay has been used to determine the presence of helper virus. Helper virus has not been detected in a background of 1 x 106 vector particles. Dr.Lotze assured Dr. Miller that the supernatant from transduced autologous fibroblasts will be assayed for helper virus prior to administration to the patient. Vector production and testing will be performed at the University of Pittsburgh.

Dr. Lotze summarized the preliminary results of his ongoing IL-4 protocol. A total of 9 patients have been treated, 5 with melanoma, 1 with renal cell carcinoma, and 3 with colorectal carcinoma. Between 80,000 and 100,000 units of IL-4 are expressed by cells injected at the primary and secondary vaccine sites. Tumor specific T cell responses, increased numbers of infiltrating T lymphocytes, expression of vascular cell adhesion molecules (VCAM) by endothelial cells, and migration of infiltrating lymphocytes out of the vessels has been observed in response to this treatment. These responses are consistent with the proinflammatory effects of IL-4 observed in preclinical experiments. Early expression of IL-4 gene has been demonstrated by RT/PCR assays. These assays demonstrate that 7 days after vaccination, the IL-4 copy number per ng total RNA is substantially reduced in biopsies taken at the injection site. Only the IL-4 producing vaccine sites are associated with T cell growth. Patients entered on the IL-4 vaccine protocol have evidence of specific T cell proliferation in their tumors. Another 11 patients will be entered on this protocol prior to completion of the study.

Given the unique aspects of IL-12 and IL-4 biology, these 2 cytokines should be tested separately. The major difference between the IL-12 and IL-4 studies is that the IL-12 fibroblasts will be injected directly into the tumor. The IL-4 protocol involves subcutaneous injection oftransduced cells into the subject's back. The proposed IL-12 trial is a natural extension of the IL-4 study.

Dr. Lotze presented additional data demonstrating tumor regression in response to systemic IL-12 administration in mice. Dr. Miller noted that the vector construct used for themurine studies is different from the vector proposed for the human study. Themurine vector has the *neo*R gene at the end of the*p40* and *p35* subunit genes rather than between these genes. Dr. Miller expressed his dissatisfaction with the Southern blot data provided in response to his concerns about vector rearrangement. Drs.Saha and Post

suggested that additional data should be requested as a stipulation for approval. Dr. Miller stressed that the vector construct to be administered to the patients should be adequately characterized. There were several statements that failure of the investigators to comply with the RAC timetable for submission of data made the approval questionable. Dr. Parkman suggested that the *NIH Guidelines* should be amended such that data submitted less than 2 weeks before the meeting at which the protocol will be reviewed should not be permitted. Ms. Wilson explained that such a provision is included in Appendix M-III-B-3 of the *NIH Guidelines*. Several RAC members stated that it is RAC's obligation to proceed with the review of the late data. The consensus of the RAC was that the protocol should be reviewed since it was on the table.

Committee Motion

The RAC approved a motion made by Dr. Post and seconded by Dr. Motulsky to approve the protocol submitted by Dr. Michael Lotze of the University of Pittsburgh, Pittsburgh, Pennsylvania, by a vote of 10 in favor, 0 opposed, and 3 abstentions. Approval of the protocol is contingent on the review and approval of the following by Drs. Miller and Parkman: (1) complete vector sequence, including a detailed restriction enzyme digestion map relevant to the vector backbone; and (2) Southern blot analysis of cells transduced with the vector constructs with and without the gene inserts, demonstrating concordance with the restriction enzyme analysis and sequence data.

XVIII. CONTINUED DISCUSSION OF THE AMENDMENT TO PART I-D, INFORMED CONSENT, OF THE *POINTS TO CONSIDER OF THE NIH GUIDELINES/DR. ZALLEN*

Dr. Walters stated that the RAC's previous comments and suggestions on the draft revision of Part I-D, Informed Consent, of the *NIH Guidelines*, have been incorporated into a revised document. Under the section on *Reproductive Considerations*, the statement about pregnant and lactating women has been moved from last to the first sentence. Dr. Motulsky suggested that this section be revised to include male contraception. Dr. Smith suggested that the first sentence should become the last sentence for clarity. Dr. Smith suggested that Section I-D-2-a-(5) should be revised to clarify the use of descriptors. The consensus of the RAC was that the revised language will read:

"If verbal descriptors (e.g., *rare*, *uncommon*, or *frequent*) are used to express quantitative information regarding risk, these terms should be explained."

Committee Motion

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Miller to accept minor editorial changes to Part I-D, Informed Consent, by a vote of 13 in favor, 0 opposed, and no abstentions.

The amended version of Part I-D, Informed Consent, of the *Points to Consider* of the *NIH Guidelines* reads:

"Part I-D. Informed Consent"

"In accordance with the requirements of DHHS regulations for the Protection of Human Subjects (45 CFR Part 46), investigators should indicate how subjects will be informed about the proposed study and the manner in which their consent will be solicited. They should also indicate how the Informed Consent document makes clear the special requirements of gene transfer research."

"Part I-D-1. Communication About the Study to Potential Participants"

"Part I-D-1-a. Which members of the research group and/or institution will be responsible for contacting potential participants and for describing the study to them? What procedures will be used to avoid possible conflicts of interest if the investigator is also providing medical care to potential subjects?"

"Part I-D-1-b. How will the major points covered in Parts I-A through I-C be disclosed to potential participants and/or their parents or guardians in language that is understandable to them?"

"Part I-D-1-c. What is the length of time that potential participants will have to make a decision about their participation in the study?"

