U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting -- March 1-2, 1993
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

March 1-2, 1993

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-third meeting at 9:00 a.m. on March 1, 1993, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Constance E. Brinckerhoff, Dartmouth Medical School
Nancy L. Buc, Weil, Gotshal, and Manges
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Mariann Grossman, University of Michigan Medical Center
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin

Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington Medical School
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University
Executive secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment).

Non-voting agency representatives:

Bernard Greifer, Department of Commerce

National Institutes of Health staff:

Leon Baltrucki, NHLBI
Bobbi Bennett, OD
Roscoe Brady, NINDS
Steven Brody, NHLBI
Jan Casadei, NCI
Ken Cowan, NCI
Chin-Shyan Chu, NHLBI
Judy Fradkin, NIDDK
MaryEllen Franko, NCI
Barry Goldspiel, CC
Michael Gottesman, NCI
Roberta Haber, NIDDK
Jeffrey Hoeg, NHLBI
Christine Ireland, OD
Sachiko Kajigaya, NHLBI
Becky Lawson, OD
Charles Link, NCI
Fred Lombardo, NHLBI
Kathryn McKeon, NIDDK
Arthur Neinhuis, NHLBI
Melissa Rosenfeld, NHLBI
Gene Rosenthal, NHLBI
Nava Sarver, NIAID
Toshihiro Soma, NHLBI
Brian Sorrentino, NHLBI
Bob Wersto, NHLBI

Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
Nicholas Akdemir, Genetic Therapy, Inc.
French Anderson, University of Southern California
Arthur Bank, Columbia University
Jack Barber, Viagene, Inc.
James Barrett, Genetic Therapy, Inc.
Edward Beecham, Genetic Therapy, Inc.
Arie Belldegrun, University of California, Los Angeles
Anton Berns, Somatix Therapy Corporation
Richard Boucher, University of North Carolina, Chapel Hill
Liz Bowie, The Baltimore Sun
G'dali Braverman, Act Up
Malcolm Brenner, St. Jude Childrens Research Hospital
Lou Bucalo, Ingenex, Inc.
Barrie Carter, Targeted Genetics, Inc.
Jan Chappell, Genetic Therapy, Inc.
Ben Cheng, Act Up
Yawen Chiang, Genetic Therapy, Inc.
Shirley Clift, Somatix Corporation
Larry Cohen, Somatix Corporation
Kenneth Culver, University of Iowa
Timothy Darrow, Duke University
Krista Delviks, Hood College
Wanda de Vlaminck, Avigen Corporation
Ross Donehorner, Johns Hopkins University
Jianyun Dong, University of Alabama
Ron Dorazio, Genetix, Inc.
Glen Dronoff, Johns Hopkins University
Anne Driscoll, Novus Group
Michelle Durand, The French Embassy
David Ennist, Genetic Therapy, Inc.
Susan Falen, Genetic Therapy, Inc.
Mitchell Finer, Cell Genesys, Inc.
Stacy Fitzsimmons, Cystic Fibrosis Foundation
Suzanne Forry-Schaudies, Genetic Therapy, Inc.
Jeffrey Fox, ASM News and Biotechnology
Ray Frizzell, University of Alabama
Siqang Fu, MD Anderson Cancer Research Center
Ram Gamgavalli, Viagene, Inc.
Richard Giles, University of Illinois
Steve Goff, Columbia University
Beth Gregory, Johns Hopkins University
Kurt Gunter, Food and Drug Administration
Ingo Haertel, Georgetown University
Karen Hauda, Johns Hopkins University
Charles Hesdorffer, Columbia University

Helen Heslop, St. Jude Childrens Research Hospital
Tom Horiagon, Chimerix Corporation
Zafer Iqbal, University of Illinois
Liz Jaffae, Johns Hopkins University
John Jaugstetter, Genentech, Inc.
Dorothy Jessop, U.S. Department of Agriculture
Larry Johnson, University of North Carolina, Chapel Hill
Douglas Jolly, Viagene, Inc.
John Kavanagh, MD Anderson Cancer Research Center
Rachel King, Genetic Therapy, Inc.
Don Koho, Childrens Hospital of Los Angeles
Toshi Kotani, Genetic Therapy, Inc.
Mike Knowles, University of North Carolina, Chapel Hill
Maryann Krane, Genzyme Corporation
Edward Lanphier, Somatix Corporation
Kyle Legg, Legg Mason
Ronald Leonardi, R & R Registrations
Hy Levitsky, Johns Hopkins University
Fray Marshall, Johns Hopkins University
Michael McCaughan, The Blue Sheet
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Bruce Merchant, Viagene, Inc.
Nanette Mitterder, Genetic Therapy, Inc.
Robert Moen, Genetic Therapy, Inc.
Lisa Morris, Genetic Therapy, Inc.
Steve Mueller, Genetic Therapy, Inc.
Richard Mulligan, Massachusetts Institute of Technology
William Nelson, Johns Hopkins University
Albert Owens, Johns Hopkins University
Drew Pardoll, Johns Hopkins University
Seth Pauker, Food and Drug Administration
Steven Piantadori, Johns Hopkins University
Rick Pickles, University of North Carolina, Chapel Hill
Eckhard Podack, University of Miami
Bob Powell, University of Miami
Paul Recer, The Associated Press
Rex Rhein, Biotechnology News Watch
Bob Riley, Genetic Therapy, Inc.
Joseph Rokovich, Somatix Therapy, Inc.
Ivor Royston, San Diego Cancer Research Center
H. F. Seigler, Duke University
G. Terry Sharrer, Smithsonian Institution
Rachel Sheppard, Genetic Therapy, Inc.
Tomiko Shimada, Ambience Awareness International
Jonathan Simons, Johns Hopkins University
Sharon Smith, Hood College
Robert Sobol, San Diego Cancer Research Center
Eric Sorscher, University of Alabama
Kasi Sridhar, University of Miami

Jim Timmins, Agricetus Corporation
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Carol Trapnell, Food and Drug Administration
John Van Gilder, University of Iowa
Lucy Vulchanova, Hood College
Michael Walsh, Robertson Stephens & Company
John Warner, Viagene, Inc.
Lisa White, The Blue Sheet
Jim Wilson, University of Michigan
Robert Wilmott, Children's Hospital in Cincinnati
Jim Zabora, Johns Hopkins University
Zhaoqing Zhou, University of North Carolina, Chapel Hill

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of this meeting was published in the *Federal Register* as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the protocol, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters welcomed the following new members of the Recombinant DNA Advisory Committee (RAC): Ms. Abbey Meyers of the National Organization for Rare Disorders, New Fairfield, Connecticut; Dr.Arno Motulsky of the University of Washington Medical School, Seattle, Washington; Dr. Brian Smith of Yale University School of Medicine, New Haven, Connecticut; Dr. Stephen Straus of the NIH, Bethesda, Maryland; and Ms. Mariann Grossman of the University of Pennsylvania, Philadelphia, Pennsylvania.

II. MINUTES OF THE DECEMBER 3-4, 1992, AND JANUARY 14, 1993, MEETINGS

Dr. Walters called on Dr. DeLeon to review the minutes of the December 3-4, 1992, RAC meeting. Dr. DeLeon stated that the minutes were an accurate reflection of the committee's deliberations. Minor corrections were submitted by Dr. Geiduschek and Ms. Meyers. The RAC unanimously approved a motion made by Dr. DeLeon and seconded by Dr. Krogstad to accept the December 3-4, 1992, RAC minutes with the inclusion of minor grammatical changes by a vote of 19 in favor, 0 opposed, and no abstentions.

Dr. Walters called on Dr. Chase to review the minutes of the January 14, 1993, RAC meeting. Dr. Chase stated that the minutes were an accurate reflection of the committee's deliberations. Minor corrections were submitted by Drs. Chase, Geiduschek, Zallen, and Carmen. The RAC unanimously approved a motion, made by Dr. Dronamraju and seconded by Dr. Zallen to accept the January 14, 1993, RAC minutes with the inclusion of minor grammatical changes by a vote of 19 in favor, 0 opposed, and no abstentions.

III. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: GENE THERAPY FOR THE TREATMENT OF MALIGNANT BRAIN TUMORS WITH IN VIVO TUMOR TRANSDUCTION WITH THE HERPES SIMPLEX-THYMIDINE KINASE GENE/GANCICLOVIR SYSTEM/DRS. CULVER AND VAN GILDER*

Review--Dr. Hirano

Dr. Walters called on Dr. Hirano to present her primary review of the protocol submitted by Dr. Kenneth W Culver of Iowa Methodist Medical Center, Des Moines, Iowa, and John C. Van Gilder of the University of Iowa, Iowa City, Iowa.

Dr. Hirano provided a brief overview of the protocol. This study is similar another protocol that was submitted by Dr. Edward Oldfield, and approved by the RAC at its June 1992 meeting. A retrovirus vector will be used to integrate the gene coding for herpes simplex-thymidine kinase (HS-tk) into the brain tumor cells of patients. In turn, the HS-tk gene renders these cells sensitive to the antiviral drug ganciclovir (GCV). HS-tk will be delivered to the patient's brain tumor cells by direct injection of the murine vector producing cells (VPC), PA317. Animal model studies performed in mice, rats, and nonhuman primates indicate that no significant toxicity is observed from the direct injection of VPC into the brain.

This study is designed to assess the safety and efficacy of the repeated administration of VPC followed by GCV treatment in patients with malignant brain tumors. In addition, patients will be monitored for systemic immunity resulting from the repeated injection of murine cells. A total of 40 patients will be entered into this study: 20 patients at the University of Iowa and 20 patients at Iowa Methodist Medical Center. Patient eligibility is limited to those individuals who have surgically accessible brain tumors.

The investigators propose to administer 3 injections of the VPC, each dose containing approximately 1 x 10 cells in a maximum volume of 10 milliliters (ml). Dr. Hirano asked the investigators to explain their reason for keeping the number of cells constant for all patients rather than proposing a dose escalation. On day 1, patients will undergo surgical debulking of their tumor and receive the initial injection of VPC. The second injection of cells will be administered on day 7 followed by GCV treatment on day 14; the original schema proposed an entire treatment cycle of 2 weeks. However, the investigators now propose a revised treatment schema which includes a third injection of VPC and subsequent GCV administration.

Dr. Hirano inquired whether the revised treatment schema is absolute for all patients or whether there is some degree of flexibility in the administration schedule. What is the possibility that any or all of the patients will require a third injection of VPC and subsequent GCV administration? Dr. Culver responded that the treatment schema for the first two injections is absolute; however, there will be flexibility in the administration of the third injection. If there is evidence of tumor recurrence, then the patient will receive the additional treatment. Dr. Hirano said that the schema described by Dr. Culver is not clearly outlined in the protocol.

Dr. Hirano said that there is no evidence that the third treatment is necessary. How long does GCV persist in the body? Is there data to demonstrate that the proposed concentration of GCV will not kill the VPC before the HS-tk gene has been integrated into the patient's brain tumor cells? Regarding the safety of the VPC, what is the minimum concentration of VPC per ml that will be produced per production lot? Will all of the proposed safety studies be performed on only a portion of each production lot? Are the assays employed for the detection of replication-competent retroviruses (RCR) sensitive enough to detect less than one RCR particle per volume, i.e., 200 ml? Are the VPC cryopreserved? If not, are these cells stable after several weeks in culture? What is the timetable from the production of the VPC to the time that they are administered to the patient? She noted that the investigators did not include the possibility of a third treatment in the informed consent document. She requested that the investigators provide an update regarding Dr. Oldfield's approved glioblastoma protocol, on which Dr. Culver is a co-investigator.

Review--Dr. Geiduschek

Dr. Geiduschek stated that the investigators had provided adequate responses to his concerns regarding the preclinical summary and the informed consent document; however, their response regarding the safety of the PA317 packaging cell line was inadequate. Dr. Culver stated in his written response that no breakout of RCR has occurred with PA317, and that there is no alternative packaging cell line that can be used for this protocol. Dr. Geiduschek said that although breakout of RCR in PA317 occurs at a relatively low frequency, it is possible. In addition, there are alternative packaging cells lines that could be used for this protocol.

Dr. Geiduschek suggested that the problems associated with RCR breakout in PA317 might be minimized by implementing the following procedures: (1) limit the period of time that the PA317 cells are expanded in culture, (2) cryopreserve the PA317 cells while a portion of these cells is grown in culture for a defined period of time to monitor for RCR breakout, or (3) use a new generation of packaging cells, i.e., Y-CRIP producer cells.

Other Comments

Dr. Miller stated that new packaging cell lines are being developed with split genomes in which the *gag* and *env* portions are separated. A canine packaging cell line is currently being developed that would eliminate the problems associated with murine helper virus production. However, the developer of this canine cell line recently reported an incident of helper virus production. Therefore, using a split genome packaging cell line does not necessarily guarantee safety. Since there is no method available to determine whether helper virus will be produced in the patient's brain, the RAC must determine that the seriousness of the disease justifies this treatment; and that there is a reasonable expectation that this procedure will be therapeutic. Dr. Haselkorn inquired whether the risk of helper virus production could be quantitated. Dr. Miller said that although the probability of detecting helper virus in a production lot could be determined, there are mitigating factors in the human; such as complement which inactivate these viruses.

Dr. Zallen noted that there was a discrepancy between the Iowa Methodist Medical Center and the University of Iowa Informed Consent documents regarding patients' financial obligations. The investigators should address the issue of patient liability in their response.

Dr. Post asked the investigators to respond to the following questions: (1) What is an Ommaya reservoir and what is the history of its use? and (2) What is the status of the glioblastoma protocol which has already been approved by the RAC? Has an immune response to the NIH3T3 cells been observed?

Presentation--Dr. Culver

Dr. Walters called on Dr. Culver to respond to the questions and comments of the RAC members. Dr. Culver explained that one of the main principles of successful tumor therapy is to treat tumor cells that are entering into the proliferative phase. Glioma cells demonstrate a very heterogeneous rate of division; therefore, this therapy will be administered over a period of 60 days in order to optimize gene transfer into the greatest number of dividing tumor cells. Patients who have evidence of recurrent tumor following the initial therapy can be retreated through the Ommaya reservoir if that recurrence is on the margin of the resection.

Dr. Culver said that the half-life of GCV is approximately 12 hours in humans. Data derived from rat and monkey animal models indicates that the producer cells survive for approximately 2 weeks. If the PA317 cells remain viable in humans for this period of time, the proposed treatment schema will allow for

maximum gene transfer and provide the greatest opportunity for the bystander effect to occur.

Dr. Culver stated that the description of the third injection of VPC into the Ommaya reservoir had been inadvertently omitted from the informed consent document; the third injection will be included in a revised version. He said that Dr. Gerard McGarrity of Genetic Therapy, Inc. (GTI), the vector supplier, would respond to the committees questions regarding safety testing of the VPC.

Dr. Culver provided a brief update on the Oldfield glioblastoma protocol. To date, 5 patients with surgically inaccessible brain tumors have received a single injection of VPC into various locations at the site of their tumor: 1 patient is currently receiving GCV therapy, and 4 patients have completed treatment. No instances of toxicity have been observed in response to either VPC administration of GCV therapy. Dr. Culver noted that initial concerns about the possibility of thrombocytopenia and neutropenia in patients recovering from chemotherapy have not been observed. Efficacy data is unavailable at this time.

Dr. Hirano inquired whether the VPC used in Dr. Oldfield's protocol are ever cryopreserved prior to administration to the patient. Dr. Culver responded that the VPC are cryopreserved and thawed immediately before they are to be administered to the patient. A minimal amount of time is allowed to count the cells and examine them for microscopic contamination. Animal studies demonstrate that cryopreserved cells are as effective as cultured cells in eliciting an anti-tumoral response.

With regard to the issue of financial responsibility, GTI is sponsoring this protocol; therefore, patients will not be responsible for any costs associated with the research aspects of this protocol. If surgery is already indicated, as it most likely will, then the costs associated with the surgical procedures will be billed to third party payers. There are differences between the two institutions regarding compensation for research related injury. The Iowa Methodist Medical Center will provide care and compensation for any acute injury related to the gene therapy procedure; there is no mechanism for long-term care. Dr. Culver introduced Dr Van Gilder to explain the compensation policy of the University of Iowa and to explain the use and history of the Ommaya reservoir.

Presentation--Dr. Van Gilder

Dr. Van Gilder stated that the University of Iowa would cover all costs associated with the research aspects of the protocol, and third party carriers will be billed for costs associated with routine surgical procedures.

Dr. Van Gilder explained that an Ommaya reservoir is a small tubing, approximately 2 millimeters in external diameter, that is placed into the center of the surgical resection. This tubing leads to a cap which is implanted directly under the skin of the skull, allowing for the direct injection of the cells through the skin and into the reservoir.
Dr. Miller asked if virus particles can pass through this reservoir? Dr. Culver answered that experiments have been performed demonstrating that high concentrations of virus particles can be passed through the reservoir with no observed decrease in viability. In addition, VPC have been passed through the reservoir and shown to exhibit the same anti-tumor effect as control cells. Dr. Van Gilder explained that Ommaya reservoirs have been used as standard treatment for the administration of chemotherapeutic agents into the cerebral spinal fluid and ventricular system of cancer patients for many years.

Dr. Leventhal asked for a clearer definition of the endpoints for the proposed study. How will efficacy by assessed? How did the investigators arrive at the proposed accrual of 20 patients? Dr. Van Gilder responded that patients will be evaluated on the basis of their Karnofsky score and by magnetic resonance imaging (MRI) and computerized tomography (CT) scans. Dr. Leventhal said that these

procedures will indicate whether the tumor is growing; however, nothing will be known about the efficacy of the treatment. If treatment failure will be indicated by growth of the tumor, how will you know if the treatment has been successful? Is patient eligibility limited to those patients who have measurable tumors on their post-operative CT scan? Dr. Van Gilder said that measurable tumor post surgery would be included as an eligibility requirement.

Dr. Van Gilder explained that it is difficult to distinguish between post-operative and therapeutic effects; therefore, sequential scanning is important. Although responses to treatment may vary, there is a consistency that is observed in response to surgery. Immediately following surgery, it is difficult to distinguish between residual tumor and edema. By the time a patient has completed their chemotherapy regimen, approximately 6 weeks post surgery, the edema has usually disappeared. Changes are generally observed in the enhanced volumes of CT scans at approximately 2 months post surgery. Sequential scanning is essential in evaluating the efficacy of the treatment. Dr. Leventhal asked whether it is possible that patients will be rejected from this study if they have no demonstrable tumor on their post-operative CT scan. Dr. Van Gilder explained that this rejection would be an extremely rare occurrence because malignant gliomas cannot be completely resected.

Dr. Leventhal asked how the residual tumor would be measured. Dr. Van Gilder explained that residual tumor is determined based on the blood brain barrier, i.e., the enhanced volume on the CT scan. Dr. Leventhal stated that the eligibility requirements should be expanded in the informed consent document to include only those patients who have measurable tumor as demonstrated by an immediate post-operative imaging procedure.

Discussion

Dr. Krogstad said that it is unclear how the investigators will determine if the patient's positive or negative response is a direct result of the gene therapy portion of the protocol. Dr. Leventhal agreed with Dr. Krogstad's conclusion and stated that the protocol should include a statistical section which outlines how the objectives of the protocol correlate with the number of patients proposed, i.e., 40 patients. Dr. Culver said that data derived from 14 patients should provide sufficient statistical information to distinguish between false positive and negative responses. Dr. Leventhal explained that 14 patients will statistically prove if there is a 95% chance that 20% of the patients may respond to the treatment. The maximum level of response has to be defined. If this therapy is toxic, then a 20% response rate may be unacceptable. If the first 14 patients demonstrate no response, will the protocol be terminated? If a positive response is observed in 1 of the first 14 patients, will the remainder of the 40 patients be treated? Dr. Culver agreed to include an expanded section regarding the definition of endpoints as suggested by Dr. Leventhal.

Dr. Chase inquired about the standard of statistical analysis for human gene transfer protocols that are approved by the RAC. Dr. Leventhal stated that investigators should be required to include a section that outlines the objective of the protocol and to describe how "X" number of patients will statistically determine that the objective has been achieved. The investigator is responsible for defining that objective, i.e., toxicity, efficacy, etc.

Dr. Haselkorn asked if the Ommaya reservoir could be used for sampling as well as for the administration of the VPC. Are there biochemical markers which can be used to determine the efficacy of the treatment using reservoir sampling? He asked Dr. Culver to respond to Dr. Post's question regarding immune status of patients that have been treated on the Oldfield protocol. Dr. Culver answered that Ommaya reservoirs are not designed for sampling purposes. The tubing is thin and flexible, aspiration would be difficult.

