U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH

MINUTES OF THE RECOMBINANT DNA ADVISORY COMMITTEE

December 3-4, 1992

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The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-first meeting at 9:00 a.m. on December 3, 1992, at the National Institutes of Health, Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Barbara E. Murray (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:

John H. Barton, Stanford Law 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
Roy H. Doi, University of California
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Susan S. Hirano, University of Wisconsin
Brigid G. Leventhal, Johns Hopkins Hospital
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Barbara E. Murray, University of Texas
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Moselio Schaechter, Tufts University School of Medicine
Marian G. Secundy, Howard University College of Medicine
LeRoy B. Walters, 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).

Ad hoc consultants:

Harold Ginsberg, Columbia University
Abbey S. Meyers, National Organization for Rare Disorders

Non-voting agency representatives:

Henry I. Miller, Food and Drug Administration

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

Tyrone Banks, NHLBI

Susan Banks-Schlegel, NHLBI
Bobbi Bennett, OD
Michael Blaese, NCI
Fred Bonkovsky, OD
Bryan Brewer, NHLBI
Steve Brody, NHLBI
Betty Brown, NHLBI
David Brown, NHLBI
Mary Rose Burnham, NHLBI
Jan Casadei, NCI
Ronald Crystal, NHLBI
Chin-Shyan Chu, NHLBI
Pascal Denoby, NHLBI
Evan Deremzo, OD
Bassam Doujaiji, NHLBI
Cindy Dunbar, NHLBI
Steven Ficca, NHLBI
Mary Ellen Franko, NCI
Kateri Gabriele, NHLBI
Barry Goldspiel, CC
Jay Greenblatt, NCI
Sungkoo Han, NHLBI
John Hay, NHLBI
Jim Higginbotham, NHLBI
Joseph Higgins, NINDS
Edward Hirschowitz, NHLBI
Jeffery Hoeg, NHLBI
Tom Horiagin, NCI
Patrick Hwu, NCI
Christine Ireland, OD
Ari Jaffe, NHLBI
Clara Jolley, NHLBI
Stefan Karlssron, NINDS
Shuichi Kato, NHLBI
Steven Kishter, NCI
Nobuyuki Kobayashi, NHLBI
Robert Korst, NHLBI
Becky Lawson, OD
Hiroyuki Maeda, NHLBI
Muneharu Maruyama, NHLBI
Andree Mastreugeli, NHLBI
Kevin McDonagh, NHLBI
Noel McElvaney, NHLBI
Kathryn McKeon, NIDDK
Akira Miyashita, NHLBI
Jim Mulay, NCI
Arthur Neinhuis, NHLBI
Brian O'Connell, NIDR
Joyce O'Shaughnessy, NCI
Herby Pollard, NIDDK

William Ramsey, NCI
Melissa Rosenfeld, NHLBI
Gene Rosenthal, NHLBI
Silvia Santamarina, NHLBI
Eva Scherer, NHLBI
Prem Seth, NHLBI
Yasuhiro Setogudri, NHLBI
Brian Sorrentino, NHLBI
Diane Striar, NHLBI
Robert Walker, NIAID
Susan Wallace, NHLBI
Robert Wersto, NHLBI
Debra Wilson, OD
Louise Williams, NHLBI
Jeehong Yoo, NHLBI

Others:

Paul Aebersold, Food and Drug Administration
French Anderson, University of Southern California
James Barrett, Genetic Therapy, Inc.
Robert Beall, Cystic Fibrosis Foundation
John Bishop, Food and Drug Administration
Hal Broderson, Hilman Medical
Richard Boucher, University of North Carolina
Anthony Calandra, Amgen
Barrie Carter, Targeted Genetics, Inc.
Gwladys Caspar, Harvard University
Yawen Chiang, Genetic Therapy, Inc.
Jan Chappell, Genetic Therapy, Inc.
Ed Chen, Los Angeles Times
Larry Cohen, Somatix Therapy
Francis Collins, University of Michigan
Declan Conroy, Biotech Daily
Matt Cotten, Institute for Molecular Pathology
Mike Courtney, Transgene, Inc.
Andrew Cuthbertson, Genentech
Ann Daigle, Immune Response, Inc.
Kevin Davies, Nature Genetics
Brian Davis, Medicines Control Agency
Albert Deisseroth, MD Anderson Cancer Research Center
Ronald Dorazio, Columbia University
Lori Doyle, University of Pennsylvania Medical Center
Anne Driscoll, Fox, Bennett and Turner
William Egan, Food and Drug Administration
John Engel, University of Michigan
Suzanne Epstein, Food and Drug Administration
Carol Ezzell, Science News
Susan Falen, Genetic Therapy, Inc.
Mitchell Finer, Cell Genesys, Inc.

Mark Fischetti, Freelance Writer
Stacy Fitzsimmons, Cystic Fibrosis Foundation
Terry Flotte, Johns Hopkins Hospital
Bernard Fox, University of Michigan
Jeffrey Fox, ASM News & Biotechnology
Wayne Galbright, Public
Victor Garcia, St. Jude Childrens Hospital
Lisa Giglio, The Pink Sheet
Scott Graham, University of Iowa
Richard Gregory, Genzyme Corporation
Mariann Grossman, University of Michigan
Bill Guggino, Johns Hopkins Hospital
Kurt Gunter, Food and Drug Administration
Elie Hanania, MD Anderson Cancer Research Center
Ingo Hartel, Georgetown University, Kennedy Center
Mark Hausmann, National Museum of American History
Russell Herndon, Genzyme Corporation
David Holzman, Bioworld Today
Susan Jenks, Journal of the National Cancer Institute
Steve Josephs, Baxter Health Care
Nancy Kan, Canji, Inc.
Arifa Khan, Food and Drug Administration
Michael Knowles, University of North Carolina
Rebecca Kolberg, United Press International
Hitoshi Kotani, Genetic Therapy, Inc.
Maryann Krane, Genzyme Corporation
Alex Kuta, Food and Drug Administration
Karen Lee, National Museum of American History
Pierre Lehn, Assistance Publique, Paris
Melvin Long, Abbott Laboratories
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Bruce Merchant, Viagene
Robert Moen, Genetic Therapy, Inc.
Lisa Morris, Genetic Therapy, Inc.
Richard Moscicki, Genzyme Corporation
Rod Morrison, Canadian Cystic Fibrosis Foundation
Stephen Mueller, Genetic Therapy, Inc.
David Mulligan, Abbott Laboratories
Hien Nguyen, National Museum of American History
Philip Noguchi, Food and Drug Administration
Jeffery O'Brian, Dupont-Merck Pharmaceuticals
John Olsen, University of North Carolina
Jeff Ostrove, Microbiological Association, Inc.
Edward Otto, Genetic Therapy, Inc.
Michel Perricaudet, National Center for Scientific Research, Paris
Janet Peterson, University of Maryland, Baltimore
Anne Petruska, The Blue Sheet
Bruce Pratt, Genzyme Corporation
Raj Puri, Food and Drug Adminstration

Judy Randal, Freelance Writer
Abdur Razzarque, Food and Drug Adminstration
Paul Recer, Associate Press, Inc.
Peter Reinecke, United States Senate staff
Devra Rich, University of Iowa
Steve Richieri, Immune Response, Inc.
Joseph Rokovich, Somatix Therapy
Ivor Royston, San Diego Cancer Center
John Russick, National Museum of American History
Laurence Schaffar, Association Francaise de Lutte Contre La Mucoviscidose
Steven Shak, Genentech, Inc.
Tomiko Shimada, Ambiance Awareness International
Nina Siegler, Rock Creek Research
Richard Simon, University of Michigan
Jonathan Simons, Johns Hopkins University
Alan Smith, Genzyme Corporation
Herb Simith, Food and Drug Adminstration
Patti Sowa, Dupont-Merck Pharmaceuticals
Mark Stewart, University of Virginia
Henri Termeer, Genzyme Corporation
Larry Thompson, Science Magazine
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Dominick Vacante, Microbiological Association
Petri Vainio, Siera Ventures
Thierry Velu, Brussels University
Mel Wabuke, Baxter Health Care
Michael Welsh, University of Iowa
Jim Wilson, University of Michigan
Savio Woo, Baylor College of Medicine
Yiping Yang, University of Michigan
Joseph Zabner, University of Iowa
Gregory Zale, Prince Ventures
Beth Ziemann, Bristol-Myers Squibb Company

I. CALL TO ORDER

Dr. Murray (Chair) called the meeting to order. She noted that the notice of meeting was published in the *Federal Register* 15 days prior to December 3 as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. The RAC serves as advisor to the NIH Director. The Director may accept, reject, or send the RAC's recommendations back to the committee for further deliberation.

Dr. Murray stated that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their reviews of the protocol, followed by responses from the principal investigators of the protocols. The Chair will then recognize other RAC 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.

II. MINUTES OF THE SEPTEMBER 14-15, 1992, MEETING

Dr. Murray called on Dr. Doi to review the minutes of the September 14-15, 1992, meeting of the RAC. Dr. Doi stated that the minutes were an accurate reflection of the committee's deliberations.

For the purpose of clarification, Dr. Doi inquired about the final review of protocols that have received approval contingent on the submission of additional information. Do the RAC members have the primary responsibility for the review of this additional information before the protocol can be initiated? Dr. Wivel responded that the primary reviewers of the protocol are responsible for the review and approval of the additional material. Dr. Wivel stated that additional information could be forwarded to all RAC members if requested. Dr. Dronamraju concurred with Dr. Doi regarding the accuracy of the minutes noting one minor correction.

A motion was made by Dr. Doi and seconded by Dr. Dronamraju to approve the September 14-15, 1992, RAC minutes. The motion passed by a vote of 16 in favor, 0 opposed, and no abstentions.

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

Review--Dr. Leventhal

Dr. Murray called on Dr. Leventhal to present her primary review of the protocol submitted by Dr. Joyce O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland. Dr. Leventhal summarized the objectives of the protocol. This protocol is designed to determine: (1) the effect of high dose chemotherapy and multi-drug resistance-one (MDR-1) transduced hematopoietic stem cells on autologous bone marrow (ABM) engraftment, (2) the potential of MDR-1 to be used as a dominant selectable marker, and (3) if MDR-1 expression in ABM cells provides protection against chemotherapy-induced myelosuppression. Patient eligibility will be limited to individuals with biologically documented metastatic breast cancer who have demonstrated either complete or partial response to conventional chemotherapy and who have no demonstrable tumor cells in their bone marrow.

Patients will initially receive a single dose of both Cytoxan and granulocyte colony stimulating factor (G-CSF). When the patient's peripheral blood cell count reaches 2 x 10 cells per milliliter (ml), peripheral blood stem cells (PBSC) will be harvested daily until 10 nucleated cells per kilogram body weight have been harvested. Two weeks following PBSC harvest, the ABM cells will be harvested. Seventy percent of these ABM cells will be cryopreserved, and the remaining 30% will be transduced with the MDR-1 gene. Following ABM harvest, patients will receive maximally tolerated doses of the chemotherapy agents ifosfamide, carboplatin, and etoposide (ICE) with MESNA. Following chemotherapy, patients will be reinfused with their cryopreserved cells; and the MDR-1 transduced cell population. Patients who demonstrate residual disease will receive either taxol or vinblastine. ABM sampling will be performed periodically to monitor for the presence of the MDR-1 gene. Any recurrent tumor will be assayed for the presence of the MDR-1 gene. In summary, the investigators will attempt to induce a higher level of chemotherapy resistance in ABM cells than in tumor cells.

Dr. Leventhal stated that the majority of her questions and those of Dr. Brinckerhoff had been addressed by the investigators; however, there are still concerns about the assays that will be performed to determine MDR-1 expression. Although polymerase chain reaction (PCR) will provide useful information

regarding gene transduction, no information will be obtained regarding gene expression. Since these patients have already received intensive chemotherapy, their tumor cells may already possess the MDR phenotype. Not enough animal experiments have been performed to support the scientific basis for this protocol. Also, gene expression should be addressed more thoroughly. An additional concern is the possibility of transducing residual tumor cells remaining in the ABM. How will the investigators distinguish between naturally occurring MDR resistance resulting from prior drug exposure and MDR resistance induced by the inserted gene?

Other Comments

Dr. Parkman asked about the patient eligibility criteria. Dr. Leventhal responded that patients will possess Stage IV breast cancer. Dr. Parkman addressed the issue of possible tumor cell transduction. Immunohistochemical staining can detect approximately 1 in 10 tumor cells. If patients demonstrate no detectable tumor cells by this method at the time of biopsy and at the time of ABM harvest, then the likelihood of tumor cells being transduced is extremely low. Clinically, disease reoccurs at the site of previous bulk disease.

Dr. Parkman stated that the optimal method for detecting MDR-1 gene expression is to grow the transduced cell population in the presence of a selective agent and then to perform PCR on the individual resistant clones. Double marking will distinguish endogenous MDR-1 expression from MDR-1 expression based on the differences in ribonucleic acid (RNA) length.

Dr. Doi asked if a high level MDR-1 expression is essential to the success of this protocol and whether the investigators plan to modify the level of expression. Are there *in vitro* experiments that could be performed that would provide information regarding expression, i.e., the *in vitro* maintenance an expansion of MDR-1 transduced hematopoietic cells. He inquired how long ABM cells would have to survive *in vivo* to provide a therapeutic effect during high dose chemotherapy.

Mr. Barton said that since the purpose of this study is to permit the use of a more intense chemotherapy regimen in the event of relapse, the risk versus benefit section of the informed document should be expanded to more thoroughly address this issue.

Dr. Post asked about the number of transduced cells that will be administered to these patients. Since the cells will be enriched by several logs due to the CD34(+) selection, what is the level of sensitivity of the immunohistochemical staining?

Dr. D. Miller explained that the proposed vectors are similar to LNL6 and G1Na with the addition of the MDR-1 gene. However, human complementary DNA (cDNA) will be used instead of murine cDNA. What are the differences between human and mouse cDNA with regard to drug sensitivity? Some of the supporting data for this protocol was derived from murine experiments using a vector different from those being proposed for this study. How does the vector used for the animal experiments compare to the vecto proposed for the human study? Will the investigators introduce additional cDNA mutations to reduce inappropriate splicing?

Dr. Leventhal noted that the investigators propose to increase the dose of taxol 10-fold. Is this a procedure that is normally part of the standard clinical protocol, or is this increased dose unique to the gene transduction protocol?

Ms. Meyers stated that the informed consent should be revised to include the following: (1) a statement that the patient is not pregnant or planning to become pregnant, (2) a statement that results of the study

will be made known to the patient, and (3) a section describing how the media will be handled.

Presentation--Dr. O'Shaughnessy

Dr. O'Shaughnessy responded to Dr. Leventhal's questions regarding MDR-1 expression. Following transplantation, bone marrow sampling will be performed on days 14, 28, and 42. If the MDR-1 gene is no expressed in the cells obtained at these time points then additional sampling will be performed at 3 month intervals. Patients receiving vinblastine or taxol due to relapse will receive sampling after cycles 1 and 3 to monitor augmentation in the number of MDR-1 expressing cells. *In situ* PCR will be performed to determine P-glycoprotein (Pgp) expression in ABM cells, and rhodamine efflux assays for the detection of antibody against Pgp. Cell cultures will be grown with and without the presence of taxol in order to determine any differential effect, and the resulting colonies will be assayed by PCR.

Dr. O'Shaughnessy addressed the issue of tumor cell contamination in both bone marrow and peripheral blood cell populations. Initially, the patient's cells will be subjected to routine hematoxylin and eosin staining in addition to immunohistochemical staining with a panel of 4 antibodies. The specificity of the antibodies has been extensively documented by E. J. Shpall, et. al. Data demonstrates that CD34(+) selection results in a 2-5 log reduction in the number of breast cancer cells. In the event of relapse, patients will receive biopsies at the sites of recurrence. It is unlikely that MDR-1 transduced breast cancer cells will contribute to relapse; however, if this result was observed, the quality and quantity of the patient's life should not be influenced. Due to prior drug treatments, the tumor cells tend to be MDR-1 positive and express Pgp. There is no evidence that insertion of the MDR-1 gene will make these breast cancer cells more biologically aggressive. In the worst case scenario, MDR-1 transduced cells could contribute to relapse and the patient may not respond to taxol or vinblastine. In this event, the patient would be treated with another chemotherapy agent to palliate symptoms. Since patient survival at the time of relapse is approximately 6 months, the objective is primarily to alleviate symptoms; prolongation of survival is not the critical issue.

In response to Dr. Leventhal's question regarding the administration of taxol, Dr. O'Shaughnessy stated that the 10% increase in dosage is a standard procedure for the clinical protocol and not unique to the gene therapy protocol. Dr. O'Shaughnessy referred Dr. Doi's question regarding ABM transplant survival to her co-investigators Drs. Arthur Nienhuis and Kevin McDonagh. The changes to the informed consent document suggested by Ms. Meyers will be incorporated in addition to the terminology *Stage IV* to describe the stage of disease.

Dr. Dronamraju asked what proportion of the patient population will be in the lactation/pregnancy age range? Dr. O'Shaughnessy answered that 10 to 20% of the proposed breast cancer patients would be in this age range.

Dr. Post asked about the total number of CD34(+) cells that would be reinfused. Dr. O'Shaughnessy said that the patients will receive between 1 and 5 x 10 CD34(+) cells. These cells are a pool of both ABM and peripheral blood cells. Dr. Post asked if immunohistochemical staining will be performed prior to reinfusion. Dr. O'Shaughnessy responded that the immunohistochemistry information would not be available until after the patient is treated, because the staining will be performed simultaneously with reinfusion.

Dr. Geiduschek asked if drug selection of nontransduced ABM cells will result in the enrichment of tumor cells. Dr. O'Shaughnessy noted that this protocol does not include drug selection of the ABM cells; however, she noted that tumor cell enrichment would depend on the specific drug. For example, 4-hydroxy cyclophosphamide preferentially kills tumor cells.

Dr. Leventhal asked if MDR can be induced in ABM cells at a level that exceeds natural tumor cell resistance resulting from previous drug exposure. Dr. O'Shaughnessy stated that the level of MDR Pgp expression required to induce clinical drug resistance in human breast cancer patients is unknown; however, sufficient levels of Pgp expression can be obtained in transduced hematopoietic cells to confer resistance. Dr. Leventhal noted that since the absolute levels of MDR expression are unknown, it is unclear how the protocol design will determine this answer. Dr. O'Shaughnessy stated that Drs. Nienhuis and McDonagh will address MDR expression in primate and murine systems. Dr. Parkman informed the RAC members that the investigators have chosen the breast tumor model because these cells are known to express MDR naturally as a result of chemotherapy exposure. The scientific question that needs to be addressed is whether the transduction of hematopoietic stem cells results in levels of MDR-1 expression that are sufficient to induce a biological effect. If a significant clinical effect is observed, than this procedure may be applied to the treatment of a variety of tumors.

Dr. Leventhal said that it is unclear that the protocol is designed to demonstrate anything other than gene expression. How will the protective effect of this gene expression be demonstrated? Dr. O'Shaughnessy explained that the protocol is designed to determine the level of MDR expression at steady state following ABM transplant. Patients who have received taxol or vinblastine after transplant will be monitored for MDR expression to determine if selection is occurring. If protection is being conferred by MDR expression, a decrease should be observed in the duration and depth of the neutropenia/thrombocytopenia. Dr. O'Shaughnessy noted that there is an ongoing gene transfer protocol in which patients receive high dose ICE therapy with neomycin resistant (neo) transduced ABM stem cells. Patients who relapse and are treated with taxol or vinblastine will be used as controls for the MDR study.

Dr. D. Miller said that this protocol should be directed at patients with tumor types that would benefit from this therapy. Since breast cancer patients already demonstrate MDR resistance following drug therapy, this therapy will probably not provide a therapeutic effect. Dr. O'Shaughnessy explained that 20 to 30% of breast cancer patients should benefit from the subsequent taxol or vinblastine therapy. Dr. Leventhal asked if eligible patients will have limited bone disease. Dr. O'Shaughnessy stated that patients with £ 3 metastases on a bone scan will be eligible for this protocol. Metastatic breast cancer refers to metastases in the liver, lung, and soft tissues.

Dr. Carmen inquired whether mixing experiments have been performed with tumor cells and transduced ABM cells. Dr. O'Shaughnessy stated that these experiments have been performed by Dr. E. J Shpall. Dr. Shpall's data demonstrates that transduced ABM cells are detectable at concentration between 1 x 10 and 1 x 10 cells.

Presentation--Dr. Nienhuis

Dr. Nienhuis addressed the issue of *in vitro* expression of transduced cells and evidence of gene transfer. The DNA from MDR-1 transduced cells has been analyzed by PCR and reverse transcriptase (RT) PCR has been employed to determine RNA levels. Rhodamine efflux assays have been performed. Rhodamine is a dye that is released by cells as a function of protein expression. Transduced cells have been grown in selective culture, and colonies are screened for drug resistance.

What is the evidence for *in vivo* gene expression? Dr. Nienhuis presented fluorescence activated cell sorter (FACS) analysis data using an antibody that is specific for an external epitope of the MDR protein. In the murine model, approximately 10 to 12% of the bone marrow cells were positive by FACS analysis. This antibody detection method would be used to monitor patients. Dr. Parkman asked about the level of

expression following long-term *in vitro* culture. Dr. Nienhuis responded that the long-term culture experiments have not been performed. Dr. Parkman asked ifMDR expression has been observed in human MDR-negative cells. Dr. Nienhuis stated that these experiments have not been performed.

Dr. Nienhuis responded to the RAC's concerns regarding the protective effect oftransduced ABM cells during taxol administration. Quantitative PCR will be employed to determine the proportion of ABM cells that contain the transduced MDR gene. Increased levels of MDR expression will indicate amplification of the genetically-modified population of cells.

Dr. Nienhuis discussed the current status of vector development. The vector that was used for the animal experiments was a Harvey-based vector that contained extraneous sequences, i.e., the entire VL30 coding region. The Moloney-based vectors are being proposed for the human experiments because the extraneous sequences have been minimized. The amphotropic producer clone G1 MD1-15 made by Dr. McDonagh was used for the rhesus animal experiments. ThecDNA derived from this clone exhibits a functional splice donor and splice acceptor site that are part of theMDR cDNA in different exons. Although these sequences probably do not function as splice sites in the natural *in vivo* transcript, this splice occurs in a proportion of the transcripts. The originalcDNA has a valine substitution in codon 185 that increases colchicine resistance and decreases vinblastine resistance. This observation was critical in the development of the vector. Dr.McDonagh created the AB-1 vector where splice donor and acceptor sites were eliminated and glycine was conservatively substituted for valine.

AB-1 was the vector that was originally proposed for use in the human studies; however, several problems were encountered. First, sequencing of this vector revealed that there was a frame shift mutation in the C-terminus of the protein. Second, although the gene could be expressed in primarytransfected cells in which the DNA had been introduced, the retrovirus vector did not provide efficient transfer of the gene due to the corrected splice sites.

The second vector, G1 MD3, contained the corrected frame shift but the splice sites remained deleted. Due to the deleted splice sites, this vector was ineffective at providing gene transfer. The final vector proposed for this protocol contains the naturalcDNA. This vector contains the splice donor and acceptor sites and incorporates a glycine substitution for valine. Although this amino acid substitution eliminates the C-terminus frame shift,vinblastine resistance is enhanced. Amphotropic producer clones are currently being characterized that will prove satisfactory for clinical purposes, i.e., capable of CD34(+) cell transduction and producing a titer of approximately 5 x 10. Dr. Parkman asked for a comparison between the Harvey-based vector and the new vector in an animal model. Dr.Nienhuis asked Dr. McDonagh to respond to Dr. Parkman's question.

Presentation--Dr. McDonagh

Dr. McDonagh explained that only 5 animals have been studied using a head-to-head comparison of the Harvey-based and Moloney-based vectors. Dr. D. Miller asked about the specificMoloney-based vector used for the *in vivo* experiments. Dr. McDonagh explained that the original vector containing the splice donor and acceptor sites and the codon 185 C-terminus mutation was used for the animal experiments. Two of the 5 animals demonstrated a 3 to 4-fold increase in copy number with theMoloney-based vector, whereas 3 of six animals showed an increase in copy number using the Harvey-based vector. These experiments have been performed using only fibroblast cell lines, not primary hematopoietic cells. Dr. D. Miller asked whether all of the Moloney constructs contain human cDNA. Dr. McDonagh said that all of the Moloney constructs contain human cDNA as well as the Harvey constructs.

Dr. Murray asked if human bone marrow cells have beentransduced and selected with chemotherapeutic

agents to demonstrate gene expression. Dr. McDonagh said that these human experiments have not been performed; however, gene expression has been demonstrated in rhesus cells. Rhesus and human cells probably function in a comparable manner.

Dr. Nienhuis said that the preliminary data is adequate to support the protocol. Murine data demonstrates that the MDR gene can be expressed in murine stem cells and that protective immunity can be conferred. In the *in vitro* primate system, MDR-1 transduction and expression have been demonstrated. Long-term reconstitution (up to seven months) with gene modified cells has also been demonstrated. At this point in time, it is appropriate to proceed with the human study.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Zallen to defer approval of the protocol. Approval is deferred until the investigators return to the RAC with the following: (1) data demonstrating that human cDNA CD34(+) cells can be transduced *in vitro* with the actual vector that will be used for the human clinical protocol, (2) a description of the assays that will be performed to monitor gene expression, (3) the methods by which gene expression will be compared in tumor and ABM cells, and (4) data demonstrating the clinical endpoint for bone marrow recovery, i.e., what is the period of time required for the detection of polymorphonuclear leukocytes? What is the standard error for this recovery period?

Dr. Post asked the investigators how long it will take them to prepare for human clinical trials. Dr. Nienhuis responded that 6 to 8 weeks would be required to complete the characterization of the producer clone and probably 3 to 4 months before Food and Drug Administration (FDA) approval is obtained.

Dr. Nienhuis responded to Dr. Leventhal's concerns regarding the detection of clinical benefit to patients. Will there be an amplification of the retrovirally-transduced cells as the patients receive taxol and vinblastine and undergo bone marrow suppression and regeneration? Amplification will be the primary determinant as to whether there is an incremental increase in the number ofretrovirally-marked cells. In the murine system, MDR-1 amplification can be compared to neo. In the human study, MDR-1 amplification can be compared to patients receiving neo transduced cells. The issue of bone marrow protection, however, remains to be determined. Dr. O'Shaughnessy agreed that each patient will act as his/her own control. Repeated taxol administration clearly results in cumulative bone marrow toxicity. If no improvement is observed in the neutrophil nadir or its duration following subsequent cycles of taxol administration, important information will be obtained. Dr. Leventhal said that the evaluation criteria should be formalized. Dr. Nienhuis suggested that a positive response could be considered an increase from 1 to 10% retrovirally-transduced cells following 3 cycles of treatment.

Dr. D. Miller asked the investigators if they will continue to enroll patients into the neo protocol that has already been approved by the RAC. Dr. Nienhuis said that the gene marking protocol will continue. The objective of the neo marking protocol is to compare reconstitution by marking peripheral blood and bone marrow cells with different vectors. This breast cancer protocol uses only one vector, theMDR vector, to transduce both peripheral blood and marrow cells. The comparison of the patient populations between the two protocols will be critical in interpreting the data. Dr. D. Miller asked the investigators if they anticipate the generation of data in the next 3 months demonstrating the ability to mark stem cells. Dr. Nienhuis explained that the first patient on the neo marking protocol was just recently treated. Since there will be variability in the levels of transduction between patients, it is appropriate to initiate both protocols concurrently.

The motion to defer approval of the protocol passed by a vote of 17 in favor, 0 opposed, and no

abstentions. Approval of the protocol was deferred until the investigators submit the following additional information and data to the RAC: (1) data will be provided demonstrating that human CD34(+) cells have been transduced *in vitro* with the vector that will be used in the clinical protocol; (2) description of all of the assays that will be used to measure gene expression, and demonstrate how this expression will be monitored in bone marrow and tumor cells; and (3) a description of the endpoint for determining efficacy (evaluation criteria), i.e., comparison of gene amplification and the rate of white blood cell recovery following taxol treatments 1 and 3.

Dr. Murray welcomed Ms. Abbey Meyers of the National Organization of Rare Disorders and Dr. Harold Ginsberg of Columbia University as *ad hoc* consultants to the RAC.

IV. DISCUSSION REGARDING COST ASSOCIATED WITH TREATMENT FOR NON-NEGLIGENT RESEARCH-RELATED INJURY/DR. ZALLEN AND MS. MEYERS

Presentation--Dr. Zallen

Dr. Murray called on Dr. Zallen to initiate discussion on the issue of costs associated with the treatment of non-negligent research-related injury. Dr. Zallen explained that the informed consent documents of many previously approved human gene therapy protocols include the following language, *You or your insurance company are expected to pay the medical costs arising out of any research-related injuries* If this statement is a trend for future protocols, then the only patients who would be able to cover the costs associated with untoward research-related injuries would be those individuals who possess high-level medical coverage or have substantial personal financial resources. This trend is in direct conflict with NIH policy, namely, the inclusion of women and minorities in study populations. Traditionally women and minorities have had less access to jobs that provide health care benefits.

Dr. Zallen noted that Dr. Charles McCarthy, formerly Director of the Office for Protection from Research Risks, NIH, presented a historical review during the September 1992 RAC meeting regarding the political factors and institutional events that resulted in a failure to act on either the 1977 Health, Education, and Welfare report or the subsequent President's commission report that recognized an obligation to compensate subjects for research-related injury.

Gene therapy is a novel area in which the potential risk to patients is not entirely understood. As time and experience are gained, the risks will be more clearly understood. However, sufficient knowledge has not been gained regarding the possible adverse effects associated with gene transfer. If injury is incurred to patients participating in these protocols, it is unlikely that insurance companies will compensate for medical costs associated with such injury. The RAC should propose that the NIH Director convene a panel to examine this issue and to create policy that will guide Institutional Review Boards (IRBs) and the RAC in future decisions.

Dr. Zallen and Ms. Meyers submitted a draft letter to the NIH that addressed the aforementioned issue, Dr. Zallen suggested that the RAC should make additions or changes to the letter as appropriate.

Presentation--Ms. Meyers

Ms. Meyers noted that the short-term and long-term implications of gene therapy are unknown. Patients who enroll in these protocols should receive special consideration and should be monitored for life. The letter that is presented for discussion specifically addresses gene therapy because of the uniqueness and uncertainty of the technology.

The broader issue is that there are many American citizens who are being denied the opportunity to participate in research protocols because they are required to pay for numerous procedures. If these patients suffer untoward consequences as a result of the research, they are currently responsible for these additional costs associated with the treatment of these injuries.

Discussion

Ms. Buc suggested that the draft letter should be expanded to discuss the issue of payment for non-negligent research injury in the broader sense. If the NIH Director is advised to convene a panel to propose policy, this issue should not be limited to gene therapy. The issues are generalizable to all other areas of research. Gene therapy should not be the specific target.

Dr. Zallen agreed that the proposed issue is not necessarily unique to gene therapy; however, the RAC has a responsibility to bring this issue to the NIH Director's attention. Gene therapy is separate from other areas of research because it is a novel technology. The potential short-term and long-term side effects cannot be anticipated as with other therapies, e.g., drug therapy. Dr. Secundy spoke in support of Dr. Zallen's assertion that gene therapy is significantly unique.

Dr. Leventhal stated that gene therapy is probably more accessible to those patients who have access to major procedures that are normally used as vehicles for gene therapy, e.g., bone marrow transplant. Gene therapy protocols are often submitted as an addition to pre-existing clinical protocols. For example, if patients can afford bone marrow transplants, they will have access to the gene therapy at no cost. In terms of risk, gene therapy probably does not present any more risk than any other Phase I study.

Dr. Parkman said that the mandate of the RAC is to review gene therapy; however, the committee is obliged to consider any aspect of medical research within this context. He explained that as a pediatrician, the same questions arise regarding possible long-term untoward effects resulting from the chemotherapeutic reagents given to children with cancer. To mandate that the long-term effects of these treatments would be reimbursable by the government may lead to numerous problems. If the Federal government starts to indemnify particular groups of potential research subjects, clinical research will become limited rather than expanded to large numbers of subjects. There will always be a degree of uncertainty about research. The purpose of the informed consent document is to inform patients of the definable and undefinable risks of that research.

Dr. Post said that he supports the concept that gene therapy is no more predictable than any other type of drug research. In fact, gene therapy is probably more predictable because the mechanism is known and assays such as PCR are available to monitor trafficking and expression.

Dr. Walters suggested that the draft letter submitted by Dr Zallen and Ms. Meyers should be revised to state that in the course of reviewing gene therapy and gene transfer protocols, the RAC has ascertained that there is significant variation among institutions regarding the question of research-related non-negligent injury. The RAC recommends the formation of a panel to establish policy regarding this issue. Mr. Barton noted that the proposed panel would generate declaratory policy rather than real policy. Mr. Barton said that since there is a possibility that gene therapy will result in untoward effects, any costs associated with such effects would more appropriately fall on the Federal government rather than on the individual or the institution. However, these liabilities should be dealt with in a way that will not deter research. This issue should be considered within the context of health care policy that will be developed by the incoming Administration. Proposed policy that will be incorporated into the new health care system should not discriminate against research in any unintended way.

Dr. Schaechter stated that he supports the draft letter because it is important that people of all socio-economic segments of society have access to new therapies. Dr.Geiduschek said that he is in support of the letter. It is inadequate for the RAC to deny that gene therapy has no special status. Dr. Geiduschek proposed that the following recommendation should be included in the draft letter to theNIH Director, *at a minimum, research sponsors or their institutions should be responsible for such costs*

Dr. Murray said that while patients are often screened to ensure that they can pay for the clinical therapies such as bone marrow transplant, there is no financial screening to guarantee that they would be able to pay for any complication resulting from untoward effects.

Ms. Meyers said that gene therapy patients are potentially accepting debt for the rest of their lives if there are research-related injuries, because no insurance company will cover a person who has undergone gene therapy. Compensation is not the issue here. The issue is guaranteed medical care for life if no insurance company will cover these patients.

Ms. Buc noted that the issue has become confused during the discussion. The issue is reimbursement for costs associated with non-negligent research-related injury, not differential access due to the cost of the procedure. With regard to informed consent, there appears to be an underlying notion that some patients are able to consent to procedures but not others. This hypothesis is unacceptable. If a patient is not capable of consenting to the risks of a protocol, then he/she should not participate in it. The financial status of a patient does not determine their ability to provide consent.

Dr. Murray recommended that Drs. Walters,Zallen, Geiduschek, and Ms. Meyers should take the committee's comments and suggestions into consideration in preparing a revised version of the letter to the NIH Director. This revised letter will be discussed later on in the meeting.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: GENE THERAPY OF THE RESPIRATORY MANIFESTATIONS OF CYSTIC FIBROSIS USING A REPLICATION DEFICIENT RECOMBINANT ADENOVIRUS TO TRANSFER THE NORMAL HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR cDNA TO THE AIRWAY EPITHELIUM/DR. CRYSTAL*

Review--Dr. Parkman

Dr. Murray called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Ronald G Crystal of the National Institutes of Health, Bethesda, Maryland. Dr. Parkman summarized the objectives of the protocol. This protocol is uniquely different from other protocols that have been previously reviewed by the RAC because: (1) the first time that cystic fibrosis (CF) has been proposed as a target disease for human gene therapy, and (2) the first time that an adenovirus has been proposed as a vector for the*in vivo* transduction of human cells.

Cystic fibrosis is the most common serious genetic disorder among whites and Hispanics; 1 in 10 individuals are carriers of this gene and 1 in 3,000 live births result in the manifestation of the disease. Three major organ systems are involved: the lungs, liver, and gastrointestinal tract. The gastrointestinal tract is affected due to the decreased production of enzymes that are necessary for food digestion; therefore, many individuals are malnourished. Patients exhibiting gastrointestinal effects traditionally undergo enzyme replacement therapy. The major clinical manifestation of cystic fibrosis is thick mucous secretions resulting from the defective cystic fibrosistransmembrane conductance regulator (CFTR) gene. The thickened secretions cause the bronchi and bronchioli to become obstructed. Infection of these secretions often leads to severe scarring of the patient's airways.

Since the discovery of the CFTR gene, investigators have demonstrated that the normal CFTR gene can be inserted into the cells of CF patients *in vitro*, and that these transduced cells are capable of normal function. Specifically, the corrected cells have the capacity to pump chloride ions, resulting in the normalization of the export of water. This observation suggests that correction of the defective CFTR gene *in vivo* would result in thinning of the patient's secretions. The objective of this protocol is to normalize the genetic defect in a significant number of bronchial cells so that the secretions obtain normal viscosity. Ultimately, the incidence of pulmonary infection would be decreased, and the patient's life would be prolonged.

This protocol is very restricted in its clinical application. Ten patients will be enrolled, each receiving only a single vector application. Since bronchial epithelial cells have a short life span, only surface epithelial cells will be affected. Therefore, the benefits of this therapy, if any, will be restricted to the life span of these surface epithelial cells.

Patients will be treated in groups of two. Each consecutive pair of patients will receive a 1 log increase in dose of adenovirus vector; the lowest dose will have a titer of 10 plaque forming units (PFU) and the highest titer will be 10 PFU. The objectives of this study are to demonstrate: (1) the efficient transduction of bronchial epithelial cells with the CFTR gene, and (2) the length of time that gene expression will persist and in what proportion of the cells. The information obtained from this study is necessary before the investigators can submit a multiple administration protocol.

The investigators have a large amount of preclinical data to support this study. While their original experiments were performed using two adenovirus constructs, the most recent experiments have focused on one vector. Using this construct, the investigators have demonstrated the *in vitro* transduction of bronchial epithelial cells and nasal mucosal cells obtained from CF patients. *In vivo* expression of the CFTR gene has been demonstrated in both the monkey and cotton rat models.

The protocol is divided into 3 parts: (1) the initial baseline period, (2) the vector control period, and (3) the experimental period. The baseline period is the timeframe that individuals are observed in order to determine if they have mild, moderate, or severe disease. Patients with severe disease will probably not be able to tolerate some of the procedures such as bronchoscopy; therefore, they will be ineligible for the protocol. Once a patient has been determined to satisfy the inclusion and exclusion criteria, he/she will enter the vector control phase. During this phase, patients will receive repeat administration of the media (vehicle) that will contain the vector. This vehicle control will be applied initially to the nares; and finally, a 20 ml administration will be applied to the left main stem bronchus. If no adverse effects are observed as result of the vehicle challenge, the patient will be qualified to proceed to the experimental portion of the protocol. During this phase of the study, the patient will receive a 0.4 ml application of the adenovirus vector to their left nare. If no adverse reactions are observed 24 hours after this preliminary application, the patient will receive 20 ml of the adenovirus vector into the left main stem bronchus. The right side of the patient will not be treated so that it will function as a control.

Following vector administration, the patient will remain in a negative pressure room in order to minimize aerosols. Daily cultures will be obtained of the patient's sputum, stools, urine, and nasopharynx until there is no longer evidence of vector excretion or secretion. In the unforseen circumstance that a patient requires intensive care unit (ICU) level of care, there are 3 negative pressure ICU rooms available. Patient care personnel will wear gowns, gloves, masks, and take the same precautions required for infectious viruses. Once the patient is no longer secreting or excreting virus, he/she will be removed from the negative pressure room. This period of isolation is estimated to be approximately 10 days.

Patients will continue to undergo further testing following this period. These studies will include monitoring of specific parameters relating to the patient's pulmonary function. Individuals will undergo nasal mucosa and bronchial cell sampling by either scraping of the nasal mucosa or intermittent bronchial lavage. The cells obtained from these procedures will be assayed for: (1) DNA of the CFTR gene, (2) RNA resulting from the CFTR gene, (3) protein expressed as a result of CFTR gene expression, and (4) cell conductivity changes. In summary, the investigators will be looking for *in vivo* evidence of electrophysiological improvements and *in vitro* evidence of gene insertion and expression.

Dr. Parkman discussed the proposed adenovirus vector. Retrovirus vectors, which have been traditionally used for gene therapy, require cell division in order to become effectively integrated. Since bronchial epithelial cells do not divide indefinitely, efficient transduction with retrovirus vectors would be almost impossible. For this reason, the investigators have developed an alternative vector based on a Type 5 adenovirus.

Naturally occurring Type 5 adenovirus is capable of producing upper respiratory symptoms in humans. This vector has had all of the E1a and most of the E1b genes removed. E1a and E1b are the genes that are required for the regulation of transcription; although this vector has the capacity to infect and transduce non-dividing cells, it is incapable of replicating. The proposed vector has a relatively consistent point of integration; therefore, some of the concerns accompanying the use of retrovirus vectors (e.g., random integration and oncogenic potential) are minimized in this setting.

The major concern regarding the use of this adenovirus vector is that Type 5 adenoviruses exist naturally in our population. What will be the effect on a person whose cells have been transduced with this adenovirus? Could a wild-type infection rescue this replication-incompetent vector and create replication-competent Type 5 adenovirus containing the CFTR gene? Dr. Parkman explained that the investigators have responded to these concerns by including the criteria that only patients with serum antibodies to Type 5 adenovirus will be used for this study. The presence of serum antibodies eliminates concerns regarding transmission of the CFTR gene to the germ cells of the patient. Rescued Type 5 adenovirus would be neutralized and eliminated from the patient's circulation. For this reason, the requirement that patients should be beyond reproductive age or have evidence of sterility are probably excessive criteria.

As an added safety procedure, patients will undergo pulmonary cell sampling in order to screen for the presence of E1a and E1b sequences. Patients who exhibit positive PCR to these sequences will be excluded from the study. In summary, only those individuals who have had pre-existing Type 5 adenovirus infections (as demonstrated by serum antibodies) and who have no evidence of persistent adenovirus elements (E1a or E1b) will be eligible for this protocol.

The E3 sequences that are capable of suppressing the host immunological response have been removed from the proposed vector. Therefore, in the event that a replication-competent recombinant virus were formed, the recombinant virus would be less pathogenic to the patient than the wild-type virus.

Dr. Parkman commended the investigators for their excellent responses to his preliminary reviews. He noted that he still has concerns regarding the possibility that a patient who has remaining transduced epithelial cells after 3 to 4 months may acquire a wild-type adenovirus infection that could cause the recombinant virus to be rescued. The question also remains about the bronchial levels in which the CFTR gene will be expressed. If the vector is instilled into the upper bronchi, how far down will the expression occur, i.e., first, second, or third order bronchioles?

Review--Dr. Ginsberg

Dr. Murray called on Dr. Harold Ginsberg, an expert on adenoviruses and *ad hoc* consultant to the RAC, to provide his review of Dr. Crystal's protocol, specifically the vector issues.

Dr. Ginsberg explained that there are tremendous advantages to using adenovirus vectors that have the E1a and E1b regions deleted; except for a high multiplicity of infection (MOI), the virus is incapable of replication. E1a is critical for enhancing the transcription of other early genes. Adenoviruses, like all DNA viruses, have genes that are expressed early. These early genes are required for DNA replication and the production of infectious virus. If DNA replication does not occur, then neutralizing antibodies will not be made. Another unique feature of E1a is that this region contains the two most dominant cytotoxic T lymphocyte (CTL) antigens. Therefore, the marked production of CTL in response to the vector is very unlikely. There is a gene within the E1a region that is required to terminate protein synthesis. Host protein synthesis is inhibited by preventing the host messenger RNA (mRNA) from being transported into the cytoplasm. Consequently, CFTR mRNA will continue to be expressed.

Within the E3 region is a gene that codes for a 19 kilodalton (kd) glycoprotein (gp). The gp prevents Class I major histocompatibility complex (MHC) antigens from being transported to the cell surface, and it is critical for CTL recognition. When the gene coding for this gp is deleted, *in vivo* pathology is augmented. This increased response is due to the fact that the significant part of the disease is a product of the expression of early genes. Viral replication is unnecessary for disease manifestation; pathology is the result of the expression of early genes.

Dr. Ginsberg responded to Dr. Parkman's question regarding the persistence of adenovirus in the host. Adenoviruses do not persist in infected epithelial cells, because these cells eventually die. Dead cells are shed by the host, not lysed. Although adenovirus DNA is capable of persisting in the lymph node, it is unlikely that DNA will exist anywhere other than the epithelial cells.

Regarding possible recombination, it is very unlikely that a recombinant will result from an E1a wild-type gene and the CFTR gene, because the resulting construct would be too large to be assembled.

Dr. Murray asked if other RAC members had questions for Dr. Ginsberg. Dr. Doi asked that if the DNA does not persist in epithelial cells, will the CFTR be present for only a short period of time? Dr. Ginsberg said that gene persistence will be a limiting factor. Dr. Schaechter asked if transfection of the CFTR gene would cause an increased turnover in these cells. Dr. Ginsberg responded that there would not be an increase in cell death; turnover will be based on the normal half-life of the epithelial cells.

Dr. D. Miller asked if adenoviruses are lytic to cultured cells. Dr. Ginsberg noted that this statement is a universal misconception. Adenoviruses are not lytic. The only means of obtaining the virus from infected cells is to lyse the cell by another method. Dr. D. Miller asked about an appropriate assay for the detection of adenovirus helper. Dr. Ginsberg stated that HeLa and KB cells are the best choice for performing a plaque assay. Plaques will indicate the presence of dead cells that are killed by replication-competent adenovirus.

Dr. Post asked if there is data regarding the enteric administration of an E3 minus adenovirus to humans. Has an E3 minus adenovirus ever been given to a human via the respiratory route? Dr. Ginsberg responded that he was unaware of any instance where these experiments have been performed.

Dr. Post asked for further information regarding long-term persistence of non-integrated adenovirus. Dr. Ginsberg stated that both E1a and E1b are required for adenovirus integration; therefore, E1a and E1b

mutants will not transform cells because there is no integration. Dr. Ginsberg noted that persistent infection of cultured lymphoid cells has been demonstrated for up to 3½ years without any evidence of integration.

Dr. Hirano asked if it is more advantageous or disadvantageous to retain the E3 segment of the vector. Dr Ginsberg answered that the advantages of retaining this segment outweigh the disadvantages. Since there will be only a 5% increase in the size of the genome, only assembly will be facilitated.

Dr. D. Miller asked if the packaging cells contain other viruses that could be transferred in addition to the vector. Dr. Ginsberg said that the cultures could possibly be contaminated withadeno-associated virus (AAV); however, testing will be performed to monitor for such contaminating agents. There are no naturally occurring contaminants.

Review--Dr. Schaechter

Dr. Schaechter said that he is reassured by the information provided by Dr. Ginsberg; specifically, that the ability of the adenovirus vector to replicate and recombine is very small. However, it would be useful for the RAC to discuss the outcome if the virus actually replicated. What would be the outcome ifCFTR is overexpressed in the cells of any tissue? What are the conductance and permeability problems that would arise? In murine experiments, overexpression of the CFTR gene produces no untoward effects; however, this is not a homologous experiment. Overexpression of the protein is a highly regulated step that requires phosphorylation by a highly regulated mechanism. In theory, overproduction ofCFTR should not produce a deleterious effect.

Dr. Schaechter said that he had concerns regarding the efficacy of this protocol. What percent of the bronchial epithelial cells will have to be transduced in order to achieve a temporary therapeutic effect? Is lavage the best method for accomplishing this transduction? What about aerosols? The chances of this single administration treatment being effective does not seem to be overwhelming. How will data derived from this experiment provide assurance that future studies will result in a therapeutic modality?

Review--Dr. Walters

Dr. Walters complimented the investigators on a well designed protocol. However, he noted that there were still a few remaining concerns following his communications with the investigators. The protocol and the informed consent document would be more accurately titled,*A Phase I Study of the Effects of A Single Administration of...*. In addition, the early introductory sections of the consent form should clearly state it is unlikely that there will be any clinical benefit to the patient from a single administration of this vector.

Dr. Walters questioned the exclusion of fertile individuals from this study. The background provided by the investigators explained that most male CF patients are infertile. What fraction of females are infertile due to their disease? While concern regarding unintended germ line effects is understood, if potential subjects are willing to commit themselves to the practice of contraception, then it is discriminatory to exclude individuals solely on the grounds of their fertility. The investigators need to discuss the possible effects of transmission of the vector to a third party bystander. Although this protocol is well designed, the responses to the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* are often inadequate. The majority of responses were primarily references to the protocol. All investigators should take the time to allow the reviewers to form an adequate conception of the protocols from reading through the*Points to Consider*. References to other sections do not provide much useful information.

Dr. Walters said that he consulted a pulmonologist regarding this protocol. The pulmonologist noted the requirement for 11 bronchoscopies. Upon further examination, Dr. Walters documented 9 mandatory bronchoscopies and 11 optional procedures. What is the experimental necessity for the proposed number of bronchoscopies? The pulmonologist inquired whether any *in vitro* or *in vivo* studies have been performed regarding the effect oflidocaine on gene transduction or expression.

Other Comments

Dr. Post asked if the administration of 20 ml of vector to a patient's bronchus would cause any pain or discomfort? Could a portion of this volume be coughed up by the patient and subsequently swallowed? What would be the result of a CFTR enteric infection?

Dr. D. Miller asked if there is any justification for the use of a negative pressure room for these patients? The RAC should decide on the appropriate level of containment for these procedures. An investigator can always increase the level of containment; an unnecessary precedent may be set if the RAC requires negative pressure containment. Dr. Leventhal agreed that the RAC must establish a minimum standard. Dr. D. Miller explained that negative pressure containment is an unnecessary precaution, because the virus is debilitated so that it cannot replicate or spread. Does CFTR present a unique concern? Dr. Leventhal asked if the investigators plan to perform any follow-up studies on the health care providers of these patients regarding the transmission of the virus.

Presentation--Dr. Crystal

Dr. Murray called on Dr. Crystal to respond to the questions and comments presented by the RAC members. Dr. Crystal provided an overview on CF, the most common lethal hereditary disorder of Caucasians and, to a lesser extent, Hispanics. CF pulmonary disease causes a thick, sticky mucus that results in chronic infection, inflammation, and airway obstruction. CF is a progressive disease; the average life span of a CF patient in the United States is approximately 29 years. The problems associated with the gastrointestinal tract are manageable. The majority of CF male patients are infertile. Current therapy for CF disease involves mechanical approaches to airway clearance, i.e., antibiotics, pancreatic enzyme replacement, and nutritional supplementation.

The mutations in the CFTR gene of these patients causes epithelial ion channel abnormalities that result in disease; however, the mechanisms are not clearly understood. An adenovirus vector was chosen for the transfer of the corrected gene because high titers of virus are obtainable and host cell proliferation is not required. The receptor for the adenovirus vectors has not been identified. The vector enters the endosome which has a low pH. It is in this endosome that the virus probably sheds it's coat and interacts with the nucleus; however, the majority of the vector probably works extra-chromosomally.

Dr. Crystal presented *in vivo* data from the cotton rat model regarding transmission of the vector to the various parts of the airways. Histological examination demonstrated 100% gene expression as far as the terminal bronchioli and alveoli in these animals.

The proposed vector, AdCFTR, was constructed by Drs. Michel Perricaudet and Chin Chu. This vector is based on an E3-deleted, E1-deleted adenovirus. Approximately 35 to 40% of the E1b region is remaining in addition to the CFTR cDNA.

Dr. Crystal addressed the issue of vector safety. Historically, replication-competent adenoviruses have been administered to humans for a variety of reasons. In the early 1960s, adenovirus infections were a major problem for closed populations and the military; therefore, live Type 4 and Type 7 vaccines were

developed and marketed to the military. It is estimated that over 5 million doses of live adenovirus vaccine have been administered to date with no epidemiologic consequences. Other investigators have documented the intra-muscular, intra-cervical, intra-arterial, intravenous, and intra-nasal administration of live adenoviruses. Results indicate that in patients with pre-existing anti-adenovirus serum antibodies, clinical sequelae are mild and rare. Therefore, the administration of replication-incompetent adenovirus should not present any apparent safety concerns.

Dr. Crystal addressed the issue of repeat administration that would be proposed for future protocols. If a patient developed IgA or IgG antibodies as a result of a single administration of this vector, would these immunoglobulins interact with the adenovirus and block receptor binding--therefore, interfere with efficacy? Although administration of this vector invokes serum anti-AdCFTR immunoglobulins within one week, there are two reasons why this response may not present a dilemma for repeat administration protocols: (1) the clinical experience is that immunity is not permanent, and (2) the mucosal immunity does not correlate with serum immunity. He presented *in vivo* neutralizing antibody titer data derived from rhesus monkeys. These titers were determined for both serum sampling and bronchiallavage. No neutralizing antibodies were detected in the airways of these animals. Mucosal immunity does not necessarily correlate with circulatory immunity. He presented *in vivo* cotton rat data demonstrating the ability to give multiple administrations of the vector, and that long-term gene expression is possible.

Dr. Crystal discussed the patient inclusion and exclusion criteria. Eligible patients will be E1a negative in their respiratory epithelium and serum antibody positive. Epithelial cell samples will be obtained in order to demonstrate *in vitro* transduction by the vector and that the vector does not replicate. Safety will be demonstrated in the nasal epithelium first. If no adverse effects are observed, the vector will be administered to the patient's lung. It is probable that efficacy will be observed at the higher vector titer; however, the effect will probably be transient over a period of several months.

What are the risks associated with this treatment? One concern is whether the vector is capable of replicating in human airway epithelial cells? Data demonstrates that the vector is incapable of replication even at 5 times the MOI of the maximum proposed dose. Will there be germ line transfer? Primate data demonstrates that CFTR mRNA is expressed only in airway epithelial cells; there was no evidence of expression by PCR in any other tissues, including gonads.

Dr. Crystal agreed with the RAC members' concerns regarding the sterility criteria. There is no indication that there will be germ line transfer; therefore, the appropriate changes could be incorporated into the informed consent document. However, women would be asked to exercise appropriate birth control.

In regard to the effects of vector administration and bronchiallavage on the patient's lung, Dr. Crystal stated that although no damage occurs to the lung, there is an inflammatory response to the vector. However, this inflammation is mild and transient compared to the endogenous inflammation.

Are there risks associated with the repeat administration of the vector? Dr. Crystal noted that no evidence of untoward effects was observed in either the cotton rat or non-human primate models. Does shedding occur? Shedding is infrequent and transient in both the cotton rats and non-human primates. The vector is often present in the stool for several days, but then disappears. Is recombination possible? Although recombination is possible, the risks are very minimal with the precautions that will be taken. Is there a risk of malignancy? There is no evidence of malignancy associated with this virus serotype. The possibility of genome integration is very remote. In response to concerns regardingcDNA regulation and CFTR overexpression, Dr. Crystal said that no clinical signs or symptoms have been observed in 250 cotton rats for as long as 14 months or in the 16 rhesus monkeys out to 240 days.

Dr. Crystal discussed the E3 region in further detail. The region deleted from the proposed vector is approximately 3.5 kilobases and codes for 6 proteins. The most important of these proteins gp 19, blocks the Class I MHC antigens from being expressed on the epithelial surfaces. There are two other proteins coded by this region which down-regulate the EGF receptor. E3 was removed from the vector for the purpose of reducing the size of the vector and to increase the stability of the vector. Although there is a greater inflammatory response when E1 is present and E3 is deleted, if there is a recombinational event, this vector is safer because it would be recognized and eliminated by CTL. Data suggests that E3 minus mutants shed less than E3 positive mutants.

Dr. Crystal responded to Dr. Parkman's question regarding the theoretical possibility that a patient who is no longer shedding the vector becomes infected with adenovirus. Experiments were described in which cotton rats received either E1 minus or E3 minus mutants. When animals received the replication-deficient virus, no shedding was observed. When these animals were rechallenged with the wild-type vector 7 days later, no shedding was observed. The same results were obtained in the rhesus model. In response to Dr. D. Miller's question regarding other viruses present in the 293 cells, Dr. Crystal said that the 293 cells will be assayed for many adventitious viruses, including AAV.

Dr. Crystal stated that 20 ml was chosen as the administration dosage because this is the volume that is required to reach the entire airway. Can any predictions be made regarding efficacy? Efficacy is expected at the higher titers, although it will probably be transient over a period of months. *In vivo* animal data suggests that immunity will not be a problem in terms of repeat administration.

Dr. Crystal agreed with Dr. Walter's suggested change in the title of the protocol and suggested the addition of the word *lung* after the words *conductance regulator*. Dr. Crystal agreed to move the clinical benefit section to the beginning of the informed consent document. In response to concerns regarding third party casual contact, Dr. Crystal stated that the transfer probably would not occur through casual contact.

Regarding the number of bronchoscopies, Dr. Crystal stated that he has had experience with CF patients who have received more than 40 bronchoscopies over a period of time. In order to demonstrate biological efficacy and to proceed to future protocols, the number of bronchoscopies outlined in the protocol are probably necessary. There will be a minimum of 5 and a maximum of 9 obligatory bronchoscopies. The language describing the bronchoscopy procedures will be clarified. Dr. Crystal stated that there is no evidence that lidocaine has any effect on gene transduction or expression.

Discussion

Dr. Post asked if the 20 ml volume of vector will be removed. Dr. Crystal acknowledged that the 20 ml will be removed after the appropriate period of time. Dr. Post asked if patients tend to cough when this volume of material is administered. Dr. Crystal said that the local anesthetic causes the patient to lose their cough reflex. Patients are observed while they retain the 20 ml volume, and they are not allowed to eat during this period.

Is negative pressure isolation absolutely critical for these patients? Dr. Crystal said that the rational for this level of containment was to use the same criteria required for adenovirus laboratory work. He added that they were trying to be extremely conservative, and that negative pressure containment would be employed regardless of the RAC's decision.

Dr. D. Miller asked for further information regarding E1a assessment. Dr. Crystal said that they are able to detect E1a with a level of sensitivity of less than 2 copies per 10 PFU. Dr. D. Miller asked if a bioassay

will also be performed? Dr. Crystal said that vector replication assays will be performed on freshly isolated normal human airway epithelium at an MOI of 1000. Dr. Crystal noted that assays are not performed with 10 epithelial cells, because this experiment would be technically impossible. Dr. D. Miller asked why HeLa cells are not used for the replication-competent assays. Dr. Crystal explained that the *gold standard* is the *in vivo* cells, which is the airway epithelium.

Dr. Geiduschek inquired if the safety margin of the vector could be improved by increasing its length to the packaging limit; therefore, decreasing the likelihood of acquiring a sequence that would increase replication-competence of other properties. Dr. Crystal explained that as the 100% limit is approached, the construct probably becomes less stable, and it is harder to obtain the same levels of titer. AdCFTR has been passaged over 15 months. The sequence of the construct after that length of time has remained unchanged.

Dr. Walters noted that the informed consent document states, *Participation in this study does not mean automatic inclusion in subsequent studies*. Would an individual who participates in this protocol be less eligible for participation in later studies? Dr. Crystal stated that participation in this protocol would not affect participation in subsequent protocols. Dr. Walters also asked if aerosol administration of vector would be more comfortable to the patient than bronchial lavage. Dr. Crystal responded that adenoviruses can certainly be aerosolized; however, an unnecessary safety risk may be introduced particularly to the health care workers. Aerosol administration may be proposed for future protocols, but it is not an appropriate choice for this early study.

Dr. Zallen asked if health care workers would be monitored in any way. Dr. Crystal said that there are no plans to monitor the health care workers. This decision is based on recommendations from both epidemiologists and hospital consultants.

Dr. D. Miller asked if reconstruction experiments were performed to demonstrate that 1 in 10 particles of adenovirus are detectable. Dr. Crystal said that these experiments have been performed. *In vitro* assays will be performed on normal allogeneic human airway epithelium in addition to their own airway epithelium at an MOI that is 5 times the maximum dose. If 200 wild-type adenovirus particles are distributed among 10 cells, the actual MOI is approximately 10. Dr. D. Miller asked if a CF patient were to obtain 100 particles of replication-competent adenovirus, what complications would be observed? Dr. Ginsberg said that 100 particles instilled into a CF patient could theoretically produce disease.

Dr. Schaechter asked what percentage of the patient's lung would have to become transfected in order to realize a therapeutic benefit. Dr. Crystal explained that the gene product is expressed at extraordinarily low levels. Very little expression is required to improve health. The mRNA expression in the airway surface cells is probably due to only 1 or 2 copies. This number may be somewhat higher for mucosal cells. Dr. Chu has demonstrated that exon 9 is a critical exon in the first nucleotide binding fold. Dr. Chu determined that the 3 CF patients studied who exhibited a 90% reduction in exon 9 were completely normal. This data suggests that all the patient's cells do not have to have the normal CFTR gene. Data from Dr. Richard Bouchet's laboratory suggests that a 7 to 10% transduction of the epithelial sheet is all that is required to correct the biology of these cells. Dr. Crystal suggested that cell-to-cell communication is a possible explanation for this result.

Ms. Meyers suggested that the investigators should add a paragraph regarding protection of the patient from the media. Dr. Crystal agreed to add a section regarding protection from the press to the informed consent document.

Dr. Post asked for a description of the generation of the clinical grade vector preparations. Dr. Crystal

explained that his laboratory has had several years experience making adenovirus vector lots that have highly reproducible titers and that are free of contaminants. Dr. Post asked about the degree of experience with the detection of helper virus. Dr. Crystal stated that helper virus assays have been performed routinely for a long period of time. Dr. Post asked if an E1a positive recombinant virus has ever been detected. Dr. Crystal said that his laboratory has detected positive E1a recombinant virus on several occasions, and that these lots were rejected. Dr. Post asked how often these positive recombinants are detected. Dr. Crystal said that the critical question is where is the recombinant virus coming from? In most cases, the E1a positive virus comes from the 293 cells. The recombinant virus probably results from pieces of DNA, not the entire virus. All vector preparations that have been used for the *in vitro* and *in vivo* experiments have been E1a negative. Dr. Post asked if recombinants have ever been detected, not E1a sequences. Dr. Crystal said that recombinants have never been detected.

Dr. D. Miller inquired whether E1a positive samples were tested in bioassays to determine the presence of replication-competent helper virus. Dr. Crystal said that these bioassays have not been incorporated as part of the routine production scheme due to concerns about HeLa cells, etc. However, bioassays have been performed at random and replication-competent viruses have never been detected.

Dr. Geiduschek said that he is aware that there are practical limits to biological assays for the detection of replication-competent virus posed by the ability of E1a minus virus to replicate at high MOIs. However, it is possible to change the lower contamination limit by performing serial plaque purifications. Therefore, it should be possible to specify precisely that specific lots of virus will be at a contamination level of *X* or lower, where *X* is defined and consistent between the various lots. Dr. Crystal said that he would perform these assays if required to do so by the RAC. However, Dr. Crystal explained that the assays that are outlined in the protocol are the experiments that are most appropriate. It is preferable to perform only the assays listed.

Dr. Ginsberg agreed with Dr. Geiduschek's recommendation that serial plaque purifications be performed on a non-permissive cell line and suggested that the investigators include this experiment in the vector testing procedures. Dr. Crystal agreed that his laboratory would perform the suggested assays. Dr. Ginsberg suggested that the seed virus used for the production lots should be assayed in addition to a sample obtained after 6 cycles of production.

Dr. D. Miller suggested that a stipulation be included that there be less that 1 adenovirus helper particle per 20 ml of vector preparation. Since the investigators cannot obtain enough airway epithelial cells to test 10 particles, how will the biological assay be performed? Dr. Crystal stated that 10 cells will be used with a MOI of 1000. Dr. D. Miller explained that the assay described by Dr. