DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
September 9-10, 1993

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The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-fifth meeting at 9:00 a.m. on September 9, 1993, at the National Institutes of Health (NIH), Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Nancy L. Buc, Weil, Gotshal, and Manges
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
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
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

A committee roster is attached (Attachment I).
Non-Voting Agency Representative:

Kurt Gunter, Food and Drug Administration

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health Staff:

Bobbi Bennett, OD
Michael Blaese, NCI
Brian Brewer, NHLBI

Sandra Bridges, NIAID
Diane Bronzert, NCI
Chin-Shyan Chu, NHLBI
Sheila Compton, NHLBI
Kenneth Cowan, NCI
Cynthia Dunbar, NHLBI
Maryellen Franko, NCI
Jay Greenblatt, NCI
Barry Goldspiel, NCI
Christine Ireland, OD
Hiroyuki Ishii, NCI
Calvin Jackson, OD
Sachiko Kajigaya, NHLBI
Masako Kawase, NHLBI
Becky Lawson, OD
Rachel Levinson, OD
Julanna Lisziewicz, NCI
Kevin McDonald, NHLBI
Catherine McKeon, NIDDK
Richard Morgan, NCHGR
Koichi Moyamura, NHLBI
Carla Pettineli, NIAID
Joanne Riley, NCI
Nava Sarver, NIAID
Erasmus Schneider, NCI
Lisa Seachrist, NCI
Joyce O'Shaughnessy, NCI
Tom Shih, OD
Brian Sorrentino, NHLBI
Daisy Sun, NCI
Frances Taylor, NINDS
Rosemary Torres, OD
Debra Wilson, OD
Haiping Wu, NHLBI

Others:

W. French Anderson, University of Southern California
Bridget Binko, Cell Genesys, Inc.
G'dali Braverman, Act Up
Shannon Brownlee, U.S. News and World Report
Rachel Carle, Genzyme Corporation
Mike Casey, Genetic Therapy, Inc.
Peter Cassileth, University of Miami School of Medicine
Jan Chappell, Genetic Therapy, Inc.
Fred Chang, University of Michigan
Henry Chang, Shared Medical Research Foundation
Yawen Chiang, Genetic Therapy, Inc.
Edward Cohen, University of Illinois at Chicago
Wanda deVlaminck, Avigen, Inc.

Anne Driscoll, Cowen & Company
Michelle Durand, The French Embassy
James Economou, University of California, Los Angeles Medical Center
Susan Falen, Genetic Therapy, Inc.
Mitchell Finer, Cell Genesys, Inc.
Diane Fleming, MID-Atlantic Biosafety Association
Danielle Foullon, The Pink Sheet
Joyce Frey, Food and Drug Administration
Morgan Gale, National Narrowcast
Richard Giles, MD Anderson Cancer Center
Douglass Given, Progenitor, Inc.
Phillip Greenberg, University of Washington
David Holzman, BioWorld
Thomas Horiagon, Chimerix, Inc.
John Jaugstetter, Genentech, Inc.
Susan Jenks, Journal of the National Cancer Institute
Michael King, Alex Brown & Sons
Toshihiko Komori, Chugai Pharma U.S.A., Inc.
Steven Kradjian, Vical, Inc.
John Krauss, University of Michigan Medical Center
Larry Kun, St. Jude Children's Research Hospital
Donald Longenecker, Viagene, Inc.
Daniel Maneval, Canji, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Bruce Merchant, Viagene, Inc.
Noel Messenger, Applied Immune Sciences, Inc.
Robert Moen, Genetic Therapy, Inc.
Elaine Morikawa, TV Asahi
Arthur Nienhuis, St. Jude Children's Research Hospital
Jeffrey Ostrove, Microbiological Associates, Inc.
Robert Overell, Targeted Genetics Corporation
Seth Pauker, Parexel International
Liz Pennisi, Science News
Anne Petruska, FDC Reports
Stephen Pijar, University of Maryland, Baltimore
Doros Platika, Progenitor, Inc.
Eckhard Podack, University of Miami School of Medicine
Eric Poeschia, University of California, San Diego
Lisa Raines, Genzyme Corporation
Urbin Ramstebt, Virus Research Institute
Paul Recer, Associated Press
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
John Richards, University of Illinois at Chicago
Phil Richards, Public
Stanley Riddell, Fred Hutchinson Cancer Research Center
Bruce Schackman, Furman Selz, Inc.
Bill Schwieterman, Food and Drug Administration
Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International, Inc.

Marcus Stern, Copley Newspapers
Frank Sturtz, Progenitor, Inc.
Nevin Summers, Massachusetts Institute of Technology
Colleen Sundstrom, Howard University
Gary Temple, Life Technologies, Inc.
Larry Thompson, Medical News Network
George Thornton, The R.W. Johnson Pharmaceutical Research Institute
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Joseph Van Houten, The R.W. Johnson Pharmaceutical Research Institute
Christine Vanderpol, Rhone-Poulenc Rorer
Ashley Wivel, Public
Flossie Wong-Staal, University of California, San Diego
Mang Yu, University of California, San Diego

I-A. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting was published in the *Federal Register* on August 18, 1993 (58 FR 44098), 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.

I-B. UPDATE ON THE LETTER SENT TO THE NIH DIRECTOR REGARDING COMPENSATION FOR RESEARCH-RELATED INJURIES/DR. WALTERS

Dr. Walters summarized the events surrounding the RAC's recommendations to the NIH Director regarding the provision of medical care to subjects who may be injured in the course of their participation in clinical research. Dr. Walters explained that at the time of the June 7-8 RAC meeting, he had not received a response to his letter sent to Bernadine Healy, former NIH Director on January 6, 1993; therefore, the committee recommended that a follow-up letter should be sent to the NIH Director regarding this issue.

Dr. Walters described the correspondence that had been received since the June 7-8 meeting: (1) a letter from Dr. Bernadine Healy dated June 3, and (2) a letter from the Acting NIH Director, Dr. Ruth Kirschstein, dated August 6. Dr. Healy's letter stated the following:

"I understand the RAC's present interest in seeing greater policy consistency among research institutions in this area, but I do not believe we can achieve a more uniform approach without also addressing the central issues involved in an expanded Federal role. Consequently, before another panel is formed to study this issue anew, I believe it is necessary to establish that the need in this area is greater today than 10 years ago. Have the frequency and incidence of non-negligent injuries increased? Is the number of research subjects who are not being treated or compensated on the rise? Are research subjects more concerned about and reluctant to participate in research today?

"Further review of the compensation question will be included in the responsibilities of the NIH Science Policy Studies Center. Recently established to enhance NIH's capacity to identify and analyze the social, legal, ethical, and economic implications of biomedical and behavioral research, the Center is planning a

priority setting conference in the fall to establish criteria for addressing pending and emerging issues. This broad assessment of current issues may give the NIH a better understanding of the compensation issue's relative pervasiveness and importance. By the end of the year, we may also have a clearer picture of the national health care reform effort and whether it might result in health insurance or other changes that will be relevant to this issue. As we continue to gather more information, the NIH would welcome any additional information or insights the RAC may have in this matter."

Dr. Kirschstein's letter addressed the concerns of the RAC in the following statement:

"Given NIH's peripheral role in the reform effort, I am transmitting the RAC's recommendation to the Assistant Secretary for Health with a request that the specific issue of covering research injuries be brought to the attention of the National Task Force on Health Care Reform."

I-C. EXECUTIVE SECRETARY REPORT ON POSSIBLE ADVERSE EFFECTS/ DR. WIVEL

Dr. Wivel noted that a written report of possible adverse effect was submitted by Dr. Edward Oldfield, NIH, Bethesda, Maryland, in a patient enrolled in his human gene transfer protocol entitled: "Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir." Dr. Wivel summarized a verbal report by Dr. Crystal to the NIH Institutional Biosafety Committee (IBC) regarding a possible adverse effect in a patient enrolled in his human gene transfer protocol entitled: *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung*.

I-D. RECOGNITION FOR SERVICE ON THE RAC/DR. WALTERS

Dr. Walters recognized several members of the RAC whose term has expired. He expressed the gratitude of the committee for their years of service, dedication, and careful review of recombinant DNA research. The outgoing members of the RAC are: Drs. Carmen, Geiduschek, Hirano, Krogstad, and Post. Outgoing RAC members will continue to serve on the committee until future appointments have been finalized by the Department of Health and Human Services.

II. MINUTES OF THE JUNE 7-8, 1993, RAC MEETING

Dr. Walters called on Dr. DeLeon to review the minutes of the June 7-8, 1993, RAC meeting. Dr. DeLeon stated that the minutes were an accurate reflection of the June meeting. Minor corrections were submitted by Drs. DeLeon, Smith, and Parkman.

Committee Motion

The RAC approved a motion made by Dr. DeLeon and seconded by Dr. Parkman to accept the June 7-8, RAC minutes with the inclusion of minor grammatical changes by a vote of 18 in favor, 0 opposed, and no abstentions.

III-A. CHAIR REPORT ON RAC-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters summarized the human gene transfer protocols that have been approved by the RAC to date. Of the 52 protocols approved by the RAC, 46 of these studies (24 gene therapy and 22 gene marking)

have been approved by the NIH Director. Listed below is a breakdown of the NIH- and RAC-approved protocols (Attachment II):

* 18/Cancer
* 2/Human immunodeficiency virus (HIV)
* 9/Genetic diseases (5/cystic fibrosis, 1/adenosine deaminase deficiency (ADA), 1/familiar hypercholesterolemia, and 2/Gaucher disease)
* 21/Others

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

Dr. Walters stated that a total of 18 minor modifications have been approved to date. He summarized three modifications that were approved since the June 7-8, RAC meeting: (1) Dr. James Wilson has been granted permission to transfer his 2 protocols from the University of Michigan to the University of Pennsylvania, and (2) Dr. Scott Freeman has been granted permission to transfer his protocol from the University of Rochester to Tulane University School of Medicine (Attachment III).

IV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *PHASE I STUDY OF TRANSFECTED CANCER CELLS EXPRESSING THE INTERLEUKIN-2 GENE PRODUCT IN LIMITED STAGE SMALL-CELL LUNG CANCER* **/DRS. CASSILETH, PODACK, SRIDHAR, AND SAVARAJ**

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol resubmitted by Drs. Peter A. Cassileth, Eckhard Podack, and Kasi Sridhar of the University of Miami, and Dr. Niramol Savaraj of the Miami Veterans Administration Hospital, Miami, Florida. This protocol was deferred at the March 1993, RAC meeting until the investigators returned to the full RAC with additional data and a revised protocol. The intent of this protocol is to culture small-cell lung carcinoma (SCLC) cells from patients and infect them with a bovine papilloma virus (BPV) vector expressing the gene for interleukin-2 (IL-2). Following lethal irradiation, IL-2 producing tumor cells will be reimplanted into patients to stimulate proliferation of tumor-specific cytotoxic T-lymphocytes (CTL) capable of destroying the reimplanted and established tumors. One primary concern of Dr. Miller was whether irradiation will prevent growth of the modified tumor cells after injection into patients. This issue is critical because the addition of BPV transforming genes into tumor cells could possibly increase the tumorigenicity of these cells. Data demonstrate that no detectable cells (<1 in 7 x 106 cells) survive following lethal irradiation. In his primary review, Dr. Miller questioned whether it is adequate to irradiate tumor cells at 6,000 rad for the human trial while in preclinical studies, data were obtained using 12,000 rad irradiation. The investigators responded to Dr. Miller's concerns stating that 12,000 rad will be used for the human study. Dr. Miller expressed satisfaction with the PI's response.

Review--Dr. Haselkorn

Dr. Haselkorn stated that the investigators have provided data demonstrating that the irradiation schedule for the transfected cells is sufficient to inhibit proliferation of clonogenic cells while retaining their ability to express IL-2. Dr. Haselkorn said that he had solicited the expert opinion of Dr. Lou Laimins of the University of Chicago, regarding the BPV vector. Dr. Laimins questioned the investigators' assumption that the BPV vector is maintained as an episome at high copy number in transfected human tumor cells

and that high level IL-2 production is facilitated. Dr. Laimins notes that while BPV plasmids transfected into mouse cells can be maintained as episomes, BPV usually integrates into human chromosomes. Therefore, the BPV vector demonstrates no advantage over retrovirus vectors. Dr. Haselkorn questioned whether the BPV vector actually expresses higher levels of IL-2 than retrovirus vectors.

Review--Dr. Secundy

Dr. Secundy stated that her comments were primarily confined to the Informed Consent document. Most o her initial concerns about the discrepancy between the gene therapy description in the protocol versus the Informed Consent document have been corrected. One remaining concern is the statements regarding third party payer reimbursement for research related injuries in Section 4.7 of the protocol and the Informed Consent document. The responsibilities for the financial burden must be clarified.

Other Comments

Dr. Walters summarized written comments submitted by Dr. Smith concerning: (1) a discrepancy between the dose of radiation used for the transfected cells versus the dose proposed for the clinical study, and (2) a discrepancy in the data regarding the success rate of growing SCLC cells *in vitro*. One statement refers to a 70% success rate versus a 30% success rate stated elsewhere in the protocol.

Dr. Geiduschek expressed his concern about the episomal status of the BPV vector within transfected cells and the levels of IL-2 production. Dr. Miller stated that the issue of whether the vector is episomal or integrated is irrelevant since the investigators have demonstrated high level IL-2 production in transfected cells. Dr. Post suggested that the RAC should stipulate a minimum level of IL-2 production for cells to be administered to the patients.

Investigator Response--Drs. Podack and Cassileth

Responding to the question of IL-2 production, Dr. Podack stated that in one human cell line studied, the level of production was between the range of 6,000 and 9,600 units of IL-2/ml/106 cells/24 hours; and in another cell line, the level of expression was between 4,000 and 5,000 units of IL-2/ml/106 cells/24 hours. These levels are at least one order of magnitude higher than the same cell lines transfected with retrovirus vectors. After irradiation, the transfected cells continued to produce the same level of IL-2 for 2 days; however, IL-2 expression declined over the next 6 days. Regarding the episomal status of the BPV vector, he explained that the vector is maintained episomally at approximately 16 copies per cell in human HeLa cells. Data derived from murine experiments suggest that there is a strong correlation between copy number and the levels of IL-2 expression.

Dr. Podack stated that Dr. Savaraj, a co-investigator on this protocol, has established cell lines from fresh tumor specimens at approximately a 70% success rate. Dr. Podack explained that most of the preclinical data was obtained using a cell line that has been grown for a relatively short term *in vitro*. This cell line was established in approximately 2 months, and aliquots of these cells were cryopreserved. All experiments were performed using cells grown from the cryopreserved stock. Dr. Podack stated that more comprehensive data would be obtained from a broader range of cell lines established from fresh tumors.

Dr. Cassileth responded to questions relating to the clinical aspects of the protocol. The financial responsibility for costs associated with the treatment of adverse effects arising from the research study wil be the responsibility of the patient, third party payers, or both. He explained that his local Institutional Review Board (IRB) requires this statement in the Informed Consent document. He said he is of the opinion that compensation for research related injury is an issue that should be addressed in a broader sense.

In regard to their success rate for establishing fresh tumor cell lines, Dr. Cassileth stated that they have demonstrated a success rate of between 70 and 80%. Overall, approximately 30% of the patients initially entered onto the protocol will be eligible to receive the transfected cells due to the exclusion of some patients based on entrance criteria.

Mr. Capron remarked that the terms "therapy" and "treatment" in the Informed Consent document are inappropriate for a Phase I study. Dr. Cassileth agreed to revise this inappropriate language.

Drs. Parkman and Post requested that a minimum level of IL-2 production should be demonstrated prior to reimplantation of the transfected cells into patients. Dr. Miller suggested that 10 units of IL-2/ml/106 cells/24 hours is an acceptable minimal level. Dr. Podack stated that 50 units of IL-2/ml/106 cells/24 hours is an acceptable minimal level of IL-2 expression.

In regard to Dr. Haselkorn's concern about whether BPV is maintained episomally in human cells, Dr. Podack stated that data derived from human cell lines, in addition toHeLa, indicate episomal presence based on high level IL-2 production. The level of IL-2 expression is a more pertinent parameter for the present study. Dr. Miller agreed with Dr. Podack's statement. Dr. Miller said that the risk ofepisomal DNA inadvertently infecting other cells, even if the cells carrying the DNA have been lethally irradiated, is quite remote.

Committee Motion

A motion was made by Dr. Miller and seconded by Mr. Capron to approve the protocol contingent upon review and approval of data demonstrating that cells obtained from patients can be successfully transduced with the proposed vector and that thesetransduced cells secrete 50 units of IL-2/ml/106 cells/24 hours. The motion passed by a vote of 18 in favor, 0 opposed, and no abstentions.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *RETROVIRAL MEDIATED TRANSFER OF THE HUMAN MULTI-DRUG RESISTANCE GENE (MDR-1) INTO HEMATOPOIETIC STEM CELLS DURING AUTOLOGOUS TRANSPLANTATION AFTER INTENSIVE CHEMOTHERAPY FOR BREAST CANCER* **/DR. O'SHAUGHNESSY**

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol resubmitted by Dr. Joyce A. O'Shaughnessy, NIH, Bethesda, Maryland. This protocol was deferred at the December 1992 RAC meeting until the investigators could return to the full RAC with the following: (1) data demonstrating that human CD34(+) cells have been transduced *in vitro* with the vector that will be used in the clinical protocol; (2) a description of the assays that will be used to measure gene expression, and demonstrate how this expression will be monitored in bone marrow and tumor cells; and (3) a description of the endpoint for determining efficacy (evaluation criteria), i.e., comparison of gene amplification and the rate of white blood cell recovery following Taxol treatments one and three.

Dr. Parkman stated that the transplantation procedure is part of a freestanding clinical protocol that deals with autologous transplantation for patients with responsive Stage IV breast cancer following chemotherapy with ifosfamide, carboplatin, and etoposide. Patients will be transplanted with a combination of bone marrow and peripheral blood stem cells and will receive subsequent Taxol administration. The gene transfer portion of the proposed study is the only aspect of the protocol that

requires RAC review.

CD34(+) stem cells will be isolated from bone marrow and peripheral blood. If adequate numbers of these cells are obtained, a portion will be transduced *in vitro* with a retrovirus vector expressing the MDR-1 gene. Both transduced and untransduced CD34(+) cells will be reinfused back into the patients. Patients demonstrating subsequent progressive disease will receive Taxol administration on a dose-escalation schedule. Although the protocol has adequate preclinical data, a major problem raised in the last review was that most preclinical data were obtained employing a vector based on the Harvey murine sarcoma virus, which was different from the vector proposed for the clinical trial. In this resubmission, the PI has addressed all the significant questions raised in the last review including: (1) construction and evaluation of the Moloney murine leukemia virus based vector, G1MD, (2) demonstration of the ability of this vector to transduce the target human CD34(+) cells, (3) demonstration of the transduced MDR-1 gene in primary hematopoietic cells, (4) demonstration of expertise in the polymerase chain reaction (PCR) assay of MDR-1 gene expression, and (5) clinical evidence for the ability to transduce CD34(+) cells with a vector containing a neomycin resistance (neoR) gene. The investigators have deleted one of their previous objectives, namely, demonstrating the therapeutic benefit of MDR-1 transduction and have focused instead on demonstration of persistence and proliferation of MDR-1 transduced cells following Taxol administration. Dr. Parkman recommended approval of the revised protocol.

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

In her written comments, Dr.Brinckerhoff explained that this revised protocol is improved over the original submission and directly addresses most of the RAC's previous concerns. She noted two reservations: (1) Since breast cancer patients demonstrate a high incidence of bone marrow metastasis, what percentage of patients will be ineligible to participate in this study? Dr. Parkman explained that breast cancer cells do not express the CD34 marker; therefore, isolation of CD34(+) bone marrow cells employing a CD34 monoclonal antibody column will eliminate most contaminating metastatic breast cancer cells. Dr. Parkman noted that this issue has been raised during the review of previously approved protocols. (2) There is a lack of preclinical data demonstrating long-term expression of the transduced MDR-1 gene in an animal model.

Review--Mr. Capron

Mr. Capron stated that the investigators have adequately responded to the suggested changes in the Informed Consent document. Except for a few typographical errors, the revised Informed Consent is acceptable. Mr. Capron recommended approval of the protocol.

Other Comments

Dr. Miller mentioned that some preclinical data were not included in his review materials and asked the investigators to present their data. Dr. Post asked the investigators to explain in detail the problem of MDR-1 mRNA splicing and the two different gene products coded for by these spliced MRNA species. Dr. Post noted that the stem cell factor used for most of the preclinical studies will not be used in the clinical protocol. Will omission of this factor affect the experimental outcome?

In regard to the issue of compensation for research-related injury, Dr. Walters noted that a statement is included in the Informed Consent document explaining that the NIH Clinical Center will provide short-term medical care, but not long-term care, for physical injuries resulting from a patient's participation in research. This statement is a modification over the usual statement that no care or compensation will be provided.

Investigator Response--Drs. Neinhuis and Sorrentino

In response to Dr. Post's comments about the use of stem cell growth factor, Dr. Neinhuis stated that stem cell growth factor will be used in the human clinical trial due to the fact that it has recently become available. Responding to the question of MDR-1 MRNA splicing, Dr. Neinhuis said that the protein encoded by the spliced MRNA is a small truncated variant gene product and is presumably nonfunctional. Approximately 50% of the MDR-1 gene transcript from the DNA construct based on either the Harvey or Moloney vector is spliced. Both full length and spliced RNA species are co-packaged as virions and are transferred to target cells. mRNA expression of the normal genomic MDR-1 gene, however, is not similarly spliced.

Dr. Sorrentino summarized preclinical studies with the murine model. While earlier studies were performed using a Harvey-based vector, recent results were obtained with the new Moloney-based construct, G1MD. A high titer amphotropic producer clone of the G1MD vector has been isolated from the PA317 packaging cell line. Transfer of the MDR-1 gene to the target cells was assayed by the rhodamine efflux assay. Expression of the transduced MDR-1 gene yielded a "dull" phenotype as compared to a "bright" phenotype for untransduced cells as observed under a fluorescence microscope. These two types of cells were fractionated by a fluorescence activated cell sorter (FACS). MDR-1 gene expression by the G1MD construct in mouse hematopoietic cells was demonstrated. In the *in vivo* murine model, Taxol conferred drug resistance to hematopoietic cells. Data also demonstrated MDR-1 gene expression after transduction with the clinical grade G1MD vector in human CD34(+) cells. MDR-1 proviral DNA was also demonstrated. Quantitative assays for the transduced MDR-1 gene were performed in primates.

Dr. Sorrentino said that similar mRNA splicing of the MDR-1 gene probably occurred in constructs used in other previously approved protocols. Mutations made to eliminate the splice donor and acceptor sites resulted in a construct that expressed the encoded MDR-1 gene, but the vector RNA was unable to be packaged in the producer cell. Presumably all packaging cells produced viral particles containing both RNA species.

Responding to Dr. Miller's question about the replication competent retrovirus (RCR) testing of the clinical grade vector, Dr. Neinhuis said that Genetic Therapy, Inc., has performed extensive testing to confirm the absence of RCR.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Miller to approve the protocol. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

VI. MAJOR AMENDMENT TO APPENDIX D-XXVII OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED:** *PHASE I STUDY TO EVALUATE THE SAFETY OF CELLULAR ADOPTIVE IMMUNOTHERAPY USING GENETICALLY MODIFIED CD8(+) HIV-SPECIFIC T CELLS IN HIV SEROPOSITIVE INDIVIDUALS***/DRS. GREENBERG AND RIDDELL**

Review--Dr. Straus

Dr. Walters noted that Dr. Miller will abstain from discussion and voting on this protocol due to a conflict of interest (employed by the same institution). Dr. Walters called on Dr. Straus to present his primary review of the request for a major amendment to the human gene transfer protocol submitted by Drs. Phillip Greenberg of the University of Washington and Stanley R. Riddell of the Fred Hutchinson Cancer

Research Center, Seattle, Washington. This protocol represents a modification of a previously approved Phase I study to evaluate the safety of adoptive immunotherapy of autologous genetically-modified CD8(+) HIV-specific T cell clones in HIV infected individuals with lymphoma following bone marrow transplantation (BMT). Apparently, the accrual from that study has been slower than expected; but the preliminary data suggest that competent cells can be detected in the recipients. The present modification will allow the investigators to accrue HIV-seropositive individuals who lack opportunistic infections, have CD4 cell counts between 200 and 500, do not have lymphoma, and who are not undergoing BMT.

As in the previously approved protocol, the patient's T cells will be modified by a retrovirus containing a hybrid insert of the hygromycin resistance (HyR) gene for marking as well as the herpes simplex virus thymidine kinase (HSV-tk) gene. The HSV-tk suicide gene will provide a safety feature that allows the transduced cells to be eliminated with the antiviral drug, ganciclovir, if adverse effects are encountered. This latter concept will be tested in a group of 3 patients enrolled in this study. Dr. Straus stated that this protocol is well developed with the vector and the strategy thoroughly described. He recommended approval of this modification.

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

Dr. Leventhal's written comments raised several questions that have been adequately addressed by the investigators. The investigators accepted her suggestion that ganciclovir should be administered in the hospital for the first two days in the event that the treatment exhibits an unanticipated toxic effect. She asked about the necessity for lymph node biopsies prior to treatment. The procedures described for assessing neurologic and pulmonary toxicity are inadequate and more extensive evaluations should be performed. Any adverse effects, if encountered, should be reported to the RAC as well as the IRB. She questioned the future plans for evaluation of this technique. How will the investigators proceed if they find that the treatment is neither toxic nor effective? She noted that her comments were minor, and she recommended approval of this major amendment.

Review--Dr. Carmen
Dr. Carmen commented that the Informed Consent document is lucid and well written. He suggested a minor revision that would more succinctly inform the patients about this study. The investigators agreed to incorporate Dr. Carmen's suggestion.

Other Comments

Dr. Walters summarized Ms. Meyers' written comments about the Informed Consent document: (1) Will a request for autopsy be included? and (2) Will the patient's medical records be made available to other physicians, NIH, and others? Mr. Capron asked the investigators why they did not apply for a certificate of confidentiality under Section 301(d) of the Public Health Service Act as suggested by their IRB. Dr. Post asked the investigators to summarize the status of patients who were enrolled in the previous study.

Mr. G'dali Braverman from ACT UP expressed his disappointment that patients with CD4 counts below 200 will be excluded from participation in this study, but he was pleased that patients receiving antiviral treatment will not be excluded.

Investigator Response--Dr. Greenberg

Dr. Greenberg explained that the existing protocol has very stringent entrance criteria, i.e., only patients with HIV-related lymphoma receiving BMT are eligible. A total of 8 patients have been evaluated to date, and only 4 have had human leukocyte antigen (HLA) matches for BMT. Of the 4 eligible patients, only one

was treated. The other 3 patients had rapidly progressive lymphoma relapse which prevented their entrance into this study. This problem of low patient accrual is the reason for the present amendment. Preliminary data on the one treated patient indicates that CTL activity to HIV can be reconstituted. Similar activity directed toward cytomegalovirus (CMV) infection has been demonstrated previously in a much larger study. Unfortunately, the single HIV patient who received this treatment, died of severe graft-versus-host (GVH) disease as a consequence of the transplantation procedure. No long-term efficacy data are available.

Dr. Greenberg explained that the rationale behind selecting HIV seropositive individuals, who have CD4 counts between 200 to 500, is that this group of patients elicit a better CD8 response and demonstrate fewer complications associated with disease progression. Therefore, a more definitive evaluation of safety and toxicity is possible. In regard to the issue of patient confidentiality, he cannot guarantee that every patient will be codified; however, he is applying for a certificate of confidentiality as noted by Mr. Capron.

Dr. Chase inquired about the rationale for selecting the sample size of 15. Dr. Riddell said that this number was chosen based on other similar Phase I toxicity studies. The primary objective of this study is to determine a dose range that is safe and biologically relevant. Dr. Parkman inquired about the number of cells that were used for the previous CMV study. Dr. Riddell answered that the highest dose of cells administered was 1 x 109 cells/m2, and this dose was well tolerated. With regard to the future course of this study, Dr. Riddell explained that if no toxicity or positive antiviral effects are observed, the study will progress to a Phase II efficacy trial that will involve a statistically meaningful cohort of patients.

Mr. Capron suggested the addition of a sentence to the Informed Consent document that explains the use of the suicide gene more clearly. The investigators appreciated Mr. Capron's comments and agreed to incorporate the suggested change.

Committee Motion

A motion was made by Dr. Straus and seconded by Mr. Capron to approve this major amendment. The motion passed by a vote of 16 in favor, 0 opposed and 2 abstentions (Drs. Miller and Motulsky abstained from voting because they are employed by the University of Washington).

VII-A. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *GENE THERAPY FOR RECURRENT PEDIATRIC BRAIN TUMORS***/DRS. KUN, SANFORD, BRENNER, HEIDEMAN AND OLDFIELD**

Review--Dr. Geiduschek

Dr. Walters called on Dr. Geiduschek to present his primary review of the protocol submitted by Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the NIH, Bethesda, Maryland. This gene therapy protocol involves HSV-tk gene transduction of brain tumor cells followed by administration of the antiviral drug ganciclovir. This Phase I trial will involve 6 children > 3 years of age with progressive or recurrent malignant supratentorial brain tumors that are resistant to standard therapy. Patients will receive multiple intratumoral injections of between 108 and 109 murine cells (PA317) in a volume of 1 to 20 milliliters. Patients will be injected with the PA317 vector producing cells (VPC) containing the G1TKSvNa retrovirus vector. Seven days post injection, patients will receive ganciclovir administration for the ablation of transduced tumor cells. Anti-tumor responses will be monitored for more than one year by magnetic resonance imaging (MRI). This Phase I toxicity study is similar to other protocols that have

previously been approved by the RAC except for a variation in the surgical procedures that are proposed to deliver the VPC. Dr. Geiduschek noted his concern about the repetitiveness of such studies. Is the public and the progress of gene therapy at this stage best served when a substantial fraction of the still small group of investigators focus quasi-repetitively on a single idea?