"Part I-D-1-d. If the study involves pediatric or mentally handicapped subjects, how will the assent of each person be obtained?"

"Part I-D-2. Informed Consent Document"

"Investigators submitting human gene transfer proposals for Recombinant DNA Advisory Committee review must include the Informed Consent document as approved by the local Institutional Review Boards. A separate Informed Consent document should be used for the gene transfer portion of a research project when gene transfer is used as an adjunct in the study of another technique, e.g., when a gene is used as a "marker" or to enhance the power of immunotherapy for cancer."

"Because of the relative novelty of the procedures that are used, the potentially irreversible consequences of the procedures performed, and the fact that many of the potential risks remain undefined, the Informed Consent process should include the following specific information in addition to any requirements of the DHHS regulations for the Protection of Human Subjects (45CFR 46). Indicate if each of the specified items appears in the Informed Consent document, or, if not included in the Informed Consent document, how those items will be presented to potential subjects. Include an explanation if any of the following items are omitted from the consent process or the Informed Consent document."

"Part I-D-2-a. General Requirements of Human Subjects Research"

"Part I-D-2-a-(1). Description/Purpose of the Study"

"The subjects should be provided with a detailed explanation in non-technical language of the purpose of the study and the procedures associated with the conduct of the proposed study, including a description of the gene transfer component."

"Part I-D-2-a-(2). Alternatives"

"The Informed Consent document should indicate the availability of therapies and the possibility of other investigational interventions and approaches."

"Part I-D-2-a-(3). Voluntary Participation"

"The subjects should be informed that participation in the study is voluntary and that failure to participate in the study or withdrawal of consent will not result in any penalty or loss of benefits to which the subjects are otherwise entitled."

"Part I-D-2-a-(4). Benefits"

"The subjects should be provided with an accurate description of the possible benefits, if any, of participating in the proposed study. For studies that are not reasonably expected to provide a therapeutic benefit to subjects, the Informed Consent document should clearly state that no direct clinical benefit to subjects is expected to occur as a result of participation in the study, although knowledge may be gained that may benefit others."

"Part I-D-2-a-(5). Possible Risks, Discomforts, and Side Effects"

"There should be clear itemization in the Informed Consent document of types of adverse experiences, their relative severity, and their expected frequencies. For consistency, the following definitions are suggested: side effects that are listed as mild should be ones which do not require a therapeutic intervention; moderate side effects require an intervention; and severe side effects are potentially fatal or life-threatening, disabling, or require prolonged hospitalization."

"If verbal descriptors (e.g., "rare", "uncommon", or "frequent") are used to express quantitative information regarding risk, these terms should be explained."

"The Informed Consent document should provide information regarding the approximate number of people who have previously received the genetic material under study. It is necessary to warn potential subjects that, for genetic materials previously used in relatively few or no humans, unforeseen risks are possible, including ones that could be severe."

"The Informed Consent document should indicate any possible adverse medical consequences that may occur if the subjects withdraw from the study once the study has started."

"Part I-D-2-a-(6). Costs"

"The subjects should be provided with specific information about any financial costs associated with their participation in the protocol and in the long-term follow-up to the protocol that are not covered by the investigators or the institution involved."

"Subjects should be provided an explanation about the extent to which they will be responsible for any costs for medical treatment required as a result of research-related injury."

"Part I-D-2-c. Specific Requirements of Gene Transfer Research"

"Part I-D-2-c-(1). Reproductive Considerations"

"To avoid the possibility that any of the reagents employed in the gene transfer research could cause harm to a fetus/child, subjects should be given information concerning possible risks and the need for contraception by males and females during the active phase of the study. The period of time for the use of contraception should be specified."

"The inclusion of pregnant or lactating women should be addressed."

"Part I-D-2-c-(2). Long-Term Follow-Up"

"To permit evaluation of long-term safety and efficacy of gene transfer, the prospective subjects should be informed that they are expected to cooperate in long-term follow-up that extends beyond the active phase of the study. The Informed Consent document should include a list of persons who can be contacted in the event that questions arise during the follow-up period. The principal investigator should request that subjects continue to provide a current address and telephone number."

"The subjects should be informed that any significant findings resulting from the study will be made known in a timely manner to them and/or their parent or guardian including new information about the experimental procedure, the harms and benefits experienced by other individuals involved in the study, and any long-term effects that have been observed."

"Part I-D-2-c-(3). Request for Autopsy"

"To obtain vital information about the safety and efficacy of gene transfer, autopsies are to be performed, i feasible. Subjects should be informed that at the time of death, no matter what the cause, permission for an autopsy will be requested of their families. Subjects should be asked to advise their families of the request and of its scientific and medical importance."

"Part I-D-2-c-(4). Interest of the Media and Others in the Research"

"To alert subjects that others may have an interest in the innovative character of the protocol and in the status of the treated subjects, the subjects should be informed of the following: (1) that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy, and (2) that representatives of applicable Federal agencies (e.g., theNIH and the FDA), representatives of collaborating institutions, vector suppliers, etc., will have access to the subjects' medical records."

XIX.FUTURE MEETINGS OF THE RAC

XX. ADJOURNMENT

Dr. Walters thanked Dr. Post for extending his service on the RAC to this meeting. He adjourned this meeting of the RAC at 1:00 p.m. on June 10, 1994.

Nelson A. Wivel, M.D.
Executive Secretary

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

LeRoy B. Walters, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health