DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
June 7-8, 1993 TABLE OF CONTENTS

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-fourth meeting at 9:00 a.m. on June 7, 1993, at the National Institutes of Health (NIH), 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 except for the closed session to discuss the expedited approval of a human gene transfer protocol submitted by Drs. Ivor Royston and Robert Sobol which included confidential information. The following were present for all or part of the meeting:

Committee members:

Constance E. Brinckerhoff, Dartmouth Medical School
Alexander M. Capron, University of Southern California
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Mariann Grossman, Hospital of the University of Pennsylvania
Robert Haselkorn, University of Chicago

Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington Medical School
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
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, VA Polytechnic Institute & State University

Executive secretary:

Nelson A. Wivel, National Institutes of Health
Debra J. Wilson, National Institutes of Health (Acting)

A committee roster is attached (Attachment I).

Non-Voting Agency Representative:

Kurt Gunter, Food and Drug Administration

National Institutes of Health staff:

Mohammad Amiri, NINDS
Leon Baltrucki, NHLBI
Norman Barton, NINDS
Alicia Bazzano, CC
R. Michael Blaese, NCI
Maritza Blanco, NINDS
Roscoe Brady, NINDS
Peter Bressler, NIAID
Steve Brody, NHLBI
Diane Bronzert, NCI
Jan Casadei, NCI
Chin Chu, NHLBI
Marinee Chuah, NHLBI
Ken Cowan, NCI
Kenneth Culver, NCI
Cindy Dunbar, NCI
Tony Eissa, NHLBI
MaryEllen Franko, NCI
Edward Ginns, NIMH
Marion Glick, NIAID
Barry Goldspiel, CC
Jeffrey Hoeg, NHLBI

Christine Ireland, OD
Ari Jaffe, NHLBI
Sachiko Kajigaya, NHLBI
Stefan Karlsson, NINDS
Masako Kawase, NHLBI
Becky Lawson, OD
Charles Link, NCI
Fred Lombardo, NHLBI
Catherine McKeon, NIDDK
Koichi Miyamura, NHLBI
Rubin Moreno, NCI
Craig Mullen, NCI
Pat Newman, NCI
Masahiro Ogasawara, NCI
Colette Parker, NINDS
Don Ralbovsky, OD
William Ramsey, NCI
Craig Reynolds, NCI
Yukio Sakiama, NCI
Nava Sarver, NIAID
Raffi Schiffmann, NINDS
Tom Shih, OD
Ellen Sidransky, NIMH
Toshihiro Soma, NHLBI
Stephanie Stahl, NINDS
Mary Sullivan, OD
Francis Taylor, NINDS
Thierry Vandendriessche, NHLBI
Keoki Williams, NCI
Matt Yeatman, NICHD

Others:

Paul Aebersold, Food and Drug Administration
French Anderson, University of Southern California
Nevin Andrews, National Narrowcast Network
Don Anthony, Case Western Reserve University
Alfred Bahnson, University of Pittsburgh
Arthur Bank, Columbia University
Jack Barber, Viagene, Inc.
John Barranger, University of Pittsburgh
James Barrett, Genetic Therapy, Inc.
Steve Bauer, Food and Drug Administration
William Benedict, MD Anderson Cancer Research Center
Bridget Binko, Cell Genesys, Inc.
John Bishop, Food and Drug Administration
G'dali Braverman, Act Up
Louis Bucalo, Ingenex, Inc.
Barrie Carter, Targeted Genetics, Inc.
Yawen Chiang, Genetic Therapy, Inc.

Edward Cohen, University of Illinois
Barbara Culliton, Nature Magazine
David Danar, Lehman Brothers
Timothy Darrow, Duke University
Tapas DasGupta, University of Illinois
Wanda de Vlaminck, Avigen, Inc.
Albert Deisseroth, MD Anderson Cancer Research Center
Michelle Durand, The French Embassy
Suzanne Epstein, Food and Drug Administration
Mason Essif, CNN
Susan Falen, Genetic Therapy, Inc.
Therese Falk, The Swedish Embassy
Ron Feldbaum, Financial Times
Mitchell Finer, Cell Genesys, Inc.
Bruce Fink, CNN
Bernie Fox, University of Michigan
Jeffrey Fox, BioTechnology
Ralph Freedman, MD Anderson Cancer Research Center
Siqing Fu, MD Anderson Cancer Research Center
Morgan Gale, Hearings-on-the-Line
J. David Gallagher, Barksdale Ballard
Jeffrey Galpin, Viagene, Inc.
Cyril Gay, Department of Agriculture
Lisa Giglio, The Pink Sheet
Richard Giles, University of Illinois
Robert Gilley, CNN
Steve Goff, Columbia University
T. Venkat Gopal, Clonexpress, Inc.
Cheryl Graham, Biometric Research Institute
Aileen Griffin, Viagene, Inc.
John Griffin, Viagene, Inc.
Janet Grun, Advanced Biosearch Associates
Elie Hanania, MD Anderson Cancer Research Center
Susanna Hegewisch, MD Anderson Cancer Research Center
Russell Herndon, Genzyme Corporation
Patrick Herve, CRTS Besancon, France
Charles Hesdorffer, Columbia Presbyterian Medical Center
Minoru Hirama, Viagene, Inc.
Sharon Hoff, Department of Agriculture
David Holzman, BioWorld
Joseph Ilan, Case Western Reserve University
John Jaugstetter, Genentech, Inc.
Susan Jenks, Journal of the National Cancer Institute
Joel Jessee, Life Technologies, Inc.
William Johnston, Baxter Health Care
Douglas Jolly, Viagene, Inc.
George Kalf, Thomas Jefferson University
Steve Kanzer, The Castle Group
Kathy Kaufmann, Theragen, Inc.
John Kavanagh, MD Anderson Cancer Research Center

Donald Kohn, Childrens Hospital of Los Angeles
Toshi Kotani, Genetic Therapy, Inc.
Alex Kuta, Food and Drug Administration
Donald Longnecker, Viagene, Inc.
Richard Lundberg, Biogen, Inc.
Daniel Maneval, Canji, Inc.
Carol Marcus-Sekura, Food and Drug Administration
Michael McCann, The Blue Sheet
Gerard McGarrity, Genetic Therapy, Inc.
Steven Mento, Viagene, Inc.
Bruce Merchant, Viagene, Inc.
Teri Merrill, Oncology News
Noel Messenger, Applied Immune Sciences
Fred Miller, Food and Drug Administration
Robert Moen, Genetic Therapy, Inc.
Richard Moscicki, Genzyme Corporation
Annemarie Moseley, Applied Immune Sciences
Gary Nabel, University of Michigan
Philip Noguchi, Food and Drug Administration
Terry O'Hanlon, Food and Drug Administration
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Microbiological Associates, Inc.
Enzo Paoletti, Virogenetics Corporation
Stewart Parker, Targeted Genetics, Inc.
Liz Pennisi, Science News
Anne Petruska, The Blue Sheet
Cary Pfeffer, Biogen, Inc.
Stephen Pijar, University of Maryland
Chris Platsoucas, Temple University
Tom Porter, Theragen, Inc.
Raj Puri, Food and Drug Administration
Corey Raffel, Childrens Hospital of Los Angeles
Judy Randall, Economists Magazine
Robert Ratcheson, Case Western Reserve University
Michael Ravitch, Treatment Action Group
Rex Rhein, Biotechnology Newswatch
Jon Richards, University of Chicago
Paul Robbins, University of Pittsburgh
Renee Rockman, Genetic Therapy, Inc.
Igor Roninson, University of Illinois
H. John Roth, Department of Agriculture
Bruce Schackman, Furman Selz, Inc.
Alain Schreiber, Vical Inc.
Hilliard Seigler, Duke University
G. Terry Sharrer, Smithsonian Institution
Joseph Sherwin, Thomas Jefferson University
Tomiko Shimada, Ambience Awareness International
Thomas Storch, Tulane University Medical Center
Nevin Summers, Massachusetts Institute of Technology
Gary Temple, Life Technologies, Inc.

Pierre Tiberghien, CRTS Besancon, France
Paul Tolstoshev, Genetic Therapy, Inc.
Arvilla Trag, Virogenetics, Inc.
Mark Tykocinski, Case Western Reserve University
Christine Van de Pol, Rhone-Poulenc Rorer, Inc.
Trish Waitschies, MD Anderson Cancer Research Center
John Warner, Viagene, Inc.
Jody Waugh, The Novus Group
Julie Werner, The Novus Group
Clyde White, Carolina Biological Supply Co.
Hong-Ji Xu, Baylor College of Medicine

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notices of the meeting were published in the *Federal Register* on May 4, 1993 (58 FR 26676) and May 28, 1993 (58 FR 31045) as required by the *National Institutes of Health 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 protocol, 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, followed by the public at large.

Dr. Walters thanked Dr. Wivel and his staff for handling a large volume of protocol materials and the RAC members for their timely review of the proposals. Dr. Walters reminded everyone to adhere to the schedule as outlined in the agenda. Dr. Wivel introduced a new staff member of the Office of Recombinant DNA Activities (ORDA), Dr. Thomas Y. Shih. Dr. Shih is a senior NIH research scientist who is well recognized for his work on *ras* oncogenes. His scientific background is most pertinent to his new position in ORDA.

II. MINUTES OF THE MARCH 1-2, 1993, MEETING

Dr. Walters called on Ms. Grossman to review the minutes of the March 1-2, 1993, RAC meeting. Ms. Grossman stated that the minutes were an accurate reflection of the March meeting. Minor corrections were submitted by Drs. Hirano and Miller. The RAC unanimously approved a motion made by Dr. Dronamraju and seconded by Dr. Smith to accept the March 1-2, 1993, RAC minutes, with the inclusion of minor grammatical changes, by a vote of 19 in favor, 0 opposed, and no abstentions.

III. DATA MANAGEMENT REPORT--REVIEW OF SEMIANNUAL REPORT FORMS FOR NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. LEVENTHAL

Dr. Leventhal summarized the semiannual report on RAC-approved human gene transfer protocols (Attachment II). The report period was for the 6 months prior to April 1, 1993. A total of 92 patients have been entered into the 36 RAC-approved protocols. The median time for these protocols to be activated was 8 months after the date of RAC approval. Of these 36 studies, 33 protocols are currently open. She noted that one of the report questions asking for evidence of gene expression was ambiguous. This question will be clarified to indicate that the PI must provide evidence of *in vivo* gene expression in the patient. *In vivo* evidence of gene transfer has been observed in 10 of the protocols; however, gene expression was not demonstrated in all cases. Two possible adverse

effects were reported. In one protocol, the patient's transplanted bone marrow failed to engraft; and in the other, a patient developed asymptomatic gliosis of the brain following treatment. It is unclear whether these two effects are related to gene transfer. Several patient deaths were reported; however, most of them were related to advanced cancer or complications arising from other clinical procedures. There is no evidence that any deaths have been directly related to the gene transfer procedures. Overall, the record on patient safety is reasonable.

Ms. Meyers raised two questions: (1) Why have fewer patients been accrued in these protocols than were approved by the RAC? (2) Why are there more patient deaths in the cancer protocols than the other studies? Dr. Leventhal explained that most of the protocols have been recently approved and are still open, which accounts for the apparent low accrual rate. Dr. Geiduschek suggested that the RAC consider implementing an expiration date for the protocol if no patients have been treated after a specified period of time. Regarding efficacy, Dr. Leventhal explained that most of the cancer protocols are Phase I trials for patients with large tumor burdens; therefore, demonstration of treatment efficacy is not expected. The report also specifically omitted the efficacy question based on the fact that full disclosure of research data might infringe on the ability of investigators to publish their results in peer-reviewed journals. Dr. Walters concurred with this policy. Dr. Parkman added that an interim statement of efficacy on a small number of patients is not statistically significant. The question of efficacy can be addressed in the renewal applications based on data from previous trials. In future data reports, published efficacy results will be included. Dr. Anderson stated that the June 1993 Data Management Report would be published in the journal *Human Gene Therapy*.

IV. MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

Dr. Walters summarized the minor modifications that have been approved to NIH-approved human gene transfer protocols. A total of 13 minor modifications have been approved, and are summarized in the form of a table (Attachment III). In response to Ms. Grossman's question on the requirements for a minor modification, Dr. Walters explained that a minor modification is a change that does not significantly alter the design of a protocol and that does not increase risk to the patient. A minor modification must be approved by the Institutional Biosafety Committee (IBC), Institutional Review Board (IRB), and the Chair of the RAC (in consultation with other RAC members as necessary).

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PHASE I TRIAL OF HUMAN GAMMA INTERFERON-TRANSDUCED AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH DISSEMINATED MALIGNANT MELANOMA* /DR. SEIGLER

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol resubmitted by Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina. This protocol was deferred at the March 1993 RAC meeting. Dr. Parkman briefly recapitulated the protocol. This Phase I trial will administer human gamma interferon (-IFN)-transduced autologous tumor cells to patients with disseminated malignant melanoma. Autologous tumor cells will be grown in short-term culture and transduced with a vector expressing human -IFN. If these cells express a minimal level of -IFN, they will be irradiated with 10,000 rads and readministered to patients. The two endpoints to this study are: (1) clinical regression of tumors, and (2) the generation of cytotoxic T lymphocytes (CTL). Responding to previous concerns, the PI has provided data demonstrating that: (1) -IFN transduced cells induce increased cytolytic activity in peripheral blood, (2) the cytolytic activity is due to CTL as well as natural killer (NK) cell activity, (3) production of -IFN by transduced cells

results in the up-regulation of Class I major histocompatibility (MHC) antigens, and (4) stimulation by transduced tumor cells results in increased CTL activity. Dr. Parkman stated that most of the scientific questions raised in the previous review have been addressed and recommended approval of the protocol.