Review--Dr. DeLeon

Dr. DeLeon stated that since the proposed vector, protocol, and target cells have been previously reviewed and approved by the RAC for other protocols, there are no major new issues for the RAC to discuss. Dr. DeLeon emphasized that the RAC should consider quality control standards and adopt guidelines for conducting similar types of trials at multiple sites.

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

Ms. Meyers' written comments raised several questions about the Informed Consent documents. Although they are generally well written, they should include information about the additional medical costs associated with participation in this study, long-term follow-up, other available experimental therapies, and a request for autopsy.

Other Comments

In his written comments, Dr. Smith raised the question of possible adverse effects in relation to the "asymptomatic gliosis" detected by MRI on one of the patients enrolled in Dr. Oldfield's protocol. This observation should be discussed in connection with the present protocol.

Mr. Capron commented that the use of the term "you/your child" in the parent copy of the Informed Consent document is confusing. The way the document reads, it is unclear whether the parent or the child is undergoing treatment. Such documents would be more appropriately written separately in language that is understandable to each person signing the document, i.e., the parents, children 7-14 years of age, and children 15 years of age.

Dr. Chase remarked that the investigators' response to comments by Ms. Meyers and Dr. Smith were inadequate. Dr. Parkman asked the investigators to elaborate on the inclusion criteria, i.e., the size of tumors and treatment of single versus multiple lesions.

Investigator Response--Dr. Kun

Responding to Dr. Geiduschek's question regarding the similarity of this proposal to other RAC-approved protocols, Dr. Kun explained that a given therapy may have different responses and toxicities depending upon the age of the patient. The present protocol targets childhood instead of adult tumors. This proposal differs from the previously approved pediatric brain tumor protocol submitted by Dr. Culver, et. al., in that the VPC will be stereotactically injected into the tumor mass rather than into anintracystic tumor cavity. Anatomical differences of the injection sites may produce different responses.

In response to several Informed Consent document issues, Dr. Kun said that patients 18 years of age can legally give consent to participate in a protocol. "You/your child" would mean "you/yourself" for patients over 18, and for patients below this age, it would mean "your child". The simpler assent form is to be signed by children under 18 with their parents. Mr. Capron and Ms.Buc questioned this practice. Dr. Secundy stated that she is not satisfied with the consent process for minors. Dr. Miller suggested that the forms should be separated for patients under or over 18 years of age. Dr. Kun agreed in principle to

change the Informed Consent document stipulated by the RAC; however, such a change would be subject to local IRB approval. This issue of complexity and conformity of Informed Consents evolved into a lengthy discussion to be summarized below (see VII-B: RAC Recommendation Regarding the Establishment of a Working Group to Develop Guidelines for Informed Consent Document).

Responding to Dr. Chase's comment, Dr. Kun noted that all the pertinent changes have been made in the protocol and consent documents. Regarding Dr. Parkman's question about inclusion criteria, Dr. Kun clarified that patients with nonresectable single foci > 1.5 cm and < 5 cm will be included in the study. As to the question of quality control in this multicenter trial, Dr. Kun said that the same standards will apply as for Dr. Oldfield's protocol at NIH. Dr. Kun agreed to include a request for autopsy into the revised Informed Consent document.

Dr. Kun responded to Dr. Smith's inquiry about the changes observed on an MRI obtained on a patient enrolled on Dr. Oldfield's study. Dr. Miller stated that these preliminary results have been overinterpreted in the media, raising false expectations for prospective patients. Dr. Krogstad said the RAC may have to deal with the issue of how preliminary results of gene therapy trials are reported by the media. Dr. Chase shared the same sentiment and urged that the Informed Consent document should properly convey the experimental nature of this kind of therapy to the patients. Dr. Kun said that the present protocol is a very limited Phase I toxicity trial; it is not designed to assess efficacy, which would require a statistically meaningful cohort of samples. The scientific results will be evaluated in concert with the NIH investigators and will avoid conflict of interest with the sponsoring company. Dr. McGarrity of Genetic Therapy, Inc., agreed that independent evaluation of the scientific results is essential.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Motulsky to approve the protocol. The motion passed by a vote of 17 in favor, 0 opposed and no abstentions. A friendly amendment was proposed by Dr. Miller and accepted by Drs. Post and Motulsky. This amendment stipulates that the RAC strongly recommends that the Informed Consent document should be divided into separate documents: (1) a guardian assent form for patients < 18 years of age, (2) a patient assent form for patients < 18 years of age, and (3) a patient consent form for patients 18 years of age.

VII-B. RAC RECOMMENDATION REGARDING THE ESTABLISHMENT OF A WORKING GROUP TO DEVELOP GUIDELINES FOR INFORMED CONSENT DOCUMENTS

Discussion

During the review of Dr. Kun's brain tumor protocol, several questions regarding Informed Consent documents and the informed consent process were raised.
Dr. W. French Anderson, University of Southern California, Los Angeles, California, stated that the RAC possesses unique expertise and is qualified to propose policy on scientific and biotechnology issues, as well as informed consent issues relating to human gene transfer. Dr. Anderson suggested that the RAC formulate informed consent recommendations to be communicated to the local IRBs through NIH's Office for Protection from Research Risks (OPRR). Dr. Parkman agreed that such a process would be a useful mechanism. Ms. Buc commented that it would be impractical to develop a universal Informed Consent document that would be applicable to all protocols. A set of common guidelines would be more feasible. Dr. Motulsky suggested that some social science studies should be conducted to obtain practical information on the effectiveness of the informed consent process for complicated genetic diseases.

Dr. Zallen commented on the importance of the Informed Consent document. She agreed that the RAC is

in a position to provide useful suggestions based on its extensive expertise in the review of such documents.

Committee Motion

A motion was made by Dr. Krogstad and seconded by Dr. Zallen that the RAC establish a working group to frame pertinent questions relevant to Informed Consent documents and that the Director of OPRR, NIH, should be invited to the December 1993 RAC meeting to address these issues. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

VIII. AMENDMENT TO THE *GUIDELINES FOR THE SUBMISSION OF HUMAN GENE TRANSFER/THERAPY PROTOCOLS FOR REVIEW BY THE RAC* **OF THE** *POINTS TO CONSIDER/NIH GUIDELINES***/DR. WIVEL**

Dr. Wivel presented amendments to the *Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC* (*Federal Register*, February 18, 1993, page 9104). These amendments will establish consistency in the submission of human gene transfer protocols for RAC review and require PIs to focus their oral responses to the RAC's questions and comments. The Title and Section I will read:

"Guidelines for the Submission of Human Gene Transfer Protocols for Review by the Recombinant DNA Advisory Committee.

"I. Investigator Submitted Material:

"Written proposals must be submitted in the following order: (1) scientific abstract--1 page; (2) non-technical abstract--1 page; (3) IBC and IRB approvals; (4) Points to Consider--5 pages; (6) protocol--20 pages excluding appendices; (7) Informed Consent document--approved by the IRB; (8) appendices including tables, figures, and manuscripts; and (9) CVs--2 pages in Biosketch format. When a proposal has been submitted previously, there should be a short section (200 words) immediately following the abstracts that summarizes the major revisions since the last review. Data provided....

"...written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to ORDA 2 weeks before the RAC meeting.

"Oral Responses to the RAC. Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted 2 weeks prior to the RAC meeting is prohibited."

Committee Motion

A motion was made by Dr. Krogstad and seconded by Dr. Parkman to accept the amendments to the *Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC*. The motion passed by a vote of 16 in favor, 1 opposed, and no abstentions.

IX. WORKING GROUP REPORT ON CATEGORIES OF HUMAN GENE TRANSFER EXPERIMENTS THAT ARE EXEMPT FROM RAC REVIEW/DR. PARKMAN

Presentation--Dr. Parkman

Dr. Parkman said that the RAC is currently realizing a proliferation in the number of gene transfer protocols submitted for review due to the evolution and maturation of the field of gene therapy. The RAC formed a working group to formulate categories of gene transfer protocols that may not require full RAC review or are considered exempt from RAC purview. Dr. Parkman reported on a tentative consensus that resulted from a working group telephone conference call that was held on July 8, 1993. The working group proposed two categories of protocols that may qualify for the accelerated review process: (1) multiple site protocols under the umbrella of the original RAC-approved protocol. Under such protocols, the "original" PI will have full responsibility for quality control and data reporting at satellite sites, and (2) protocols that are similar to other RAC-approved protocols; however, minor modifications have been introduced that do not involve gene transfer aspects of the studies. He stated that a check list is being developed for use in screening protocols that may qualify for the accelerated review process.

One major issue that remained unresolved by the working group is the uncertainty about how to proceed with the issue of Informed Consent documents that are different from those that were previously approved by the RAC. Dr. Parkman noted that Dr. Bruce Merchant of Viagene, San Diego, California, submitted a letter dated August 12, 1993, requesting that the RAC consider certain categories of vaccine protocols as "exempt" from the *NIH Guidelines* and, therefore, exempt from RAC review.

Discussion

Dr. Zallen said that it is not practical to have a standardized Informed Consent document for all different institutions and protocols. Instead, she suggested 6 essential elements that should be considered when developing the Informed Consent documents: (1) recommendations about the use of birth control, (2) financial costs to patients, (3) the necessity for long-term follow up, (4) a request for autopsy, (5) timing of press releases relating to research, and (6) how patient confidentiality will be maintained.

In her written comments, Ms. Meyers stated that it is premature for any gene transfer protocols to proceed without prior RAC review. The number of patients treated to date is relatively small, and the issues of long-term safety and clinical benefit of gene transfer are largely unresolved.

Dr. Post agreed with the working group's recommendations and suggested an additional three categories of protocols that could qualify for exemption from RAC review: (1) vaccine protocols, including those using vaccinia viruses, adenoviruses and retroviruses, (2) those in which transduced cells (using a RAC-approved vector) are lethally irradiated prior to administration to human subjects, and (3) gene marking protocols with RAC-approved vectors.

Dr. Straus noted that the *NIH Guidelines* definition of "vaccines" as described in footnote 21 is unclear. Footnote 21 states that:

"Section III-A-4 covers only those experiments in which the intent is to modify stably the genome of cells of a human subject. Other experiments involving recombinant DNA in human subjects such as feeding of bacteria containing recombinant DNA or the administration of vaccines containing recombinant DNA are not covered in Section III-A-4 of the Guidelines."

Dr. Straus explained that although vaccinia viruses do not persist in the host, other viruses such as herpes viruses, adenoviruses, papovaviruses, and retroviruses persist either through integration into the host genome, or stably within host cells. He suggested that a distinction should be made as to the categories of vaccines that are considered exempt from the *NIH Guidelines* and RAC review.

Ms. Buc asked the RAC if it might consider relinquishing its purview over human gene transfer protocols

entirely. Mr. Capron explained that the RAC should not relinquish its responsibilities since there are many ethical questions for the RAC to consider, i.e., germ-line intervention. Dr. Chase added that the RAC serves to inform the public about broad issues involving recombinant DNA research in an unbiased and scientifically informed manner. Dr. Miller noted that the Food and Drug Administration's (FDA's) review of protocols is not held in a public format; and at this stage of gene transfer research, public review by the RAC is very important.

Dr. Parkman said that the RAC review of individual protocols is essential to the development of science policy concerning gene therapy. Dr. Walters commented that the first cystic fibrosis (CF) protocol reviewed by the RAC in December 1992 is an example that illustrates the importance of public deliberation by the RAC. Not only did the RAC consider a new disease for the first time, but also a new vector (adenovirus). The public discussion of the CF protocols was very productive and useful in establishing policy for the review of other protocols.

There was a lengthy discussion about the interpretation of the Footnote 21 of the*NIH Guidelines* in regard to the types of vaccine protocols that are considered exempt. Dr. Merchant stated that although submission of protocols for RAC review is on a voluntary basis for commercial industry not associated with NIH-funded investigators, it is not realistic to conduct serious commercial development efforts without engaging NIH-funded investigators or institutions. For commercial research development, the time required to complete the review process is critical in order to bring the product to market. An exempt or accelerated review process would facilitate the process.

Dr. Walters requested that Drs. Parkman, Post, Carmen, and Straus form a Working Group on Categories for Accelerated Review to report back later on in the meeting. The RAC tabled their discussion until the next day to allow the working group to develop a revised list of possible categories of accelerated review protocols.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *PILOT STUDY OF TOXICITY OF IMMUNIZATION OF PATIENTS WITH UNRESECTABLE MELANOMA WITH INTERLEUKIN-2 SECRETING ALLOGENEIC HUMAN MELANOMA CELLS*I
DRS. DAS GUPTA AND COHEN

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol resubmitted by Drs.Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine. This protocol was deferred at the June 1993 RAC meeting until the investigators could return to the full 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* effect of irradiated transduced cells (either 5,000 or 10,000 rads) in the murine model; (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.

Dr. Smith stated that this study is a Phase I trial using a well characterized human melanoma cell line that is transduced with the human IL-2 gene as an immunogenic vaccine. Twelve patients with advanced stage melanoma will be accrued on the study. The IL-2 secreting melanoma cells will be administered to patients who differ in at least 3 out of 6 alleles from the melanoma cell line at the Class 1 major histocompatibility locus. The transduced cells will be lethally irradiated with 5,000 rads. The major endpoint of the study will be toxicity. Other minor endpoints include measurement of induction of B and T cell responses to tumor cells and any potential clinical anti-tumor effect. The investigators have

addressed most of the previous concerns raised by the RAC. Additional data on the irradiation of the tumor cells and production of IL-2 have been provided. Further information has been provided regarding the efficacy of the therapy in themurine model. The Informed Consent document has been revised and addresses the issue of financial responsibility for research expenses and routine care. Results for detection of RCR have been provided. Dr. Smith recommended approval of the present protocol.

Review--Ms. Buc

Ms. Buc commented primarily on the Informed Consent document. She suggested several changes to avoid misleading patients that any efficacy will be expected from this Phase I toxicity study. She expressed uneasiness about a statement that participation in the study may be halted at the sole discretion of the PI should contraception be interrupted.

Other Comments

Ms. Meyers raised several questions in her written comments about financial responsibility and patient privacy that have been addressed by the investigators. Dr.Haselkorn's written comments requested identification of the investigator who will be responsible for conducting the clinical study since Dr. Richards, a co-investigator on the protocol, is no longer employed by the University of Chicago.

Dr. Miller asked about the adequacy of the experiment to test viability of the cells after irradiation. The number of cells tested were far below that to be administered to patients. He questioned adequacy of the RCR assays for detecting viruses that might be activated in the irradiated human cells.

Dr. Walters raised several concerns about the Informed Consent document. The request for post-mortem should not be stated as if agreement to this procedure is a condition for entering this study. The *Purpose of the Research Study* section should begin with a sentence that informs patients that this protocol is a toxicity study, and that no therapeutic benefit is expected.

Ms. Buc remarked that under the Uniform Anatomical Gift Act, patients have the right to agree to donate organs or tissues for medical studies. A lengthy discussion ensued regarding whether families can override the patient's wishes. Mr. Capron stated that a patient can legally donate his/her body for a scientific study over the objection of family members under the Uniform Anatomical Gift Act. Dr. Chase suggested that a joint Informed Consent process in which patient's assent and the relatives' intent for autopsy could be obtained simultaneously and would avoid later discord on this matter.

Investigator Response--Dr. Cohen

Dr. Miller stressed that the viability test on the irradiated cells should be performed using cell numbers that correlate to a patient dose, i.e., 108 cells. Dr. Cohen agreed to comply with this stipulation. Dr. Miller suggested RCR safety testing should be performed by co-cultivation with human target cells rather than mouse cells that may be resistant to infection by humanRCR. The activated RCR can be assayed by a neoR colony assay since it will package the vector with the neoR gene. Dr. Cohen agreed to this suggestion.

In response to Dr. Haselkorn's question about Dr. Richards no longer being at the University of Chicago, Dr. Cohen stated that Dr. Richards has joined the faculty of the University of Illinois and will continue to collaborate on this project.

On the Informed Consent document issue, Dr. Cohen agreed to theRAC's recommendation about a

request for autopsy.

Committee Motion

A motion was made by Dr. Smith and seconded by Ms.Buc to approve the protocol contingent on the review and approval of the following stipulations by the primary reviewers and by Dr. Miller: (1) submit data demonstrating that the proposed dose of radiation effectively inhibits viability using the number of cells proposed for a single patient dose, (2) submit colony assay data demonstrating lack ofRCR using irradiated transduced melanoma cells, and (3) submit revised language for the Informed Consent document that explains how consent for autopsy will be obtained, i.e., invoking the Uniform Anatomical Gift Act or requesting assent from the patient's relatives. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *A PHASE I CLINICAL TRIAL TO EVALUATE THE SAFETY AND EFFECTS IN HIV-1 INFECTED HUMANS OF AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A RIBOZYME THAT CLEAVES HIV-1 RNA* **/DRS. WONG-STAAL AND POESCHLA**

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California, San Diego, La Jolla, California. This protocol is a Phase I study of a retrovirus construct containing aribozyme insert that when expressed is capable of cleaving HIV transcripts and diminishes virus replication and spread. Ribozymes are potentially therapeutic RNA molecules that contain antisense sequences for specific recognition and RNA-cleaving enzymatic activity. The investigators have demonstrated that human T cell lines and primary peripheral blood T cells transduced with the hairpin ribozyme that cleaves HIV-1 RNA in the 5' leader sequence are intracellularly immunized against challenge with HIV. The ribozyme acts at two steps in the viral life cycle by cleaving: (1) afferent genomic viral RNA, and (2) efferent viral mRNA expressed from integrated provirus. The investigators propose to evaluate the safety and efficacy of gene transfer in 6 patients with HIV-1 infection byreinfusing autologous CD4(+) T cells that have been transduced *ex vivo* with a retrovirus vector that expresses the hairpinribozyme. The *in vivo* kinetics and survival of ribozyme-transduced cells will be compared by limiting dilution polymerase chain reaction (PCR) with cells transduced with a control vector. Dr. Straus stated that the PI is a renowned and accomplished retrovirologist, who has enlisted qualified clinical collaborators to facilitate the project. The protocol relies on a vector and a packaging cell line that are well defined. Dr. Straus raised the following concerns about the protocol: (1) What assays will be performed for the detection ofRCR and who will perform these assays? (2) Is rabbitpyrogen testing of clinical materials necessary? (3) Isazidothymidine (AZT) antiviral therapy required for subjects entering this study? and (4) Will 4 or 6 patients be accrued onto the study? Dr. Straus said that this study is creative and with clarification of these issues, he recommends approval of the protocol.

Review--Dr. Hirano

Dr. Hirano stated that the results of the preclinical studies and the objective of this protocol are very exciting, but there are five concerns that should be addressed: (1) Although the vector and packaging cell lines have previously been approved, production of vector supernatant is currently being performed in a different laboratory. Results ofRCR testing in the new laboratory should be provided. (2) There is a lack of PCR data comparing the survival oftransduced T cells in the background of a large excess of untransduced cells as a function of time in tissue culture. (3) Transduction efficiency and the ability to

expand these transduced cells to numbers that correlate to a patient dose has not been demonstrated. (4) The transduction procedure described in the preclinical studies is different from that proposed in this protocol. (5) Data are not provided regarding testing ofribozyme toxicity *in vivo* in the severe combined immunodeficiency (SCID)-Hu mouse model. (6) The effectiveness of theribozyme in cleaving HIV RNA in lymphocytes obtained from HIV patients with uncloned virus strains has not been demonstrated.

Review--Dr. Chase

Dr. Chase raised four major issues: (1) The investigators are proposing to use FDA criteria forRCR testings, which have not been totally agreed upon by the RAC. (2) The necessity for AZT administration, which was mandated by IRB, is questionable. Dr. Chase recommended that AZT administration should be at the discretion of the treating physicians. (3) The financial risks to patients for treatment procedures are not clearly stated in the Informed Consent document. (4) The kinetics of cell survival, which compares ribozyme-transduced with control cells, have not been adequately described. Pending clarification on these issues, Dr. Chase recommended approval of the protocol.

Other Comments
Ms. Meyers indicated several concerns in her written comments about the Informed Consent document language. The financial responsibility of patients is not clearly stated. The request for autopsy includes a statement that the patient's permission for autopsy may not be rescinded by relatives. Mr. Capron said tha he would defer to the opinion of the legal counsel of the University of California to determine whether this language is enforceable without invoking the Uniform Anatomical Gift Act.

Dr. Carmen suggested several specific language changes in the Informed Consent document to convey more clearly the recombinant DNA aspects of this protocol to patients. Dr. Smith expressed concern abou the ambiguous statements of financial risks to patients and the lack of request for autopsy in the Informed Consent document. He stated that theIRB stipulation of mandatory AZT is unnecessary. Also, there is a question about the possibility of generating RCR with an altered host range by administering a retrovirus vector to HIV infected patients.

Regarding the issue of AZT use in acquired immunodeficiency syndrome (AIDS) patients, Dr. Straus said that there is a changing opinion in the medical community resulting from new studies. In patients with CD4 counts below 200, AZT appears to have beneficial effect on patient survival. But for patients with CD4 counts between 250 and 600, which is the target patient population of this study, the current data are conflicting as to the beneficial effect of AZT. Dr. Straus was concerned about the mandatory use of AZT as suggested by the IRB. The decision to use antiviral drugs is best left to the primary care physicians. Dr. Post also expressed his concern about the requirement for AZT in healthy HIV(+) individuals.

Dr. Walters requested a clearer statement in the Informed Consent document as to whether there will be any clinical benefit to patients in this Phase I trial. Mr.Braverman commented on the proposed study. He expressed his reservation that the IRB has mandated the use of AZT for patients in this trial. He raised several points regarding inclusion and exclusion criteria for patients in this trial. Overall, he was pleased to see this gene therapy protocol reviewed by the RAC and applauded such an advancement in AIDS research. Dr. Nava Sarver, National Institute of Allergy and Infectious Diseases,NIH, expressed her support for this protocol to evaluateribozyme treatment in HIV(+) individuals.

Investigator Response--Drs. Wong-Staal and Poeschla

Regarding the question of RCR testing, Dr. Wong-Staal said that they are in the process of seeking an established laboratory to test clinical grade preparations. Preliminary results from her own laboratory

indicate no RCR contamination. Responding to questions raised by Drs. Hirano and Chase regarding the detection of ribozyme transduced cells in the presence of untransduced cells, Dr. Wong-Staal presented data demonstrating that the PCR assay is capable of detecting at least one transduced cell in the background of 5,000 untransduced cells. Dr. Chase pointed out that the real question is how to analyze the differential survival rates of ribozyme transduced versus untransduced cells in HIV patients, and this question is not adequately addressed by this experiment. As to transduction efficiency of the retroviral vector, Dr. Wong-Staal said the initial transduction rate is only a few percent. However, after G418 selection, the ribozyme-transduced T cells are resistant to challenge by two HIV isolates. Dr. Parkman commented that other investigators have encountered some technical difficulties in performing G418 selection of transduced cells obtained from patient samples due to non-specific toxicity to lymphocytes, particularly, in the scaling up of this procedure to obtain large quantity of cells for patient use. Dr. Wong-Staal agreed that a rehearsal of this isolation procedure at the clinical scale will be performed. Dr. Poeschla addressed questions on the assays that will be performed to detect HIV in the transduced lymphocyte preparations that will be administered to patients. Any preparation positive in the p24 HIV test will be discarded.

Dr. Poeschla explained that there are differing opinions between the investigators and their IRB regarding the mandatory requirement for AZT administration. Not only is there controversy about the use of AZT, but the IRB has mistakenly assumed that AZT is useful for inhibiting murine RCR in the event of RCR contamination (AZT was used in Dr. Nabel's previously approved HIV protocol). Mr. Capron suggested that if there is a misunderstanding on the part of the IRB, the RAC should clarify the issue. Dr. Parkman agreed that the risk/benefit ratio argues against mandatory AZT treatment for patients who do not need the drug. Dr. Poeschla stated that the rabbit pyrogen test required by his IRB is neither sensitive nor specific. Although the activated lymphocytes may produce cytokines that are pyrogenic, the fever experienced by patients can be readily treated with medication. Regarding how the number of patients was selected, Dr. Poeschla said that is not critical because this is a Phase I trial to compare survival of lymphocytes within the same individual.

Regarding questions about the Informed Consent document, Dr. Poeschla agreed to the changes suggested by Dr. Carmen. As to the directive made by patients to consent for autopsy irrespective of a relative's objection, Dr. Poeschla said that this language is requested by most of his HIV(+) patients. In regard to the question of any potential benefit to the patients, Dr. Poeschla said that considering the small number of cells to be administered to patients, the clinical benefit is very small, and it will be stated clearly in the Informed Consent that there will be no clinical benefit. In future trials, the use of stem cells may have a greater potential to affect disease progression.

Dr. Hirano stated that a rehearsal scale-up experiment must be performed to ensure that lymphocytes obtained from HIV(+) patients are transduced with the vector, enriched by G418 selection, checked for lack of HIV, and grown up to clinical quantity.

Dr. Straus suggested that a letter be forwarded from ORDA to the IRB recommending that AZT administration and rabbit pyrogen testing should not be mandatory.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Chase to approve the protocol contingent on the following: (1) a letter will be forwarded from ORDA to the IRB recommending that AZT administration and rabbit pyrogen testing should not be mandatory, (2) the RAC will be notified of the name of the laboratory that will conduct RCR testing on the master cell bank and a detailed list of the tests that will be performed, and (3) the investigators will demonstrate that they can perform the transduction procedure on a clinical

scale using cells from HIV-infected patients. The motion passed by a vote of 17 in favor, 0 opposed and 1 abstention (Dr. Geiduschek abstained from voting because he is employed by the same institution).

XII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *GENETICALLY ENGINEERED AUTOLOGOUS TUMOR VACCINES PRODUCING INTERLEUKIN-2 FOR THE TREATMENT OF METASTATIC MELANOMA***/DRS. ECONOMOU AND GLASPY**

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California. This protocol is a study of patients with metastatic melanoma who have failed standard therapy. In an attempt to increase the patient's immune response to the tumor, the IL-2 gene will be introduced into a human melanoma cell line (M-24). The gene-modified melanoma cell line producing IL-2 will then be mixed with tumor cells obtained from the patient. This mixture of cells will be lethally irradiated and injected subcutaneously into patients. This injection is expected to augment the immune response of the patients to tumor cells through the immune stimulatory effects of IL-2 secreted by the gene-modified cells. Animal models have shown that injection of gene-modified cells has important anti-tumor effects. To determine the safety of the procedure, a constant number of tumor cells will be mixed with escalating numbers of IL-2 producing cells. The study will involve 30 patients in 3 dose-escalation groups. The following endpoints will be measured: (1) toxicity, (2) generation ofCTL precursors, lymphokine activated killer (LAK) cells and enhanced natural killer (NK) activity, (3) immunohistochemistry and delayed-type hypersensitivity skin tests using injection of irradiated autologous tumor cells, and (4) clinical anti-tumor responses. Dr. Motulsky stated that most of his initial concerns have been adequately addressed by the investigators. Several questions remain: Have experiments been performed in which IL-2 is injected locally to observe any effect on tumor growth? What is the rationale of mixing theallogeneic M24 cells in the vaccine cocktail? Would other IL-2 producing cells, such as transduced B-lymphocytes, serve the same purpose? Will HLA typing of autologous and allogeneic cells be performed?

Dr. Motulsky provided the following comments in regard to the Informed Consent document: (1) abbreviations should be avoided since they may not be understood by laypersons, (2) the use of the term "vaccine" is inappropriate and misleading, (3) the statement that describes the patient's consent as "received" should be more appropriately be described as "given", (4) the issue of financial risk to patients for costs associated with an investigational procedure should be addressed, and (5) the statement that addresses the commercial value of cell lines should be clarified. The investigators should provide the RAC with information about the source of the proposed cell line. In conclusion, the study may demonstrate the potential to reduce human tumor growth, and there do not appear to be any new problems associated with the gene transfer portion of the proposal.

Review--Dr. Doi

Dr. Doi stated that the investigators have responded to most of his initial concerns; however, he noted tha the use of the term "vaccine" in the title, throughout the protocol, and in the Informed Consent documents is misleading to patients and others. The proposed material to be used in the treatment is not a "vaccine" in the classical sense. The investigators should respond to the following questions: (1) How many transduced M24 cells are required to produce between 103 and 105 picograms of IL-2? (2) Are transduced cells stable during cryopreservation? (3) Will patients react immunologically to the allogeneic melanoma cell line after biweekly and monthly injections over a period of one year? Dr. Doi said that the overall experimental design appears to be based on sound and reasonable procedures, the study is

straightforward, and the endpoints are well defined. Dr. Doi recommended approval of the protocol.

Review--Dr. Zallen

Dr. Zallen explained that her questions are directed to three areas. In regard to the animal and preclinical studies, most of the preclinical studies were performed with a different melanoma cell line, M14. Very little data was submitted using the M24 cell line that will be used for the clinical trial. The relevant animal studies described in a preprint have not been included as part of the proposal. The expected bystander effect has not been defined in the animal studies. In the area of experimental design and patient selection, HLA typing should be performed prospectively to confirm that there is a match with the M24 cell line before patients are enrolled onto the study. In most preclinical studies, cells were irradiated with 10,000 rads, yet 5,000 rads is proposed for the clinical study. Is 5,000 rads a sufficient dose to lethally irradiate the M24 cells?

In regard to the Informed Consent process, Dr. Zallen asked the investigators to elaborate on how patient consent will be obtained. Several specific changes were suggested to the Informed Consent document: (1) the use of the term "vaccine" is misleading to the patients, (2) it is unclear as to the number of biopsies that will be performed and the associated risks, (3) the section that describes financial risks to patients that could result from research-related injuries should be clarified, and (4) an exaggerated number of $100,000 was originally assessed as the patient's upper limit of financial costs. That figure should be removed from the Informed Consent document.

Other Comments

Dr. Smith asked whether there are data demonstrating that 5,000 rads is sufficient to lethally irradiate the M24 cells. Dr. Parkman said that HLA typing of patients will facilitate interpretation of research results because differences in patient responses between those who share HLA antigens with the M24 melanoma cell and those who do not will be interpretable. Dr. Parkman asked the investigators to summarize their earlier results from studies using the M24 cell with and without systemic IL-2 administration. Dr. Geiduschek asked whether the IL-2 producing M24 cells will be derived from a clonal cell line or mass cell culture transduced by the vector? Will the cell line be selected for high IL-2 secretion?

Ms. Meyers raised concerns in her written comments about the use of the term "vaccine" and the statement about the financial costs for patients participating in this trial. The Informed Consent document does not include a request for autopsy. Dr. Walters questioned if the first sentence of the Informed Consent document adequately states the purpose of the study.

Investigator Response--Dr. Economou

Dr. Economou explained that the present study combines the merit of two approaches of immunotherapy of cancer, i.e., transduction of an IL-2 expressing vector in patients' autologous tumor cells and an allogeneic cell line. Studies in animal models suggest that a "bystander effect" is produced by this immunization. Reduced tumorigenicity and enhanced immunogenicity is achieved by mixing untransduced tumor cells with immunologically irrelevant cytokine-producing cells. The local continuous production of cytokines by cells adjacent to autologous tumor cells is sufficient to provoke immunological responses to tumors. In regard to the rationale for selecting the M24 melanoma cell line for transduction by the IL-2 vector, he explained that the IRB urged him to use M24 because it is a well characterized cell line that has been used extensively in previous clinical trials, and it appears to be safe for human use.

Clarifying the question about the source of funding for the present study, Dr. Economou said that the present study will be funded by NIH through an implementation program project grant. Genetic Therapy, Inc., will provide scientific collaboration but no direct financial support. The IL-2 producing cell line will be made in the investigator's laboratory that is funded by NIH.

Responding to a question about lack of direct data on M24 cells, Dr. Economou said that in preclinical studies the M14 cell line was used, and similar results were obtained with the M24 cell line although those data were not included in their published paper. Dr. Zallen said those relevant data should be presented in this proposal. Dr. Miller asked whether the investigators have already isolated a high IL-2 producing cell line. Dr. Economou responded that they have not isolated a high producer to date. The investigators are waiting for the biosafety testing requirements from FDA. As to the question of whether 5,000 rads irradiation is sufficient to inhibit cell proliferation, Dr. Economou said that 5,000 rads is the dose used for this cell line in other tumor vaccine studies at the University of California, Los Angeles. No tumor growth has ever been observed at the inoculation site in other clinical trials. Responding to Dr. Doi's question about possible adverse effects from repeated immunization with M24 cells, Dr. Economou said that no adverse immunological reaction has been observed in the last 10 years of clinical trials with these cell lines. Dr. Economou agreed to perform HLA typing prior to entering patients onto the study. There was a lengthy discussion about the scientific justification for approving this type of clinical immunotherapy trial, i.e., using cytokine producing cells and autologous tumor cells. Several RAC members expressed their reservation about the lack of any definitive result from other RAC-approved clinical trials that were similar to this proposal.

Committee Motion

A motion was made by Dr. Motulsky and seconded by Dr. Doi to approve the protocol contingent on review and approval of the following stipulations: (1) submission of transduction data on the M24 cell line, (2) an indication of the PI's intent to perform HLA typing prior to enrolling the patients onto the study, and (3) submission of data demonstrating the biological activity of IL-2 of transduced M24 cells in the animal model. The motion passed by a vote of 11 in favor, 0 opposed, and 6 abstentions.

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

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the proposal resubmitted by Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland. This request was deferred at the September 14, 1992, and June 8, 1993, RAC meetings. The proposal was deferred during the September 1992 RAC meeting until the investigators return to the full RAC with data regarding the following: (1) the frequency of recombination that produces replication-competent virus, using cell numbers analogous to the laboratory setting (e.g., 1 x 109 cells), and (2) acquire data regarding the frequency of seropositivity among personnel previously exposed to SFV. The proposal was deferred during the June 1993 RAC meeting until the investigators could return to the full 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 fo 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.

Dr. Miller stated that the investigators are requesting a reduction in the physical containment level from

Biosafety Level (BL) 3 to BL2 for Life Technologies' SFV/Helper 2 Gene Expression System. The investigators have produced a Users' Manual as recommended by the RAC. This manual contains the following information: (1) a summary of possible health risks associated with the use of the cloning system, (2) a description of practical and effective means to inactivate SFV, (3) a simple protocol for the detection of replication-competent SFV, (4) a description of symptoms associated with SFV infection and procedures to be followed in the event of laboratory infection, (5) warnings applicable to BL2 guidelines, (6) a "Biosafety Clearance Agreement" to be signed by the user and his/her IBC, and (7) a listing of BL2 practices advisable for use with the cloning system. Dr. Miller commended the investigators for the Users' Manual and stated that the investigators have adequately responded to all of the RAC's previous concerns. Dr. Miller recommended approval of the request to reduce the physical containment level from BL3 to BL2.

Review--Dr. Krogstad

Dr. Krogstad commented on the well prepared Users' Manual. This manual addresses most of the concerns raised during the previous RAC review. He suggested that the important areas of the manual should be highlighted in boldface type. The only issue still of concern is how laboratory personnel will be informed of the potential risks. Every individual in the laboratory, even if they do not use the SFV system, should sign the "Biosafety Clearance Agreement" as an acknowledgement that they have been informed of the potential risks associated with the SFV expression system. SFV is an infectious agent that can spread by aerosol. He recommended reducing only the containment level for the expression system from BL3 to BL2; however, for the parent virus, SFV, which is a Class 3 agent, the containment level should not be reduced. There is still substantial risk of serious disease among people who become infected as a result of laboratory accidents or as a result of natural transmission.

Review--Dr. Post

Dr. Post stated that the Users' Manual prepared by the investigators addresses all the previous concerns raised by the RAC, and he recommended use of this SFV/Helper 2 Gene Expression System at BL2 containment. Dr. Post still expressed his concern about the discrepancy between the classification of Sindbis virus (Class 2) and SFV (Class 3). The scientific justification for this distinction is weak.

Other Comments

Dr. Miller said that requiring all laboratory personnel to sign the Biosafety Clearance Agreement will be unworkable. It should be the PI's responsibility to ensure that all laboratory personnel have been adequately informed. Dr. Geiduschek and Dr. Straus considered that it is appropriate for all individuals who are likely to be exposed to this agent to acknowledge the risks in writing.

Investigator Response--Dr. Temple

Dr. Temple stated that in compliance with the *NIH Guidelines*, everyone entering a BL2 facility must be informed of the activities within the laboratory. The PI is responsible for ensuring compliance with the *NIH Guidelines*. For practical reasons, it is difficult to keep track of all individuals who are entering and exiting the facility. Dr. Temple explained that according to the *NIH Guidelines*, when an infectious agent is in use in a laboratory, there are special entry requirements, i.e., a hazard warning sign must be posted, and the access to the laboratory is limited to those individuals authorized by the PI. In cases of mixed-use facilities, the containment requirement for the highest level of activities should be observed by all individuals within the same facility.

Dr. Straus expressed his concern about commercial distribution of the SFV/Helper 2 Gene Expression System even if all the BL2 safety rules are observed. He said he is still concerned about the pathogenicity of SFV, noting that an incidence of fatality was previously reported. The rationale for safe use of this agent in the laboratory is based on experiences of a very limited number of individuals (approximately 50). This sample size of individuals is too small to predict risk for the wider use that would occur if this product becomes commercially available. An example of this risk is polio virus which causes paralysis in 1 out of 1,000 infected individuals. Although it has been handled safely by qualified investigators in the laboratory, its use as a cloning vector would not be justified in an unvaccinated population. Dr. Temple said that over 400 laboratory workers have successfully worked with SFV over the last decade, and there have been no instances of symptomatic disease.

Dr. Miller agreed with Dr. Krogstad that a stipulation for approval should be added that would require that the PI obtain signatures from all laboratory personnel who are regularly present in the laboratory and agreed that this stipulation adequately addresses the safety concern.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Krogstad to approve the request to reduce the level of physical containment for SFV/Helper 2 Gene Expression System to be distributed by Life Technologies, Inc., from BL3 to BL2. Approval of the request is contingent on the requirement that the PI must obtain signatures from all laboratory personnel certifying that they have been informed of the possible risks associated with this expression system and that they have read the Users Manual. The motion to approve the request passed by a vote of 13 in favor, 2 opposed, and 1 abstention.

XIV. AMENDMENT TO APPENDIX B OF THE *NIH GUIDELINES* REGARDING UPDATING APPENDIX B: CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD/DR. FLEMING

Presentation--Dr. Fleming

Dr. Walters called on Dr. Fleming of the Mid-Atlantic Biological Safety Association, Bowie, Maryland, to present her updated listing of Appendix B: Classification of Microorganisms on the Basis of Hazard. Dr. Fleming explained that the current Appendix B is based on biohazard information about etiologic agents obtained in the 1970s. Since that time, there have been several updated publications from the Centers for Disease Control (CDC) and the NIH. An updated classification was published in 1980, and was incorporated into a book entitled: *Biosafety in Microbiological and Biomedical Laboratories*, published in 1984. The latest edition of the NIH/CDC publication was published in May 1993 and is available from the Government Printing Office. The NIH/CDC publication contains quantitative risk-assessment information; however, this information has not been developed into a simplified list, i.e., updated Appendix B. The proposed Appendix B has been compiled from information derived from the NIH/CDC publication. Dr. Fleming stated that a copy of proposed Appendix B has been forwarded to the CDC for review.

Discussion

Dr. Miller noted that SFV is listed as both a Class II and a Class III agent, and it is unclear which containment level should be used. Dr. Post expressed concern about the interpretation of the information in the CDC/NIH publication and how this information has been transformed into the proposed Appendix B. Dr. Post stated that the RAC does not have the expertise or time to assess the accuracy of this interpretation. Many RAC members expressed similar lack of expertise to properly assess the accuracy of this list, and deferred voting on this proposal until expert opinions have been obtained from the CDC and

NIH Division of Safety.

Committee Motion

The RAC recommended by consensus that the current classification of etiologic agents described in *Biosafety in Microbiological and Biomedical Laboratories* , 3rd edition, May 1993, U. S. Department of Health and Human Services, should be endorsed by the RAC; however, the RAC retains the option to adopt any modifications to the CDC listing. The RAC recommended that the summary list (proposed Appendix B) should not be adopted by the RAC until the subcommittee receives letters of concurrence from both the CDC and NIH Division of Safety.

XV. AMENDMENTS TO SECTIONS III, IV, V AND APPENDIX C AND F OF THE *NIH GUIDELINES* REGARDING THE CLONING OF TOXIN MOLECULES/DR. WIVEL

Dr. Walters called on Dr. Wivel to present his request for amendments to Sections III, IV, and V, and Appendices C and F regarding the review process for experiments involving the cloning of toxin molecules. Dr. Wivel stated that these amendments will establish a new category of review entitled: Experiments that Require NIH (ORDA) and IBC Approval Before Initiation. Under this new category of review, experiments involving the cloning of toxin molecules that are lethal for vertebrates at an LD50 of < 100 nanograms per kilogram of body weight will be reviewed by NIH (ORDA) in consultation with *ad hoc* toxin experts. Sections III, IV, V and Appendix C and F are proposed to read:

"Section III. Guidelines for Covered Experiments.

"Part III discusses experiments involving recombinant DNA. These experiments have been divided into five classes:

"III-A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

"III-B. Experiments which require NIH (Office of Recombinant DNA Activities/ORDA) and Institutional Biosafety Committee (IBC) approval before initiation of the experiment;

"III-C. Experiments which require IBC approval before initiation of the experiment;

"III-D. Experiments which require IBC notification at the time of the experiment;

"III-E. Experiments which are exempt from the procedures of the Guidelines.

"IF AN EXPERIMENT FALLS INTO BOTH CLASS III-A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III-A MUST BE FOLLOWED. If an experiment falls into Class III-E and into either Class III-C or III-D as well, it can be considered exempt from the requirements of the Guidelines. Changes...."

Section III-A-1 will be moved to a new Section III-B-1. New Section III-B is proposed to read:

"Section III-B-Experiments That Require NIH (ORDA) and IBC Approval Before Initiation.

"Section III-B-1. Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin

molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight....

"Section III-B-1-(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH through ORDA. The containment conditions for such experiments will be determined by ORDA in consultation with *ad hoc* experts. Such experiments also require the approval of the IBC before initiation (see Section IV-C-1-b-(3)-(f)."

Sections III-A-2, III-A-3, III-A-4 will be renumbered to III-A-1, III-A-2, III-A-3 respectively. Sections III-B, III-C and III-D will be renumbered to III-C, III-D, and III-E respectively.

The new Section III-C-2 is proposed to read:

"Section III-C-2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.

"Section III-C-2-a. ...Many experiments in this category are exempt from the Guidelines (see Section III-E-4) and III-E-5). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH (ORDA) approval (see Section III-B-1) or must be carried out under NIH specified conditions as described in Appendix F."

Section IV-B-5-b-(3) is proposed to read:

"Section IV-B-5-b-(3). Petition NIH (ORDA), with concurrence of the IBC for approval to conduct experiments specified in Sections III-A and III-B of the Guidelines;"

Section IV-C-1-b-(3)-(f) will be deleted which reads: "Approving the cloning of toxin genes in host-vector systems other than *E. coli* K-12 (see Appendix F); and]"

Section IV-C-1-b-(3)-(g) will become the new Section IV-C-1-b-(3)-(f).

The new Section IV-C-3-a is proposed to read:

"Reviewing and approving experiments involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD50 100 nanograms per kilogram body weight in organisms other than *E. coli* K-12 (see Section III-B-1 and Appendices F-I and F-II)."

Sections IV-C-3-a and IV-C-3-b will be renumbered to become Sections IV-C-3-b and IV-C-3-c respectively.

Section V-2 is proposed to read:

"...In the cases falling under Sections III-A through III-D, this judgment is to be reviewed and approved by the IBC..."

Appendix C is proposed to read:

"Appendix C. Exemptions Under Section III-D-5.

"...Appendix C-I. Recombinant DNA in Tissue Culture...

"...Exceptions. The following categories are not exempt from theNIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents, and (iv) experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-II. Experiments Involving *E. coli* K-12 Host-Vector Systems...

"...Exceptions. The following categories are not exempt from theNIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-III. Experiments Involving *Saccharomyces* Host-Vector Systems...

"...Exceptions. The following categories are not exempt from theNIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-IV. Experiments Involving *Bacillus subtilis* Host-Vector Systems...

"...Exceptions. The following categories are not exempt from theNIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms...

"...Exceptions. The following categories are not exempt from theNIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F.)"

Appendix F is proposed to read:

"Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules

Toxic for Vertebrates.

"Appendix F-I. General Information.

"Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD50 of <100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under Section III-B-1 of the Guidelines and require NIH (ORDA) and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD50 of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxin molecules with an LD50 of < 100 micrograms and > 100 nanograms per kilogram body weight require registration with ORDA and IBC approval prior to initiating the experiments. A list of toxin molecules classified as to LD50 is available from ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(e))...

"Appendix F-III. Containment Conditions for Cloning Toxin Molecule Genes in Organisms Other Than *E. coli* K-12.

"Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight in host-vector systems other than *E. coli* K-12 will be evaluated by NIH (ORDA) in consultation with *ad hoc* toxin experts (see Sections III-B and IV-C-1-b-(3)-(f)

"Appendix F-IV. Specific Approvals.

"An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Appendix F-IV-A through Appendix F-IV-K would be deleted. [A list of these specific approvals will be maintained in ORDA.]

Committee Motion

The RAC approved a motion made by Mr. Capron and seconded by Dr. Post to accept the amendments to Sections III, IV, and V and Appendix C and F of the *NIH Guidelines*. The motion passed as proposed by a vote of 16 in favor, 0 opposed, and no abstentions.

XVI. AMENDMENTS TO SECTION III AND APPENDIX D OF THE *NIH GUIDELINES* REGARDING ACTIONS TAKEN UNDER THE GUIDELINES/DR. WIVEL

Dr. Walters called on Dr. Wivel to present his request for an amendment to Section III and Appendix D. Dr. Wivel stated that these amendments would eliminate the requirement for publication of Appendix D (Actions Taken Under the Guidelines) in the *Federal Register* and to allow distribution of these actions by the Office of Recombinant DNA Activities. Section III-A and Appendix D is proposed to read:

"Section III-A--Experiments that Require RAC Review and IBC Approval Before Initiation.

"...Specific experiments already approved in this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

"Appendix D--Actions Taken Under the Guidelines.

"As noted in the subsection IV-C-1-b-(1), the Director,NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Committee Motion

The RAC approved a motion made by Mr. Capron and seconded by Dr. Chase to accept the amendments to Section III and Appendix D of the *NIH Guidelines*. The motion passed as proposed by a vote of 16 in favor, 0 opposed, and no abstentions.

XVII. WORKING GROUP REPORT ON CATEGORIES OF HUMAN GENE TRANSFER EXPERIMENTS THAT ARE EXEMPT FROM RAC REVIEW/DR. PARKMAN

Dr. Walters called on Dr. Parkman, Chair of the Working Group on Categorization of Protocols, to summarize the conclusions of the working group. Dr. Parkman stated that the working group proposes three possible categories of experiments that may be eligible for accelerated review by the RAC: (1) protocols conducted at satellite institutions under the responsibility of the PI, (2) protocols that are identical to another NIH-approved human gene transfer protocol under the responsibility of a new PI, (3) protocols that are similar to NIH-approved protocols; however, minor changes have been introduced that do not affect the gene therapy aspects of the study, and (4) protocols that use lethally irradiated cells (the RAC did not reach a consensus about the kinds of vectors that would qualify).

Dr. Parkman proposed the following: (1) ORDA would assume the responsibility for making determinations whether protocols would qualify for accelerated review status and qualifications of any new investigators, (2) a summary of approved accelerated review protocols would be presented to the full committee at scheduled RAC meetings, and (3) semi-annual data reports would be required for all accelerated review protocols in order to monitor for evidence of gene transfer and possible adverse effects.

Dr. Walters proposed that the working group continue to refine the criteria for accelerated review protocols and to develop draft language for inclusion in the *NIH Guidelines*. This material will be reviewed by the full RAC at its December 1993 meeting. The RAC will also reconsider the definition of recombinant DNA vaccines with the view that Footnote 21 in Section V of the *NIH Guidelines* may need to be revised.

XVIII. FUTURE MEETINGS OF THE RAC

Dr. Walters announced that the next meeting of the RAC will be December 2-3, 1993,NIH, Building 31C, Conference Room 6.

XIX. ADJOURNMENT

Dr. Walters adjourned the meeting at 3:30 p.m. on September 10, 1993.

Nelson A. Wivel, M.D.
Executive Secretary

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

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