Dr. Culver responded to Dr. Post's question about the immune status of patients in the ongoing

glioblastoma protocol. Cell and serum samples have been cryopreserved for all patients who have received VPC and ganciclovir therapy. These samples will be evaluated in the future for immune responses to PA317. Data indicates that patients receiving 32 milligrams per day of dexamethasone are completely anergic based on immunologic analyses. Dr. Post inquired about the number of cells that patients have received to date on the Oldfield protocol. Dr. Culver answered that 5 patients have received one of the following numbers of VPC: 1.7 x 10, 5 x 10, or 1 x 10 cells. There has been no evidence of toxicity in response to any of these doses of VPC. Dr. Walters asked if any unexpected effects had been observed in the 5 patients treated to date. Dr. Culver responded that no unexpected effects have been observed as a result of the implantation of these VPC in patients.

Dr. Smith reiterated that Phase II studies such as this one should include a clearer definition of the criteria for responsiveness. The investigators need to clarify the injection schedule of the VPC. If all patients receive 1 injection of VPC, what are the criteria for more than 1 injection? Dr. Culver said that patients must have demonstrated an initial response. If progressive tumor is observed soon after the first injection, e.g., less than 3 weeks, the patient will not be eligible for further injections. However, if tumor recurrence occurs several months following the initial treatment, another injection of VPC would be administered.

Dr. Hirano inquired about the production and testing procedures for the VPC. Dr. McGarrity explained that there is a master cell bank of the VPC that contains several hundred cryopreserved ampules. Working cell banks are developed from the cryopreserved ampules. A working cell bank contains several hundred ampules. The final production lot of cells is taken from the working cell bank. Extensive quality control testing is performed at every step of this process. The VPC used in the proposed study will probably require two separate production runs. Since the cells are derived from the same working cell bank and the quality control standards are identical, there should be no differences between production runs. Dr. McGarrity explained that Genetic Therapy, Inc., is in the process of expanding the size of its production runs; therefore, alleviating any concerns associated with multiple processes.

Dr. McGarrity addressed the issue of RCR testing. Depending on the production lot, between 2.5 and 5% of each lot is removed for testing purposes. Supernatants are assayed by SL and NIH3T3 amplification on *Mus dunni* cells. A portion of the VPC are cultured for 3 weeks to ensure that no breakout of RCR occurs.

Committee Motion

A motion was made by Dr. Hirano and seconded by Ms. Meyers to approve the protocol with the following stipulations: (1) Patient eligibility will be limited to those patients with measurable residual tumor on immediate post-operative imaging studies, i.e., CT or MRI scans. (2) Patient enrollment will be limited to 15 patients divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Dr. Geiduschek asked for clarification regarding the number of VPC that were removed for extended culture. With the recently approved compassionate use exemption for a human gene transfer protocol, the RAC will have to scrutinize the underlying safety issues more than they have in the past. Dr. McGarrity stated that approximately 2.5% of each production lot undergoes extended culture for 3 weeks in order to monitor potential RCR breakout. Dr. Post inquired as to how this percentage of the production lot compares to a patient dosage. Dr. McGarrity responded that 2.5% of a production lot represents approximately 4 to 5 patient doses. Each production lot contains between 1 x 10 and 1 x 10 VPC.

Dr. Miller asked why the RAC was including the stipulation about demonstration of residual tumor if this disease is uniformly fatal. Dr. Leventhal responded that the stipulation would probably not exclude very

many patients because it is almost impossible to remove all of the patient's tumor by surgery. If the investigators proposes to establish efficacy based on stable disease for "X" period of time, then they must be able to define the "X". Stable disease for a period of time is not necessarily evidence of a response; however, stable disease could be a criteria for approving additional injections. There should be a minimum period of disease stability after the third injection before proceeding to a fourth injection.

Dr. Straus inquired about the practicality of determining residual tumor on a post-operative scan. How is residual tumor distinguished from edema? Dr. Van Gilder explained that it is often difficult to determine residual tumor on a post-operative scan; however, sequential scans are beneficial. Dr. Straus agreed with Dr. Miller that demonstration of residual tumor on a post-operative scan may be a moot issue in this uniformly fatal disease. Dr. Leventhal asserted that there are very well defined criteria for distinguishing between residual tumor and edema. However, performing a scan immediately following surgery is critical. Dr. Leventhal stated that if the investigators employ the criteria established by the Brain Tumor Study Group for evaluating residual tumor versus edema, the stipulation would be satisfied.

Dr. Krogstad said that he would be satisfied with the approval to treat 2 or 3 patients; however, the end points of this study and how efficacy will be defined should be addressed before the approval is granted to treat additional patients. Perhaps the approval of additional patients could be handled as a minor modification.

Ms. Meyers asked Dr. Culver how he would respond to any patients who requested to receive this therapy on a compassionate use basis. Dr. Culver said that all patients must meet the eligibility requirements of the protocol. If patients do not meet these requirements or the study is closed, they will be referred to othe research protocols in the U.S.

Dr. Leventhal suggested a third stipulation for approval as follows: (3) After the initial 3 injections of the VPC and GCV therapy, patients only will be eligible for additional treatments if they have demonstrated stable disease for a minimum of 6 months. Dr. Hirano and Ms. Meyers accepted Dr Leventhal's additional stipulation as part of the motion for approval of the protocol. The protocol was approved with stipulations by a vote of 19 in favor, 0 opposed, and no abstentions.

IV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: PHASE I STUDY OF GENE THERAPY FOR BREAST CANCER/DR. BANK*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Arthur Bank of Columbia University, New York, New York.

Dr. Parkman provided background information about the multi-drug resistance (MDR) gene. The MDR gene encodes a protein that can export chemotherapeutic agents out of cells. High concentrations of MDR are present in hematopoietic stem cells; however, the level of MDR expression is greatly reduced following differentiation. Since the protection provided by MDR is diminished in differentiated cells, chemotherapy usually produces a significant decrease in a patient's white blood cell and platelet counts.

The objective of this protocol is to insert the gene coding for MDR, which is linked to a promoter that maintains gene expression, into hematopoietic stem cells. The patient's cells would be relatively resistant to the effects of certain anti-tumor drugs which allow them to receive increased doses of these chemotherapeutic agents. Increasing the doses of these agents should increase the likelihood that the

patient's tumor cells will be ablated.

The investigators propose to use a Harvey murine leukemia virus vector; they have submitted preclinical data based on a murine model that demonstrates their ability to transduce hematopoietic stem cells with MDR and to confer a protective effect against several chemotherapeutic agents, particularly Taxol.

Patient eligibility is limited to women with Stage IV breast cancer who are undergoing autologous bone marrow (ABM) transplantation. Approximately one-third of the patient's bone marrow will be enriched for CD34(+) cells, transduced with the MDR retrovirus vector, and cryopreserved. At the time of transplantation, the patient will receive a mixture of transduced and untransduced ABM cells. The untransduced portion of the patient's ABM cells is sufficient for hematopoietic engraftment. Following transplantation, the patient will be monitored by polymerase chain reaction (PCR) for the MDR gene. The patient's cells will be examined for colony growth, i.e., resistance to the effects of various chemotherapeutic agents.

Patients who demonstrate persistent or progressive disease at day 50 will receive Taxol administration. Following Taxol, patients will be monitored for any increase in the proportion of MDR-expressing cells resulting from the ablation of cells that do not express the MDR gene.

Dr. Parkman said that there are still several remaining questions about this protocol. A hard copy of the complete vector sequence was not made available to all of the members of the committee although Dr. Miller has screened the sequence by computer analysis and has assured the committee that the vector is acceptable. The investigators have not provided any long-term culture or animal data demonstrating that the MDR gene has been incorporated in relatively immature hematopoietic progenitors. The submitted data is based on days 12 and 14 short-term cultures. The investigators have not demonstrated that they can transduce the gene into immature hematopoietic cells and that the progeny of these cells express the MDR gene. In addition, the investigators have not stated the length of time that they expect to observe gene expression in these patients. How long will patients have to be followed after ABM transplantation to demonstrate the presence of resistant colonies and PCR positive cells, which is indicative of transduced committed progenitors?

The administration of Taxol is another aspect of this protocol that should be considered by the RAC. Taxol is not routinely administered following ABM for breast cancer at Columbia University. Since the administration of Taxol is a procedure that is not independent of the gene transfer procedure, then the committee has a responsibility to review all aspects of the protocol. Dr. Parkman stated that his written review asked if patients tolerated 175 milligrams per meter squared (mg/m) of Taxol 50 days following ABM transplantation. The investigators responded that one patient had received this dose of Taxol, 175 mg/m; however, the drug was administered on day 90 instead of day 50. He stated that data obtained from 1 patient treated on day 90 following ABM does not justify the treatment of many patients on day 50 following ABM.

Dr. Parkman summarized the questions that the investigators should respond to during their oral presentation: (1) Is there preclinical data demonstrating gene transduction into immature hematopoietic progenitors as opposed to committed progenitors? (2) What are the endpoints of the study? and (3) Is there any additional clinical data regarding the safety of Taxol administration at the dose proposed for this study?

Review--Dr. Krogstad

Dr. Krogstad stated that the investigators had responded adequately to his concern about the use of the

term MDR-1 for both the genomic DNA message and the gene product. It is unclear how gene expression resulting from the transduced gene will be distinguished from the natural expression of MDR from genomic DNA. Since 1 of the objectives of this study is to select for MDR-1 producing cells or cells that exhibit greater levels of p-glycoprotein, the investigators should provide further clarification on this issue.

One of the specific objectives of this protocol is to assess the toxicity of the treatment. Due to the large number of variables within this protocol, i.e., ABM transplantation and Taxol administration, it is likely that any toxicity resulting from the gene transfer portion of the protocol will be missed.

Dr. Krogstad said that there is concern about the possibility that malignant cells in the patient's bone marrow might be inadvertently transduced with the MDR gene in addition to the normal cells. Published data in the scientific literature indicates that between 30 and 50% of breast cancer patients have bone marrow involvement; however, the extent of involvement is unclear. How effectively will tumor cells be removed by the CD34(+) selection process? If tumor cells fail to be removed by the selection procedures, will they survive the *in vitro* culture conditions and inadvertently become transduced? These questions must be satisfactorily answered before patients can be allowed to participate in this protocol.

Other Comments

Dr. Brinckerhoff agreed with Dr. Krogstad's concerns about the possible transduction of tumor cells which would render them resistant to chemotherapy. There are no techniques available at the present time that can detect breast cancer cells that may contaminate the bone marrow.

Ms. Meyers stated that she has several concerns about the informed consent document. All references that gene therapy or gene transfer is safer should be deleted. Gene therapy is still in its very early stages, and all possible outcomes are not yet known. The statement, "Taxol is a new drug which has been shown to be effective in the treatment of breast cancer" should be deleted. Taxol has been approved for the treatment of ovarian cancer, but it is still considered experimental in the treatment of breast cancer. Patients should not be responsible for the experimental costs either personally or through their insurance. This procedure is very experimental, not only the gene therapy portion, but also the bone marrow transplantation procedure. The informed consent document should include a statement recommending that the patient should not be pregnant or planning to become pregnant. A statement should be included about a request for autopsy. The investigators should explain how they would respond to patients who might request this treatment on a compassionate use basis.

Dr. Parkman said that he had requested that the informed consent document be revised in his written comments to the investigators and was disappointed that the revised document was not submitted. With regard to the issue of contaminating breast cancer cells in the bone marrow, this issue has been previously discussed during the review of Dr. Joyce O'Shaughnessy's protocol at the December 1992 RAC meeting. During the December 1992 meeting, Dr. Cynthia Dunbar stated that the majority of breast cancer cells are already MDR positive, and that data from other laboratories has demonstrated that there is a significant reduction in the number of breast cancer cells after CD34(+) selection. The discussion should not focus on concerns of transducing non-MDR expressing tumor cells, but only on the possibility of over-expression of the gene. The CD34(+) selection procedure reduces the total number of cells that are to be transduced, therefore, reducing the relative proportion of contaminating breast cancer cells. Dr. Brian Sorrentino, a co-investigator on Dr. O'Shaughnessy's protocol agreed with the comments made by Dr. Parkman. Dr. Sorrentino noted that one of the differences between the two protocols is that Dr. O'Shaughnessy's protocol included a salvage therapy with 5-fluorouracil (5-FU) and other chemotherapeutic agents, which are not MDR pump drugs; therefore, their effectiveness would not be affected by MDR gene transduction.

Dr. Carmen explained that he had been assigned as a primary reviewer of Dr. Bank's protocol before it was withdrawn for review from the September 1992 RAC meeting. How does the current protocol differ from the previously submitted protocol other than the fact that ovarian cancer is no longer the targeted disease? What is the status of the murine immunodeficiency model? Dr. Carmen then submitted suggested language for inclusion in the informed consent document.

Dr. Post noted that the proposed vector includes 6 additional kilobases which are upstream from the MDR cDNA. What are these additional sequences? Does it make *gag*? Are any additional retrovirus proteins made? Does this MDR vector have the same point mutation and frame shift that Dr. O'Shaugnessy's vector had? What is the status of characterizing the performance of this vector in the proposed packaging cell line? What is the status of Institutional Review Board (IRB) approval of this protocol? The RAC received a document which granted approval in principle; what are the specifics of approval?

Dr. Leventhal stated that all investigators submitting human gene transfer protocols to the RAC should grow and/or purify the target population of human cells and transduce these cells with the same vector proposed for the study. There is no data demonstrating that purified human CD34(+) cells have been transduced with the proposed vector.

Since the target cell population exhibits MDR expression, which decreases as the cells mature, the investigators must demonstrate that the transduced CD34(+) cells express higher levels of MDR than the cells produce spontaneously. What is the effect of transduction?

Dr. Geiduschek stated his concern about the quantitative expression of MDR in the transduced population. The investigators have not submitted data to address the critical question, namely, a comparison between Taxol-selected transduced and untransduced cells. Does Taxol selection cause an increase, decrease, or sustained expression of MDR? The section of the protocol that describes the CD34(+) selection procedure should be clarified. The investigators propose that 30-50% of the total number of bone marrow cells harvested will be removed for CD34(+) selection, depending on the total cell harvest yield. What factors will influence this percentage? The following statement needs to be clarified by the investigator: "We will evaluate the safety of gene transfer by looking for the presence of intact retrovirus in the bone marrow; this is the major risk." The presence of intact retrovirus would be for only a brief duration. Are there other parameters that will be used to monitor the safety of this procedure in patients?

Dr. Miller stated that he has screened the proposed vector. The oncogenes have been removed, and the MDR cDNA extends through approximately 50% of the vector. No other additional open reading frames were identified. The investigators should describe the additional sequences that are at the tail of the vector. Dr. Post inquired about the sequences that are upstream from the MDR cDNA. Dr. Miller said that the upstream sequences are a derivative of a leukemia virus and contain the packaging signal. Dr. Post asked if there was any *gag* upstream from the cDNA. Dr. Miller responded that no *gag* sequences are present. The vector is approximately 9,000 base pairs (bp) from the 5' long terminal repeat (LTR) to the 3' LTR. The MDR cDNA is approximately 4,000 bp.

Dr. Miller explained that the investigators provided data derived from only 1 experiment demonstrating their ability to transduce long-term reconstituting stem cells in mice. This experiment was performed by co-cultivation of the marrow cells with the virus producing cells. However, the human study will use virus supernatant for transfection as opposed to co-cultivation with producer cells. Virus supernatant has been demonstrated to be much less efficient than co-cultivation for transferring genes to bone marrow cells. The virus titer projected for this vector is 10 colony forming units (CFU) per ml. This concentration is much less

than other investigators have reported as required for successful transduction of bone marrow cells. The *in vivo* transduction data is insufficient to support the proposed study. Dr. Parkman said that the investigators should perform additional long-term *in vitro* human experiments with the proposed vector. Dr. Miller asked the investigators if there is a large animal model that would support this study.

Presentation--Dr. Bank

Dr. Bank introduced his collaborators: Drs. Steve Goff and Charles Hesdorffer of Columbia University, New York, New York, and Dr. Michael Gottesman of NIH, Bethesda, Maryland. Dr. Bank explained that he had reviewed the comments made by the RAC in response to Dr. O'Shaugnessy's protocol, and that this protocol addresses all of the RAC members recommendations. Dr. Bank said that he has PCR data demonstrating that human CD34(+) cells can be transduced with the proposed vector.

In response to Dr. Geiduschek's concerns about MDR expression, Dr. Bank explained that gene expression will be monitored by fluorescence activated cell sorter (FACS) analysis using the 17F9 antibody. When unselected bone marrow cells are transduced with the MDR vector, 10-20% of the cells demonstrate a 5-10-fold increase in MDR expression. One CFU-granulocyte/erythrocyte/megakaryocyte/monocyte (GEMM) resistant colony assay was performed using CD34(+) transduced cells in which 2 of 7 resistant colonies grew. Dr. Bank stated that he is currently in the process of defining the endpoints of this study to determine efficacy.

In response to Dr. Carmen's question about the status of the immunodeficient mouse model, Dr. Bank explained that CD34(+) cells have been transferred to severe combined immunodeficient (SCID) mice; however, this procedure is difficult and the results are inconsistent.

With regard to Taxol administration, Dr. Bank explained that numerous breast cancer patients have received Taxol administration at Columbia University; however, they have not received bone marrow transplants. Dr. Parkman stated that the addition of ABM presents a very different biological setting than Taxol administration alone. Data must be submitted from patients that have received both Taxol and ABM transplantation. This data does not necessarily have to be derived from breast cancer patients. Data from 3 patients does not provide sufficient justification for this protocol.

Other Comments

Dr. Parkman explained that in order to draw a conclusion about the protective effect of the proposed therapy, there must be criteria established for defining a response to Taxol administration alone. Taxol response must be considered in addition to gene transduction and expression. Dr. Hesdorffer responded that patients will continue to be accrued onto Taxol protocols who have had ABM transplantation without transduction. Of the 3 patients who received ABM transplantation and Taxol administration, 1 ovarian cancer and 1 breast cancer patient demonstrated a response. Data is still being accrued on the third patient. Dr. Parkman explained that in order for the RAC to approve this protocol, the risk to the patient must be assessable. Since the response to Taxol administration and untransduced ABM cells alone is unknown, the effect of the inserted MDR gene cannot be evaluated.

Dr. Leventhal stated that the investigators are obligated to define a stopping rule based on their clinical experience if adverse effects occur, i.e., aplasia, resulting from this treatment. Dr. Bank responded that they have defined toxicity criteria for giving patients recurrent doses of Taxol after ABM transplantation, and that Taxol will not be administered to patients until their white counts have reached 300-500.

Dr. Bank responded to Dr. Krogstad's question about distinguishing between gene expression resulting

from the transduced gene and natural expression of MDR from genomic DNA. The inserted MDR gene can be distinguished from natural MDR by the following: (1) there is a single mutation that is distinguishable by reverse PCR, (2) glycine is substituted for a valine at position 185, and (3) there are different sequences in the 5' end.

With regard to the assessment of toxicity, Dr. Bank responded that if any patient develops a lymphoma or other type of tumor, the investigators will look forinsertional mutagenesis. In addition, vector producing cells will be assayed for replication competent retrovirus. This treatment should not present any added risk to the patient, i.e., administration ofMDR-resistant breast cancer cells. In order to impose increased risk to the patient, transduced tumor cells would have to demonstrate biologic virulence that would cause them to repopulate the marrow. Otherwise, there is very little consequence; because it is rare that the bone marrow is the site of tumor recurrence.

Ms. Meyers inquired about the financial responsibility for the costs associated with the proposed study. Dr. Hesdorffer stated that arrangements will be made with third-party carriers to cover the costs associated with the ABM transplantation procedures. Any costs specifically related to the transduction of the cells will not be covered by the institution. Additional procedures such as blood tests, bone marrow harvest, and any pre- and post-transplantation procedures will be billed to third-party carriers.

Ms. Meyers asked who would be responsible for the costs associated with adverse side effects should any arise as a result of the gene transfer procedure. Dr. Bank answered that the patient's third-party carrie would be billed for such costs. Dr. Hesdorffer stated that if toxic side-effects occur as a result of theTaxol administration, a third-party carrier would be charged for costs associated with treatment; however, adverse effects relating to the gene transduction procedure would be covered by Columbia University. Ms. Meyers inquired if the institute would cover any injury not related toTaxol administration or bone marrow transplantation. Dr. Bank agreed that the institution would cover these injuries.

In response to the issue of whether the investigators would seekNIH approval for compassionate use of this protocol for patients who do not meet the eligibility requirements, Dr. Bank said that Columbia University would probably not pursue such a request, because there are numerous breast cancer research protocols to which patients could be referred. He noted that fullIRB approval has been obtained and is available.

Dr. Post asked if the investigators have data using the exact packaging cell line and vector that is being proposed of this study. Dr. Bank responded that the only data available with the proposed vector are reverse transcriptase (RT) assays on supernatant from thetransduced bone marrow cells. Dr. Bank stated that S L assays and co-cultivation with *Mus dunni* cells have not been performed. The proposed packaging cell line has been used with other vectors in over 300 laboratories throughout the world without any incidence of RCR breakout.

Dr. Miller asked whether any of these laboratories have assayed for helper virus. Dr. Bank said that approximately 20 of the 300 laboratories had tested for helper virus. Dr. Miller said that experiments must be performed with the proposed packaging cell line and vector in order to demonstrate that helper virus has not been produced. With regard to the vector titer, Dr. Bank stated that the titer is approximately 5 x 1 as assayed by colchicine resistance; however, this number may be an underestimate.

Dr. Parkman noted that the eligibility criteria for patients who would receive Taxol treatment is not clearly stated in the informed consent document. Dr. Bank responded that he would clarify this section of the informed consent document.

Dr. Leventhal noted that patients will have 3 x 10 ABM cells harvested; 1 x 10 of these cells will be transduced. What procedures will be followed if the proposed number of cells are not obtained in the original harvest? Dr. Hesdorffer answered that if 3 x 10 ABM cells are not obtained then either a lower number of cells will be transduced or no cells at all.

Dr. Bank presented additional data demonstrating an increase in gene expression following Taxol administration. PCR amplification showed that the signal changed from negative to positive after Taxol and a 10-fold increase in MDR expression was observed by FACS analysis 8 months post-transduction in the murine model. FACS analysis of human marrow demonstrated that MDR expression in untransduced early cells is negligible. However, 15-20% of the cells demonstrated a log increase in MDR expression following transduction and 5 days in culture.

Dr. Bank introduced his co-investigator, Dr. Steve Goff, to respond to the committee's questions regarding the vector sequences. Dr. Goff explained that the additional sequences at the end of the vector are derived from the Harvey sarcoma virus genome which has part of VL30 and *env* before the LTR. None of this portion of the vector contains open reading frames. Dr. Post noted that during the review of Dr. O'Shaugnessy's protocol, there was some confusion about the frame shift at the end of the MDR cDNA. Can you assure the RAC that your vector does not contain the same frame shift? Dr. Bank stated that his vector does not contain a frame shift at the end of the MDR cDNA.

Dr. Parkman stated that approval of this protocol should be deferred because it lacks necessary information. There are basic requirements that a protocol should meet prior to submission: (1) investigators should demonstrate the lack of replication competent virus with the proposed vector. The minimum standard for RCR testing is the S L assay. (2) transduction and expression should be demonstrated using the proposed vector and target cell. Transduction does not always have to be performed in an animal model; *in vitro* long-term cultures may also be acceptable. (3) the clinical protocol must include extensive detail regarding all proposed procedures and assays. This protocol does not meet any of the aforementioned criteria.

Committee Motion

A motion was made by Dr. Parkman and seconded by Ms. Grossman to defer approval of the protocol. There was a brief discussion between RAC members in which they agreed that Dr. Bank had not demonstrated transduction in the reconstituting cell population that would ultimately be administered to the patient. Although transduction data was submitted using the murine stem cells, the same transduction protocol was not used. Dr. Bank noted that he has successfully transduced CFU-GEMM cells. Dr. Leventhal said that the CFU-GEMM data was not submitted to the RAC. Dr. Parkman stated that additional data should have been submitted demonstrating the effect of the proposed dose of Taxol following ABM transplantation. Safety must be demonstrated with the proposed vector. Dr. Leventhal stated that data should be obtained demonstrating transduction of CD34(+) cells in a "macro" system.

Dr. Michael Gottesman, a co-investigator, commented that there is an implicit assumption that the MDR gene has to be inserted into stem cells in order to provide protection. Short-term protection by active cells might be equally as effective in providing protection against the chemotherapeutic agents as stem cells; particularly since stem cells already exhibit a slight degree of MDR protection. One of the goals of this protocol is to determine whether the MDR gene will be inserted into stem cells. Therefore, failure to demonstrate transduction of stem cells is not a valid reason for disapproval of this protocol. Dr. Parkman stated that the objectives of the protocol had not been presented in this manner. If the protocol was designed as a trafficking study to determine which cell populations are transduced by this procedure, the RAC may have approved the study as a legitimate protocol. The protocol has not been written with this

intent. Dr. Leventhal agreed with Dr. Parkman and noted that endpoints are the real issue. If stem cell transduction is demonstrated, the patients could probably receive several courses of Taxol. If committed progenitor cells are transduced, patients may require ABM transplantation after each course of Taxol.

Approval of the protocol is deferred until the investigators provide the following additional information for full RAC review: (1) safety data, e.g., S L assays demonstrating the absence of RCR using the proposed vector, PHaMDR1/A and the amphotropic retrovirus packaging line, GP+envAM-12; (2) toxicity data demonstrating the effect of Taxol, at doses comparable to those proposed in the protocol, in patients undergoing ABM transplantation; and (3) transduction of CD34(+) bone marrow cells in a large animal model or long-term bone marrow culture.

The motion to defer approval of the protocol was approved by a vote of 20 in favor, 0 opposed, and no abstentions.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: ADMINISTRATION OF NEOMYCIN RESISTANCE GENE MARKED EBV SPECIFIC CYTOTOXIC T LYMPHOCYTES TO RECIPIENTS OF MISMATCHED-RELATED OR PHENOTYPICALLY SIMILAR UNRELATED DONOR MARROW GRAFTS/DRS. HESLOP, BRENNER, ROONEY*

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Drs. Helen E. Heslop, Malcolm K. Brenner, and Cliona Rooney of St. Jude Children's Research Hospital, Memphis, Tennessee.

Dr. Straus stated that the objective of the proposed study is to mark Epstein Barr Virus (EBV)-specific cytotoxic T lymphocytes (CTL) with the gene encoding neomycin resistance (neo). The rationale for this protocol is based on the fact that recipients of mismatched related or unrelated bone marrow cells are at a substantial risk for developing EBV-associated lymphoproliferative disorders. The investigators propose to obtain EBV-specific CTL from the donor, transduce these cells with a retrovirus vector encoding neo, and adoptively transfer the transduced cells to the recipient. The recipient will be monitored for reconstitution of the EBV-specific CTL response, which would prevent the outgrowth of EBV-transformed T cells and the persistence of marked donor cells. The investigators have demonstrated their ability to generate EBV-specific CTL with a 5-25% transduction efficiency.

Intravenous administration of transduced CTL will be initiated day 45 post-bone marrow transplantation and continued weekly for a total of 4 weeks. Three groups of patients representing 3 to 6 patients per group will receive one of the following dose-escalations: (1) 1 x 10 to 5 x 10 cells, (2) 5 x 10 to 1 x 10 cells, or (3) 1 x 10 to 5 x 10 cells.

Survival of the neo CTL and the level of EBV-specific DNA from peripheral blood and saliva samples will be monitored by PCR amplification. Immunologic responses will be monitored *in vitro* by CTL assays and lymphocyte phenotyping. Dr. Straus stated that the investigators have extensive experience with all aspects of the proposed study, and that they have addressed the reviewers questions and comments adequately.

Review--Dr. DeLeon

Dr. DeLeon noted that the investigators had satisfactorily responded to her comments regarding the

informed consent document and recommended that the protocol be approved.

Review--Dr. Carmen

Dr. Carmen stated that this protocol is very well designed. In particular, the investigators have provided a discussion about the inappropriateness of using the *herpes simplex*-thymidine kinase/ganciclovir system for this study. The comparison of methodologies that was included in the submission was informative.

Other Comments

Ms. Meyers stated that the informed consent document is very well written and that her comments are minor. The parent and patient informed consent documents should include the statement, "The patient will not derive any personal benefit from this protocol; however, knowledge will be gained which will benefit others." The document should include a statement regarding patient follow-up. All gene transfer patients should be followed for life and suggested that the NIH establish a patient registry. A request for autopsy in the event of accidental death should be included in the parent informed consent document. Statements should be included about financing, confidentiality, access to records, and statements about contraception and pregnancy, i.e., for teenagers.

Dr. Zallen responded to Ms. Meyers comment about the establishment of a gene transfer registry. Dr. Zallen inquired about an appropriate course of action that the RAC could take regarding the establishment of gene transfer registry. Dr. Walters reminded the RAC members that Dr. Fred Ledley, Texas Medical Center, Houston, Texas, previously submitted a proposal to the RAC regarding a human gene transfer patient registry for information purposes. Dr. French Anderson, University of Southern California, Los Angeles, California, stated that he has worked with Dr. Ledley for approximately 10 years in an effort to obtain financial support for a patient registry. However, because of the current budget cuts, the registry may not be supported by the NIH. Dr. Leventhal said that it is not appropriate to discuss the future of a patient registry during the discussion of Dr. Heslop's protocol, and the RAC should focus their comments on the EBV-specific CTL study.

Dr. Walters inquired as to whether this protocol is considered a therapeutic or a trafficking study. Dr. Heslop responded that this protocol is a Phase I study; therefore, efficacy will not be determined. Dr. Heslop stated that although it would be encouraging if an effect was observed from the adoptive transfer of these EBV-specific CTL, this protocol is solely a marking study.

Dr. Leventhal explained that human gene transfer and therapy protocols should be categorized by the function of the gene that is inserted. Since the gene transferred for this study encodes for neo, which has no therapeutic potential, this protocol should be considered gene transfer.

Committee Motion

A motion was made by Dr. DeLeon and seconded by Dr. Leventhal to approve the protocol. The protocol was approved by a vote of 19 in favor, 0 opposed, and no abstentions.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: ASSESSMENT OF THE EFFICACY OF PURGING BY USING GENE-MARKED AUTOLOGOUS MARROW TRANSPLANTATION FOR CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA IN FIRST COMPLETE REMISSION/DRS. BRENNER, KRANCE, HESLOP, SANTANA, AND IHLE*

Review--Dr. Chase

Dr. Walters called on Dr. Chase to present his primary review of the protocol submitted by Drs. Malcolm K Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle of St. Jude Children's Research Hospital, Memphis, Tennessee.

Dr. Chase explained this is a marking study in which two different retrovirus vectors, LNL6 and G1Na, will be used to compare the efficacy of two bone marrow purging techniques, 4-hydroxyperoxicyclophosphamide (4-HC) and interleukin-2 (IL-2) activation of cytotoxic effector cells. Data from previous studies indicates that gene-marked malignant cells have been identified in the marrow of remission patients and can contribute to recurrent disease. Therefore, the current purging methods probably do not eliminate all of the malignant cells in the reinfused marrow.

Review--Dr. Doi (presented by Dr. Chase)

In Dr. Doi's absence, Dr. Walters asked Dr. Chase to summarize Dr.Doi's written comments regarding this protocol.

Dr. Chase noted that the investigators had responded to all of Dr.Doi's written comments and questions. Dr. Doi had inquired whether it was possible to determine the percentage of relapsed cells that contained marker genes in the 2 marked relapse patients from the previous study. The investigators' response to this question was that 2% of the relapse cells were marked; however, since only one-third of thereinfused marrow was transduced, the total number of marked cells could be as much as 6%.

Dr. Chase commended the investigators for their responses regarding the patient sample size. A detailed discussion and published article about the sample size and power computations has been included. Dr. Chase suggested that all investigators should submit an analysis section according to these standards. He complemented Dr. Brenner on his clear explanation of the issues and recommended that the protocol be approved.

Review--Ms. Meyers

Ms. Meyers stated that this protocol is the logical next step in a series of protocols that have been initiated by the investigators. The initial concerns about the informed consent document have been adequately responded to, except for the addition of a request for autopsy in the parent consent form. Dr. Brenner had stated that it is the policy of St. Jude Children's Research Hospital to verbally notify all patients and their parents that an autopsy will be requested in the event of death; however, this statement is not included in the informed consent document. Ms. Meyers stated that it is important that this statement be reflected in the parent consent form.

Other Comments

Dr. Parkman inquired whether the IL-2 purging procedure will be performed in Vancouver; and if so, will the bone marrow cells be shipped? If the cells are shipped, what are the risks involved? In addition, there will be a 6 hour delay between cell isolation and 4-HC purging due to the transduction procedure. Is the sensitivity of the leukemic or normal cells to 4-HC affected by the long incubation period?

Dr. Post asked the investigators to provide information about the patients who have receivedtransduced cells and not relapsed on their previously approved protocols. Do they have marked cells? Do you have data regarding the efficacy of transduction, etc.?

Dr. Haselkorn noted that other investigators have utilized other purging methods such as magnetic bead/antibody selection procedures. Why has 4-HC and IL-2 activation been chosen over other selection procedures?

Presentation--Dr. Brenner

Dr. Brenner stated that 12 patients will be entered onto this protocol per year. At the end of each year the results of the protocol will be assessed. The ongoing study was initiated in September 1991, and 12 patients have been entered onto the protocol. Two of these patients have relapsed; both of these have been marked. In addition, a third patient has relapsed as demonstrated by cytology; however, this patient has not been clinically determined to be in relapse. A maximum number of 35 patients will be enrolled only if no marked relapses have been observed. A significant number of marked relapses will indicate reconsideration of the protocol.

In response to Ms. Meyers' concerns about the request for autopsy in the informed consent document, Dr. Brenner said that he did not believe that the St. Jude'sIRB will ever approve such a statement. Dr. Brenner noted that there have been 4 patient deaths to date that have not been associated with the gene marking procedure, and that all 4 patients underwent autopsies at the time of death. This standard reques is entered into the patient's chart at the time the family is contacted.

Ms. Meyers asked if all of the deaths occurred at St. Jude. Dr. Brenner responded that 3 of the 4 patients died while at St. Jude, and that all of the autopsies were performed at St. Jude.

Dr. Brenner explained that the IL-2 purging procedures will be performed in Vancouver. Four bone marrow preparations have been shipped back and forth from St. Jude to Vancouver, and the cells have remained viable. After the cells are purged in Vancouver, they will be sent back to St. Jude to be grown in culture for 7 days and then cryopreserved. The purged cells have retained their viability upon thawing.

Dr. Parkman inquired if any patients have received ABM transplantation with IL-2 purged cells that have not been gene-marked. Dr. Brenner answered that no patients have received IL-2 purged ABM cells at St. Jude; however, 14 patients have undergone this procedure at another institution. Dr. Brenner stated that there is no evidence that the sensitivity of these cells to 4-HC is altered following the 6 hour incubation period.

Dr. Brenner stated that all of the patients who have not relapsed areevaluable for engraftment. Of these 18 patients, 16 demonstrated marked cells which are hematopoietic progenitor cells, lymphoid cells, or a combination of both. The level of marking ranges between 1 and 12%, which is much greater than was originally anticipated.

Dr. Brenner explained that the reason that the magnetic bead separation procedures have not been used for these studies is that the technique requires an antibody that specifically recognizes theleukemic cells. These cells do not usually possess a unique phenotype. The 4-HC and IL-2 purging methods were chosen because effective purging will probably prove to be a combination of these two techniques. To date, the most successful treatment of leukemia has proven to be a combination of therapies which do not cause excessive toxicity.

Dr. Parkman asked Dr. Brenner whether the St. JudeIRB was concerned that the shipment of cells to Vancouver imposed any increased risk to the patient. Dr. Brenner said that two-thirds of the marrow remains cryopreserved at St. Jude; therefore, any unforeseen loss of the shipped cells should not affect

engraftment of the untransduced cells. Dr. Brenner agreed; however, that the shipment issue should be included in the informed consent document.

Dr. Post noted that all patients will receive both purging procedures. He inquired if there would be any additional benefit if the patients were divided into groups that received only a single treatment of either 4-HC or Il-2. Dr. Brenner responded that although the patients may benefit from a single treatment, it is unknown which is the most efficacious method. There is no data demonstrating that either of these treatments alone or in combination is effective. Dr. Leventhal said that parallel studies with single vectors and purging methods would require a larger number of patients than this simultaneous labelling experiment.

Dr. Leventhal asked how the investigators are interpreting the data which demonstrates 80% gene marking in patients who are still in remission. Dr. Brenner responded that they are transducing a long-lived progenitor cell, if not a pluripotent stem cell. This assumption is based on GEMM colonies growing out at 1 year as well as marking of T and B cells. Four of these patients have an A21 translocation. Other patients have demonstrated CD34 and CD 56 double positive cells. In the neuroblastoma study, 2 patients have relapsed, 1 of which was marked. Dr. Parkman asked if the neuroblastoma relapse was at the original site of disease. Dr. Brenner said that biopsies are currently being analyzed; therefore, the only known site of relapse at this time is the marrow.

Committee Motion

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

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

Review--Dr. Leventhal

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina.

Dr. Leventhal provided a brief overview of the proposed study. This protocol is a Phase I trial of human gamma interferon (g-IFN)-transduced autologous tumor cells in patients with disseminated malignant melanoma. Autologous tumor cells will be grown in short-term culture and transduced with the gene encoding for g-IFN. Patients will receive subcutaneous injections of 3 x 10 transduced irradiated tumor cells every 2 weeks for 3 months and 1 injection per month for a total of 24 injections.

Animal data has been submitted demonstrating that transduced cells grow at a slower rate than untransduced cells, and that a CTL response is generated. The investigators propose that the delayed growth rate of the tumor cells is a secondary effect of the CTL. Dr. Leventhal noted that her written comments to the investigators referred to the fact that the data did not demonstrate a diminution or maintenance of tumor growth in an immune-competent animal. The data submitted by Dr. Seigler showed that g-IFN transduced B16 melanoma cells grow more slowly than untransduced tumor cells in nude mice. Dr. Leventhal stated that the murine *in vivo* data suggests that the slowed growth rate of the tumor cells may not be due to the generation of CTL, but to natural killer cells or some other mechanism.

Based on this *in vivo* data, there is concern about the specific parameters that will be monitored in terms of patient response, i.e., the generation of CTL. The investigators have not provided data demonstrating that the injection of transduced tumor cells at 1 site affects the growth of untransduced tumor cells at other sites. Although the murine data suggests that transduced cells prevent the occurrence of tumor at other sites when injected intravenously, they have not demonstrated any effect on established tumor nodules.

The investigators have agreed that patients must have measurable lesions following the removal of the tumor cells for transduction. Dr. Leventhal said that she had requested that a statement be included in the informed consent document which explains to the patient that tumor cells will be removed in an attempt to perform this experiment; however, there is a possibility that they may not receive any injections.

The endpoints of this study have not been well-defined. The investigators have stated that toxicity will be the endpoint; it is unclear how toxicity is defined. They state that the development of a granuloma or other vigorous immune response at the site of the injection is the desired endpoint. If the generation of CTL will be used as the endpoint, the animal data is insufficient to justify this evaluation criterion.

Review--Dr. Parkman

Dr. Parkman said that he was concerned about the lack of data demonstrating an anti-tumor effect on established tumor. The investigators have indicated that tumor-specific CTL can be induced by *in vitro* stimulation of peripheral blood lymphocytes (PBL) using autologous or human leukocyte antigen (HLA)-matched tumors. However, the data which was submitted was generated using a cell line. In addition, there is no independent control included to demonstrate the nonspecific effect of g-IFN.

Dr. Parkman stated that his written review asked if there is a minimum amount of g-IFN that is necessary to achieve the desired effect. The investigators answered that a minimum of 2 units of g-IFN/1 x 10 cells/day would be required. Dr. Parkman noted that the investigators did not provide a dose-response curve comparing the amount of g-IFN to the induction of Class II expression, which is one of the proposed mechanisms of the anti-tumor response.

Review--Ms. Meyers

Ms. Meyers stated that the revised version of the informed consent document submitted by the investigators is much improved over the original; however, it still does not include a request for autopsy. The investigators should describe the financial obligation of the patient for the treatment of any adverse effects which might possibly result from the gene transfer procedure.

Other Comments

Dr. Zallen said that the informed consent document is poorly written. When the tumor cell transduction process is described, the investigators should explain that this procedure will be conducted *in vitro*, not *in vivo*. This section should also include a statement explaining that if the *in vitro* procedure is unsuccessful, then the patient will not receive any cell injections. The *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* should be clarified with regard to potential benefits and possible adverse reactions. The investigators should explain the entire informed consent process at Duke University Medical Center.

Dr. Post asked the investigators to provide further information about the proposed vector and packaging cell line. Is there homology and overlap between them? What is the investigators' experience with this vector and this packaging cell line?

Dr. Miller presented a brief description of the proposed vector. This vector has a g-IFN coding sequence and a mutated *gag* start codon, ATT, which prevents *gag* translation. There is an LTR, a packaging signal that contains portions of *gag*, and the g-IFN gene. Then there is an SV40 neo sequence at the 3' end that will be used for selection purposes.

Dr. Miller said that one possible concern is that this vector produces a protein that is made in the *gag* region upstream from the CTG start codon, or glycosylated *gag* protein, which has been altered in other vectors previously been approved by the RAC. It is unknown what effect this protein would have on the protocol. The investigators have provided safety studies using this vector which indicate that this extra open reading frame should not be a serious concern.

Dr. Miller said that one of the packaging cell lines that is mentioned in the protocol has been demonstrated to generate helper virus in the past; however, the investigators will probably propose to use another version of that packaging cell line which is more contained. The RAC must keep in mind that any packaging cell line will generate helper virus at some rate, and that the breakout that occurred was a nonhomologous recombination. There was no overlap in the *env* region between the vector and the packaging cell line.

Dr. Anderson stated that the precise mechanism of the breakout is unclear; the issue of homologous versus nonhomologous recombination has not been absolutely determined. Dr. McGarrity stated that if homologous recombination occurs, it will probably occur within the vector and packaging system. There is an overlap in the *gag* region of approximately 50 base pairs; however, there is no overlap in the *env* region. Dr. Geiduschek stated that although the recombination event should not be categorized prematurely, it is extremely important to understand the mechanisms of these rare events in order to gain an understanding of the risks. When the mechanism of recombination is determined, the RAC should be notified of this information immediately.

Presentation--Dr. Seigler

Dr. Seigler responded to the RAC members questions regarding the eradication of established tumor in the murine model. The murine experiments have not been performed because they do not accurately represent the human situation. Murine and human g-IFNs are species-specific; therefore, the research has focused on the human situation.

Data was presented demonstrating decreased tumor growth in response to transduced B16 melanoma cells in immune-competent mice. Additional data indicated that a decrease in the growth rate of L33 tumor cells was due in part to CTL based on the fact that tumors do not develop in normal mice but do occur in nude mice.

Dr. Seigler stated that endpoints will be determined by two measurements: (1) tumor status, and (2) the generation of CTL. Patients with stable disease will remain on the study, and these who demonstrate progressive disease will not continue in the protocol. If 10 patients demonstrate no CTL response, the study will be terminated unless there is clinical evidence of tumor response, i.e., stable or regressing tumor.

Dr. Parkman asked if there are data demonstrating CTL responses from autologous PBL to tumor cells. Dr. Seigler said that CTL responses were generated *in vitro* to tumor cell lines. Dr. Parkman stated that established cell lines are very different from autologous fresh tumor cells. Dr. Darrow of Duke University Medical Center, Durham, North Carolina, stated that the induction of melanoma-specific CTL has been

demonstrated with both transduced and untransduced cells against tumor cell lines and fresh autologous tumor cells. Transduced tumor cells demonstrate a significant increase in the level of cytolytic activity. Dr. Parkman said that this data should have been provided to the RAC. Dr. Darrow said that these CTL responses have been melanoma antigen restrictive. Dr. Darrow said that he regrets that this data had not been included in their submission. As a point of clarification, Dr. Leventhal asked whether PBL from tumor-bearing patients demonstrate greater cytolytic activity against transduced autologous tumor cells than untransduced tumor cells. Dr. Darrow said that data indicates these transduced cells do generate a greater level of CTL activity than untransduced cells.

With regard to Ms. Meyers's concern about the informed consent document, Dr Seigler said that the document now states, "However, if incorporation of the virus into the tumor does not occur you will not be able to continue with the study." This statement along with the inclusion of *in vitro* transduction should provide clarification. Ms. Meyers suggested that the term "laboratory setting" be used in place of *in vitro*. Dr. Seigler agreed to the suggested change.

Dr. Seigler stated that the patient will not be responsible for any of the costs associated with the experimental procedures. All other costs, including costs associated with treatment, will be the responsibility of the patient and/or the third-party insurance carrier. Immediate medical care will be provided to the patients in the event that injury occurs as a result of participation in the protocol. However, there is no provision for free medical care or monetary compensation for such injury.

The informed consent process involves the PI, the patient, a family member, and one other neutral party, i.e., a nurse clinical specialist. Dr. Zallen inquired about the time frame that is involved in the informed consent process. Dr. Seigler stated that the patient has a minimum of 1 full day to consider the informed consent document and usually has several weeks to reconsider their decision before the procedure is initiated.

Discussion

Dr. Parkman asked the investigators if there are data demonstrating that 2 units of g-IFN is adequate to induce Class II expression. Dr. Seigler stated that 2 units is a minimum level of production. Of 5 tumor cell lines that have been transduced, there is an average of 45 units of g-IFN produced per 1 x 10 cell per day. Dr. Parkman asked Dr. Seigler what he would do if a patient's transduced cells produced only 2 units of g-IFN/1 x 10 cells/day. Dr. Seigler said he would not inject cells that demonstrated this minimum level of g-IFN expression. Dr. Parkman stated that the investigators should provide a dose-response curve demonstrating g-IFN production verses Class II expression.

Dr. Parkman inquired if S L assays have been performed. Dr. Seigler said that these assays have been performed, and that Dr. Jack Barber of Viagene, Inc., would provide a summary of these data. Dr. Leventhal asked what would be considered as dose-limiting toxicity in these patients. Dr. Seigler responded that toxicity is no longer being considered as an endpoint. The endpoints will be tumor and CTL responses. In addition, if no CTL activity is demonstrated after 10 patients, the study will be terminated unless tumor regression is observed.

Dr. Leventhal asked how toxicity will be monitored and evaluated as a stop criterion. If a patient demonstrates fever, chills, and shock, there must be a point at which the protocol will be terminated. Dr. Seigler agreed to include the suggested stop criteria in the protocol.

Ms. Meyers asked if the patient would be liable for any costs associated with adverse effects associated with an overdose of g-IFN. Dr. Seigler stated that the patient would not be financially responsible for the

cost of treatments that resulted directly from the research.

Dr. Haselkorn inquired about the responsibilities of the co-investigators and if Dr. Seigler is the only investigator who would have contact with the patient. Dr. Jolly of Viagene, Inc., responded that he and Dr. Barber will produce the vector at Viagene; Drs. Seigler and Darrow will perform the laboratory experiments at Duke University. Dr. Seigler will have direct contact with the patients.

Dr. Barber of Viagene, Inc., addressed the RAC members' questions regarding the vector and packaging cell line. The proposed vector and packaging cell have not produced helper virus breakout; the breakout that the committee members referred to was with another packaging cell line. The proposed system has been extensively characterized. All of the RCR testing will be repeated after the master cell bank and working cell banks have been established. The testing of the cell banks is currently in progress.

Dr. Post asked further questions regarding the helper virus breakout. If the breakout did not occur with the proposed packaging cell line and virus, did it occur with the same packaging cell line and another virus? Dr. Barber stated that the breakout occurred with a different virus and a different packaging cell line. Dr. Post asked about the difference between the two systems. Dr. Barber explained that the gene expression vectors are the same; however, everything else is different. The cell line that had the breakout of helper virus was the canine fibroblast cell line, CF2. The current packaging cell line is based on the canine cell line, D17. The only similarity between the 2 systems is the expression of the Moloney structural gene products. Dr. Barber noted that the reason that the RCR safety data had not been included in the original submission was that the master and working cell banks had not yet been established.

Dr. Leventhal said that approval of this protocol should be deferred until the investigators submit data demonstrating the following: (1) regression of established tumor, (2) generation of CTL from human PBL from tumor-bearing patients in response to autologous tumor, (3) a quantitative correlation between Class II antigen expression and the amount of g-IFN, and (4) a quantitative correlation between the number of CTL generated per unit of g-IFN.

Dr. Barber stated that it would not be appropriate to use the human vector in the animal model system because g-IFN is species-specific. Dr. Leventhal suggested that quantitative data should be submitted demonstrating the correlation between the generation of CTL from human PBL and the amount of human g-IFN produced. In addition, the investigators should identify a safe threshold of minimal expression. Dr. Leventhal said that this *in vitro* data would be acceptable in place of the *in vivo* data.

Dr. Leventhal stated that she is not convinced that there is sufficient justification to conduct this protocol based on the data that was submitted. Dr. Parkman said that the important issue is whether the RAC should approve every protocol in which a cytokine gene is inserted into melanoma cells. There has to be evidence that the cytokine gene augments the immune response.

Presentation--Dr. Darrow

Dr. Darrow presented additional data to the RAC which was not included in the meeting materials. Blastogenesis data indicated a substantial increase in thymidine incorporation in response to g-IFN transduced tumor as compared to untransduced tumor. Dr. Parkman noted that the data was derived using established tumor cell lines. Dr. Darrow said that the cell line was derived from a melanoma patient, and that it had been cultured for a maximum of 5 weeks. Dr. Darrow presented a representative experiment with several different cell lines which demonstrated increased efficacy of CTL generation with g-IFN transduction. Dr. Darrow stated that these assays are performed after 4-5 weeks in culture, because CTL activity peaks at this time and nonspecific killing is absent. This *in vitro* data substantiates the

hypothesis that transduced cells induce a significant increase in the level of CTL activity; therefore, a similar response should be achieved *in vivo*.

Discussion

Dr. Leventhal said that the data is insufficient to satisfy her concerns. Data must be derived from multiple experiments and statistically analyzed. Dr. Miller said that he would recommend approval of the protocol contingent on the submission of additional data. Dr. Leventhal stated that contingent approval of this protocol would not be consistent with the standards that have been used for other investigators.

Dr. Miller said that most protocols have been deferred based on the lack of safety data about the proposed vector and packaging cell line. Dr. Seigler has demonstrated safety; however, he is lacking data regarding the immune response. Dr. Miller said that if the RAC members insist that the investigators submit the required data, they should be allowed the opportunity to submit the data in response to a contingent approval. Dr. Motulsky agreed with Dr. Miller stating that the RAC should not stand on ceremony and precedence.

Dr. Haselkorn asked the RAC members to specify the exact information that should be submitted. Dr. Miller said that the CTL response data should be determined to be statistically significant. Dr. Geiduschek said that Dr. Motulsky is making the assumption that the RAC would be completely satisfied with the submission of additional data. Dr. Leventhal said that it is in the investigators' best interest to resubmit their data for full RAC review.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Ms. Meyers to defer approval of the protocol. Dr. Miller asked the investigators if they had an anticipated time frame in which the vector would be available for transduction. Since the master and working banks have not been established and tested, additional review at the June RAC meeting should not significantly delay the process. Dr. Jolly responded that an additional 3 months could introduce a slight delay. Dr. Leventhal reminded the investigators that the Food and Drug Administration (FDA) and RAC approvals are parallel processes; therefore, a delay in RAC approval will not affect their ability to obtain FDA approval. Dr. Miller asked the investigators if they have submitted their Investigational New Drug (IND) application to the FDA yet. Dr. Seigler responded that they had not yet submitted their application to the FDA.

The motion to defer approval of the protocol was approved by a vote of 16 in favor, 1 opposed, and 1 abstention. Approval of the protocol was deferred until data is submitted for full RAC review of the following: (1) generation of CTL from human PBL from tumor-bearing patients in response totransduced autologous tumor, (2) quantitative correlation between Class II antigen expression and the amount of g-IFN, and (3) quantitative correlation between the number ofCTLs generated per unit of g-IFN.

VIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: PHASE I STUDY OF NON-REPLICATING AUTOLOGOUS TUMOR CELL INJECTIONS USING CELLS PREPARED WITH OR WITHOUT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR GENE TRANSDUCTION IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA/DR. SIMONS*

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. Jonathan

Simons of Johns Hopkins Oncology Center, Baltimore, Maryland.

Dr. Smith provided a brief overview of the proposed study. This protocol is a Phase I vaccination study for the treatment of metastatic renal cell carcinoma for patients who have failed conventional therapy. Patients will receive transduced or untransduced cells in a randomized dose-escalation fashion.

The investigators have submitted extensive data from an *in vivo* murine model. The rationale for this protocol is based on published scientific data demonstrating that granulocyte-macrophage colony stimulating factor (GM-CSF) significantly enhances tumor regression. Safety and tolerance of the vaccination procedure, as well as any adverse effects associated with gene transfer, will be critically evaluated. Long-term potential toxicity will also be monitored, i.e., autoimmune disease. In addition, the investigators will monitor immune response to the transduced and untransduced cells.

The proposed retrovirus vector has a high efficiency of transduction and does not require selection. The patient's cells will be irradiated with a maximum dose of 15,000 rads. This dose of radiation does not adversely affect GM-CSF production, but inhibits tumor cell growth. Primary cell cultures will be used instead of long-term cell culture lines.

Dr. Smith stated that his written comments to the investigators addressed their ability to evaluate immune responsiveness in these patients in the absence of clinical endpoints. The investigators provided adequate responses and explained that additional biopsies of the draining lymph nodes potentially will be taken and assayed for immune responsiveness. The investigators should describe the types of assays that will be performed on these biopsy specimens. Also, the investigators should provide additional data regarding the safety and design of the proposed vector.

Review--Dr. Brinckerhoff

Dr. Brinckerhoff stated that this protocol is very well designed and documented. The major concern is the investigators' ability to cultivate and grow the primary tumor cell cultures obtained from the patients' kidneys. Primary cell cultures are difficult to initiate and expand. The investigators responded that they have been successful in all except one attempt to develop these primary cell cultures. The investigators should comment on the ease with which they have been able to establish these cultures.

Review--Dr. Carmen

Dr. Carmen stated that this protocol is innovative and fascinating. The investigators should describe the differences between transduced and untransduced irradiated cells, and the their ability to elicit an immune response. The investigators need to explain the differences between the MFG and MFGS vectors. Dr. Carmen said that this protocol is very interesting and recommends that it be approved.

Other Comments

Ms. Meyers provided comments about the informed consent document. The document should contain an explanation of gene therapy, including a lay description of vectors and the gene transfer process. A statement should be included about patient confidentiality, and that the NIH and the FDA will have access to the patient's records. A section should be included about the time frame for patient follow-up and the procedures involved. The informed consent document provides a recommendation to women regarding the use of contraception; however, this statement should include men. A statement should be added about a request for autopsy.

Dr. Miller provided a description of the proposed vector. This vector is based on the N2 vector which has been previously reviewed by the RAC. The vector contains the *gag* region of the Moloney murine leukemia virus (M-MuLV) at the 5' end and the *env* sequences of the parental virus at the 3' end. When used in combination with the packaging cell line, there is the potential for homologous recombination and the generation of helper virus. One of the problems associated with an N2 based vector is the large *gag* open reading frame. Since the *gag* protein is antigenic, the effects of this protein must be considered.

Dr. Miller explained that the investigators have proposed a new vector, MFGS, in which deletions and mutations have been incorporated to eliminate *gag* protein synthesis. However, the RAC must consider that the animal data that has been submitted was generated using the MFG vector, not MFGS. The committee should be certain that the new animal data, generated with the new vector, correlates with that of the old vector. MFGS differs from MFG in that a cryptic splice has been included upstream from *gag*; therefore, this vector is not new. MFGS is still an N2 based vector. Past experience has demonstrated that is very easy to generate helper virus from N2.

Dr. Miller noted that the LN series was developed in response to the helper virus problems associated with N2. In the LN vectors, the *env* region was deleted in order to eliminate the possibility of homologous recombination. The investigators have not detected any helper virus associated with the use of the MFG vector; however, there is the potential for helper virus production. The MFGS vector has a mutated *gag* region; however, the *env* remains which could allow for homologous recombination.

Dr. Haselkorn asked if the RAC is being asked to consider a vector that has not been thoroughly tested. Dr. Miller responded that the proposed vector has been tested extensively; however, the committee should consider the theoretical probability of such an event. Dr. Haselkorn said that it is imperative that the investigators address the issue of helper virus in their responses to the committee.

Dr. Walters noted that two RAC members, Drs. Chase and Leventhal, have recused themselves from reviewing and voting on this protocol because they are employees of Johns Hopkins University, and do not want to present the appearance of conflict of interest. Dr. Walters called on Dr. Simons to respond the questions and comments of the RAC members.

Presentation--Dr. Simons

Dr. Simons introduced his co-investigators: Drs. Richard Mulligan, Glen Dranoff, Larry Cohen, Fray Marshall, William Nelson, Drew Pardoll, Steven Pianadori, Elizabeth Jaffe, Karen Hauda, Jim Zabora, and Beth Gregory. Dr. Simons stated that Dr. Mulligan would address the RAC members' questions and comments about the MFG and MFGS vectors.

Dr. Mulligan said that, in general, he agreed with Dr. Miller's comment that there is no packaging cell line that is absolutely safe. The vector has an open reading frame that expresses the p15 protein. One of the positive features of this vector is that it possesses extra packaging sequences. When the desired sequences are inserted into the vector, the protein which is expressed is very similar to the normal protein and is very efficiently expressed.

In terms of safety, this vector has been tested extensively in the packaging cell line, Ψcrip. Dr. Mulligan stated that there have not been any concerns about the existence of open reading frames in this vector. He said that 30 separate constructs have been made and that no helper virus production was detected in any case. The assays for the detection of helper virus have been performed in-house as well as by reliable outside laboratories.

Dr. Mulligan stated that they attempted to introduce simple changes that would increase the safety potential of the MFG vector; this safety modified vector is MFGS. A stop codon has been introduced in MFGS in order to inhibit the open reading frame from initiating p15 protein production. The key factors tha were considered in evaluating this modified vector as compared to the parent vector were: (1) the efficiency of gene transfer, (2) level of gene expression, and (3) the immune effects observed in relation to GM-CSF expression.

The modified vector has been used with approximately 30 different cytokines. In every case, the modified vector performed as well as the original vector. Recently, there has been concern among the scientific community that the introduction of multiple antigens may reduce the specificity of the T cell response. In light of these concerns, the absence of the neo coding region may offer an advantage. The modifications that have been introduced are simple and offer a theoretical safety advantage over the original vector. Data was presented demonstrating that there are no significant differences in gene transfer or GM-CSF expression between the 2 vectors.

Dr. Post asked whether animals that receive MFG transduced cells develop an immune response to the GM-CSF. Dr. Mulligan said that the immune response to this protein in animals has not been examined in a careful fashion. Dr. Geiduschek asked Dr. Mulligan to describe the particular advantages of the new vector in relation to geometry, concentration, and the detection of helper virus breakout. Dr. Mulligan said that the new vector allows for very high virus titers using relatively low numbers of producer cells. This system allows for the possibility of preserving all of the producer cells in order to test for helper virus generation. These producer cells can be maintained in culture for 7 days.

Presentation--Dr. Simons

Dr. Simons responded to Dr. Smith's questions about the parameters that will be used to monitor immune response. Dr. Simons explained that immune response will not be used as an endpoint for this study since this protocol is Phase I. The immunologic assays outlined in the protocol are merely for information purposes. The assays that will be used to analyze immune function in future studies are still in the developmental stages. The immune parameters have not yet been defined.

In regard to the ability to establish primary cultures from these patients, Dr. Simons explained that Drs. Jaffe and Marshall have developed a special tissue culture medium which allows for the specific expansion of these primary cells. These studies have been performed in collaboration with Somatix Therapy, Inc. Somatix has been responsible for the early feasibility studies involving these primary cultures. Patients' cells have been routinely shipped from Johns Hopkins to Somatix, cultured, and transduced. Dr. Simons said that they were able to culture 11 out of 12 renal cell carcinomas and grow these cultures to sufficient numbers that would make the patients eligible for this study.

Dr. Simons responded to Dr. Carmen's comments about the effect of radiation on both transduced and untransduced cells. When non-irradiated tumor cells are injected, these cells outgrow the inoculation site. The rationale for irradiation is patient safety. Data demonstrates that irradiated tumor cells are equally effective as non-irradiated cells at evoking an anti-tumor response.

In response to Ms. Meyers' concerns about the informed consent document, Dr. Simons explained that the Johns Hopkins IRB will not approve an informed consent document similar to those approved by the RAC, because the reading level is too high. Johns Hopkins requires the document be worded at a sixth grade reading level. An attempt will be made to incorporate statements about confidentiality, contraception, and request for autopsy at the sixth grade reading level; however, there is no guarantee that the IRB will approve these additions.

Ms. Meyers said the informed consent document that was approved by the Johns Hopkins IRB is outrageous. The language is not written at the sixth grade level. The sentence that explains that the patient will receive an "injection of cancer" is not an accurate description of the procedure that will be performed. Dr. Simons explained that the IRB has very strict standards as to length of the document and the reading level. It is unlikely that the IRB will accept any major revisions or additions to the document.

Dr. Simons explained that the informed consent document is the product of "field testing." The Johns Hopkins IRB asked that the document be distributed to outpatients for comments and suggestions regarding the clarity of the document. The current document has been improved over the original version. Ms. Meyers suggested that the investigators ask the IRB if the document could be expanded to provide a more detailed description of the gene therapy procedures. This protocol is not a drug study but a novel approach which requires further explanation.

Dr. Wivel reminded the members of the RAC that recommendations about IRB informed consent documents are not binding. The IRB has the authority to accept or deny any changes to the document; therefore, the RAC should not include any such contingencies in a motion for approval. Ms. Meyers stated that if a contingency is not included as part of the formal motion for approval of this protocol, she will not vote for approval based on the opinion that patients will be misinformed. Dr. Parkman suggested that changes to the informed consent document should be recommended to the IRB; however, it is inappropriate to disapprove a scientifically sound protocol because the RAC does not approve of the IRB's standards. Dr. Simons stated that he would incorporate the changes suggested by Ms. Meyers, submit these changes to the IRB for approval, and modify the current document to the extent that the Johns Hopkins IRB will allow.

Committee Motion

A motion was made by Dr. Brinckerhoff and seconded by Dr. Carmen to approve the protocol using the MFGS vector. Approval of this protocol is contingent on the submission of relevant safety studies with the MFGS vector. The motion was approved by a vote of 15 in favor, 0 opposed, and 3 abstentions (Leventhal, Carmen, Meyers).

IX. ADDITION TO THE *POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING SEPARATION OF THE GENE MARKING INFORMED CONSENT DOCUMENT FROM THE THERAPEUTIC INFORMED CONSENT DOCUMENTS/DR. POST*

Presentation--Dr. Post

Dr. Walters called on Dr. Post to present the proposed amendment to the *Points to Consider*. Dr. Post explained that the proposed amendment is intended to clarify the informed consent process for patients considering participation in a human gene transfer protocol. When one informed consent document is used for two independent procedures the patient may be given the impression that participation in one form of therapy is contingent on participation in the gene transfer protocol. Therefore, a new sentence will be added to Section I-D-Informed Consent of the *Points to Consider*:

> "When gene transfer is a procedure separate from the therapeutic protocol, informed consent documents should be submitted for both the gene marking and therapeutic procedures."

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Krogstad to approve the proposed amendment t the *Points to Consider*. Dr. Leventhal objected to the use of the word "marking" and suggested tha "transfer" is a more appropriate term because the word "transfer" encompasses marking as well as therapeutic procedures. Also, Dr. Leventhal recommended that the phrase "therapeutic procedures" b replaced with "other clinical protocols." Drs. Post and Krogstad accepted Dr. Leventhal recommendations as a friendly amendment to the motion.

Dr. Walter stated that the revised language of the proposed amendment will read as follows:

> "When gene transfer is a procedure separate from the clinical protocol, informed consent documents should be submitted for both the gene transfer and the clinical protocol."

The motion to approve the revised amendment was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

X. ADDITION TO *THE POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDING TH SAFETY OF DELIVERY/EXPRESSION SYSTEMS AND REPORT ON MURINE RCR ASSAYS/ MILLER*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to initiate discussion of the document entitled: *Safety of Delivery/Expression Systems and Report on Murine RCR As*, submitted by Dr. W. French Anderson of the Southern California School of Medicine, Los Angeles, and Drs. Gerard J. McGarrity and Rober Moen of GTI , Gaithersburg, Maryland

Dr. Miller asked if the report was being considered for inclusion in the *Points to Consider* or for endorsement by the RAC as a state-of-the-art guide for investigators about the recommended procedures for RCR testing. Dr. Wivel explained that the report was included as a proposed action to *Points to Consider* and published in the *Federal Register* to provide the RAC with the option to include specific RCR assay requirements and minimal levels of RCR detection if necessary. Addition of language to *Points to Consider* by the RAC is an option, not a directive.

Dr. Miller explained that the investigators originally submitted an RCR report to the RAC at the Decemb 1992 meeting. At that meeting, the RAC made several recommendations regarding additions and corrections to the report. This revised report incorporated all of the RAC's suggestions and is comprehensive summary of murine RCR assays. The report should not be included in *Points to Consider* due to the rapidly evolving nature of the field. This report should be used as a guide for those investigators who are considering submission of human gene transfer protocols which involve murine retrovirus vector.

Review--Miller (for Dr. Geiduschek

In Dr. Geiduschek's absence, Dr. Miller summarized Dr. Geiduschek's written critique of the report. Geiduschek's comments indicated that he is satisfied with the revised version of the report. In particula he was pleased to see that a section was included that analyzed the dynamics of RCR breakout events Dr. Geiduschek states that, "The report is to be regarded as a review of status, not as a final resolution the safety issues." Dr. Geiduschek outlines the following stipulations for endorsement of the report: (1)

the experiment cited on pages 191 and 193 (RAC mailing) are to be repeated sufficiently to establish a time criterion for breakout events, and (2) the fraction of each production lot that will be monitored should be large, i.e., a minimum of 5%. Dr. Geiduschek's written comments indicate that the investigators hav agreed to these stipulations, and that these provisions coincide with their own activities and plans.

Dr. Geiduschek recommends that the report be accepted as guidance for investigators, and that it shou not be included in the *Points to Consider*.

Presentation-- Dr. Anderson

Dr. Anderson reiterated that fact that the report encompasses ongoing studies, and that these studies are being closely coordinated with the FDA.

Comments--Dr. Gunter

Dr. Kurt Gunter, Acting Deputy Director of the Division of Cell and Gene Therapy, FDA, stated that the FDA agrees with most of the conclusions presented in the report. The one area that the FDA is not entirely in agreement is with the quantitation of risk. The FDA feels that a number of assumptions hav been made with regard to this issue and prefers to rely on empirical data rather than assumptions.

During the December 1992 RAC meeting, Dr. Arifa Khan of the FDA presented the FDA' recommendations for a variety of assays for the detection of RCR at various time points in the producti process. The reactions of various individuals to the FDA's recommendations illustrates the problems that the FDA has had in dealing with the public. The FDA does not want to outline requirements that are not scientifically founded. However, the FDA is responsible for providing investigators with what is considered the state-of-the-art in terms of RCR testing. As a result, the FDA has devise recommendations that are based on current scientific data and common sense.

The FDA's recommendations are as follows:

o Master cell bank characterization

Supernatant test
Co-culture with permissive cell line

o Manufacturer's working cell bank characterization

Repeat test for RC
Test of transduced target cells (high M

o Release test of production lots

Supernatant test
Co-culture with permissive cell line

o Post production run culture and testing

o Lot release test of transduced cells (*in vivo*)

Discussion

With regard to Dr. Gunter's presentation, Dr. Post asked if 5% refers to a production lot. Dr. Gunter responded that 5% of the production lot is the recommended testing volume. Dr. Post inquired whether there is any correlation between the size of a production lot and a patient dosage. Dr. Gunter stated that the FDA is not correlating dosage with the size of a production lot at this time. However, as investigators progress to Phase II and Phase III trials and the size of the production lots increases, the FDA will reevaluate these criteria.

Dr. Parkman noted that there are clinical settings in which *ex vivo* testing of transduced cells would not possible prior to administration, i.e., transduced hepatocytes . There are instances in which either patient is undergoing a surgical procedure or cell survival is limited *ex vivo*. Dr. Gunter agreed with Dr. Parkman's comments and said that there will be instances where there will be no choice but to proceed with administration of the transduced cells prior to the completion of the proposed assays. Dr. Gunte stated that the FDA will consider any valid, rational, scientific argument against any of the FDA's recommendations.

Dr. Parkman asked about the proposed limit of RCR detection. If one production lot is equal to 5 patier doses, what is the level of detection? Dr. Anderson answered that the limit of detection would be 4 RCR particles. Dr. Miller suggested that the limit of RCR detection should approach 0. Dr. Anderson said tha the theoretical limit of RCR detection is 0 particles per production lot, assuming that the lot will be cultu for a sufficient period of time after harvest. However, the practical limits of detection will actually be less than 1 RCR particle per production lot. If a single RCR particle is detected in any assay, the lot will discarded. Dr. Miller said that the calculations do not accurately reflect the level of detection because the testing volume will be only 5% of an entire production lot. Testing this volume only guarantees that there are less than 20 RCR particles per production lo

Dr. Chase stated that the calculations should not be interpreted based on sample volume, because this is not a solid object. The probability of contamination is not the same as the fraction of the area that is sampled. Dr. Anderson agreed with the statement made by Dr. Chase and added that carrying a post-production run out for twice the period of time in which an RCR breakout would occur, brings th level of detection to 0 per production lot. Dr. Chase stated that although the desired goal of detection is 0 particles per production lot, you can never be certain that it is absolutely 0. However, one can approach the 0 particle limit by judicious sampling.

Dr. Gunter stated that the dose of RCR which is disease-causing is still unknown and cited the R breakout which resulted in disease in the primate setting, i.e., lymphomas. Until the limits of RCR tha cause disease are ascertained, the FDA does not feel that it can define absolute limits for sensitivity of RCR assays. Dr. Gunter said that he is personally uncomfortable with assessing the overall safety o RCR

Dr. Miller said that Dr. Gunter's statement is not fair to investigators, and that this issue is the basis for which the report was developed. The report provides useful significant detail about the RCR event whi occurred in the monkeys and how the monkey situation translates to breakout levels of RCR . Dr. Gunte said that he differs with Dr. Anderson's interpretation because there have been only 5 monkeys that have been tested rigorously for RCR . The monkey database is too small at the current time to draw accurat conclusions.

Dr. McGarrity described a reconstruction experiment designed to reproduce an RCR breakout. Afte days in culture, the supernatant was removed from 10 RCR producing cells. The cells and th

supernatant were then assayed separately for RCR breakout. Breakout was simulated in 2 to 3 days w this method. These studies are currently being refined to achieve a sensitivity level of detecting 1 RCR particle. When assays are perfected at this level, statistical analysis will probably translate into what actually happens when a breakout occurs, i.e., do the number of RCR particles increase tremendously remain at low levels? These reconstruction experiments are the first attempt to develop a quantitative method for the detection of RCR

Dr. McGarrity described an RCR breakout that recently occurred in the laboratory setting. The brea was detected when the RCR titer was approximately 200 particles per ml. This breakout was readil detected by the standard S L and NIH3T3 amplification assays.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Haselkorn to accept the report as a state-of-the- guidance for investigators who are preparing retrovirus vectors for clinical use. The motion was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

XI. ADDITION TO *THE POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDIN PROCEDURES FOR EXPEDITED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS*

Discussion

Dr. Walters summarized the deliberations of the Working Group on Expedited Review. The working group (Drs. Leventhal , Post, Zallen , and Walters) held a telephone conference call in order to develop the document entitled: *Procedures for Expedited Review*; the document was distributed to all of the members of the RAC for discussion. These *Procedures for Expedited Review* were originally proposed by Ms. Buc at the January 14 RAC meeting. The working group has incorporated several minor changes to the originally proposed procedures; however, these have not been substantive changes.

Dr. Krogstad reminded the RAC that he had originally suggested that protocols approved through th expedited review process should be formally reviewed at the next RAC meeting. Specifically, the expedited approval that was granted by the NIH Director in December 1992. This step would provid feedback from the RAC members to the individuals who are involved in the interim review process. This requirement does not appear in the draft document. There was a clear consensus of the RAC members on this issue. Dr. Leventhal agreed with Dr. Krogstad's comments and said that all of the RAC mem should have access to the materials on which an expedited review approval is based.

Dr. Parkman said that the RAC would serve an important function by reviewing expedited approvals at the next regularly scheduled meeting. Approval or disapproval of an NIH -approved expedited review proto would serve as a check and balance on the quality of the review process. Since the *Procedures for Expedited Review* were not incorporated as a major action to the *Points to Consider* of the *NIH Guidelines* at the time that the December approval was granted, the requirement for full RAC review at the next meeting could not be enforced.

Dr. Walters asked if the RAC intends to review expedited review protocols using the same standards of review as for other protocols. Dr. Parkman agreed that expedited review protocols should be reviewed by the same standards used for other protocols. Dr. Krogstad explained they would be evaluated for bot scientific and medical merit. Dr. Wivel reminded the committee members that they had specified tha investigators must also provide all of the materials that were submitted to the FDA in order to obtain approval.

Dr. Chase said that review of any protocol that receives expedited approval by the NIH Director i important information with regard to the rights of these patients. There is a chance that a decision for approval of an expedited review protocol by the Director could be contradicted by the RAC. This information would be invaluable to those individuals who might be tempted to believe that any treatment approved by the government is considered scientifically sound and efficacious.

Dr. Leventhal said that the RAC should not refer to expedited approval as compassionate ple exemptions. The term compassionate indicates that there is some inherent value to the procedure. Expedited approvals may have no experimental value; therefore, they should not be considered compassionate. She cautioned the committee to consider the thousands of terminal cancer patients who will hear about this expedited approval process and have their hopes raised that they may also qualify for such an exemption. Dr. Parkman said that the *Procedures for Expedited Review* includes the provision that expedited approval will be granted only for a single patient; this provision should obviate any concerns about raising false hope for terminal patients. Dr. Walters concurred with the statement made by Dr. Parkman and emphasized that the onus is on the investigator to provide a valid argument for why the patient cannot wait until the next scheduled RAC meeting. The expedited review process is not viewed as a mechanism for accruing multiple patients onto a single protocol.

Dr. Leventhal reminded that committee that Dr. Wilson is an example of an investigator who identifie several patients who did not fit the inclusion criteria for RAC approval. However, Dr. Wilson had sufficient time to request a minor modification to his approved protocol and to present the unique set of circumstances to the RAC at its regularly scheduled meeting. Dr. Miller reminded the committee that Dr. Wilson's request was not for expedited review, but was submitted as a minor modification to a previously approved protocol.

Dr. Parkman said that the objective of the *Procedures for Expedited Review* is to ensure that the scientific quality of the protocol is as good, if not better, than the quality of those protocols approved through the regular review process.

Cover Sheet for Expedited Review

Dr. Parkman presented a draft checklist of critical information that should be completed at the time that an initial request is made by an investigator for expedited review of a single patient protocol. This *Cover Sheet for Expedited Review* will be used by the Office of Recombinant DNA Activities (ORDA) t determine whether the investigator has minimal requisite preclinical data which would warrant expedited review. If the requesting individual cannot complete the entire *Cover Sheet for Expedited Review* than their request will not be forwarded based on the fact that the requisite minimal data to evaluate the protocol does not exist. If the investigators do not have the vector sequence, gene transduction or expression data, or safety data, then it is not appropriate to evaluate the protocol.

Dr. Post expressed concern that this 1 page document may send a signal to investigators that they can submit less information for an expedited review protocol than for a protocol reviewed through the normal process. Investigators should be required to submit the *Points to Consider*. Dr. Parkman said that the *Cover Sheet for Expedited Review* is not intended to outline all of the documentation that is required for submission; it is intended as a screening process.

Dr. Haselkorn stated his objection to the use of the word "therapy" in item number 4 of the *Cover Sheet for Expedited Review*. Dr. Wivel suggested that the word "transfer" be substituted for the word "therapy". I Haselkorn agreed to this substitute wording. Dr. Haselkorn suggested that expedited review should

be considered for post-Phase I trials. Phase I studies are inappropriate for expedited review since there is no demonstrated therapeutic value.

Dr. Zallen suggested that the RAC should consider the public's perception and whether they will vie these investigators as trying to take advantage of desperate individuals who can afford to pay for medical interventions. If the public develops this perception, the integrity of gene therapy research may be called into question. She suggested that a statement be included in the *Procedures for Expedited Review* requiring that all costs associated with the experimental protocol will not be borne by the patient or the patient's family. Inclusion of this criterion would maintain public confidence. Ms. Meyers agreed that the provision outlined by Dr. Zallen should be included in the final document. Dr. Leventhal reminded RAC that this requirement would not preclude patients or their families from making monetary donations to an institution.

Dr. Chase stated that the RAC should remain confident that making this document "air-tight" will develop into long-term implications. One important factor that will be the peer pressure of responsible scientists in the area of gene therapy. Eventually, there may be an attempt to discredit those investigators who repeatedly make these types of requests. The other factor that will weigh heavily is the parallel recommendation by the RAC following NIH Director's determination. Eventually, the media and othe individuals will influence public opinion. The public will realize that some of the experiments will have no medical or scientific value. It may take several years for the public to actually realize the implications of these expedited approvals. Dr. Krogstad recommended that item number 6 of the *Procedures for Expedited Review* include extramural scientists in the review of these protocols. In light of previous discussions about the possibility of political pressure being imposed on the review process, the inclusion of extramural scientists' review would avoid the appearance of such pressure being imposed.

Dr. Leventhal suggested that a question should be included in the *Cover Sheet* which requires that the investigators define the endpoint of the study, i.e., what will be measured? A clear example of the importance of endpoints is the patient who received expedited approval in December 1992. The RAC was told that the patient required emergency treatment because the tumor was growing rapidly. However, the RAC was informed that the patient received hyperfractionated radiation prior to receiving th injections of gene- transduced cells. In addition, the patient's response was going to be measure compared to pre-radiotherapy. What would actually be measured in this situation? Dr. Parkman agreed that he experimental design must be valid in order accurately measure any potential response. Dr. Brinckerhoff said item number 1 of the *Procedures for Expedited Review* reads, "The NIH will strongl emphasize that the standard method of protocol submission is preferred." The words "strongly emphasize" are not forceful enough.

Dr. Anderson recommended that investigators should be required to indicate that the completed protocol, the *Points to Consider* document, and the vector sequence are attached as part of the completion of the *Cover Sheet for Expedited Review*. He said that this document should become a checklist of "yes or no" answers that can easily be completed by the requesting individual.

Dr. Leventhal suggested that the statement, "patient privacy will be maintained," should be included a part of item number 7 of the *Procedures for Expedited Review*. Also, the statement, "Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review," will sometimes be difficult to determine. For example, Drs. Sobol and Royston had a protocol that wa deferred. However, these investigators contended that the expedited review protocol submitted was a new protocol. In actuality, the differences between the 2 protocols are not very substantive. Investigators will always submit their request as a new protocol to satisfy this criterion.

Dr. Sobol asked about a scenario in which the investigator has data that would support an expedite review protocol. Dr. Leventhal said that the investigator should present the data at the next RAC meeti Dr. Sobol asked about a situation in which the patient could not wait until the next meeting. Dr. Lever responded that the investigator would have to convince the RAC there are extenuating circumstances surrounding the experiment that are so specific that the opportunity will never present itself again to perform the experiment.

Dr. Sobol said that a situation could arise in which approval of a protocol is deferred by the RAC due t insufficient data, and the investigators obtain the requested data several weeks following the meeting. For example, under extraordinary circumstances a patient could meet the eligibility requirements of the deferred protocol, but the disease is progressing at such a rate that he/she was unable to wait until the protocol is reviewed at the next RAC meeting. Dr. Leventhal urged the RAC to keep this contingency a part of the *Procedures for Expedited Review*. The majority of protocols that are deferred by the RAC are missing significant data that cannot be obtained in a relatively short time frame. Protocols that are missing minor bits of data or information are normally approved with contingencies.

The members of the RAC continued to make minor modifications to the*Procedures for Expedited Review* and the *Cover Sheet for Expedited Review*. Dr. Walters suggested that a revised version of these two documents should be presented for final approval later on in the agenda.

Before presenting the next agenda item, Dr. Walters suggested that the RAC should form a working group to analyze and develop different categories for the classification of human gene transfer and therapy protocols. The purpose for this exercise is to assist the RAC in developing certain categories of protocols that may be eligible for decentralized review.

XII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GEN THERAPY PROTOCOL ENTITLED: A PHASE I STUDY OF GENE THERAPY OF CYSTIC FIBROSIS UTILIZING A REPLICATION DEFICIENT RECOMBINANT ADENOVIRUS VECTOR TO DELIVER THE HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR cDNA TO AIRWAYS/DRS. WILMOTT , WHITSETT , AND TRA*

Review--Ms. Grossman

Dr. Walters called on Ms. Grossman to present her primary review of the protocol submitted by Drs. Robert W. Wilmott and Jeffrey Whitsett of Children's Hospital Medical Center, Cincinnati, Ohio, and Bruce Trapnell of GTI , Gaithersburg, Maryl

Ms. Grossman provided a brief overview the proposed study. This protocol is a Phase I study to assess the safety and biological efficacy of a recombinant adenovirus delivered to the nose and lungs of patients with cystic fibrosis (CF). The investigators proposed to treat a total of 15 adult patients, who will be divided into 3 groups of 5 patients. Each group of patients will receive 1 x 10, 1 x 10, or 1 x 10 plaque forming units (pfu) of recombinant adenovirus

This protocol is more complex than the other CF protocols that have previously been reviewed by the RAC. This study involves the administration of vector to the nose and lung of the patients, followed by subsequent challenge with an equivalent dose of virus 2 months after the initial treatment. The proposed vector is an E1 and E3-deleted type 5 adenovirus. The cystic fibrosis transmembrane conductanc regulator (CFTR) cDNA is expressed from a Rous sarcoma virus (RSV) prom

Ms. Grossman said that the investigators responded to the majority of her written comments; however,

there is still one remaining question regarding the sequencing of the vector. The investigators indicate that they will confirm the sequence of the viral backbone. They should describe the exact portions of the vector that will be sequenced. Also, the investigators need to present their quantitative PCR dat demonstrating that they are able to detect 1 wild-type virus particle per 1 x 10 recombinant adenovirus particles.

Ms. Grossman said that there are several questions that the investigators should address during their oral presentation. What fraction of each dose will be evaluated for sterility, mycoplasma an replication-competent adenovirus? How will *in vivo* toxicity be determined? Toxicity studies have been performed in cotton rats; however, it would be preferable if these studies would be performed in a larger animal model. She stated a concern that the investigators are attempting to assess biological efficacy and safety of a single administration of recombinant adenovirus, and then they propose to rechallenge th patient with a second dose of virus 2 months later. The second administration of recombinant adenovirus may not be feasible and/or necessary. This second dosage may be putting patients at an additional risk since the effects of a single administration are unknown. She inquired whether recombinant adenovirus has ever been administered to a large animal model and an attempt made to obtain CFTR -expressin cells? Is there data demonstrating the investigator's ability to administer the recombinant adenovirus and recover gene- transduced cells from brushings

Ms. Grossman noted that the inclusion/exclusion criteria limits eligibility for this protocol to patients greate than 18 years of age; however, the experiments will be performed at the Cincinnati Children's Hospital. Are there any necessary measures that must be taken to treat adults at a pediatric hospital? Who will be the primary physician?

Review--Dr. Haselkor

Dr. Haselkorn stated that he was originally concerned about the necessity for frequent biopsies and th degree of discomfort associated with these procedures. The investigators did satisfactorily respond to these concerns by reducing the frequency of the bronchoscopies and biopsie

Review--Dr. Zalle

Dr. Zallen said that she had the same initial concerns as Dr. Haselkorn regarding the frequency biopsies, and that she was satisfied with the revised schedule. For clarification purposes, the investigators need to explain the necessity for the large number of x-rays and to explain their ability to reliably detect improvement in a lung that is already severely damaged.

Dr. Zallen stated that her written comments requested that a diagram be included to assist the patient i understanding the sequence of events that they would undergo. A diagram has been submitted; however, the scale of the diagram is not proportional to time and should be revised. The investigators were commended for providing a comprehensive education program for health care workers that has been implemented for this study.

Other Comments

Dr. Parkman asked on what basis 56 days was chosen as the optimum time point for administration of the second dose of recombinant adenovirus. The reason that other investigators have proposed only a single administration is that the time frame for CFTR expression in humans is unknown. Although differen adenovirus vectors will elicit varying degrees of immune response, Dr. Crystal has reported that the cotton rats have exhibited increased inflammatory responses for longer durations, in response to subsequent

challenges of virus. What *in vivo* data exists with regard to the nature and duration of the inflammatory response with the proposed vector? Does the second challenge elicit a greater response than the first administration? Does the magnitude of the response increase with each consecutive administration?

Ms. Meyers noted that the informed consent document should include statements about long term follow-up and a request for autopsy. Dr. Walters asked the investigators to explain the role of GTI in th proposed study.

Presentation--Dr. Trapnel

Dr. Trapnell described the progress that has been made in the sequencing of the adenovirus vector Restriction mapping has been performed to evaluate the structure of the virus. Sequence analysis has been initiated for both the plasmid and the backbone of the parental virus. GTI will sequence the entir vector; however, there is uncertainty about the necessity of sequencing the entire vector. If the RAC requires the entire sequence, GTI will comply with the request

With regard to the fraction of the dose that will evaluated for sterility, Dr. Trapnell stated that the exac fraction has not yet been determined because GTI is still in the process of scaling up the productio volume. GTI's goal is to test an entire patient dosage. Ms. Grossman asked about the size of a product lot. Dr. Trapnell responded that a production lot will be equal to several patient doses; however, the ex number of doses has not yet been determined.

Dr. Miller asked if 1 helper virus particle can be detected in a 1 x 10 vector particles, which is the maximum proposed dosage. Dr. Trapnell said that he would first like to summarize the possible metho for detecting replication-competent particles. The first method is to evaluate the biologic titer of infected cells. The second method is to look for cytopathic effects and to evaluate for characteristic cel morphology which would indicate wild-type infection. The third method, which is more sensitive than the other 2 methods, is metabolic labeling of DNA in infected cells. The metabolic labeling method identifies the presence of wild-type genomic sequences, i.e., E1 sequences.

Dr. Trapnell presented data demonstrating metabolic labeling of genomic DNA sequences in bronchia epithelial cells that were obtained by bronchoscopy . Following DNA extraction, enzyme restrictio analysis is performed using agarose gel electrophoreses and autoradiography. The wild-type an recombinant virus have distinguishable restriction fragment patterns. PCR analysis is being used t detect the presence of replication-competent adenovirus particles. The PCR method is several logs mo sensitive than the other methods. A reconstruction experiment was presented in which wild-type virus was mixed with the recombinant virus at different ratios. The cells are heat denatured, and PCR amplificatio is performed with both the E1a and E2b primers. Next, these samples are subjected to Southern blot analysis. The PCR amplification method demonstrates that 1 wild-type virus particle can be detected in x 10 vector particles. This assay sensitivity is the current level.

Dr. Miller said that patients will receive as much as 1,000 times the number of vector particles used in the reconstruction experiment. Dr. Parkman said that with this level of sensitivity, the investigators can only say that there is less than 1,000 replication-competent particles per patient dose. Dr. Trapnell said tha plaque purification will be performed; therefore, the theoretical number of replication-competent particles will be much less than 1,000. Ms. Grossman inquired if there are other cell-based assays that could be performed to increase the level of sensitivity. Dr. Trapnell responded that the cytopathic effect assa be performed; however, the necessary dilutions would require thousands of plates, which would make assay prohibitive.

Dr. Trapnell presented data from other trials in which wild-type adenovirus was delivered to th respiratory tract of a large number of individuals. This trial was conducted between 1953 and 1970. These individuals received as much as 4 x 10 wild-type particles. The most severe symptoms consisted of mild upper respiratory illness, i.e., coughing and rhinorrhea . These symptoms were consistent with thos observed in patients with a typical adenovirus induced cold. For individuals who demonstrated preexisting neutralizing antibody from a prior infection, there was an inverse relationship between the amount of antibody and the development of asymptomatic infections. Patients enrolled in this study will have demonstrated neutralizing adenovirus antibody. There has never been any instance of malignancy associated with adenovirus infection in humans.

Dr. Motulsky inquired if these trials have been followed long-term. Dr. Trapnell said that some of th studies have been studied long-term, and that 4 x 10 adenovirus particles is considered to be a safe dose. Dr. Post commented that this number of particles are safe to administer to a normal patient. What would the effect be in CF patients? Dr. Wilmott responded that CF patients are exposed to adenovirus particl at the same rate as normal individuals. In general, CF patients are hyperimmune and demonstrate hig levels of gamma globulin, particularly IgG . There is no reason to believe that these patients will manife any problems specifically associated with adenovirus infection.

Presentation--Dr. Whitset

Dr. Whitsett responded to Ms. Grossman's concerns about *in vivo* toxicity. The cotton rat was chosen as the *in vivo* model because of its unique susceptibility to adenovirus. Data was presented in which cotton rats were given 3 x 10 pfu intratracheally . Substantial gene expression was observed out to 7 days. animals demonstrated lymphocyte and macrophage peribronchiolar and perivascular responses response to vector administration. Recently, this same response has been observed in the hamster model. Dr. Whitsett explained that rabbit experiments are currently in progress; 3 animals have bee treated to date. These 3 rabbits demonstrated responses similar to those observed in the cotton rats and hamsters. The animals did not become ill. They did not breath rapidly or have cyanosis. However, pathology demonstrated significant lymphocytic and monocytic infiltration by hematoxylin and (H&E) staining. There was no evidence of pneumonia or hemorrhage.

Dr. Whitsett explained that the investigators are concerned about demonstrating the safety of multipl administrations, and that they will continue to perform repeat dose experiments in large animals. The human protocol will not be initiated until these large animal experiments have been completed and evaluated.

Dr. Whitsett explained that repeat administrations will be necessary because of the turnover of th respiratory epithelium. The time frame involved in cell turnover is between 80 and 100 days. Conditions will be optimized such that CFTR expression can be achieved throughout this entire period. The dat chosen for the second administration, 56 days, is based on data which demonstrates that this period is where one-half of the original mRNA is detectable within the lung. All patients will have neutralizing antibodies to the adenovirus which should obviate any concern about an aggressive immune response to the vector.

Dr. Whitsett stated that he is compelled to proceed with this protocol as a feasibility study in terms of th repeat administration of the adenovirus vector. The entire strategy of CFTR transduction in the lung i dependent on multiple administration of the vector. Ms. Grossman said that patients should not be exposed to a second administration of the vector if the effect of a single dose is unknown. There is a possibility that patients may not demonstrate a clinical response at the lower concentrations; therefore, why would you subject a non-responsive group of patients to a second administration? Dr. Whitsett

explained that if the objective of the protocol was to demonstrate efficacy, then there would be a flaw in the experimental design. However, the objective of the protocol is to determine toxicity and whether a second dose of vector is feasible. This study is designed to determine safety, not efficacy.

Dr. Parkman said that it is difficult to approve a protocol in which a second administration of vector has no been tested in a large animal model. He said that he would more readily vote for approval of the protocol i it involved only a single administration of the vector. Dr. Whitsett stated that a single dose would be fall-back position; however, it would be preferable to proceed with the protocol as it was originally proposed. Ms. Grossman said that she would prefer that only a single administration be approved by the RAC until such time that *in vivo* preclinical data was available in a large animal model. Dr. Whitsett requested permission to change the design of the protocol to include a single administration of the vector instead of 2 doses.

Dr. Miller inquired about the effect of neutralizing antibodies on the adenovirus vector. Dr. Trapnell stat that *in vivo* data demonstrates that adenovirus infection is possible in the presence of overwhelming levels of serum antibody. The lymphocytic and monocytic immune responses that are observed transient regardless of whether animals are immune.

Dr. Trapnell responded to questions regarding the ability to detect gene expression. A technique wa developed for evaluating gene expression in the respiratory epithelium using bronchoscopies to obtai brushing samples. Millions of ciliated and secretory cells can be obtained in this fashion. Gen expression is evaluated by quantitative PCR . Gene expression has been demonstrated in respiratory t cells from the nose through the bronchus; however, gene expression is depressed in the pharynx. Comparison between quantitative PCR and the amount of hybridization will provide an estimate as to t copy number per cell.

Ms. Grossman asked the investigators to describe the*in situ* hybridization assays that have been performed. Dr. Whitsett explained that the investigators have been successful in distinguishing both th endogenous and transferred gene by *in situ* hybridization. Dr. Whitsett stated success at detecting seve copies of CFTR per cell by this metho

In response to Dr. Zallen's concerns, Dr. Whitsett said that a revised flow chart has been prepared assist the patient in understanding the various procedures and time points; however, the second administration phase will be deleted. Patients will be followed by Dr. Robert Baughman who is the Director of the adult program of the Cystic Fibrosis Center.

Dr. Whitsett stated that all patients will be followed long-term, and that an autopsy will be requested in event of death. With regard to the number of x-rays that will be required, the number of x-rays will be greatly reduced because the second dose of vector will now be deleted. As the protocol was originally written, the proposed dosage of radiation was within the National Radiation Safety Council's Guidelines. The new dosimetry will be half of what was outlined in the original protocol. With regard to the ability t measure potential differences (PD), data was presented demonstrating a nasal PD of 80 millivolts in a patient as compared to 10-20 millivolts in normal individual

Dr. Whitsett concluded that the investigators' intent is to formally remove the rechallenge portion of protocol. The second dose protocol will be resubmitted to the RAC when the *in vivo* data is complete and the clinical efficacy of the initial trials has been evaluated.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Motulsky to approve the protocol as am to include a single administration of the adenovirus vector.

Dr. Miller said that the RAC has always required that there be less than 1 adenovirus helper virus particle per patient dose; however, this requirement may be unnecessary. The problem is that there is no solid data with regard to the number of particles that will produce complications in a CF patient. Dr. Trapnell explained that CF patients are routinely exposed to adenovirus infections at the same rate as normal individuals, and both groups demonstrate similar clinical responses as demonstrated by gross examination.

Ms. Grossman asked if the patients enrolled into this protocol will have be sicker than the average CF patient. Dr. Wilmott responded that the eligibility criteria includes patients who demonstrate mild t moderate disease. Initial patient accrual will include patients with mild disease.

Dr. Parkman asked about the threshold dose to get a wild-type adenovirus infection. Dr. Whitsett answered that one-half of all individuals get a cold when exposed to concentrations of between 1 x 10 and 1 x 10 adenovirus particles. It is unknown how many adenovirus particles are transmitted in a human cough. Dr. Miller noted that 1 x 10 particles is not an enormous dose; therefore, it is reasonable to allow this upper limit.

Dr. Straus explained that the argument that CF patients have normal immunity to adenovirus is somewhat flawed, because all individuals acquire these infections at a young age. Data has demonstrated that 100% of the population is exposed to adenovirus types 1, 2, 5, and 6 by the age of 3 years. This early exposure occurs at a time when the lungs are still relatively normal in CF patients. The protocol is designed to treat CF patients whose pulmonary function in no longer normal. Dr. Miller explained that advanced CF patients do not demonstrate clinical symptoms in response to adenovirus exposure. Dr. Straus acknowledged that these patients do not demonstrate bronchiolitis or pneumonia. However, the patients are being exposed to low titers and do demonstrate evidence of mucosal immunity. These patients have never had large quantities of adenovirus particles delivered into their respiratory tract. The capacity of these patients is still unknown; therefore, the RAC should not prematurely approve excessive levels of wild-type exposure.

Dr. Parkman said that the criteria for this protocol are similar to those standards established for the other CF protocols approved by the RAC. Dr. Miller said that the RAC should exercise consistency and ask investigators to demonstrate the ability to detect 1 wild-type particle per patient dose. Dr. Post said that there is reason to believe that this requirement is unnecessary. Dr. Miller suggested that he would recommend approval of the protocol with the stipulation that the investigators try to increase the level of sensitivity of their assay for the detection of wild-type adenovirus; however, the current standards are acceptable at this time.

Dr. Haselkorn suggested that perhaps the level of PCR sensitivity could be increased by the additio biotinylated nucleotides in the late steps and using radiolabelled avidin to detect the product. An option is to perform a reconstruction experiment with 1 x 10 vector particles and identifying replication-competent virus following serial passage. Dr. Trapnell explained that one of the difficulties i identifying replication-competent particles is the number of cells that must be inoculated. This procedure could require as many as 2 x 10 plates to perform 1 assay. Even if this number of plates could technically be inoculated, it would be difficult to believe 1 plaque in 2 x 10 plates.

Dr. Trapnell said that it is reasonable to believe that the level of PCR sensitivity will be improv However, even in the worst case scenario, these patients would not be exposed to levels of wild-type

virus that are any higher than the natural situation. Dr. Post asked if assays have been performed with A549 cells. Dr. Trapnell said that assays have been performed with A549, HeLa , human embryo kidney, and 293 cells. There are problems associated with each of these cells. These systems reach their capacity in terms of the number of vector particles per cell; therefore, dilutions are still necessary.

Dr. Parkman reminded the RAC that the limit of 1 x 10 is the worst case scenario of replication-competent particles; the actual number could be 0. Dr. Trapnell agreed with Dr. Parkman noting that the initial gro of patients who will receive 1 x 10 vector particles will receive vector preparations that have been demonstrated to have less than 1 replication-competent particle. Dr. Haselkorn asked if the current P amplification utilizes a nested procedure with multiple rounds of amplification. Dr. Trapnell explained th the investigators are in the process of optimizing the nested procedure, and that this method should increase the level of sensitivity.

Dr. Leventhal suggested that if the number of infectious particles is a real concern that the response of initial 3 groups of patients can be used to evaluate toxicity. A stopping rule for toxicity should be based on the criteria that have already been developed. This study will provide valuable data about the number of infectious particles a CF patient can tolerate. Dr. Parkman responded that a direct correlation cannot be made since the actual number of infectious particles cannot be quantitated . Dr. Leventhal said that committee should be confident that the experimental design of this protocol will not produce any extreme adverse reactions such as anaphylaxis.

Dr. Parkman moved the question. The motion to approve the protocol is contingent on the following stipulation: (1) that the second administration of the adenovirus vector, Ad1CF2, and associate clinical procedures will be eliminated from the protocol and the informed consent document. The RAC recommends that the investigators attempt to obtain a level of sensitivity adequate to detect 1 replication-competent virus particle per patient dose. The protocol was approved by a vote of 16 in favor, 0 opposed, and 2 abstentions.

XIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GEN TRANSFER PROTOCOL ENTITLED: GENE TRANSFER FOR CYSTIC FIBROSIS USING E1 DELETED ADENOVIRUS: A PHASE I TRIAL IN THE NASAL CAVITY/DRS. BOUCHER AND KNOWLES*

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the protocol submitted by Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina.

Dr. Post explained that this protocol involves the intranasal administration of an adenovirus vector containing the gene coding for CFTR . The advantages of nasal versus lung administration is that th epithelium is easily accessible for biopsies and electrochemical analyses.

This protocol will use the same adenovirus vector that was approved for Dr. Wilson's CF protocol at the December 1992 RAC meeting. Dr. Wilson, a co-investigator on this study, and will supply the vector. This vector has a cytomegalovirus (CMV)-enhanced b- actin promoter with an SV40 polyadenylation s inserted into the E1 region. One important difference between this protocol and Dr. Welsh's nasal administration protocol is the proposed doses of vector. Dr. Welsh's protocol proposed doses between 2 x 10 and 5 x 10 pfu . In this protocol, patients will receive between 2 x 10 and 2 x 10 pfu . Dr. Post add similar doses of adenovirus vector have been approved for other CF protocols. The unique feature of this protocol is that the vector particles will be administered in a relatively small volume, i.e., 2 ml. The

investigators propose that this concentration of virus will result in a high multiplicity of infection (MOI), i 1 x 10 infectious viruses per cell.

Dr. Post explained that he was originally concerned that this high MOI might increase the probability o generating replication-competent adenovirus which could result in a cytopathic effect. The investigator have provided extensive discussion with regard to the *in vitro* versus *in vivo* effects of high MOIs . Th investigators have proposed a rapid dose-escalation, i.e., a 30-fold increase in concentration per group. Dr. Post asked the other RAC members to state their opinions about the proposed doses. The investigators have provided supplemental data derived from extensive baboon studies. These studies involved a total of 15 baboons that received intranasal administration of 1 x 10 virus particles. Analyses of these animals was still in progress at the time of the original submission; therefore, the investigators should provide an update on this *in vivo* data.

Dr. Post asked about the status of vector sequencing. The investigators' written responses indicated that additional sequencing information would be available at this meeting. The RAC should consider whether complete sequencing of adenovirus vectors is a requirement. The RAC has always required that an investigator submit the vector sequence, yet the committee has never provided specific details.

The investigators have proposed a very stringent assay for the detection of helper virus. In light of the discussion of Drs. Wilmott , Whitsett , and Trapnells ' protocol, perhaps the assays proposed for th are too stringent. What is the difference between helper virus assays #1 and #2? Which assay(s) will b performed? How is a determination made as to the cell types that are recovered by bronchial alveolar lavage (BAL)? Can respiratory epithelial cells be distinguished from infiltrating lymphocyte

Dr. Post complimented the investigators for submitting a well-designed, readable, and thoroughly documented protocol. He stated that if the investigators provide satisfactory responses to his questions, he would recommend approval of the protocol.

Review--Dr. Motulsk

Dr. Motulsky stated that the protocol has been thoroughly documented, and that he is very satisfied wit the overall design of the study. The investigators should respond to several issues during their presentation. What is the possibility that patients will produce antibodies in response to the modified adenovirus? Is there any possibility of homologous recombination which would result in a pathogenic virus. Is there any concern about over-expression of the CFTR gene? Is it legal to confine patients for u to 3 days if they are excreting virus if they do not want to be confined? Data demonstrates that the gene does not penetrate the submucosal glands. How important are these glands at restoring complet function?

Review--Dr. Zalle

Dr. Zallen explained that her initial written comments were regarding the source of the vector and the u of the term "gene therapy" in the informed consent document. The proposed vector will be supplied by investigators who are moving from the University of Michigan to the University of Pennsylvania. She inquired whether this transition would create any unforseen problems with regard to obtaining the vecto Also, the RAC should consider the appropriateness of the term "gene therapy" since this protocol is a Phase I study that has no therapeutic intent.

The statement, "I am unlikely to gain any medical benefit from this study" should be revised to a stronger statement such as, "I will not get any benefit from the study." It is unlikely that transduction of a small

portion of the nasal epithelium with the CFTR gene will result in any benefit to the patient's disease

The investigators' responses to the *Points to Consider* were not complete. The listing of the questions far exceeds the responses. The reader is prompted to refer to the protocol for most of the answers. The *Points to Consider* is a document that is read by a much larger audience than the RAC; therefore, the committee should encourage investigators to provide complete responses to the document. The RAC should begin to consider an issue that is not particular to this protocol. Specifically, the RAC should discuss whether a protocol that has been approved at one institution can be conducted at another institution. There may be a great deal of variability in the conditions between institutions. Dr. Walters agreed with Dr. Zallen that the RAC should begin discussion on this issue

Other Comments

Ms. Meyers noted that the investigators have provided a model statement about a request for autopsy in the informed consent document. All investigators submitting human gene transfer protocols for review by the RAC should include a similar statement.

Dr. Chase agreed with Dr. Zallen's comment about the use of the term "gene therapy." Dr. Leventh suggested that "gene transfer" is acceptable alternative wording. This language has been adopted for other protocols.

Dr. Straus stated that the investigators need to expand on the status of the baboon studies. Is it true that human adenoviruses do not replicate in baboon cells? Is the baboon a relevant animal model? Will individuals be excluded from participation in the study if they demonstrate expression of E1 gene products. This stipulation was part of the exclusion criteria for Dr. Crystal's protocol. E1 has been shown to complement adenoviruses. Dr. Parkman explained that during the course of reviewing Dr. Crystal's protocol, Dr. Harold Ginsberg (an *ad hoc* consultant during the December 1992 meeting) stated that complementation by E1 should not present any deleterious effects; therefore, this exclusion criterion is unnecessary.

Presentation--Dr. Boucher

Dr. Boucher explained that the nasal epithelium was chosen as the target for this Phase I study because it provides a defined region that can be easily assessed in order to obtain electrochemical measurements and biopsy samples. Valuable information will be obtained about the safety and efficacy of the gene transfer procedure. The respiratory epithelium of the nasal cavity is very similar to the airways; therefore, it is a very pertinent model.

He presented data demonstrating the fraction of cells in the epithelial sheet that have to be corrected in order to restore the ion transport defect that characterizes CF. Using an artificial system of immortalized epithelial cells, gene transduction correct the electrophysiological defect in approximately 10% of the epithelial sheet. This system is artificial, and it is unclear that the same results will be observed *in vivo*. A transduction rate higher than 10% will probably have to be achieved in humans to see any significant correction in the electrophysiological defect.

He presented *in vitro* data demonstrating that approximately 1 x 10 pfu are required to obtain maximu gene transfer in both nasal and bronchial epithelial cells. Therefore, the nasal cavity should represent a good model in which to study the efficacy of gene transfer in the lower airways. Experiments were presented demonstrating that the vector is capable of correcting the chloride transport defect in primary cultures of cells obtained from CF patients.

Using a murine model, the investigators have been able to demonstrate that the *in vitro* dose-response observation correlates with the *in vivo* response. Both human and murine nasal epithelial cells ar transduced at the same rate with the proposed vector. Therefore, the mouse is an appropriate *in vivo* model for efficacy studies. The *in vivo* murine model was characterized. A cylinder is inserted into th trachea which allows for the administration of a known concentration and volume of virus. This area can be isolated for a defined period of time and provides the opportunity to determine the efficacy of gene transfer in a defined surface area. Exposure to 1 x 10 pfu per ml for 30 minutes results in diffuse gen transfer, i.e., approximately 20% of the cells were transduced . This data suggests that highe concentrations of vector will probably have to be achieved in order to realize a therapeutic effect.

Assuming that very high concentrations of vector will be required, what are the safety considerations? Three days following intratracheal administration of 1 x 10 pfu , no cellular response is observed in submucosal area. However, there was a mild mononuclear infiltrate that was observed on day 7 whic diminished by day 14.

Dr. Boucher explained the baboon model. The nasal mucosa of these animals was slowly perfused particularly the inferior turbinate. Excess material drains anteriorly ; therefore, the material is no swallowed and the possibility of ectopic expression is minimized. Dose-response studies in the baboo indicate that concentrations in excess of 1 x 10 pfu per ml will be required to obtain efficient transductio

Dr. Haselkorn asked Dr. Boucher to address the issue of submucosal resistance in the mouse. Boucher explained that although these tissues are resistant to wild-type adenovirus infection, there are receptors that are expressed in the nasal epithelium. The issue of gene transfer may not be dependent on the virus going through an entire life-cycle. Dr. Boucher introduced Dr. Wilson to provide additional information about the primate model.

Presentation--Dr. Wilson

Dr. Wilson explained that the investigators have established the primate model to determine toxicity. A total of 12 baboons, 3 animals per group, received between 1 x 10 and 1 x 10 pfu per ml into their lung the contralateral side. At a concentration of 1 x 10 pfu per ml, 90% of the distal airway and alveolar were transduced . The cells of the proximal airway demonstrated sporadic gene transduction. A clea dose-response was observed. The gross necropsies were normal at all doses except at 1 x 10 pfu per On day 21, this concentration of vector demonstrated hemorrhagic reaction in localized segments of the lung. Other than the development of this infiltrate at day 21 and a slight fluctuation in their blood gases, the animals were clinically normal. These baboons have also been analyzed for the recovery of virus by BAL and nasal swabs. No virus was recovered from any of these animals.

Dr. Wilson commented on the status of the safety testing of the proposed vector. At the December 1992 RAC meeting, Dr. Ginsberg suggested that indicator cells are the method of choice for detecting replication-competent virus. However, the problem with indicator cells is that HeLa cells cannot tolerate high MOI due to cytopathic effects. The maximum tolerated dose on HeLa cells was 100 pfu Ginsberg suggested using A549 cells. Dr. Wilson said that the investigators anticipated testing a maximum of 1 x 10 pfu per production lot. A maximum of 1 x 10 pfu per plate of A549 cells were ad This large volume of plates should be manageable.

With regard to the status of sequencing, Dr. Wilson stated that the vector has been sequenced from the LTR through the promoters, through CFTR , through the polyadenylation signal, 6.l2 kilobases and 3 kilobases surrounding the E3 deletion. The LTR , CMV , b- actin , the polyadenylation

CFTR gene were intact and functional. However, the sequencing analysis revealed that there are base-pair changes. Two of these changes are identical to the rat sequence. This observation underscores the importance of sequencing the coding region.

In an attempt to discover why these base-pair changes occurred, the vector sequence was cross-checked with the plasmid and found that these changes were present in the original plasmid. Despite these changes, this CFTR allele is totally functional. Currently, the investigators are reconstructing the plasm with known sequences. The new recombinant will probably be available in approximately 4 weeks. This protocol will not proceed until the sequence of the coding region has been determined.

Dr. Miller asked how the investigators can be certain that the entire viral DNA sequence is acceptable. Dr. Wilson responded that the sequencing is being performed by a contractor in 6 different orientations. The sequencing gel has been reviewed, and the data are correct. It is unusual that 3 mutations would occur, 2 of which are homologous to another species. Dr. Miller asked if the sequence obtained by the contractor has been cross-checked with the published sequence. Dr. Wilson said that he learned about the base changes only recently and has not had the opportunity to cross-check with the published sequence.

Dr. Parkman said that the standard should be the sequence of the vector that is going to be administered to the patient. Dr. Miller explained that the retroviruses have never been sequenced, only the plasmids. Even at that, investigators generally assemble sequences from the published literature as opposed to sequencing the plasmids themselves. The data that Dr. Wilson has provided goes beyond any sequence data that has been required previously by the RAC.

Dr. Wilson explained that the investigators have monitored the presence of antibodies to E2a in a variety of species, including primates, and none have been detected. With regard to the issue of recombination, Dr. Wilson stated that even in the worst case scenario, reconstitution of an E3 deleted wild-type virus should not result in any serious adverse consequences. In response to the question about carcinogenesis, Dr. Wilson stated that although there are data which demonstrates tumorigenicity in nu mice with other serotypes, there is no evidence that type 5 adenoviruses are tumorigenic

Dr. Motulsky inquired if there is any coordination between the different laboratories that are conductin gene transfer research for the treatment of CF. Dr. Wilson responded that through the CF Foundation, there is substantial networking. The CF Foundation supports scientific meetings for the exchange of current information and data in the area of gene therapy.

Dr. Post asked Dr. Trapnell if the CFTR cDNA has been sequenced for the GTI supplied ade vector. Dr. Trapnell answered that the plasmid was sequenced and found to be different from th sequence published in *Science*. However, Dr. Lap-Chi Tsui's laboratory said that transcriptional error had been found, and that the sequence published in *Science* was not entirely correct. The *Science* paper had transcriptional errors that have since been corrected. The sequence derived at by GTI is the same the final sequence obtained by Dr. Tsui's laboratory

Dr. Post asked Dr. Wilson if he compared his CFTR cDNA sequence to the sequence published *Science*. Dr. Wilson explained that he compared the sequence to the one in GenBank . Dr. Parkman sa that the CF Foundation should identify the correct sequence of CFTR cDNA . Dr. Wilson stated that cross-check the sequence against Dr. Tsui's corrected sequenc

Dr. Straus stated that the RAC should be cognizant of the duplication of trials. Not only are these trials expensive, but they require a minimum number of subjects per trial. Each trial encompasses a certain degree of risk. With approval of 5 similar trials, these risks are encumbered at 5 centers. Although this

approach may be exciting, it may ultimately prove to be non-therapeutic. Therefore, the RAC should emphasize the importance of animal models in assessing the safety of vectors. Dr. Boucher noted that the proposed study is a safety trial.

In regard to the question of over-expression, Dr. Boucher explained that a number of studies had been conducted in which CFTR was over-expressed in human epithelial sheets that were polarized. Dat demonstrates that there is a level of saturation in the apical membrane. No evidence of expression in the contralateral membrane has been observed. CFTR seems to target in appropriate amounts. Dr. Bo introduced his collaborator Dr. Knowles to respond to questions about the informed consent document. Presentation--Dr. Knowles

Dr. Knowles addressed the issue of holding patients in quarantine. The IRB , the Institutional Biosafet Committee (IBC), and local health officials are in agreement that patients should be confined for 3 days to protect the public welfare. This statement will be included to inform patients that they will not gain any direct benefit from this particular protocol.

Dr. Knowles said that he prefers to use the term "gene therapy" because the objective protocol is to correct the cellular function of the cells in the nose. However, the terminology can be changed if required by the RAC. Dr. Miller said that the term "gene transfer" should be used instead of "gene therapy", because "gene therapy" implies that the corrected gene will restore function in the entire body.

Dr. Knowles explained that the issue of patient follow-up is very controversial at his institution. The patients will be a young adult population. There is a high probability that they will move several times within their lifetime. Is it necessary that they return to the institution after a year? Ms. Meyers stated that it is incumbent on investigators to follow these patients for life. Ms. Meyers reminded the investigators that the patients may not necessarily have to return to the institution. The long-term follow-up clause implies that the investigator will maintain a registry and contact the patient periodically with regard to their clinical status. Dr. Parkman reminded the RAC that the*Points to Consider* clearly indicates that patients will be followed for life.

Ms. Meyers commented on the previous discussion regarding the approval of duplicate or similar protocols. In the past 3 years, approximately 90% of the protocols reviewed by the RAC have been for the treatment of cancer. There have been very few protocols designed for the treatment of genetic diseases. The investigators should be applauded for pursuing multiple trials.

Dr. Post asked if there was data about the particular cell types that are transduced . Dr. Wilson respond that the majority of cells that are transduced are epithelial cells; however, there may be low leve transduction of alveolar macrophages.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Miller to approve the protocol contingent on the following: (1) The correct sequence of the CFTR cDNA must be determined prior to administration vector to patients and provide a should provide a report at the next RAC meeting regarding resolution of the CFTR cDNA sequence. (2) Replace the phrase "gene therapy" with "gene transfer" throughout document. The motion was approved by a vote of 16 in favor, 0 opposed, and 1 abstention.

XIV. PROPOSED ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMA GENE THERAPY PROTOCOL ENTITLED: PHASE I STUDY OF TRANSFECTED CANCER CELL EXPRESSING THE IL-2 GENE PRODUCT IN LIMITED STAGE SMALL-CELL LUNG CANCER/DR.*

CASSILET

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Peter A. Cassileth and Eckhard Podack of the University of Miami/ Silvester Cancer Center, Miami, Fl objective of this protocol is to enhance localized cytokine secretion in order to stimulate an immune response against cancer cells. Tumor cells from patients with limited stage small-cell lung carcinoma (SCLC) will be transfected with a bovine papilloma virus (BPV) expression vector producing IL-2 cells expressing IL-2 will be irradiated to reduce their replication potential and re-introduced into patients after chemotherapy-induced disease remission. Animal data generated by these investigators in the Lewis lung carcinoma model supports the concept that such manipulations can induce rejection of implanted IL-2-secreting tumor cells. The investigators have shown that they can grow SCLC cells from human patients, and that the cells produce relatively high levels of IL-2 after transfection with the B vector.

Dr. Miller pointed out that this protocol is the first study designed to use a potentially oncogenic B vector as a vaccine. Sixty-nine percent of the BPV genome, including all early genes but not the lat capsid genes, is included in the vector. These sequences include BPV genes with transforming pot There is a concern whether the BPV vector is indeed replication-defective and that it does not pose an biohazard or possibility of spreading to other parts of the patient's body or to the population at large. The investigators have reviewed the previous literature and concluded that because of its size and lack of capsid proteins, the BPV vector is replication-defective. In his written review, Dr. Miller asked wheth BPV vector provides improved expression levels of cytokines in the human target cells over the standa retrovirus vectors that would clearly justify its use in humans. This question was not satisfactorily answered by the investigators. Also, there is a lack of convincing evidence in the animal model that injection of irradiated IL-2-secreting mouse tumor cells can promote rejection of existing tumor cells.

Review--Dr. Secundy (presented by Dr. Dronamra

In Dr. Secundy's absence, Dr. Walters called on Dr. Dronamraju to summarize her written commen Secundy's concern was primarily with the informed consent document as follows: (1) the potential bene statement appears too strong; (2) the financial responsibility of the institution in the event of unanticipated side effects of the treatment continues to be problematic, and (3) the question of unpredictable adverse consequences related to recombination of the vector with the human genetic material. In the revised consent form, the investigators addressed these concerns and included a statement about liability for costs of medical care engendered by side effects of therapy.
Review--Dr. Dronamraj

Dr. Walters then asked Dr. Dronamraju to present his comments on the protocol. Three different types questions were raised: (1) Are the investigators proposing to use IL-2 alone or a combination of IL-2 and IL-4 therapy? In the animal model, encouraging results were obtained with a combination of IL-2 plus IL-4. (2) What is the transfection rate in human cells and the dose necessary to elicit a CTL response? (3 was the sample size of 12 patients determined.

Other Comments

Dr. Leventhal found this protocol to be extremely confusing. The investigators should address the num of tumor cells to be obtained, the end points of the study, and the evaluation of the tumor response in patients who already responded to chemotherapy. It is doubtful that any valuable information will be

obtained from this study.

Dr. Straus stated that Dr. Douglas Lowy of the National Cancer Institute has demonstrated that the 69% BPV genome contained in the vector can transform cells *in vitro* because the early viral genes interfere with cell cycle regulation. It is probable that the BPV vector will alter growth properties of tumor cells a well as normal cells.

Ms. Meyers made several comments regarding the informed consent document including the financial responsibility of institutions. She requested a statement should be included about the fertility status of patients, both males and females, and a suggestion that contraception should be used during the experiment.

This issue prompted a lengthy debate regarding the inclusion/exclusion criteria based on the fertility status of men and women. Dr. Miller and Ms. Buc questioned the scientific basis of requiring birth cont during the trial. Dr. Parkman mentioned that drug companies are very firm on this requirement out of concern of any untoward effect to the fetus. Ms. Buc pointed out that contraception clauses will preven exclusion of women from protocols. Dr. Anderson added that this safeguard is good for public perception. In conclusion, the RAC members agreed that both men and women should be allowed to participate in protocols; however, they would be encouraged to use appropriate birth control.

Presentation--Dr. Podac

Dr. Podack stated that this protocol is similar to others that have used lymphokines to increase immunogenicity of tumors in order to achieve an immune response that will be beneficial to patients. Th protocol is for the treatment of SCLC. This tumor is particularly vicious with a mean survival of about 15 months; therefore, the potential benefit of this treatment outweighs the risk of the disease itself.

One major difference between this study and other protocols approved by the RAC is that this protocol is the first to utilize a BPV vector. In responding to a question from Dr. Miller regarding the infectivity of Dr. Podack stated that the 69% fragment of the BPV genome contained in the vector deletes the ge proteins that are necessary to make an infectious particle. BPV virus is very difficult to gr*ow in vitro*, based on data reported in the literature over the past 15 to 20 years. The only way to obtain BPV is to extract virus from a cow wart. Other reports have documented the absence of BPV sequences in slaughter ho butchers who have repeatedly been exposed to bovine warts. In responding to the question about recombination of the vector with human papilloma viruses, Dr. Podack stated that based on extens literature, no recombination of approximately 60 subtypes of human viruses has been reported. Papillom viruses are very species-specific, a bovine virus will infect a cow, but not other animals. Responding to the question of transforming potential, Dr. Podack agreed that this risk is real. There are 3 potentiall transforming genes, E5, E6 and E7, in these viruses. E5 has been shown to be transforming in both bovine and human species.

Discussion

In the present protocol, tumor cells are transfected with the BPV vector. Do these tumor cells bec more aggressive? Dr. Podack stated that in SCLC cells, no morphology changes or changes in the rat cell division have been observed. Dr. Miller asked whether the critical experiment has been performed in animals, i.e., have transfected and untransfected cells been injected into the Lewis lung carcinoma Dr. Podack answered that no differences in tumorigenicity have been observed in animal surviv According to literature, it is very difficult to demonstrate transformation in tissue culture with this vector. No transformed foci were observed in cells selected for G418 resistance. However, without selection, foci

developed after long periods of time. The vector has not been conclusively demonstrated to be transforming. Dr. Nava Sarver of the National Institute of Allergy and Infectious Diseases, NIH , stated t she has shown that papilloma virus transformation can be demonstrated with the C127 cells both *in vitro* and *in vivo* in mice.

Dr. Miller commented on a question raised by Dr. Carmen that a radiation dose of 12,000 rads appears be insufficient to kill all transfected cells. Dr. Miller was concerned that a new type of cancer might aris from the residual surviving cells. After reviewing Dr. Podack's data on cell survival following severa doses of irradiation treatments, Drs. Smith and Miller agreed that 12,000 rads of irradiation does not kil the tumor cells. Dr. Parkman stated that the radiation dose is already very high. Increasing the dose might compromise the transfected cells' ability to produce IL-2. A clonogenic assay would be a prefera method for determining tumor growth after radiation. Dr. Podack stated that he would conduct that ass it is required for approval.

Further questions were raised about *in vitro* studies that were performed for other purposes, with 4 tumor cell lines previously established by Dr. Niramol Savaraj , a co-investigator. Data on IL-2 production a complete due to limited life span of these cells which already have been in tissue culture for several months. In the future, Dr. Savaraj will be responsible for establishing tumor cell lines from biopsies for t gene therapy protocol.

Dr. Dronamraju asked about Dr. Podack's contribution to a paper submitted with this protocol regar combination effect of vaccination with IL-2 and IL-4 complementary DNA transfected cells and th induction of a therapeutic immune response against Lewis lung carcinoma cells. Dr. Podack stated tha this animal model work was initiated while he visited the Japanese laboratory at the National Cancer Institute in Japan and was performed largely by scientists in that laboratory. In response to Dr. Dronamraju's question about IL-2 and IL-4, Dr. Podack stated that if IL-2 experiment is successful, the combination of IL-2 and IL-4 will be used.

Dr. Parkman summarized the discussion noting that interesting pre-clinical data have been provided, but the protocol does not have all the elements to justify approval. Dr. Leventhal criticized the protocol for l of any *in vitro* human data showing that transduced peripheral blood cells from tumor bearing patient demonstrate an enhanced anti-tumor response to autologous tumor cells from those patients. Dr Parkman concurred that additional data should be provided using freshly established cell lines from tumor biopsies. Drs. Leventhal and Miller requested that an endpoint should be included, i.e., CTL assays Podack said these studies will be performed in the future

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Parkman to defer approval of the protocol. Approval is deferred until the investigators return to the RAC with the following: (1) a definition of the clinical endpoints, (2) clonogenic assays using irradiated tumor cells, and (3) a revised informed conse document, including a statement that some patients selected for gene therapy will require a second surgical procedure to obtain material for the study. The motion to defer approval of the protocol passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XV. PROPOSED ADDITION TO APPENDIX D OF THE NIH GUIDELINES **REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED:** *USE OF SAFETY-MODIFIED RETROVIRUSES TO INTRODUCE CHEMOTHERAPY RESISTANCE SEQUENCES INTO NORMAL HEMATOPOIETIC STEM CELLS FOR CHEMOPROTECTION DURING THE THERAPY OF OVARIAN CANCER/DR DEISSEROT*

Review--Dr. Leventha

Dr. Walters called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Albe Deisseroth of the University of Texas M. D. Anderson Cancer Center, Houston, Texas. The objectives this Phase I study are: (1) to test the feasibility of introducing the MDR-1 cDNA into normal hematopo early progenitor cells in advanced ovarian cancer patients by *in vitro* transduction with a retrovirus vector, and (2) to determine the effect on hematopoietic function following ABM transplantatio

Dr. Leventhal explained that the present protocol is similar to other studies reviewed by the RAC. Th investigators will attempt to introduce the MDR-1 gene into ABM cells prior to transplantation so that higher doses of Taxol can be tolerated by the patient. Ovarian cancer rarely metastasizes to bone mar thus lessening the chance that drug-resistant tumor cells will be introduced into bone marrow. There are several concerns that should be addressed regarding this protocol. *In vivo* murine data demonstrates th mice which receive transduced marrow cells recover more rapidly than those that receive untransduc cells. Dr. Leventhal asked if *in vitro* assays have been performed using human bone marrow cells. What is the transduction rate? What is the level of MDR-1 gene expression? The investigators need to provide a more detailed description of the vector, packaging cell line, and the reproducibility of experiments using the CD34(+) stem cells. The procedure for harvesting nucleated ABM cells should be described. Can 7 x 10 CD34(+) cells be reproducibly selected from 2 x 10 nucleated cells/kg? The protocol states that a total of 4 x 10 nucleated cells/kg will be harvested so that one-half of the cells can be cryopreserved as back-up. Will harvesting this number of cells place patients at increased risk without blood transfusion? What is the relationship between G- CSF and Taxol administration? Is there any risk of toxicity in pa who have received prior anthracycline therapy with a daily dose of Cytoxan ? What is the end point protocol?

Review--Dr. Dronamraj

Dr. Dronamraju expressed concern about the informed consent document. The document does not cle state that there will be no direct benefit to the patient, however, useful information will be obtained for the treatment of ovarian cancer in the future. The document should contain a request for autopsy.

Other Comments

Dr. Parkman asked about the parameters that will be used to define recovery of hematopoietic functio following each administration of Taxol . Will the proposed dose of Taxol be tolerated by patients follo ABM transplantation? Is Taxol a standard therapy at M. D. Anderson Cancer Center for ovarian patient who have received ABM transplantation?

Ms. Meyers said that the informed consent document is not understandable to the lay person.

Presentation--Dr. Deisserot

Dr. Deisseroth responded to questions raised by Drs. Leventhal and Parkman regarding T administration in combination with ABM transplantation. Dr. Deisseroth stated that Taxol is not a sta therapy for ovarian cancer. Data demonstrates that Taxol can be administered to only 50% of al individuals because of myelosuppressive effects. The rationale for this protocol is that transduc hematopoietic cells with MDR-1 will increase the patient's tolerance to Taxol . With regard to the saf Taxol administration in combination with ABM transplantation, Drs. Deisseroth and Kavanaugh e they have had experience with patients who have received Taxol following an ABM transplant. Back-u marrow will be stored in the event that patients exhibit unusual sensitivity to Taxol

Dr. Deisseroth presente *in vitro* human data that was not previously submitted. Taxol is routinely used treat patients with advanced cancer. Unfortunately, therapeutic doses have been shown to induce severe leukopenia . At 250 mg/m, 67% of patients have demonstrated severe neutropenia . Myelosuppres the major obstacle to the use of Taxol in ovarian cancer. The goal of this study is to assess whethe introduction of the MDR gene into hematopoietic progenitor cells will decrease Taxol sensitivity. cancer was chosen as the target disease because bone marrow involvement is rare.

The MDR cDNA contained in the vector (obtained by Dr. Michael Gottesman of the National C Institute) has a point mutation which does not affect its function on drug efflux. The vector is derived from the LN vector series

In response to Dr. Leventhal's question, Dr. Deisseroth stated that the second bone marrow harves necessary. Dr. Deisseroth stated that they have successfully established a procedure for selectin CD34(+) cells using a monoclonal antibody column. Following MDR transduction, the bone marrow cel were cryopreserved . The threshold for hematopoietic recovery following the preparative regime 100,000 platelets per cubic microliter and an absolute neutrophil count of 2,000. Escalating doses Taxol will be administered every 3 to 4 weeks in combination with MDR-1 transduced ABM cells. Is a stopping rule for the trial, at doses 150 mg/m of Taxol ? GM- CSF is necessary to stimulate recove

FACS analysis will be used to monitor CD34(+) cells following transplantation. CD34(+) cells will b grown in Dexter culture. Transduced MDR sequences can be distinguished from PCR endog sequences by this method. MDR -resistant cells will be enriched following each consecutive cycle o Taxol . A correlation will be made between the number of MDR transduced cells and the lev chemotherapy-induced neutropenia . Patients will be monitored following each dose of Taxo

Dr. Deisseroth presente *in vivo* murine data. Data demonstrated that transduced bone marrow ce conferred a protective effect against Taxol . Dr. Miller noted that the vector used in the animal model i different from the vector proposed for the human study. Dr. Deisseroth acknowledged that the 2 vector are different. Another objection made by Dr. Miller is that 5FU, which was used to enrich stem cells in the animal study, will not be used for human studies, a significant difference. Dr. Deisseroth presente preliminary data showing a protective effect of the vector that will be used in the human trial in the murin model. Drs. Miller, Leventhal , and Parkman all commented that this data should have been submitted v the protocol.

Dr. Post commented that there was no quality control data on the packaging cell line. Dr. Parkman expressed his concern that the potential risk to patients has not been adequately stated in the informed consent document, i.e., the risks associated with Taxol administration alone and in combination wit transduced and untransduced ABM cells. If patients suffer from neutropenia , they may re transplantation of the cryopreserved marrow and a long costly hospital stay. Dr. Deisseroth conced the protocol may have to be revised incorporating a lower dose of Taxol in order to reduce the risk t patients.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Parkman to defer approval of the protocol Approval is deferred until the investigators return to the RAC with the following: (1) additional preclinical data on the infusion of CD34(+) ABM cells, (2) data demonstrating safety of the starting dose of Taxol , a clear description of the proposed vector, (4) a revised informed consent document using simplified language and including a statement about the potential risks associated with Taxol administratio

following ABM transplantation (5) a revised title reflecting the fact that early progenitor cells will be used rather than stem cells, and (6) provide long-term cell culture data. The motion to defer approval of the protocol passed by a vote of 16 in favor, 0 opposed and no abstentions.

XVI. ADDITION TO THE *POINTS TO CONSIDER OF THE NIH GUIDELINES REGARDIN PROCEDURES FOR EXPEDITED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS (CONTINUED)*

Discussion

The members of the RAC continued their discussion regarding the *Procedures for Expedited Review*. Dr. Walters summarized the changes that had been incorporated into the document based on the RAC's previous discussion.

A lengthy discussion ensued regarding limitation of expedited review to single patient protocols. The intent of this requirement is to define the limits for expedited review. The committee was concerned that investigators might interpret this statement as meaning that any single patient protocol will be reviewed as an expedited review protocol. The fact that a protocol involves 1 patient is not justification for expedited review.

The RAC discussed the appropriateness of using the phrase "gene transfer" for an expedited review protocol since the word "transfer" infers marking and the word "therapy" indicates an attempt to provide a therapeutic treatment. The members of the committee agreed that the term "transfer" should be used with the understanding that these are unproven Phase I trials, not marking studies. For clarification, the RAC added an additional item which specifies that the investigator must provide detailed information regarding the necessity of the expedited review.

The RAC debated whether the statement "Protocols that are deferred or not approved by the RAC in its normal review process, are not eligible for expedited review" should be deleted from the document. Since the committee was unable to come to a consensus with regard to this statement, a vote was called. A motion was made by Ms. Meyers and seconded by Dr. Parkman to include this statement as part of the *Procedures for Expedited Review*. The motion to retain this statement was approved by a vote of 12 in favor, 4 opposed, and no abstentions.

The committee agreed that extramural experts must be included in the expedited review process. The term "scientist" was changed to "expert" to reflect the importance of social and ethical review as well as scientific review.

Committee Motion

A motion was made by Dr. Chase and seconded by Dr. Miller to accept the*Procedures for Expedited Review* incorporating the minor modifications agreed upon by the RAC. The motion was approved by a vote of 16 in favor, 0 opposed, and no abstentions.

The revised version of the *Procedures for Expedited Review* reads as follows:

Procedures to be Followed for Expedited Review

1. An investigator submitting a request to the NIH for expedited review of a gene transfer protoco

must provide detailed information regarding the necessity of expedited review.

2. No protocol shall be considered without Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) approva

3. At this time, all gene transfer protocols must be considered experimental.

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

5. Review of such protocols may include intramural NIH experts but must include extramura experts.

6. Among other factors to be considered by the reviewers, is the similarity of the new protocol to previously approved protocols.

7. The NIH will report to the RAC following expedited review and will include all of the materials o which the decision was based. The RAC will formally review the protocol at its next scheduled meeting. Patient privacy will be maintained.

8. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for expedited review. No protocol shall have more than one patient approved under expedited review.

9. As requested in the context of non-expedited review, none of the costs of the experimental protocol should be borne by the patient or the patient's family.

10. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure.

Discussion

The RAC resumed discussion of the revised version of the*Cover Sheet for Expedited Review (Cover Sheet)*. This working document will be used solely by ORDA as a checklist for the minimum amount o information that is required for submission of an expedited review protocol for RAC review. Unlike the *Procedures for Expedited Review*, the*Cover Sheet* will not be incorporated into the*Points to Consider*. Several minor modifications were made to the*Cover Sheet* and agreed upon by the members of the RAC.

Dr. Royston questioned the intent of the RAC. He proposed the following scenario: If an investigator was approaching the deadline required for *Federal Register* notice, and the protocol in not in final form, would the request be deferred by the RAC if submitted in the incomplete format? Presuming that the protocol would be deferred due to insufficient information, should an investigator withhold submission of the protocol from the regular review process for submission through the expedited review process? Drs. Leventhal and Parkman stated that an incomplete protocol would not be accepted for either the norma expedited review processes. All protocols, whether regular approval or expedited review, need to be complete and contain all of the information necessary to make a rational decision.

Committee Motion

A motion was made by Dr. Carmen and seconded by Dr. Miller to accept the*Cover Sheet for Expedited Review* with the incorporation of minor modifications agreed upon by the RAC. The motion passed by a vote of 15 in favor, 1 opposed, and no abstentions.

The revised version of the *Cover Sheet for Expedited Review* reads as follows:

COVER SHEET FOR EXPEDITED REVIEW OF A GENE TRANSFER PROTOCOL

1. What is the gene and vector proposed for the gene transfer protocol?
    2. a. Has the vector been previously approved by the RAC?

    b. If not, attach**complete** sequence of vector.

3. What is the target cell for the gene transfer?

4. What is the transduction efficiency of the vector into the proposed target cell?
    5. a. What is the level of expression in the target cell?

    b. How was expression determined?

6. What system was used to determine pre-clinical efficacy?

7. What is the endpoint of the protocol?
    8. a. What assay was used to detect replication competent helper virus?

    b. Was replication competent helper virus detected? Yes No

    c. What is the level of sensitivity of the assay? (Attach documentation)

    9. a. What was the date of IRB approval? (Attach copy of approva

    b. What was the date of IBC approval? (Attach copy of approval)

    c. What was the date of IND submission to the FDA

    10. a. If the FDA permits the protocol to proceed, is clinical grade material available? Yes No

    b. If clinical grade material is not available, on what date will it be available?

XVII. DISCUSSION REGARDING THE HUMAN GENE TRANSFER PROTOCOL MATERIALS SUBMITTED FOR APPROVAL ON A COMPASSIONATE USE BASIS/Drs. Royston and Sobo

Background

In a letter dated December 7, 1992, Dr. Ivor Royston of the San Diego Regional Cancer Center, Sa Diego, California, requested compassionate plea approval for a human gene transfer protocol. The protocol was entitled: *Phase I Study of Cytokine Therapy of Cancer, Active Immunotherapy of Glioblastoma with Tumor Cells or Fibroblasts Genetically Modified to Secrete IL*On December 28, 1992, the NIH Director and the FDA granted approval of Dr. Royston's request on a compassionate us basis.

On January 14, 1993, the NIH Director called a special meeting of the RAC to discuss expedited revie procedures. During this meeting, Dr. Royston stated that he would provide the RAC with additional data that was supplied to the FDA. Dr. Royston said that the FDA-submitted material included the following: (1) additional safety data on the transduced cell line, (2) sterility data, (3) safety data on replication-compe virus, (4) vector identity data, and (5) data on the level of IL-2 production. The RAC requested that documentation submitted to the IRB and IBC should be include

Discussion

Dr. Post asked Dr. Sobol if the materials submitted to the RAC are the same as those submitted to th FDA? Dr. Sobol responded that it was his understanding that the general information provided to the F should be submitted. Dr. Sobol said that the most of their discussions with the FDA were by way o telephone communication. Dr. Sobol stated that the document submitted to the RAC is not an exac duplicate of the FDA submission. Dr. Sobol stated that the FDA was supplied with a more detaile description of the protocol, procedures, assays, etc. Dr. Sobol said that the information provided to th RAC is a summary of the information that the FDA had at the time that compassionate approval was granted.

Dr. Chase stated that the RAC never received the *Points to Consider* for this protocol. At the January 14 meeting, Dr. Sobol stated that the *Points to Consider* would be submitted in addition to the FDA-submission for review at the March RAC meeting. Dr. Royston stated at the January 14 meeting that the *Points to Consider* had been prepared and were back at his office. Dr. Wivel explained that th transcripts reflect the fact that Dr. Royston offered to submit the *Points to Consider*; however, the formal request was only for the FDA submission.

Dr. Parkman asked if data had been provided to the FDA regarding the level of IL-2 expression by either transduced tumor cells or fibroblasts. Dr. Sobol said that this data was submitted to the FDA. Dr. M stated that the information provided to the RAC is deficient in several areas; the protocol would never have been approved by this committee. Dr. Miller asked if data was provided to the FDA about the effectiveness of irradiation in inhibiting growth of the injected tumor cells. Dr. Sobol stated that thi information had been supplied to the FDA. Dr. Sobol apologized for omitting this data from the RA submission, and that this omission was an oversight.

Dr. Miller asked the investigators if they submitted any *in vivo* efficacy data to the FDA. Dr. Royston answered that no IL-2 efficacy data in a glioblastoma model was provided. The only efficacy data was *in vitro* colorectal carcinoma data. Dr. Miller asked how they determined the number of IL-2-secreting cells used for injection if the level of IL-2 expression *in vivo* is unknown. Dr. Royston said that the number of cells injection was based on information derived from literature and data derived from the *in vitro* colorectal carcinoma model.

Dr. Royston said that this request was supported by many oncologists and scientists of the National Cancer Institute who were of the opinion that efficacy data is unnecessary. Dr. Miller said that the RAC

does not necessarily agree about the requirement for efficacy data. Dr. Royston stated that if the RAC had not approved this protocol then he would have been forced to challenge the review system.

Dr. Leventhal stated that the protocol approved by the NIH Director and the FDA is not what would normally be considered an emergency protocol. Dr. Leventhal stated that if a protocol is planned for only one patient, then it should be designed to answer a particular question. This protocol has no endpoint. Dr. Royston said that the *Procedures for Expedited Review*, just approved by the RAC, do not include long-term plans. Dr. Leventhal reminded Dr. Royston that this document does include the statement that the single patient protocols must be consistent with the *Points to Consider* which encompasses long-term planning.

Dr. Straus expressed concern that the protocol followed by the investigators is different than the protocol submitted to the FDA. Dr. Sobol said that if the RAC perceives that there was a deviation from the protocol, the error is probably that the statements were not worded clearly enough. Dr. Sobol said that in his opinion the treatments administered to the patient were articulated in the protocol.

Dr. Chase said that the key issue is that the RAC does not have enough information to determine whether it would have voted to recommend approval of this protocol on scientific grounds at the time that the NIH Director approved the request. One of the important aspects of the RAC is the degree of confidence that the public has in the decisions made by this committee. The public deserves to know that the RAC has made an informed decision about the value of an experiment. A decision made by the RAC about a research proposal may be different from a clinical decision made by a physician. As a physician, Dr. Healy granted approval of this protocol based on compassion for the patient. However, from a public standpoint, it is important that this protocol be evaluated for scientific merit by a panel of experts.

Dr. Royston stated that the public is primarily concerned about safety. Dr. Chase agreed that the public is concerned about issues of safety; however, there are other concerns. Dr. Royston said that the FDA looks at safety as well as the scientific merit of a protocol. However, the criteria used by the FDA may be different from those of the RAC. Dr. Chase said that one of the issues that the RAC is concerned about is the possibility Drs. Sobol and Royston might return to the NIH Director with other single patient exp review requests, and that the data generated would not be interpretable by the scientific community. Dr. Chase stressed the importance that all investigators complete the *Points to Consider*. Dr. Chase stated that it is highly probable that if Drs. Sobol and Royston had completed the *Cover Sheet for Expedited Review*, the protocol would not have qualified for expedited review.

Dr. Royston stated that if the RAC retrospectively disapproved the protocol, there would be a greater understanding as to the perceived defects of the study. The RAC has already articulated their expectation that efficacy should be demonstrated in an animal model. There is disagreement about this particular requirement in the medical community. Also, there is disagreement about the extent of preclinical data that is required for a single patient expedited review protocol. Ms. Meyers reminded Dr. Royston that the RAC is not voting on this protocol; approval has already been granted by the NIH Director. Dr. Royston encouraged the RAC to retrospectively vote on this protocol to determine whether the committee's decision would have been the same as the NIH Director's decision. Ms. Grossman reminded Dr. Royston that the documentation submitted to the RAC is incomplete; therefore, it is impossible for the committee to make an informed decision. The purpose of this discussion is for the RAC, not the investigators.

Dr. Sobol stated that he would be unable to satisfy all of the criteria listed on the *Cover Sheet for Expedited Review*, even with the data that is currently available. There is no preclinical efficacy data, only historical data. Would the RAC have considered historical data in human subjects who were immunized with glioblastoma cells as an acceptable alternative to preclinical efficacy data? This proposal was bas

on data from a single uncontrolled study suggesting a survival benefit to patients who received adoptive immunotherapy of glioblastoma cells. The rationale is that gene transfer to these adoptively transferre tumor cells should enhance the survival benefit. There is no evidence that these procedures should produce any toxic side effects.

Dr. Parkman said that the fact that 1 cytokine gene has proven effective in the regression of 1 particular type of tumor does not establish preclinical efficacy for another cytokine or another tumor type. Dr. Sobo disagreed with Dr. Parkman's statement. Dr. Post noted that the uncontrolled study referenced by Dr. Sobol was reported a long time ago. Have any controlled follow-up studies been published since th original report? Dr. Sobol said that subsequent trials have been performed in which efficacy was no demonstrated.

Dr. Straus said that the urgency of illness sometimes necessitates that clinicians extend beyond the reach of existing scientific data. There are circumstances in which protocols could be justified without having demonstrated efficacy in an animal model. However, Phase I trials of this nature usually include therapies for which there is extensive data about the characteristics and safety of these therapies. Gene therapy is a novel therapeutic approach. Until such time that there is confidence in this area of research, it would be inappropriate to presume that protocols should be approved that go beyond the boundary of existing data. Dr. Sobol disagreed with Dr. Straus. Dr. Sobol stated that he does not see any distinction between considered acceptable for chemotherapeutic agents versus gene transfer.

Dr. Carmen asked the investigators how they would have responded to the question about transduction efficiency as listed on the *Cover Sheet for Expedited Review*. Dr. Sobol responded that the percentage cells transduced is not an important issue; the key factor is the amount of IL-2 that is expressed. Ms Grossman said that if an investigator cannot demonstrate reliable and efficient transduction of cells, then the protocol should not be approved. Dr. Royston asked, "If we have a selected cell line that is transduce and secreting the gene product, what is the concern?" Ms. Grossman said that the basic issue is that the RAC must be certain that investigators are capable of transducing genes into target cells. Dr. Sob agreed that gene expression by the target cell must be demonstrated; however, the efficiency of the transduction should not be a decisive factor.

Dr. Sobol said that the first time any agent is administered to a human subject, the effects of a particula dosage are unknown. Only by monitoring the patient's response can the appropriate dose can be defined. Deferring approval of a study in which the only way to obtain data is in the human subject suggests that the RAC is too focused on details. Dr. Leventhal stated that Dr. Sobol's comments confirm her opin this protocol should not have been approved. To approve any protocol, there must be an assurance that every patient who participates in an experiment, including novel therapy, will provide valuable data about that therapy. Investigators cannot discover efficacy in the course of treating a single patient. The fact that the investigators have no plan to study other patients confirms that no valuable information will be gained. Dr. Royston stated that they would be evaluating the patient's immune response to this treatment.

Dr. Leventhal said that if the study had been presented as the first of a series of patients, she would ha recommended approval of this single patient protocol. In that way, valuable scientific information could have been derived from the experiment. Dr. Royston responded that he would like to obtain approval for the treatment of additional patients; however, the FDA required that this request to be submitted as a single patient IND. Dr. Leventhal said that the RAC will certainly review single patient protocols; howev this protocol is inadequate.

Dr. Royston stated that he is concerned by the fact that if he had submitted this study as a single patient protocol through the normal RAC review process, that it probably would have been deferred by the

committee. Dr. Sobol said that he shares Dr. Royston's perspective. Dr. Walters stated that the RA would welcome the review single patient and multiple patient protocols submitted by Drs. Sobol an Royston in the future, and that the committee would attempt to be fair in its review of such protocols based on their merits.

XVIII. ADJOURNMENT

Dr. Walters adjourned the meeting at 4:02 p.m. on March 2, 1993.

Nelson A. Wivel , M.
Executive Secretary

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

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