Review--Dr. Leventhal

Dr. Leventhal stated that she was satisfied with the revised submission, and that the protocol should be approved in its present form.

Review--Ms. Meyers

Ms. Meyers raised a major concern about a statement in the Informed Consent document that requires patients to pay for any injury that occurs as a direct result of participation in the study. Dr. Carmen suggested minor changes to the Informed Consent document that would make the document more understandable to lay persons.

Investigator Response--Dr. Seigler

Dr. Seigler agreed to incorporate the minor changes in the Informed Consent document suggested by Dr. Carmen. In response to Ms. Meyers' concern about patient compensation, DrSeigler said that immediate care is available for any individual who is injured as a direct result of their participation in research. The Informed Consent document informs patients that further information about their rights is available from the Hospital Risk Management Office.

Dr. Walters stated that compensation for research injuries is a generic issue that the RAC has discussed previously. On January 6, 1993, the RAC sent a letter addressing this issue to theNIH Director, Dr. Bernadine Healy. To date, no specific response to this letter has been received. There was a lengthy discussion on this issue, and Dr. Walters asked Dr. Parkman to draft a follow-up letter to the NIH Director that could be circulated for later discussion.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Parkman to approve the protocol. The motion passed by a vote of 20 in favor, 0 opposed, and no abstentions.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *USE OF MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF OVARIAN CANCER: A PILOT TRIAL*/DRS. DEISSEROTH, KAVANAGH, CHAMPLIN

Review--Dr. Leventhal

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol resubmitted by Drs. Albert B. Deisseroth, John Kavanagh, and Richard Champlin of the University of Texas M.D. Anderson Cancer Center, Houston, Texas. This protocol was deferred at the March 1993 RAC meeting. Dr. Leventhal explained that the investigators plan to introduce the cDNA of the multi-drug resistance gene (MDR-1) into normal hematopoietic early progenitor CD34(+) cells in patients with advanced ovarian cancer in an attempt to determine toxicity and the effect of modifying these norma

stem cells. Responding to a question on the transduction procedure, the investigators have provided data demonstrating that human CD34(+) cells can be isolated and transduced, and have demonstrated adequate expression of MDR-1. The desired outcome will be that each successive incremental dose of Taxol will result in an increased percentage of MDR-positive bone marrow cells. She was concerned about the actual benefit these patients will derive from introducing the MDR-1 gene into their bone marrow stem cells. Adverse side effects of Taxol, such as neurotoxicity, will not be prevented by MDR-1 transduction; therefore, hematologic protection is the only possible beneficial outcome of this study. Dr. Leventhal stated that she is not completely satisfied that the experimental design of this protocol will provide a definitive answer about hematological protection.

Review--Dr. Dronamraju
Dr. Dronamraju stated that Dr. Deisseroth had responded to his earlier concerns about the use of primate models. Dr. Deisseroth noted the RAC stated previously that either a large animal model or long-term bone marrow cultures were acceptable models, and that MDR-1 expression was demonstrated in bone marrow cells after 35 days in Dexter culture.

Review--Mr. Capron

Mr. Capron raised concerns about the Informed Consent document. Sections 3 and 4 of this document which describe the research plan and potential risks and benefits, are poorly written and very confusing. The description is too technical (e.g., using terms such as "pumping mechanism") rather than using simplified language (e.g., "a gene for drug resistance") to refer to the MDR-1 gene. The videotape prepared by the hospital is equally confusing.

Other Comments

Ms. Meyers said that the Informed Consent document may leave patients with the impression that the experimental treatment will cure their cancer. More conditionality should be incorporated into the document. She asked whether Dr. Deisseroth has obtained enough data from his previously approved protocols to justify approval of an additional protocol?

Dr. Leventhal responded that Dr. Deisseroth's two previously approved protocols are for chronic myelogenous leukemia, and that their progress is satisfactory. This protocol is for the treatment of ovarian cancer and is a well designed study.

Dr. Parkman questioned whether the administration of growth factor during hematopoietic recovery may obscure the clinical outcome of MDR-1 gene protection of Taxol toxicity to bone marrow cells. MDR gene expression will be monitored by polymerase chain reaction (PCR) analysis of colonies derived from peripheral blood and bone marrow cells following Taxol treatment. An increase in the number of MDR-1(+) colonies following Taxol administration will be a definitive endpoint, even if any protective effects due to MDR-1 expression are normalized by hematopoietic cell growth factor administration. Furthermore, if MDR-1 transduction does not result in clinical benefit because of Taxol toxicity to cells other than the bone marrow cells, the proposed study will still provide useful information for future attempts to convey drug resistance to hematopoietic cells using other genes which may have greater therapeutic margins.

Dr. Chase stated that he primarily considers safety and the responses to the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* when reviewing human gene transfer protocols, not necessarily that the best experiment has been proposed. Dr. Walters added that the RAC should

make a threshold judgment that there is a reasonable hope of success. Dr. Leventhal agreed that it is not ethical to allow a patient to participate in an experiment that is so poorly designed that no useful information will be obtained.

Dr. Carmen suggested simplified language that would make the Informed Consent document more understandable to lay persons. Drs. Post and Smith asked the investigators to clarify the bone marrow culture and transduction procedures. Dr. Geiduschek raised a concern about the adequacy of testing a small fraction of the vector supernatant for replication-competent retrovirus (RCR), and objected to the use of the term "safety modified viruses" throughout the Informed Consent document and in the protocol title. Dr. Walters recommended that the word "stem" should be omitted from the titles of both the protocol and the Informed Consent document.

Investigator Response--Dr. Deisseroth

Dr. Deisseroth explained that he would limit his response to the issue of MDR-1 expression in hematopoietic cells followed by specific questions raised by the RAC members. He presented data demonstrating that MDR-1 transduced CD34(+) cells "pump out" the control rhodamine dye more efficiently than untransduced CD34(+) cells. Since these transduced cells express a higher level of MDR-1 gene product, protection against Taxol toxicity is conferred. Expression of mRNA by transduced cells was detected by PCR analysis. The transduced gene was distinguishable from the endogenous cellular gene by a point mutation.

Dr. Deisseroth described the schema for the proposed clinical protocol. Following collection of peripheral blood and bone marrow cell for transduction, a conditioning regimen of thiotepa-cyclophosphamide will be delivered to reduce the total body tumor burden and to eradicate the bone marrow. Following autologous bone marrow (ABM) transplantation, a fraction of which has been transduced with the MDR-1 gene, patients will undergo a period of recovery prior to Taxol treatment. He stated that in the clinical protocol that does not involve gene transfer, only 1 out of 30 patients who received the conditioning regimen experienced mild marrow failure. Responding to questions about possible toxicity associated with retrovirus transduction of ABM cells, he cited the retrovirus transduction marking data obtained from studies conducted both at MD Anderson and St. Jude demonstrates that no difference in engraftment frequency has been observed by 30 days in patients receiving either marked or unmarked ABM cells.

Dr. Deisseroth stated that patients have received granulocyte colony stimulating factor (G-CSF) as part of the Taxol treatment regimen. Therefore, G-CSF will be administered to patients receiving transduced ABM cells to allow direct comparison of results. Since patients receiving G-CSF still exhibit a certain degree of hematopoietic toxicity due to Taxol, any protective effect of MDR-1 expression will be detectable. There is a dose window in which Taxol toxicity primarily involves the bone marrow and not the neural or gastrointestinal systems; therefore, bone marrow protection can be demonstrated. Long-term protection of the bone marrow from Taxol toxicity has been demonstrated in the murine model using the same vector proposed for the human study. Responding to questions raised by Drs. Post and Smith about the stromal cells used to enhance the transduction efficiency of the CD34(+) cells, Dr. Deisseroth stated that the monolayer stromal cells are grown from a standard diagnostic bone marrow aspirate performed 2 weeks prior to the transduction procedure. In response to Dr. Geiduschek's question about the adequacy of RCR safety testing, Dr. Deisseroth stated that many safety tests have been performed, but that all of the data has not been prepared for presentation at today's meeting.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Dronamraju to approve this protocol contingent on submission of the following: (1) data on the pre- and post-productionRCR testing, and (2) a revised Informed Consent document that incorporates the changes suggested by Mr. Capron, Ms. Meyers, Dr. Leventhal and Dr. Walters. The motion passed by a vote of 21 in favor, 0 opposed, and no abstentions.

VII. AMENDMENT TO THE HUMAN GENE THERAPY PROTOCOL ENTITLED: *TREATMENT OF SEVERE COMBINED IMMUNE DEFICIENCY (SCID) DUE TO ADENOSINE DEAMINASE (ADA) DEFICIENCY WITH CD34(+) SELECTED AUTOLOGOUS HEMATOPOIETIC STEM CELLS*/DR. BLAESE

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the minor modification submitted by Drs. R. Michael Blaese and Craig A. Mullen of the NIH, Bethesda, Maryland. Dr. Post stated that this modification represents the following significant changes to the previously approved protocol: (1) an additional source of cells, i.e., cord blood and placenta, (2) an expanded eligibility criterion that includes newborn babies, and (3) the administration of transduced CD34(+) cells alone, and (4) the inclusion of additional sites at which the gene therapy procedure will be performed. This modification would have been more appropriately handled as an expedited review protocol; however, the expedited review procedures were not yet officially incorporated into the *NIH Guidelines*. He recommended approval of this minor modification based on: (1) the imminent birth of the newborns, (2) the gene therapy safety issues are the same as the original protocol, and (3) the prospective benefit to these infants.

Review--Dr. Smith

Dr. Smith agreed with the issues discussed by Dr. Post. Dr. Smith recommended the RAC should approve the proposed minor modification.
Review--Dr. Leventhal

Dr. Leventhal recommended that the proposed minor modification should be approved contingent on the following: (1) the diagnosis will be confirmed at birth, (2) there will be no maternal contamination of the cord blood, (3) polyethylene glycol (PEG)-ADA will be administered, and (4) a portion of the cord blood will be cryopreserved.

Review--Dr. Walters

Dr. Walters stated that the investigators have outlined the differences between the proposed minor modification and the major amendment that was approved by the RAC at its February 1992 meeting (1) G-CSF will not be administered to the newborns, (2) leukapheresis will not be performed to harvest CD34(+) cells, and (3) the requirement for PEG-ADA pretreatment has been omitted. He asked the investigators to respond to the following questions: (1) Will PEG-ADA be withdrawn at the discretion of the PI? (2) Is bone marrow aspiration in newborns safe and necessary? (3) Are there any preclinical studies in which newborn animals have received autologous transduced cells obtained from cord blood or placenta? (4) At what point in the pregnancy is fetal ADA deficiency detected? Are there alternative therapies? (5) Will a sufficient supply of a new stem cell growth factor be available for these patients to be treated in May 1993? and (6) Will theLASN or G1NaSvADA vector be used to treat these infants?

Other Comments

Ms. Meyers questioned whether there are separate Informed Consent documents for patients that will be treated at the additional sites outside of the NIH. Dr. Doi asked whether transduced cryopreserved CD34(+) cells have been demonstrated to be as effective astransduced fresh cells. In response to Dr. Kohn's statement that the Food and Drug Administration (FDA) has determined that the current lot ofLASN vector is unacceptable for clinical use based on new RCR testing standards, Dr. Post inquired about the accuracy of this statement.

Ms. Grossman suggested that the proposed revision is more than a minor modification and should have been treated as an expedited review protocol. She objected to the breech of patient confidentiality in this case and was concerned that the current publicity will impact on patient enrollment in other gene therapy protocols. Dr. Walters explained that this request came on April 13, 1993, and that the expedited review procedures were not officially in effect at that time. Therefore, the revision was treated as a minor modification.

Investigators' Responses--Drs. Blaese and Kohn

Responding to Dr. Post's question, Dr. Blaese explained that the FDA has determined that the previous lot of LASN may be used only for patients on the existing protocol and will not be permitted for use in this amended protocol. Subsequently, a new production lot ofLASN has been produced and will be available for use on the two newborns. Regarding Dr. Doi's question, Dr. Blaese said that fresh CD34(+) cells will be used immediately without cryopreservation. In response to the confidentiality issue raised by Ms. Grossman, Dr. Blaese stated that patient confidentiality was maintained almost 3 years for the initial ADA patients. Ultimately, the parents chose to publicize the encouraging results that are being observed in their children. In the case of these newborns, the parents voluntarily waived their confidentiality and encouraged press contact. Ms. Meyers expressed her concern on patients' families going public which could affect their ability to make proper informed consent. Drs. Miller and Leventhal agreed on the validity of Ms. Meyers' concern; however, this issue should not be the basis for disapproval of this minor modification. In regard to Dr. Walters' question about the time of diagnosis, Dr. Blaese said that these two families have previous children born with ADA deficiency; therefore, the mothers were monitored for the defect. Following a positive diagnosis, the families expressed interest in pursuing stem cell gene therapy using cord blood and placenta. Regarding Ms. Meyers's question about Informed Consent documents, Dr. Blaese said that these documents have been prepared for each of the collaborating institutions. Ms. Meyers commented that all of these documents were not submitted for RAC review. Dr. Leventhal stated that if the additional documents are essentially the same as the one approved by the RAC, then they would be acceptable.

Dr. Leventhal suggested that the withdrawal of PEG-ADA should be at the discretion of the PIs since they possess the expertise to make such a decision. She noted that bone marrow aspiration is a routine procedure for 6 month old children; therefore, the procedure is acceptable if properly described in the Informed Consent document. Dr. Kohn responded that the aspiration procedure is clearly stated in the Informed Consent document, and that the patients' parents have the right to refuse the procedure. Dr. Motulsky concurred with Dr. Leventhal's comments.

Dr. Kohn stated that only fresh cord blood will be used for transduction and reconstitution of stem cells because the effects of cryopreservation are unknown. The necessity for fresh cord blood is the basis for this minor modification.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Motulsky to approve the minor modification allowing for the treatment of ADA-deficient newborns with autologous CD34(+) cells obtained from the cord blood and placenta. Also, this modification will allow the PIs to: (1) withdraw PEG-ADA at their discretion, and (2) perform bone marrow aspirations, as necessary, to monitor for gene transduction. The motion passed by a vote of 18 in favor, 0 opposed, and 3 abstentions.

VIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *IMMUNOTHERAPY FOR CANCER BY DIRECT GENE TRANSFER INTO TUMORS* /DR. NABEL

Review--Dr. Doi

Dr. Walters called on Dr. Doi to present his primary review of the protocol submitted by Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan. This protocol is an extension of the study approved by the RAC at its February 1992 meeting. The goal of this protocol is to improve the efficacy of tumor immunotherapy by the introduction of a gene encoding a foreign Class I MHC protein. The investigators propose that enhanced production of this protein will augment a CTL response against unmodified tumor cells. This protocol incorporates modifications that were not part of the original protocol approved by the RAC. These changes include: (1) the use of more efficacious cationic liposomes in which 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethylammoniumbromide (DMRIE) is utilized with dioleoyl-phosphatidylethanolamine (DOPE) for more efficient delivery of the gene; (2) the use of an improved vector, which includes the 2 microglobulin gene that forms a complete complex with Class I MHC gene product; (3) the inclusion of a catheter-based gene delivery system for direct gene transfer into the tumor microcirculation; and (4) application of the system to different tumor types. Preliminary murine data indicate that the new DMRIE/DOPE liposome preparation: (1) improves transfection efficiency, (2) demonstrates minimal toxicity, and (3) enhances the anti-tumor effect against the foreign MHC gene. Dr. Doi posed the following questions: (1) Has liposome transfection proved to be safe and efficacious in the previous RAC-approved protocol? (2) Have anti-tumor effects been observed? (3) Have all of the previous safety and toxicity concerns been satisfactorily resolved? (4) Will the increased transduction efficiency *in vitro* with DMRIE/DOPE be reproducible *in vivo*? (5) Are there any possible adverse effects on the immune response to DMRIE/DOPE? (6) How precise is the catheter delivery? (7) Will normal cells be transduced? and (8) What is the discomfort level in patients undergoing catheter administration? If the investigators can satisfactorily respond to these questions, he would recommend approval of this protocol.
Review--Dr. Brinckerhoff

Dr. Brinckerhoff agreed with Dr. Doi's assessment of the protocol. She noted that the investigators have not provided an extensive summary of previous results. The PI should explain whether the large doses of liposome that will be administered by catheter to the patients is safe. She stated that the investigators adequately responded to her written comments and that she recommends approva of the protocol.

Review--Dr. Secundy (Presented by Dr. Brinckerhoff)
In Dr. Secundy's absence, Dr. Brinckerhoff summarized her written review. Dr. Secundy states that in the Informed Consent document should be clarified and written in simplified language. In addition, the *Alternative Therapies* section should be clarified.

Other Comments

Ms. Grossman asked whether this protocol is an extension or a replacement of the previous study, and a description of the types of tumors that will be treated. Dr. Carmen asked whether the IL-2 that will be administered refers to the gene or the gene product. Ms. Meyers suggested that the Informed Consent document should recommend male and female contraception and include a request for autopsy. Dr. Miller stated that the vector sequence was checked through GenBank, and that there are no harmful sequences or open reading frames that pose any concern. Dr. Geiduschek asked the PI to comment on the comparative merit of liposome delivery versus retrovirus vector in regard to technical issues such as transduction efficiency, mitotic state of target cells, etc. Dr. Krogstad asked the PI to elaborate on the animal data.

Investigator Response--Dr. Nabel

Dr. Nabel explained that this study is a new protocol, not the extension of the previous protocol. Intratumoral injection of liposome complexes is currently used in the treatment of melanoma. When catheter-based delivery is employed, other tumor types will be targeted that have a well-defined vascular blood supply, e.g., hepatic tumors. Animal studies indicate that DMRIE/DOPE is more efficacious and less toxic than the liposome preparation used in the previous trial. There have been no adverse effects associated with the old liposome preparations. The new liposome-foreign MHC gene preparation provides protection in animals. At 1,000-fold higher doses of the new preparation, no toxicity is observed. He explained that much higher doses of the new vector will be obtainable.

Dr. Nabel summarized previous results from 5 melanoma patients. The clinical endpoints used to evaluate the trial were: (1) gene expression, (2) toxicity, and (3) antitumor response. In 4 of 5 patients, RNA expression of the injected DNA was detected by reverse transcriptase PCR on cells obtained at the site of injection. Protein expression was assayed by immunofluorescence and immunostaining techniques in several patients. Generally, there was no toxicity regarding renal, myocardial, liver, and hematological functions. The anti-DNA immune response did not increase following DNA-liposome treatment; therefore, the primary endpoint of the trial has been achieved. CTL responses to autologous tumor, particularly against human leukocyte antigen (HLA)-B7, were observed. In at least one patient, a melanoma nodule disappeared 85 days following treatment. Several other tumor responses were observed; however, these responses could be non-specific. Dr. Nabel stated that he is encouraged by the augmented CTL responses to the injected gene product.
Discussion

Drs. Parkman and Miller expressed satisfaction in the initial trial. Dr. Nabel cautioned that only 1 patient demonstrated a significant antitumor response to the treatment. The new liposome preparation will allow delivery of 100-fold more DNA. Therefore, the proposed study may produce more efficacious results. Regarding the animal studies, the antitumor response appears to be dependent on the gene that is injected rather than the non-specific response to liposome-DNA complexes. In response to Dr. Carmen's question about IL-2, Dr. Nabel explained that patients will receive the IL-2 protein not the gene.

Dr. Nabel explained the rationale for including other tumor types. Melanoma was chosen for the initial studies because noninvasive treatment was required. In the proposed protocol, other tumor types will be treated; 12 patients will be treated by direct intratumor injection and 12 patients by the catheter delivery method. The latter will be administered to patients with colon cancer, renal cell cancer, and metastatic melanoma. Renal and liver tumors with well accessible blood supplies are preferable targets for this therapy. Following a lengthy discussion about the types of tumor that

should be approved for treatment, the RAC agreed not to place any restrictions on the tumor type. The committee members recommended that patients should have a good performance status in order to ensure the likelihood of successful treatment.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Doi to approve this protocol contingent on submission of the following: (1) a revised patient eligibility section including the provision that patients who are eligible for catheter delivery of cationic liposomes must have a performance status of 0-1, and (2) a revised Informed Consent document that includes a recommendation that male/female patients use contraception and that females not be pregnant or plan to become pregnant while participating in the study, an explanation of long-term follow-up, and a request for autopsy. The motion passed by a vote of 18 in favor, 0 opposed, and 2 abstentions.

IX. WORKING GROUP REPORT ON SUBMISSION OF HUMAN GENE TRANSFER PROTOCOLS/DR. BRINCKERHOFF

Dr. Walters called on Dr. Brinckerhoff to present the report on the Working Group on the Submission of Protocols. The working group consisted of Drs. Brinckerhoff, Miller, Krogstad, and Ms. Meyers. A telephone conference call was held on May 28, 1993. The working group discussed whether investigators submitting human gene transfer protocols for RAC review should continue to be required to submit both the clinical protocol and the *Points to Consider* as part of their submission. Due to a substantial increase in the number of protocols submitted for RAC review, many committee members expressed concern about the volume of paperwork that is required; stating that the submission materials have reached an unmanageable level.

Committee motion

A motion was made by Dr. Chase and seconded by Dr. Krogstad to endorse the recommendations of the Working Group on Submission of Human Gene Transfer Protocols and to amend the *Points to Consider* of the *NIH Guidelines* as appropriate. The motion passed by a vote of 22 in favor, 0 opposed, and no abstentions. The recommendations of the working group are as follows:

1. The RAC should more strictly enforce the page limits for the protocol (20 pages) and *Points to Consider* (4-5 pages) as required by the *Guidelines for the Submission and Review of Human Gene Transfer Protocols by the RAC* of the *Points to Consider*. References may be made to supplemental materials. Discretion should be used on the part of investigators about the amount of supplemental materials that are included in their submissions. Materials will not be accepted if typed in a font that is more than 15 characters per inch.

2. The *Points to Consider* should remain a free standing document because protocols are not always succinct. The *Points to Consider* assists investigators in focusing their answers to specific questions. The investigators should provide complete responses to each question and if necessary, specifically refer the reviewers to portions of the protocol that address certain issues more fully.

3. The investigators should focus on following the Informed Consent Section of the *Points to Consider*. The working group will develop a guidance document for investigators to use in developing appropriate Informed Consent documents.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE

THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR GAUCHER DISEASE: EX VIVO GENE TRANSFER AND AUTOLOGOUS TRANSPLANTATION OF CD34(+) CELLS* /DR. BARRANGER

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Haselkorn stated there are two protocols being presented at this RAC meeting for the treatment of Gaucher disease; therefore, there are several general comments that apply to both protocols. Gaucher disease is an ideal genetic disorder for gene therapy. It is likely that positive results could be obtained similar to those reported for the ADA protocol, with the added feature that Gaucher disease is a much more prevalent disease. Gaucher disease results from an accumulation of glucocerebroside in macrophages due to a deficiency in the glucocerebrosidase (GC) enzyme. Type I is the most prevalent form of Gaucher disease resulting in less than 25% of the normal level of enzyme activity in homozygotes. The rarer forms of the disease, Types II and III, also involve the central nervous system and can result in early death. Current therapy involves purified GC enzyme; however, this treatment is extremely expensive which justifies gene therapy as a viable alternative.

Dr. Haselkorn explained that in this proposal G-CSF will be used to mobilize CD34(+) cells in the peripheral blood. In turn, these stem cells will be harvested and transduced with retrovirus vectors encoding the absent GC enzyme. The hypothesis is that GC will enter the macrophages and alleviate the lipid storage condition. The investigators propose to treat the first two patients without bone marrow ablation. If these two patients engraft without prior ablation, the remaining patients will be treated similarly. If these two patients do not engraft, then the remaining patients will receive low doses of Cytoxan to produce partial myeloablation. He asked questions concerning the choice of cells, the collection and transduction of cells, the level of gene expression, the necessity of marrow ablation, and how the two different vectors proposed for the two different Gaucher disease protocols compare.

Review--Ms. Grossman

Ms. Grossman stated that the investigators have submitted inadequate data demonstrating GC expression enzyme using the proposed vector in the human target cells. She questioned whether the transduced cells will engraft without myeloablation. If myeloablation is necessary, does the potential risk justify the use of gene therapy since an effective alternative therapy is available?

Review--Dr. Carmen

Dr. Carmen stated that Gaucher disease appears to be a paradigm affliction made to order for gene therapy. He recognized the similarity between this disease and ADA deficiency in which the transduced cells have been observed to have growth advantage in engrafted bone marrow, therefore, minimizing the need for myeloablation. Data suggest that even a low level of GC enzyme expression may be sufficient to ameliorate the condition. He recommended that the protocol be approved.

Other Comments

Dr. Smith expressed concern about the need for myeloablation and questioned whether Cytoxan is an adequate ablative regimen for bone marrow transplantation. Dr. Leventhal commented that

patients receiving transduced CD34(+) cells without myeloablation will have other treatment options if engraftment is not successful.

In response to Ms. Grossman's critique, Dr.Motulsky stated that as a medical geneticist, he is not in agreement with her comments about the validity ofGaucher disease as a candidate for gene therapy. Gaucher disease is an ideal candidate for gene therapy since enzyme replacement is expensive and the treatment has to be continually repeated. Ms. Meyers said that the Informed Consent document should include the following: (1) a suggestion that contraception should be used by males and females, and (2) a request for autopsy. Ms. Meyers questioned why patients under 18 years old are excluded from participation in this study.

Responding to Dr. Haselkorn's question, Dr. Miller stated that the two vectors proposed for the two Gaucher protocols are functionally identical. Both vectors are based on the Moloney murine leukemia virus, and produce only the GC enzyme without other vector proteins including *gag*. Dr. Haselkorn suggested that additional *in vitro* experiments should be submitted in which the two Gaucher vectors are directly compared in human hematopoietic cells. Dr. Krogstad expressed concern that an immune response might be generated against the GC enzyme. Dr. Parkman commented that most patients will be tolerant to gene expression although there have been a few reported instances of antibody responses against the recombinant enzyme. Dr. Parkman noted that the recombinant enzyme differs from the cellular enzyme in proteinglycosylation.

Investigator Response--Dr. Barranger

Responding to the question of myeloablation, Dr. Barranger explained that the first two patients will be treated with genetically corrected CD34(+) cells obtained from peripheral blood to assess whether significant engraftment is achieved without myeloablation. GC enzyme activity will be measured in the peripheral blood leukocytes to assess the success of engraftment. Animal studies indicate that a small correction of functional GC enzyme activity results in a therapeutic response. Dr. Smith asked whether the results obtained from two patients will conclusively indicate whether myeloablation is necessary for the remaining patients. Dr.Barranger responded that the first two patients will be evaluated after the first transplantation with CD34(+) cells; and if necessary, the procedure will be repeated until sufficient GC expression is demonstrated. Animal experiments demonstrate engraftment without myeloablative therapy. Dr. Barranger stated that he plans to treat patients without myeloablation. Several RAC members questioned whether this stopping rule is explicitly written in the protocol. Dr. Barranger clarified the flow sheet in the protocol and said that if the first two patients succeed in the transplant, then the remaining patients will not receive myeloablation.
Responding to Ms. Meyers' question about the exclusion of children, Dr.Barranger said that it is desirable to obtain results in adult patients first before considering the treatment of children. Dr. Barranger agreed to revise the Informed Consent document regarding contraception for men and women, a request for autopsy, and other minor changes suggested by Ms. Meyers.

Dr. Barranger stated that some patients will receive GC enzyme replacement therapy. The exogenous enzyme will not interfere with measurement of the gene-expressed enzyme level in the peripheral blood. He agreed to include a statement in the protocol explaining that the enzyme therapy will not be terminated until engraftment has been demonstrated. Dr.Krogstad asked whether anti-GC antibodies will react with the enzyme produced by genetically corrected leukocytes? Dr. Barranger responded that this issue has not been addressed and agreed to revise the protocol to exclude patients who have anti-GC antibodies. Dr. Miller was concerned that if myeloablation is to be considered for this protocol, large animal experiments should be performed.

Dr. Smith suggested that the protocol should be amended to eliminate themyeloablation procedure. The PI should resubmit a request for RAC review if myeloablation is to be considered. Dr. Barranger agreed to this stipulation.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Motulsky to approve the protocol with the following stipulations: (1) a maximum of 5 patients will be entered onto the study, (2) patients will not receive cyclophosphamide ablation of bone marrow, (3) patients who demonstrate antibodies against GC will not be eligible for the protocol, (4) GC enzyme replacement therapy will not be discontinued until cytochemical evidence of engraftment is demonstrated, and (5) the Informed Consent document will be revised to include a recommendation that male/female patients use contraception and females not be pregnant or plan to become pregnant while participating in the study, an explanation of long-term follow-up, a request for autopsy in the event of death, protection from the media, and a statement informing patients that although they may receive no direct benefit from the protocol, knowledge may be gained that will benefit others. The motion passed by a vote of 15 in favor, 0 opposed, and 4 abstentions.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *RETROVIRAL MEDIATED TRANSFER OF THE cDNA FOR HUMAN GLUCOCEREBROSIDASE INTO HEMATOPOIETIC STEM CELLS OF PATIENTS WITH GAUCHER DISEASE*/DRS. KARLSSON, DUNBAR AND KOHN

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Drs. Stefan Karlsson and Cynthia Dunbar of the NIH, Bethesda, Maryland, and Dr. Donald B. Kohn of Childrens Hospital of Los Angeles, Los Angeles, California. Dr. Haselkorn stated that his general comments regarding gene therapy for Gaucher disease that he provided during the review of Dr. Barranger's protocol are also applicable to this protocol. This protocol has the potential to produce results as dramatic as those observed in the ADA gene therapy protocol. One major concern regarding this protocol is that it involves two institutions, the NIH and Childrens Hospital of Los Angeles. Two different protocols are described, one using bone marrow cells and the other using peripheral blood cells. Although the protocol is for 10 patients, it is unclear how many patients will be treated at each institution and how the choice of target cells to betransduced will be determined. Dr. Haselkorn stated his concern that the *in vivo* enzyme levels are not very significant with the proposed vector. Enzyme levels were significantly higher using other vector constructs. Dr. Haselkorn stated that all of his other concerns have been adequately addressed by the PIs.

Review--Dr. Motulsky

Dr. Motulsky stated that Gaucher disease is a recessive disease that affects Jewish people of European origin with a substantial frequency of 1 in 500. There is tremendous variability in the clinical manifestations of this disease. The correlation between glucocerebrosidase (GC) enzyme activity and clinical severity is not predictable. Even with the most common mutation of Type I Gaucher disease (nucleotide 1226), there is considerable variation in clinical severity. How will this variability affect patient selection? Will previous enzyme therapy interfere with determining the success of the gene therapy procedure? The investigators have explained that GC expression by transduced cells can be distinguished from the activity of the exogenous enzyme. The other concerns about preclinical studies and CD34(+) cell transplantation without myeloablation were

adequately addressed by the PI. Several specific minor suggestions in the Informed Consent documents were outlined. Overall, the protocol is well thought out and promises to be successful. Dr. Motulsky recommended approval of the protocol with the inclusion of minor revisions.

Review--Dr. Carmen

Dr. Carmen stated that animal data demonstrated that bone marrow cell ablation is not a necessary predicator for correcting enzyme deficiency. The protocol will test this hypothesis in humans. The research design is lucid and carefully crafted. The Informed Consent document correctly emphasizes the gene "transfer" rather than the "therapy" features of the project. Dr. Carmen asked the PI to explain why Childrens Hospital of Los Angeles will perform transduction of bone marrow and the NIH will transduce peripheral blood cells. Why is the vector proposed for the human study different from the vector used in preclinical studies? Dr. Carmen recommended approval of the protocol.

Investigator Response--Dr. Karlsson
Responding to Dr. Haselkorn's question regarding the choice of vector, Dr.Karlsson explained that G1Gc was chosen as the vector because it produced the highest titers and levels of gene expression by macrophages in the murine model. When the MSG vector system became available later, no additional advantage was found. Since there is little human experience with CD34(+) cell transduction and transplantation without myeloablation, a comparison will be made between bone marrow cells and peripheral blood cells. Five patients will receive transduced bone marrow cells, and 5 patients will receive transduced peripheral blood cells. Investigators at the NIH have extensive experience with peripheral blood cells, and investigators at the Childrens Hospital of Los Angeles possess expertise with ABM transplantation. If one cell source proves to be more efficacious than the other, the optimal source will be used by both institutions. Responding to Dr. Carmen's comment about the choice of PA317/G1Gc vector and packaging cell line, Dr.Karlsson stated that this packing cell line and vector is probably functionally equivalent to Dr. Barranger's packaging cell line and vector. The PA317/G1Gc system is available and efficacious based on transduction and expression data in the target CD34(+) cells. Dr. Kohn agreed with Dr.Karlsson's statement regarding the similarity between the two vectors. Ms. Meyers commented that the Informed Consent document is very well written.

Committee Motion

A motion was made by Dr. Motulsky and seconded by Dr. Haselkorn to approve the protocol. The motion passed by a vote of 14 in favor, 0 opposed, and 4 abstentions.

XII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PRELIMINARY STUDY TO EVALUATE THE SAFETY AND BIOLOGIC EFFECTS OF MURINE RETROVIRAL VECTOR ENCODING HIV-I GENES [HIV-IT(V)] IN ASYMPTOMATIC SUBJECTS INFECTED WITH HIV-1* /DRS. GALPIN AND CASCIATO

Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Drs. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dennis A. Casciato of the University of California, Los Angeles, California (sponsored by Viagene, Inc., San Diego, California). The overall goal of this study is to evaluate the use of retrovirus vector-mediated

gene transfer for the treatment of human immunodeficiency virus (HIV)-infected individuals. The N2 retrovirus vector carrying *env/rev* gene of HIV-1 will be administered to asymptomatic HIV infected individuals. The hypothesis is that the vector will express the HIV *env* protein within cells and induce an augmented CTL response. This augmented response may slow or reverse disease progression. The safety and biological effects of the vector have been assessed in mice and in nonhuman primates. These *in vivo* studies demonstrated the lack of any acute or chronic toxicity and induction of enhanced CTL and antibody responses. The specific objective of this protocol is to evaluate these parameters in human subjects. Intramuscular injections will be administered monthly for 3 months and patients will be followed for evidence of virus replication, HIV burden, and HIV-1-specific CTL responses. This will be a dose-escalation study with increasing multiplicities of the vector.

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

In Dr. Straus' absence, Dr. Hirano summarized his primary review of the protocol. Most of the questions raised by Dr. Straus have been satisfactorily answered by the PIs. Dr. Straus asked about the advantage of using a retrovirus vector to express the HIV*env* gene instead of direct injection of recombinant *env* protein. The PIs responded that the*env* protein produced by the retrovirus vector will be processed within the cell to induce cellular immunity while direct injection of *env* protein will only induce a humoral immune response. Dr. Straus asked whether theCTL responses induced by the injected HIV strain would cross react with other strains of HIV virus. Dr. Straus questioned the exclusion of patients being treated by antiviral drugs since this protocol is not an efficacy study and since antiviral drugs should not alter CTL responses. He asked why the PIs propose crossing-over with the placebo recipients in this Phase I study.

Dr. Hirano agreed with Dr. Straus' concerns about the exclusion of patients receiving antiviral drug therapy, particularly in reference to the statement thatazidothymidine (AZT) (an antiviral drug) increases CTL responses in HIV patients. Dr. Hirano noted that the PIs have stated that all safety testing on the vector/producer cell line are satisfactory. This statement is meaningless unless one knows the specific fractions of the production lot that were tested and how these aliquots correlate with the proposed patient doses. Safety testing in terms of patient-dose equivalents would be a more meaningful approach. She questioned whether the phenotypes of CD8(+) and CD4(+)CTL responses differ between mice and humans.

Review--Dr. Zallen

Dr. Zallen raised specific concerns regarding the proposed protocol. Why is patient selection restricted to those individuals who have no early signs of acquired immunodeficiency syndrome (AIDS) and who have a CD4(+) count above 400? In their written responses, PIs responded that this protocol is a Phase I study in which patients are targeted in an effort to minimize any possible masking of product-attributable toxicities due to the onset of progressive disease. The Informed Consent document is very well written, and she suggested several minor changes such as using the term "gene transfer" instead of "gene therapy." There may be a possible conflict of interest in the informed consent process; namely, since the PIs are the primary care physicians, they are not the most appropriate persons to act as negotiators for obtaining informed consent. She noted that the PIs included limited research credentials in their submission. What is the function of the Monitoring Board? How does the Monitoring Board affect PIs' decision-making? How will patient confidentiality be assured?

Other Comments

Dr. Smith proposed a hypothetical event in which an HIV virus with an altered host range could result from the retrovirustransfection protocol used in these patients. Theoretically, such an event could occur if an amphotropic *env* gene from the packaging cells is transduced by the retrovirus vector into the HIV-producing cells of the patients. An altered host range of HIV then could arise either through a recombination event between HIV and the amphotropic *env* gene or through transient production of a pseudotype virus of HIV with the amphotropic envelope. A pseudotyped HIV could result in uncertain toxicity in the patient or others. Dr. Smith inquired whether this hypothetical scenario could pose a problem in this study. Dr. Geiduschek suggested that the use of another unrelated vector, such as a DNA virus, could circumvent such a scenario.

Dr. Parkman noted that there is a threshold virus dose in eliciting CTL responses in animal experiments. The dose proposed for this human study is below the threshold for CTL responses in animal models. Ms. Meyers commented that the Informed Consent document is well written and suggested that a statement should be included about a request for autopsy. Dr. Post asked the investigators to elaborate on their statement that using a co-cultivation procedure, Viagene has detected RCR that has previously escaped detection by regular supernatant assays. He inquired whether antiviral drugs would be administered if patients' CD4(+) counts fall below 400. He noted that the formulation of the virus preparation is proprietary information; therefore, it was not submitted for RAC review. He asked why Viagene submitted this protocol for RAC review if they do not receive NIH funding.

Dr. Walters called on Dr. Wivel to explain the circumstances that led to this protocol being submitted for RAC review. Dr. Wivel explained that the *NIH Guidelines* requires RAC review only for those studies that: (1) are funded by the NIH, and (2) involve collaboration with NIH-funded investigators. Submission of this proposal is on a voluntary basis since no NIH funding is involved. As to the question of whether a vote should be taken for this protocol, Dr. Wivel answered that the standard voting procedure will be used, but the vote is not binding on the PIs if they have FDA approval to proceed with the study. Dr. Miller commented that it is an encouraging development that private companies are voluntarily submitting their human gene transfer protocols for RAC review. As to the proprietary information, RAC has previous experience with reviewing such materials in executive session.

Investigator Response--Dr. Mento

Dr. Steven Mento, Vice-President of Research and Development at Viagene, responded to the RAC's questions and comments. He explained that Viagene is currently sponsoring an ongoing protocol which was not reviewed by the RAC, which is an *ex vivo* study involving a first generation product that will be used in a limited number of clinical trials. The current protocol utilizes a direct vector product that will be used for future trials involving NIH funded institutions. Therefore, Viagene has voluntarily complied with the RAC review process. After some discussion, the RAC adopted the position that it will review this protocol with the same standard as for other protocols. It was unnecessary for the RAC to go into executive session.

Dr. Mento responded to Dr. Post's question regarding detection of RCR. The RCR safety data has been submitted to FDA as part of the master file. A co-cultivation assay using *Mus dunni* cells has been used as a sensitive method for the detection of RCR in packaging cell lines. Dr. Mento described an instance in which a producer cell line tested negative for all assays on supernatant. When the *Mus dunni* co-cultivation assay was employed, a low level of RCR contamination [1 RCR particle per 107 plaque forming units (PFU)] was detected. A high standard of quality assurance is

maintained for virus preparations; therefore, the producer line was discarded. Drs. Post and Miller inquired about the nature of the RCR breakout. Dr. Mento explained that the breakout appeared to be an amphotropic recombinant arising in the packaging cell line. Dr. Mento speculated that the packaging cell line may have a block that prevents re-infection by amphotropic virus. Such a block could prevent RCR detection by other assays such as the S+L- amplification test.

Regarding the issue of virus formulation, Dr. Mento explained that the excipient added to increase stability of retrovirus particles has previously been tested in humans. The excipient formulation is proprietary information and has not been submitted in this application. He stated that Viagene would submit the formulation on a confidential basis if necessary. Dr. Miller stated that the formulation of the excipient should not present a problem from the recombinant DNA aspect. Dr. Chase, however, was concerned approving a protocol in which information has been withheld. Dr. Parkman and Mr. Capron suggested that the formulation could be submitted to ORDA and reviewed by RAC members on a confidential basis.

Responding to Dr. Straus' question about allowing patients on the placebo group to crossover to receive the retrovirus preparations at a later time, Dr. Mento stated that this decision was made based on the fact that the placebo group was originally intended to be a control for the active treatment group in the assessment of CTL responses and other parameters. Recent studies have shown that comparisons within one group at different time points are more meaningful than comparisons to a placebo group. In the current design, the placebo group is primarily for comparing the short-term adverse effects of vector administration. Patient crossover at a later time point will not affect the investigational results.

Dr. Galpin responded to the question of excluding patients on AZT from participation in this study. Most of the patients in this study will have CD4(+) cell counts over 500; therefore, the study can be conducted without the necessity for AZT administration. On the question of confidentiality, patient identity will be known only to the physicians, not Viagene. The physicians negotiating informed consent will not be the PIs of this study.

In answering a question raised by Dr. Smith on the possibility of altering the HIV host range through the amphotropic envelope gene of the packaging cells, Dr. W. French Anderson, University of Southern California, said that such a scenario is highly unlikely. Recombination between the HIV gp120 and murine gp70 is unlikely to occur. Any recombinant would likely be less toxic than gp120 alone. If any of the HIV genome were packaged in the murine amphotropic envelope, it would be destroyed by the host complement system. Dr. Mento commented that the retrovirus backbone used in the vector does not contain the amphotropic *env* gene. The vector preparations should be safe if they are free of RCR. Dr. Miller agreed that this hypothetical risk is a highly speculative situation. Mr. G'dali Braverman from Act Up expressed his support of Viagene submitting this protocol for RAC review. It is an encouraging phenomenon that industry has voluntary established dialogue with the RAC and the public. In closing, Dr. Mento agreed to provide the excipient formulation on a confidential basis. Dr. Post proposed this formulation should be reviewed by Dr. Straus and Ms. Grossman.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Secundy to approve the protocol with the following stipulations: (1) the excipient formulation of the retrovirus vector will be reviewed by Dr. Straus and Ms. Grossman, and (2) the Informed Consent document will include a request for autopsy. The motion passed by a vote of 16 in favor, 0 opposed, and 2 abstentions.

XIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A MOLECULAR GENETIC INTERVENTION FOR AIDS - EFFECTS OF A TRANSDOMINANT NEGATIVE FORM OF REV*/DR. NABEL

Review--Drs. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. Gary J Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan. Dr. Smith stated that the underlying hypothesis of this protocol is that transduction of genes that express atransdominant inhibitory HIV protein into HIV-infected and non-infected lymphocytes may reduce productive viral replication in these cells. Such action may result in prolonged survival of the transduced cells *in vivo* in patients with HIV. In the long term, it is hoped that prolonged survival could result in a beneficial clinical effect for HIV-infected patients by prolonging the latent phase of the infection. CD4(+) cells will be obtained from the peripheral blood of HIV patients, stimulated to grow *in vitro*, and transduced with a retrovirus vector carrying atransdominant inhibitory *rev* gene of HIV (Rev M10). As a control, an aliquot of CD4(+) cells from the same patient will betransduced with a similar vector that contains a frameshift mutation at the initiation codon of *rev* that prevents its expression (Rev M10). Both sets oftransduced cells will be reinfused in the patient and observed for differential rates of survival. The endpoints of the study are: (1) to measure survival of the two differentially transduced CD4(+) cell populations, and (2) to monitor the immune status of these patients. The PI has proposed two methods of gene transfer, transduction with a retrovirus vector carrying the *rev* mutant gene and biolistic transduction of the mutant gene in a plasmid DNA construct. The latter method would avoid many of the safety issues pertaining to retrovirus vectors, but its transduction efficiency has not yet been demonstrated.

Dr. Smith said that the original protocol contained insufficient information about the number of patients, the amount of blood necessary for transduction, and an incomplete Informed Consent document, etc. Responses by the PI subsequently clarified most of these questions. The investigators must provide a more detailed description of the transduction procedure.

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

In Dr. Straus' absence, Dr. Smith summarized his review of the protocol. Dr. Straus raised similar concerns to those outlined previously. He was satisfied with the responses to his questions by the PI and recommends approval of the protocol.

Review--Dr. Zallen

Dr. Zallen explained that her initial concerns were about the number of patients that will be accrued onto this study and the criteria for patient selection. Several modifications were suggested regarding the Informed Consent document. The PI responded to one of her concerns stating that 3 patients wil be treated in each arm of this study with different transduction vectors. There are still several questions remaining about patient selection, and the Informed Consent language is too technical and needs simplification.

Other Comments

Ms. Meyers commented that the Informed Consent document should include the following: (1) a recommendation that contraception be used by males, (2) a section about compensation for

research-related injury, (3) a description of long-term patient follow-up, and (4) a request for autopsy Mr. Capron explained that the insurance company will cover the costs associated with standard treatment, and that the research grants will cover costs related to research. DrNabel agreed.

Dr. Parkman asked two questions: (1) Is there data demonstrating that transduction of the mutant *rev* gene blocks HIV production in lymphocytes infected with HIV? and (2) Since there are 2 transduction schemes and 2 perturbations of each scheme, CD3/IL-2 and CD3/CD28 lymphocyte stimulation, are there 2 or 4 patient groups in this study? Dr. Miller stated that all 3 vector sequences were screened through GenBank, and that no open reading frames or harmful sequences were identified.

Investigator Response--Dr. Nabel

Dr. Nabel explained that the objective of this study is to introduce a protective gene into the uninfected CD4(+) lymphocytes of HIV patients to prevent HIV replication when later infected with the virus. *Rev* is one of the essential HIV genes required for the transition from latent to active infection. The function of the *rev* gene product is to facilitate the transport of HIV RNA from the nucleus to the cytoplasm for completion of the virus replication cycle. The transdominant mutant form of the *rev* protein inhibits this transport and keeps HIV in the latent phase. Therefore, the ultimate goal is to protect the CD4(+) cells that are not yet infected with HIV.

In addressing the safety issues raised by Dr. Smith, Dr.Nabel stated that *in vitro* transduction by the retrovirus vector will be performed in the presence of anti-HIV drugs and *Pseudomonas* exotoxin, which kills cells that have HIV gp120 *env* protein. Such measures will minimize the generation of HIV from these cells. Dr. Nabel stated that the probability of generating a novel strain of HIV by the amphotropic vector is extremely small as discussed in Drs. Galpin and Casciato's protocol. Regarding the trial design, Dr. Nabel said that a total of 12 patients will be enrolled, 4 groups of 3 patients each. There will be 2 transduction methods; in each transduction group, there will be 2 subgroups with different lymphocyte stimulation procedures, CD3/IL-2 and CD3/CD28.

Dr. Nabel agreed to simplify the language in the Informed Consent document in order to make it more understandable to patients. Patients will not be required to pay for any costs associated with the gene transfer aspects of the protocol. Ms. Meyers mentioned that long-term follow-up of patients is important if any unforeseeable event occurred in the gene transfer trial.

Responding to Dr. Miller's question, Dr. Nabel said that in cell culture experiments, the Rev M10 mutant protects lymphocytes from producing HIV by 3 to 4 orders of magnitude. However, this protection is not absolute; it can be overwhelmed by higher titer of virus production. The proposed human study is designed to test *in vivo* protection. No animal model is currently available in which to test this hypothesis. Dr. Miller asked whether the present proposal is different from the protocol submitted by Dr. Clay Smith, Memorial Sloan Kettering Cancer Center, New York, New York, which was previously deferred by the RAC. Dr. Post mentioned that in Dr. Smith's protocol, all experiments were performed in cell lines. The present protocol has additional data that was obtained using fresh peripheral blood lymphocytes from HIV patients.

Responding to Dr. Zallen's question about the long-term planning of the present study, Dr.Nabel said future directions depend on the outcome of this initial trial. If no protective effect is observed, useful knowledge will still be obtained in terms of transducing CD4(+) cells. But if there is positive protective effect, these transduced CD4(+) cells can be isolated and expanded in tissue culture and reinfused back to patients for a more aggressive therapeutic trial. Dr. Parkman remarked that in this

initial stage of the study, the word "benefit" in the Informed Consent document is not appropriate. Dr. Smith stated that he was still concerned about the safety issues surrounding the use of an amphotropic retrovirus vector in HIV patients but felt that he could defer to those members with greater expertise in this area. He stated that he would abstain from voting on this protocol. Dr. Post noted his intention to abstain from voting on this protocol due to a conflict of interest.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Miller to approve the protocol contingent on the submission of a revised Informed Consent document including the following: (1) a recommendation that contraception be used by males as well as females, (2) a request for autopsy in the event of death, and (3) an explanation of long-term patient follow-up. The motion passed by a vote of 14 in favor, 0 opposed and 4 abstentions.

XIV. CLOSED SESSION FOR EXPEDITED REVIEW OF A HUMAN GENE TRANSFER PROTOCOL

A closed session of the full RAC was held to provide an in-depth review of the May 11, 1993, expedited approval by the NIH Director of a human gene transfer protocol submitted by Drs. Ivor Royston and Robert Sobol of the San Diego Regional Cancer Center, San Diego, California. Approval was granted for additional treatments using a new retrovirus vector, G1NaCvi2. The RAC concurred with the recommendations of the intramural and extramural reviewers and the NIH Director's decision to approve the expedited review protocol.

In open session the following day, Dr. Walters summarized the committee motion on Drs. Sobol and Royston's protocol and read a statement prepared by the RAC regarding expedited review procedures.

"The RAC recommends that ORDA urge Principal Investigators to provide timely notice when considering a minor modification to a previously approved protocol or a request for expedited review. This recommendation is intended to facilitate patient care and the quality of the review process."

XV. AMENDMENT TO SECTION IV-C-3-c OF THE *NIH GUIDELINES* REGARDING PUBLICATION OF THE *RECOMBINANT DNA TECHNICAL BULLETIN* AND DATA MANAGEMENT OF THE NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/MS. WILSON

Ms. Wilson proposed to discontinue publication of the *Recombinant DNA Technical Bulletin (Bulletin)* on the basis that most of the information is duplicated elsewhere. RAC meeting minutes are currently published in the *Journal of Human Gene Therapy* (Mary Ann Liebert, Inc.). *Federal Register* notices, including proposed and major actions to the *NIH Guidelines*, are distributed through ORDA to IBC Chairs, RAC members, and other interested parties. Ms. Wilson proposed an amendment to the *NIH Guidelines* that would replace the requirement for publication of the *Bulletin* with a statement that ORDA will serve as a focal point for data management of NIH-approved human gene transfer protocols. The *NIH Guidelines* Section IV-C-3-c currently reads:

"IV-C-3. *The Office of Recombinant DNA Activities.* ....ORDA shall be responsible for the following...

"IV-C-3-c. Publishing the *Recombinant DNA Technical Bulletin* ..."

Section IV-C-3-c will be amended to read:

"IV-C-3. *The Office of Recombinant DNA Activities*. ...ORDA shall be responsible for the following...

"IV-C-3-c. Serve as the focal point for data management of NIH-approved human gene transfer protocols as required in the Reporting Requirements section of the *"Points to Consider*."

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Secundy to accept the proposed amendment. The motion passed by a vote of 16 in favor, 0 opposed and 2 abstentions.

XVI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING *RICKETTSIA PROWAZEKI* -- TRANSFER OF A CHLORAMPHENICOL RESISTANCE MARKER TO AN AVIRULENT STRAIN/DR. POLICASTRO
Review--Dr. Krogstad

Dr. Walters called on Dr. Krogstad to present his primary review of a request submitted by Dr. Paul Policastro of the NIH, Hamilton, Montana, to introduce a chloramphenicol-resistant marker into an attenuated strain of *R. prowazeki* (Madrid E). Dr. Krogstad stated that *R. prowazeki* is a human pathogen that causes epidemic typhus. Since chloramphenicol and tetracycline are the antibiotics used in treating human infections, transfer of the chloramphenicol resistant gene in this organism is a cause for concern.

The PI has provided little information on the genetics of the avirulent strain of *Rickettsia* to be used in this study or the rationale for choosing chloramphenicol as opposed to other less critically useful antibiotics. Dr. Krogstad recommended that the RAC defer approval of this request.

Review--Dr. Post

Dr. Post stated that he originally considered this request to be reasonable since the PI proposed the introduction of an antibiotic resistant gene in an attenuated strain of *Rickettsia*. Since Dr. Krogstad (an infectious disease expert) has raised many serious concerns about this experiment, Dr. Post recommended that the RAC defer approval of this proposal.

Other Comments

Dr. Haselkorn noted that an outside expert, Dr. David O. Wood of Department of Microbiology and Immunology, University of South Alabama, Mobile, Alabama, submitted a review of this request. Dr. Wood states that introduction of an antibiotic resistance gene into a Class 3 pathogen poses major concerns. Dr. Haselkorn questioned whether the PI has explored the use of other antibiotics that would be useful in cell culture but that are not critical for the treatment of human infection.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Straus to defer approval of the request. The motion to defer approval passed by a vote of 20 in favor, 0 opposed and no abstentions. The reques was deferred until the investigator submits the following data for full RAC review: (1) data demonstrating that the construct is safe and useful, and (2) *in vitro* data demonstrating the selective

advantage of chloramphenicol resistance over other selectable markers.

XVII. AMENDMENT TO SECTION III-A-4 OF THE *POINTS TO CONSIDER* REGARDING THE TERM SUBJECTS/DR. PARKMAN

Dr. Parkman proposed an amendment to the *NIH Guidelines* regarding the use of the term "subjects". To close a potential loophole that could conceivably allow investigators to bypass RAC review for single patient protocols, he proposed that the current language should be revised to cover single or multiple patient protocols.

Section III-A-4 currently reads:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into *human subjects* [21]..."

Throughout the *Points to Consider* document the term "subject" and "subjects" are used.

Section III-A-4 will be amended to read:

"III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into *one or more human subjects* [21]..."

The term "subject" and "subjects" will be changed throughout the *Points to Consider* and the *NIH Guidelines* to "one or more human subjects."

Committee Motion

A motion was made by Dr. Motulsky and seconded by Ms. Meyers to accept the proposed amendment. The motion passed by a vote of 20 in favor, 0 opposed, and no abstentions.

XVIII. DISCUSSION REGARDING INITIATION OF NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS AT SATELLITE INSTITUTIONS

The RAC initiated discussion regarding the possible format that would be employed for the review and approval of human gene transfer protocols conducted at satellite institutions. The RAC noted several issues that would have to be examined in further detail, i.e., uniform Informed Consent documents, quality control, and the cell transduction process, such as *in vivo* versus *ex vivo*, if transduction is performed by the sponsor or at a satellite site, etc. The RAC recommended that this issue should be examined in further detail and discussed as a future agenda item.

XIX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR THE TREATMENT OF RECURRENT PEDIATRIC MALIGNANT ASTROCYTOMAS WITH IN VIVO TUMOR TRANSDUCTION WITH THE HERPES SIMPLEX THYMIDINE KINASE GENE*/DRS. RAFFEL AND CULVER
Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Dr. Corey Raffel of the Childrens Hospital of Los Angeles, Los Angeles, California, and by Dr. Kenneth Culver

of Iowa Methodist Medical Center, Des Moines, Iowa. This protocol is very similar to two protocols by Drs. Oldfield and Culver previously approved by the RAC. The approach is to use the murine vector producer cell line (PA317) to deliver the *Herpes simplex* thymidine kinase (HS-tk) by means of a retrovirus vector. Tumor cells that express the HStk gene will be killed by administration of the antiviral drug, ganciclovir. The major difference between this protocol and the other two previously approved protocols is that the proposed study will involve children, 2-18 years of age, with malignant brain tumors. Dr. Hirano asked the PIs to clarify several questions: (1) What are the statistics on treatment responses? Since this protocol is a Phase II study addressing the question of treatment efficacy, it is important to clearly state the definition of responses and criteria of efficacy. It is unclear how the "stop/proceed" criterion was derived. A total of 15 patients will be placed on this study initially. If no patients respond to treatment, then the trial will be closed. If at least 1 patient responds, then an additional 14 patients will be enrolled in this study. How were these numbers derived at statistically? What are the re-treatment criteria? Patients who demonstrate minimal, partial, or complete responses to therapy will be considered for re-treatment with 3 cycles of virus producer cells (VPC). What is considered a response, and what are the rationale for these criteria? I these questions are adequately addressed by the PIs, the protocol should be approved.

Review--Dr. Geiduschek

As a note on safety, the PI has indicated that 8 brain tumor patients have now been treated, and no evidence of toxicity has been observed. Dr. Geiduschek asked the PIs to confirm whether this statement is true. Most of the other points raised by Dr. Geiduschek concerned the wording in the Informed Consent document, such as explicitly stating that the cells to be given to patients are *mouse* cells. Dr. Geiduschek expressed his satisfaction to responses from the PIs on his remarks.

Other Comments

Dr. Zallen asked why there was no assent form included for children participating in this trial. Dr. Raffel stated that at the Childrens Hospital of Los Angeles the same form is used for consent by parents and assent by children, a practice adopted by the Committee for Clinical Investigation. Dr. Parkman, the past Chair of the Committee for Clinical Investigation, agreed with Dr. Raffel's statement. Dr. Zallen questioned whether a 10 year old is capable of understanding the consent form prepared for adults. Dr. Motulsky shared the same sentiments. Mr. Capron expressed the need to prepare a form understandable to minors. Since different institutions have their own rules for preparing these documents, Ms. Meyers proposed uniform federal regulation. Dr. Walters stated that proposing such regulation is beyond the mandate of the RAC, but a letter could be drafted addressing this issue for institutions funded by NIH.

Dr. Doi inquired whether the young animals responded similarly to adults in the animal studies. Is there additional information about the toxicity issue? Ms. Wilson noted that a report had been filed with ORDA regarding a possible adverse reaction on the Oldfield protocol. One patient demonstrated asymptomatic gliosis following treatment. Dr. Miller said that the vector used in this trial is different from the vector used in the previous protocols. Does this vector have the same activity as the vector used for the animal experiments? He asked if the PIs are planning to treat a large number of patients in this study before obtaining results from previous protocols.

Investigators' Responses--Drs. Raffel and Culver

Dr. Raffel explained the rationale of proposing this protocol for the treatment of pediatric brain tumors. The biology of astrocytoma in children and adults is different, e.g., childhood tumors do not

possess p53 tumor suppressor gene mutations. Responding to Dr. Hirano's question on the "stop/proceed" criterion, Dr. Raffel said that tumor recurrence occurs quickly without additional therapy. A failure will be considered tumor recurrence within 3 months of vector producing cell (VPC) administration. Patient with recurrent tumors will not be eligible for retreatment. Otherwise, additional rounds of 3 treatment cycles will continue. Regarding the issue of an assent form for children, Dr. Raffel agreed to make changes as permitted by his IRB. As to the animal studies, results were obtained in young rats, which may not be comparable to 2 year old humans. This study excludes patients below 2 years of age because their brain development is incomplete.

Responding to a question by Dr. Miller about differences between the proposed vector and the vector used in the 2 previously approved human trials, Dr. Culver stated that the vector and the VPC proposed for this study are the same vector used for other studies. Several vectors were compared in animal studies. Regarding the toxicity issue of the two ongoing brain tumor trials, Dr. Culver stated that no acute toxicity was encountered, but a chronic or subacute adverse reaction was reported by Dr. Oldfield. Dr. Culver stated that in this protocol, attempts will be made to select patients with rapidly growing tumors and to remove as much necrosis as possible from the tumor mass to allow for optimal "bystander" effects of the VPC on surrounding tumor cells.

In response to Dr. Hirano's question about the statistics of the stopping rule, Dr. Raffel said that statistical analysis indicates that if 15 patients are treated and no responses are observed, the chance of missing an effective treatment is less than 5%. This outcome is the cut off point. If a response is observed, then a total of 30 patients will be treated.

A lengthy discussion ensued regarding the necessity for obtaining proper assent from children. The suggestion was made that there are children's assent forms for other protocols that could be used as an example, e.g., the assent form for the ADA protocol.
Committee Motion

A motion was made by Dr. Hirano and seconded by Dr. Geiduschek to approve the protocol. An amendment was made by Dr. Zallen and seconded by Ms. Meyers that the investigators will submit an assent document that will inform children of the experimental procedures and associated risks. This document must be reviewed and approved by Drs. Zallen and Secundy. The motion to approve the amendment passed by a vote of 19 in favor, 0 opposed, and 2 abstentions. The motion to approve the protocol passed by a vote of 19 in favor, 0 opposed, and 2 abstentions.

XX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY 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. DeLeon

Dr. Walters called on Dr. DeLeon to present her primary review of the protocol submitted by Dr. Ralph S. Freedman of the University of Texas MD Anderson Cancer Center, Houston, Texas. Dr. DeLeon stated that this pilot study proposes the use of gene marking to monitor the trafficking patterns of purified tumor infiltrating lymphocytes (TIL) that are employed in the treatment of patients with epithelial ovarian carcinoma. The first objective of this study is to determine whether purified CD3(+)/CD8(+) ovarian TIL, which have been expanded in IL-2, can be transduced with the G1Na retrovirus vector carrying the neomycin resistant (neoR) gene. Secondly, the PI will determine the

distribution and survival of the transduced cells following intraperitoneal injection. The PI plans to study a total of 20 patients. TIL will be obtained from tumor specimens at the time of surgery, expanded in tissue culture, and returned to patients. At 1, 2, and 3 months post-TIL administration, a group of 6 patients will undergo surgical biopsy at the site of tumor and surrounding normal tissue to monitor for neoR by PCR. The proportion of CD8(+) and CD4(+) cells will be monitored by fluorescence activated cell sorter analysis. She stated that this proposal is a straightforward gene marking protocol and presents no major concerns since it is similar to other previously approved protocols. Dr. DeLeon posed two minor issues: (1) omission of the required written report of adverse reactions to ORDA in the *Points to Consider*, and (2) the requirement that patients must bear the cost of the investigational agents and procedures. The PI agreed that this requirement is inappropriate, and that this section of the Informed Consent document will be amended.

Review--Dr. Chase

Dr. Chase stated that the protocol is poorly written; there is no clear description of the structure of the experiment. Dr. Chase expressed great doubt about the validity of the proposed statistical considerations of the experimental results. He raised five major concerns: (1) The treatment plan is not uniform. (2) Standard oncologic treatment is indistinguishable from the experimental procedures. (3) The Informed Consent document is unclear and requires patients to bear some of the experimental costs. (4) The IBC placed a contingency on approval of the protocol, yet the PI has stated that the contingency has been met, and (5) The discussion of the expected outcome is different from the research hypothesis.

Review--Dr. Secundy

Dr. Secundy stated that the investigators had adequately responded to her concerns regarding the time frame. However, questions about the exclusion and inclusion criteria remain.

Other Comments

Dr. Parkman stressed that the risks associated with the administration of gene marked cells, which will have no therapeutic effect, should be weighed against the importance of the data that will be obtained. Dr. Geiduschek stated the critical criterion for approval of this protocol is the consensus that this study will yield knowledge that will benefit the treatment of future patients. Dr. Chase has indicated that this protocol will not yield scientifically beneficial information as it is currently constructed. Therefore, Dr. Geiduschek stated that he is inclined to defer approval of the proposal. Dr. Parkman asked the PI to elaborate on how selectivity will be demonstrated, i.e., what are the methods that will be employed to demonstrate preferential trafficking of TIL to tumor versus adjacent normal tissues? Dr. Carmen said that the gene transfer procedure is not adequately described in the Informed Consent document in language that will be easily understood by lay persons.

Investigator Response--Dr. Freedman

Dr. Freedman stated that this protocol is an extension of an ongoing study of adoptive immunotherapy for the treatment of ovarian cancer. TIL will be marked with the neoR gene to determine the trafficking pattern of transduced cells. Ovarian cancer primarily involves the peritoneal surface and serosa. The objective of this study is to determine whether TIL preferentially localize to tumors. Marked TIL will be monitored by quantitative PCR. He presented preliminary tissue culture data.

Dr. Parkman asked about the percentage of TIL that are transduced *in vitro*. Dr. Freedman responded that technical difficulties have been encountered in transducing CD8(+) cells, and no definitive result has been obtained. Dr. Miller commented that if transduction frequency is low, marked TIL will be difficult to track in tumor and surrounding normal tissues. Dr. Parkman said that if the sensitivity of detecting marked cells by PCR is unknown, the selectivity of TIL trafficking to tumor cites cannot be determined. Dr. Freedman responded that TIL will be administered intraperitoneally close to the tumor sites; therefore, a large fraction of TIL should localize to tumor. Dr. Freedman said that Dr. Deisseroth's laboratory will perform the PCR analysis. Dr. Deisseroth presented PCR data. Dr. Miller stressed the technical difficulty of performing quantitative PCR analysis and stated that adequate control experiments have not been included. Dr. Post said that this proposal has many shortcomings.

Committee Motion

A motion was made by Dr. Dronamraju and seconded by Dr. Parkman to defer approval of the protocol based on the following concerns: (1) data demonstrating efficient transduction of TIL, (2) insufficient information regarding demonstration of selectivity, i.e., specific trafficking of TIL to tumor, (3) incomplete statistical analysis, (4) the Informed Consent document must be revised in simplified language, and (5) concerns about patient responsibility for research-related costs must be addressed. The motion to defer approval of the protocol passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XXI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *IMMUNIZATION OF MALIGNANT MELANOMA PATIENTS WITH INTERLEUKIN-2-SECRETING MELANOMA CELLS EXPRESSING DEFINED ALLOGENEIC HISTOCOMPATIBILITY ANTIGENS*/DRS. DAS GUPTA, COHEN AND RICHARDS

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Chicago, Illinois, and Dr. Jon M. Richards of the University of Chicago, Chicago, Illinois. Dr. Smith stated that this protocol is a Phase I study of 12 patients with advanced stage melanoma. Patients will be injected with a melanoma cell line (Mel-4) that has been transduced with a gene encoding IL-2. These transduced cells will act as an immunogenic vaccine. The hypothesis is that IL-2 secreting allogeneic melanoma cells will induce B and T cell anti-tumor responses. The endpoint for this study is toxicity; however, minor endpoints will include measurements of the induction of antibodies against HLA and melanoma associated antigen, and where possible, induction of CTL-mediated responses and other parameters. The transduced Mel-4 cells will be irradiated with 10,000 rads. He stated his concern that the murine data was generated using viable melanoma cell immunization; however, the human study proposes using irradiated cells that could impair IL-2 secretion. Is IL-2 production in Mel-4 cells stable following irradiation? What are the levels of IL-2 production in human cell lines? Dr. Smith stated that he was originally concerned that the eligibility criteria in this protocol were too stringent, but the PI has relaxed the criteria. The Informed Consent document implies that the purpose of this trial is therapeutic, yet requires patients to pay for part of the research associated costs. This issue must be addressed by the PIs. In summary, additional information regarding the background data, particularly the transduced cell line to be used for the human study, should be provided prior to approval of this protocol.

Review--Dr. Dronamraju

Dr. Dronamraju noted that the patient population is divided into several categories according to their ethnic background and gender. What is the scientific rationale for excluding certain ethnic groups?

Other Comments

Mr. Capron stated that the Informed Consent document does not include a recommendation for male/female contraception, a description of the financial responsibility of patients, or a request for autopsy. Is this study a gene therapy or gene marking protocol? Dr. Walters commented that this immunization study has therapeutic intent. Ms. Meyers objected to the use of the word "vaccine" in the Informed Consent document and suggested several other minor changes.

Dr. Geiduschek said that the murine data do not directly correlate with the human proposal, e.g., different cell numbers and treatment regimen. Dr. Post asked the PI to clarify an earlier statement about RCR assays. Dr. Parkman said that critical data were not submitted prior to the meeting; therefore, it is impossible for the RAC to evaluate the protocol.

Investigator Response--Dr. Cohen

Dr. Cohen presented *in vivo* murine data demonstrating prolonged survival of mice that were immunized with IL-2 secreting allogeneic mouse fibroblasts expressing melanoma associated antigens. Dr. Parkman remarked that themurine experiments were performed with non-irradiated cells. Dr. Cohen agreed that the cells were not irradiated, and that all tumors eventually recurred. Responding to Dr. Smith's question about the effect of irradiation, Dr. Cohen presented *in vivo* murine data demonstrating increased survival with irradiated IL-2 cells over control animals; however, survival was less than with non-irradiated cells. Dr. Smith noted that the cells used for the animal experiments were irradiated with 5,000 rads not 10,000 rads as proposed for the human study. Experiments have not been submitted using IL-2 producing cells that have been irradiated with 10,000 rads. Responding to Dr. Post's question about RCR assays, Dr. Cohen said that these safety assays will be performed by Microbiological Associates, Inc., Rockville, Maryland

Dr. Smith expressed his concern at the lack of *in vitro* human data. He said that he is inclined to recommend deferral of this protocol until additional data is submitted. Data should demonstrate whether IL-2 is produced at 24, 48, or 72 hours following irradiation with 10,000 rads. Dr. Miller suggested that data be submitted demonstrating that 5,000 rads inactivates the growth of human cells and that these cells continue to secrete IL-2. Dr. Post recommended that RCR co-cultivation assays should be submitted using irradiated human cells. Dr. Miller added that a positive control experiment demonstrating RCR in human melanoma cells will aid in the interpretation of RCR assays on these cells. Dr. Miller said that he had screened the vector sequence through GenBank, and that no harmful sequences or open reading frames were identified.

Committee Motion

A motion was made by Dr. Smith and seconded by Ms. Grossman to defer approval of the protocol until the investigators return to the RAC with the following: (1) data demonstrating the efficiency of transduction in Mel-4 cells; (2) data demonstrating viability, IL-2 production, and *in vivo* murine effect of irradiated transduced cells (either 5,000 or 10,000 rads); (3) rationale for ethnic eligibility criteria; (4) complete responses to the *Points to Consider*; and (5) RCR testing data demonstrating safety of the vector preparation. The motion to defer approval of the protocol passed by a vote of 19 in favor,

opposed, and 1 abstention.

XXII. ADDITION OF APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *HUMAN MDR GENE TRANSFER IN PATIENTS WITH ADVANCED CANCER* /DRS. HESDORFFER AND ANTMAN

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York. Dr. Parkman stated that this protocol is a resubmission of a proposal previously submitted by Dr. Arthur Bank at the March 1993 RAC meeting. The objective of this protocol is to evaluate expression of MDR-1 in the progeny of CD34(+) bone marrow stem cells in recipients of autologous bone marrow (ABM) transplants for the treatment of advanced cancer. Patients will be followed after ABM transplantation to evaluate MDR-1 expression. At the time the original protocol was submitted, the RAC was concerned about clinical aspects of the protocol. The original protocol was approved for the non-invasive treatment of melanoma. Ovarian, brain, and breast cancer are the proposed targets of this study. The PIs have submitted additional data demonstrating the lack of RCR using co-cultivation with *Mus dunni*. The PIs have removed all aspects concerning the post-transplant administration of Taxol from the protocol. The revised protocol focuses on the transduction of CD34(+) cells rather than "humanhematopoietic stem cells." The Informed Consent document has been revised. Since Taxol administration is no longer part of the gene therapy protocol, which will be performed as part of another Phase I/II study, the RAC must discuss how the issue of toxicity related to Taxol administration will be considered.

Review--Dr. Krogstad

Dr. Krogstad stated that one major concern is the possibility of inadvertent transduction of malignant cells in the marrow. Sensitive techniques detect bone marrow metastases in 30 to 50% patients of advanced breast cancer. Originally, he suggested that a "spiking" experiment be performed to simulate the inadvertent transduction of tumor cells in bone marrow. If tumor cells are not detected following isolation of CD34(+) bone marrow cells that have been deliberately added to the initial sample, the risk of inadvertent transduction of tumor cells is minimal. The PI has performed such experiments, and the results are encouraging. The CD34(+) cell selection procedure efficiently removes contaminating tumor cells from bone marrow. The Informed Consent document should be revised to avoid the suggestion that the present procedure is "safe", and should suggest that contraception should be used by males and females. The revised protocol is substantially improved over the previous submission.

Review--Mr. Capron

Mr. Capron stated that the question of whether Taxol will be involved in the present study is somewhat ambiguous in the writing of the protocol and the consent form. The headings and some technical terms of the Informed Consent document should be revised in simplified language.

Other Comments

Dr. Miller expressed concern about the RCR testing data that was submitted. The PIs state that an extremely safe packaging cell line will be used that is incapable of producing wild-type retroviruses; and therefore, does not pose any public health hazard. The seriousness of RCR testing has been

greatly overlooked. The reverse transcriptase assay is not as sensitive as other retrovirus rescue assays for the detection of RCR that have been approved by the RAC. In this protocol, a large volume of retrovirus supernatant will be used to infect bone marrow cells; however, RCR assays were performed with 1 ml aliquots. Dr. Miller stated that these assays are unacceptable. Dr. Miller said that the Informed Consent document does not clearly state whether the study involves Taxol administration. He agreed with Dr. Parkman's concern about ambiguities in the scope of this study. Ms. Meyers said that she had the following concerns about the Informed Consent document: (1) the statement that patients are responsible for costs associated with side effects due to the treatment, (2 there should be a statement about long-term follow-up, (3) a section about patient confidentiality, and (4) a request for autopsy.

Investigator Response--Dr. Hesdorffer

In response to questions about Taxol administration in this protocol, Dr. Hesdorffer explained Taxol is an issue separate from the transduction protocol. The purpose of this study is to introduce MDR-1 into CD34(+) cells and to evaluate long-term expression following ABM transplantation. If patients relapse or have residual disease after ABM transplantation, they or their physicians can elect to enter the Taxol protocol in which Taxol will be administered by a dose escalation regime. The question of enhancement of MDR-1 expression by Taxol will be assessed in the subsequent protocol. Dr. Hesdorffer agreed to revise the Informed Consent document to indicate that Taxol treatment is not part of the initial gene transfer experiment.

Regarding Dr. Krogstad's question about metastatic disease involving bone marrow, Dr. Hesdorffer said ovarian and brain tumors rarely metastasize to the bone marrow. For breast cancer, the PI will select patients without metastatic disease. Data suggests that the monoclonal antibody selection procedure eliminates the majority of tumor cells still present in the bone marrow. Since most patients who participate in this study have advanced cancer with no other alternative therapies, the additional risk is of minimal concern. Dr. Hesdorffer said that the Informed Consent document will be revised according to Mr. Capron and Ms. Meyers' suggestions. With regard to financial responsibility for research-related injuries, this unresolved issue was discussed when the protocol was presented previously. Responding to Dr. Miller's questions about RCR assays, Dr. Hesdorffer said that *Mus dunni* co-cultivation and S+L- assays have been performed in addition to the reverse transcriptase assays. Twenty percent of the clinical grade supernatant will be assayed for RCR prior to use in patients. Dr. Miller said that the safety assays described by Dr. Hesdorffer were not included in this submission.

Dr. Miller said that data have not been submitted demonstrating the transduction of human bone marrow cells. Dr. Hesdorffer said that these data were included in the original submission. Drs. Parkman and Geiduschek said that the RAC should recommend approval of this protocol contingent on the submission of additional data. Ms. Grossman expressed her interest in reviewing the additional data.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Krogstad to approve the protocol. Approval of the protocol is contingent on the review and approval of the following: (1) data demonstrating the transduction efficiency of human CD34(+) cells, and (2) a description of assays that will be performed on the clinical grade supernatant.
The motion passed by a vote of 11 in favor, 5 opposed, and 3 abstentions.

XXIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR HUMAN BRAIN TUMORS USING EPISOME-BASED ANTISENSE cDNA TRANSCRIPTION OF INSULIN-LIKE GROWTH FACTOR*/DR. ILAN

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Dr. Joseph Ilan of Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio. Dr. Miller explained that an Epstein-Barr virus (EBV) vector will be used that directs the synthesis of antisense insulin growth factor (IGF)-1 RNA and inhibits IGF-1 synthesis in glioblastoma cells obtained from patients with incurable brain tumors. Modified cells will be lethally irradiated and injected subcutaneously to stimulate immune destruction of tumor at peripheral sites. *In vivo* studies in rats support the feasibility of this technique. An episomal plasmid-based vector, which encodes EBV nuclear antigen-1 and antisense IGF-1, will be introduced into tumor cells by liposome transfection. The cells will be lethally irradiated prior to injection; therefore, there are no vector-related safety issues. The animal data supports the potential clinical utility of this approach. He asked if the investigators have additional data demonstrating the transduction of theantisense construct and inhibition of IGF-1 in human cells. Antisense IGF-1 expression is driven by a metallothionein promoter, which is inducible by metal ions. He asked whether the level of metal ions in the patients' body is sufficient to induce the promoter to expressantisense RNA. Dr. Miller recommended that the protocol should be approved.

Review--Dr. Geiduschek

Dr. Geiduschek explained that the episomal vector used in this study is a circular piece of DNA that replicates within the cell nucleus; therefore, it is not an integral component of the chromosomes. Dr. Miller added that the reason for using an episomal vector for antisense expression is that it will replicate to high copy number when transfected into target cells and will produce high levels of antisense RNA necessary to inhibit IGF-1 production.

Dr. Geiduschek said that this protocol proposes a potential therapy of an otherwise incurable disease in a conceptually coherent and generally persuasive way. The majority of his initial concerns have been responded to with the following exceptions: (1) Does the PI have experience generating the required quantities of cells from surgically acquired specimens? and (2) Does the PI have experience in producing large quantities of antisense expressing cells for clinical use?

Review--Ms. Meyers

Ms. Meyers said that the section of the Informed Consent document that explains "sense" and "antisense" gene expression may not be understandable to lay persons. She suggested that the term "cures" should be changed to "treatments."

Other Comments

Dr. Parkman asked if the PI will select patients with gliomas that over-express IGF-1. What is the definition of over-expression? Does over-expression in tumor cells established *in vitro* correlate with primary tumors *in vivo*? What is the transduction efficiency in human cells using liposome transfection? Does the rate of transduction correlate with therapeutic responses observed in animal

studies?

Dr. Post said that this proposal is very interesting; however, important human data is missing. Dr. Ilan responded that he had indicated in his written responses that the human data would be provided at the RAC meeting. Dr. Post stated that it is not an acceptable practice to allow PIs to withhold critical data until the meeting.

Investigator Response--Dr. Ilan

Dr. Ilan presented animal data that supported the basis for the proposed human trial. Rat C6 glioma cells express IGF-1 and form rapidly growing tumors in syngeneic animals. These cells lose tumorigenicity when transfected with the antisense IGF-1 cDNA vector. Subcutaneous injection of transfected C6 cells into rats prevented tumor formation at the injection site and at distal sites. These anti-tumor effects result from a glioma-specific immune response involving CD8(+) lymphocytes. Antisense blocking of IGF-1 expression may reverse a phenotype of the tumors that allows C6 glioma cells to evade the immune system. Modified C6 cells act as a vaccine against C6-induced tumors. Dr. Ilan presented additional data on other tumor types, such as osteosarcoma and rat teratocarcinoma.

Drs. Miller and Geiduschek stated that although the animal data is encouraging, little information is known about the transduction of human cells. In response to Dr. Geiduschek's concern about the length of time required to grow a sufficient quantity of cells, Dr. Ilan explained that patients will receive radiation treatment for 2 months following surgery. During this time, tumor cells will be established in culture, transduced, and expanded to the necessary number of cells required for implantation. Dr. Ilan said that he is capable of reproducibly generating sufficient quantities of cells for treatment. Dr. Parkman inquired about the percentage of cells that are transduced. Dr. Ilan responded that the cells will be selected in hygromycin; therefore, the remaining cells will be 100% transduced. Dr. Miller asked about the IGF-1 efficiency of inhibition in these transfected human cells. Dr. Ilan answered that IGF-1 production is completely inhibited; however, he stated that he did not have data demonstrating this inhibition. Dr. Miller stated that he had requested these data several weeks before the meeting. Critical data have not been submitted for review. Drs. Miller and Geiduschek stated that they could only recommend approval of this protocol contingent on the submission and review of IGF-1 inhibition data.

A lengthy discussion ensued about the inappropriateness of investigators withholding critical data prior to a RAC meeting. The RAC members said that this practice is an ongoing procedural problem that must be addressed. Dr. Parkman suggested that data not included in the written proposal should not be allowed to be presented at the RAC meeting. Dr. Krogstad said that the *Points to Consider* states that written responses from the PI are due to ORDA 2 weeks before the meeting. Dr. Parkman requested that the *Points to Consider* should be amended to prevent the submission of data immediately prior (< 2 weeks before meeting) and during the committee meeting.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. DeLeon to approve the protocol contingent on the submission of the following: (1) data demonstrating inhibition of IGF-1 expression by the antisense construct in human tumor cells, (2) data demonstrating repeated success in establishing primary cultures from fresh human tumors, and (3) data demonstrating efficiency of the transduction procedure. This document must be reviewed and approved by Drs. Straus, Post, Miller, Geiduschek, and Ms. Grossman. The motion to approve the protocol passed by a vote of 19 in favor, 0 opposed,

and no abstentions.

XXIV ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A SEMLIKI FOREST VIRUS (SFV) VECTOR EXPRESSION SYSTEM--REDUCTION OF PHYSICAL CONTAINMENT FROM BL3 to BL2/DR. TEMPLE

Review--Dr. Krogstad

Dr. Walters called on Dr. Krogstad to present his primary review of the proposal submitted by Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland. This request is a resubmission that was previously reviewed at the September 1992 RAC meeting. The investigators are requesting a reduction in the physical containment level from Biosafety Level (BL) 3 to BL2 for their Semliki Forest virus (SFV) cloning vector. Dr. Krogstad stated that this reclassification would make Life Technologies' SFV cloning kit more widely available and marketable. Since there is no jurisdiction in this area by FDA or any other federal agency, the RAC is the only review body for this particular proposal. Dr. Krogstad provided a clinical overview of SFV infection. In Tübingen, Germany, 1978, a laboratory worker death was reported in association with SFV infection. Although the cause of death was not clearly established, the containment classification of SFV was raised from BL2 to BL3. In 1990, there was an outbreak of SFV infection among a group of French soldiers in Africa. Clinical manifestations included fever and other mild symptoms of systemic infection. Other instances of infection in laboratory workers have been reported who demonstrated seroconversion and developed antibodies against SFV. Dr. Krogstad stated that the RAC must exercise extreme caution in their consideration of this proposal since a judgement must be rendered without adequate clinical information.

Review--Dr. Miller

Dr. Miller outlined the issues that remained at the time this proposal was last reviewed by the RAC. Little information was known about the frequency of recombination yielding replication-competent SFV under conditions for using the cloning vector system. In addition, the incidence of seropositivity of laboratory workers exposed to this virus had not been determined. On this resubmission, the applicants have determined the frequency of helper virus production in the system. The results indicate that helper virus will be readily detectable. Two strategies were employed to reduce the possibility of generating helper virus. One involves the separation of helper function on different RNA molecules. The frequency of generating helper virus is 10-3 per vector infectious unit. The other strategy involves mutation of the spike protease region to prevent virus activation, and the maximum rate of helper production with this mutation is 2 x 10-4. These rates are much higher than the 10-6 that was originally proposed by the PIs. These results indicate the real potential for generating SFV helper virus in the gene expression vector product. Regarding the risk to laboratory workers from using this system, the risks are very real and must be considered. Seroconversion rates are still too low to evaluate the potential for disease following infection. The RAC must consider that a fatal infection was previously reported. Given that one can expect helper virus production at some rate, the risk to laboratory workers cannot be ignored.

Dr. Miller said that he recommends reclassification of this cloning vector from BL3 to BL2 provided that potential customers are adequately informed of the potential risks associated with the system. The investigators must provide customers with: (1) an information sheet that describes the potential health risks, (2) appropriate methods to be used for virus inactivation, (3) a simple helper virus assay to detect replication competent SFV, (4) a description of the symptoms that would be expected in the event of SFV infection, and (5) a warning indicating the potential for SFV recombination.

Dr. Miller said that the investigators' response to the RAC's initial concerns appeared to downplay the potential pathogenicity of this virus. Two *ad hoc* reviewers have also submitted written critiques of the proposal. The *ad hoc* reviewers, Dr. Alan L. Schmaljohn of U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick at Frederick, Maryland, and Dr. Dennis W. Trent of National Center for Infectious Diseases, Fort Collins, Colorado, recommended use of this system at BL2 containment, however, they also emphasized the necessity to exercise caution.

Ms. Meyers inquired whether SFV is infectious to animals. Dr. Miller responded that it is possible that SFV can be transmitted by mosquitos from humans to animals, causing the virus to become endemic in the region.

Review--Dr. Post

Dr. Post stated that there is no valid scientific reason for classifying SFV and Sindbis virus in different containment categories. It appears that the classification of SFV at the BL3 containment level resulted from a single fatal case report. This case involved a subject suffering from chronic bronchitis and a strain of SFV that was unnaturally passaged through animals. Considering the long-term safe laboratory use with SFV and the epidemiological data about the natural infection of humans, SFV should not be considered a particularly virulent agent. The vector proposed by Life Technologies, Inc., is a disabled version of the wild-type virus. He said that he would vote to approve the request to reclassify this cloning vector system for use at BL2 provided that the applicants supply proper instructions for the safe handling of this vector cloning system.

Other Comments

Dr. Parkman asked whether this virus can be easily inactivated. Dr. Straus responded that it is easily inactivated and should not be an issue; however, he has serious concern about the general use of this virus by nonvirologists who are inexperienced at handling viral agents. The history of the safe use of SFV at BL2 in the past involved virologists experienced with use of these viruses. Dr. Straus was concerned that when the cloning vector is marketed, it will be used by laboratory personnel who do not have proper virology training.

Investigator Response--Dr. Temple

In response to Dr. Krogstad's question of seroconversion, Dr. Temple said that in Dr. Robert E. Shope's laboratory at Yale University, 2 out of 15 laboratory workers demonstrated borderline conversion; and in Dr. L. Kaariainen's laboratory at the University of Helsinki in Finland, 8 out of 16 laboratory workers were seropositive for SFV. These two laboratories all performed their experiments at BL2 containment. Regarding Dr. Miller's interpretation of the recombination frequency, Dr. Temple said that it is his belief that there is a low level of replication competent SFV that could emerge from large volumes of cells and cloning vectors in the laboratory setting.

Dr. Temple stressed that the cloning vector is derived from an attenuated strain of SFV. Symptoms of SFV infection are very similar to infection by Sindbis virus. SFV is spread from animal to animal by mosquitos; human infection is incidental. The proposal specifically states that the vector is not going to be used in animal experiments. Dr. Temple said that passages of SFV through animals generally increase its virulence, as occurred in the one reported fatality.

Dr. Miller emphasized that the investigators failed to include an information sheet describing safe

usage and potential risk as was requested.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Motulsky to defer approval of the proposal. The proposal was deferred until the investigators return to the RAC with the following: (1) a product information sheet informing customers of the potential health risk of the expression system, standard methods to be used for virus inactivation, a helper virus assay to detect SFV, and a description of symptoms and procedures to be followed in the event that SFV infection occurs in a laboratory worker (including methods to prevent transfer to insect vectors and environmental spread); and (2) SFV inactivation data. The motion to defer the request passed by a vote of 16 in favor, 2 opposed, and 1 abstention.

XXV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING THE *POXVIRUS VECTORS NYVAC, ALVAC, AND TROVAC--REDUCTION OF PHYSICAL CONTAINMENT FROM BL2 TO BL1*/DR. PAOLETTI

Review--Dr. Gay

Dr. Walters called on Dr. Gay, Chief Staff Veterinarian of the Office of Veterinary Biologics, U.S. Department of Agriculture (USDA), to present his *ad hoc* review of the proposal submitted by Ms. Arvilla L. Trag (on behalf of Dr. Enzo Paoletti) of Virogenetics Corporation, Troy, New York. Dr. Gay explained that this proposal requests a reduction in physical containment for 3 recombinant pox vectors NYVAC, ALVAC, and TROVAC from BL2 to BL1. Dr. Gay said that the Office of Veterinary Biologics is responsible for licensing all veterinary biologics in the United States. License applications have previously been reviewed for Rhone Merieux, Inc., for 2 recombinant vector vaccines that were constructed with poxvirus-based vectors, TROVAC and ALVAC. TROVAC-NDV, a Newcastle Disease-Fowlpox Vaccine is based on TROVAC. ALVAC-RG, a Rabies Vaccine is based on ALVAC. The USDA Office of Veterinary Biologics has conducted risk analyses and has recommended that the physical containment levels for TROVAC and ALVAC be reduced from BL2 to BL1.

Review--Dr. Moss (Presented by Dr. Walters)

In the absence of Dr. Moss, NIH, Bethesda, Maryland, an *ad hoc* expert on poxviruses, Dr. Walters summarized Dr. Moss' written review of the request. Dr. Moss is of the opinion that there is little or no inherent risk to individuals in working with these viruses. The only possible risk that could be foreseen would be inadvertent immunization with a recombinant protein expressed by these vectors. Dr. Moss recommended the use of the proposed vectors at BL1 containment unless there is reason to believe that immunizing amounts of the gene products would provide an additional risk.

Other Comments

Mr. Capron asked about risk of inadvertent immunization alluded to in Dr. Moss' review. Dr. Straus described one example in which an individual exposed to HIV proteins produced by poxvirus vectors could be seropositive for HIV even though the individual is not infected by HIV. Another example would be respiratory syncytial virus infection in which a vaccinated individual may react more severely to viral infection than an unvaccinated child. Such risks are not due to the vectors *per se* but are due to the particular proteins expressed by these vectors.

Many other written comments had been submitted in support of the proposed reclassification based on the highly attenuated characteristics and restricted host ranges of these vectors. These written reviews were by the following scientists: Dr. W. K. Joklik of Duke University Medical Center; Dr. Peter W. Mason of the Agricultural Research Service, USDA; Dr. Joanne Maki, RhoneMerieux, Inc.; Dr. David E. Lanar, Walter Reed Army Medical Center; Dr. Bert Jacob of Arizona State University; Dr. Robert E. Shope of Yale University; and Dr. Mary Lou Clements of Johns Hopkins School of Hygiene and Public Health.

Investigator Response--Dr. Paoletti

Dr. Paoletti explained that he is requesting reclassification of three vectors that are currently classified as Class 2 pathogens. NYVAC is based on the Copenhagen strain of vaccinia virus in which 18 viral genes have been deleted; ALVAC is based on an attenuated strain ofcanarypox; and TROVAC is based on an attenuated strain offowlpox virus. Dr. Post asked what additional advantages would be gained from this reclassification since permission for field testing has already been granted by the USDA. Dr. Paoletti said that approval of this request would facilitate the transfer and production of viral materials and allow for the inexpensive disposal of vaccinated animals. If these vectors are reclassified for use at BL1, there would be substantial commercial benefit. Dr. Pos suggested one possible problem associated with the use of NYVAC (the vaccinia-derived vector) in patients with eczema. Such a vector may cause disseminated disease in these individuals. Dr. Paoletti said that in immune compromised animal models, no evidence of disseminated infection has been observed. The vector will probably be administered by subcutaneous or intramuscular routes as opposed to dermal scarification for the parentalvaccinia virus.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Carmen to approve the request. The motion was passed by a vote of 15 in favor, 0 opposed, and 1 abstention.

XXVI. REPORT FROM THE WORKING GROUP ON EXEMPT REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DR. PARKMAN

Dr. Parkman submitted a draft document entitled:*Cover Sheet for Exempt Review of Human Gene Marking Protocols*. The RAC submitted several changes to the proposed document which might be used for the decentralized review of human gene transfer protocols that are identical to studies previously reviewed and approved by the RAC. The working group will continue to refine the draft document for discussion at the next RAC meeting.

XXVII. A DRAFT LETTER TO THE NIH DIRECTOR REGARDING COMPENSATION FOR RESEARCH-RELATED INJURIES

Dr. Walters resumed discussion on a letter circulated earlier by Dr. Parkman regarding the issue of compensation for research-related injuries. This letter would be forwarded to theNIH Director as a follow-up to the January 6 letter.

Dr. Parkman suggested that compensation be incorporated in the forthcoming President's health care reform proposal. The following statement was approved by the RAC as follows:

"The sense of the Recombinant DNA Advisory Committee (RAC) is that the present discussions about national health care reform provide an opportunity for theNIH Director to address the issues

relating to providing health care for patients who are injured as a result of participating in clinical research approved by NIH-mandated IRBs. A report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, *Compensating for Research Injuries* (1982), demonstrated that other nations which provide health care coverage for al citizens avoid the problems that arise in attempting to design a separate compensation system for patients injured because of their participation in medical research. Thus, the RAC supports the inclusion in basic universal health care of coverage for injury received through participation in approved clinical research. It is the strong desire of the RAC that theNIH Director would call this issue to the attention of the individuals formulating the President's health care reform proposal."

Committee Motion

A motion was made by Dr. Carmen and seconded by Ms. Meyers to approve the letter. The motion passed by a vote of 14 in favor, 0 opposed, and no abstentions. This follow-up letter will be forwarded to the NIH Director by Dr. Walters. [Executive Secretary's Note: Dr. Walters, Chair of the RAC, forwarded the follow-up letter to Dr. Healy on June 28.]

XXVIII. ADJOURNMENT

Dr. Walters adjourned the meeting at 4:45 p.m. on June 8, 1993.

Nelson A. Wivel, M.D.
Executive Secretary

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

Date: 9/9/93

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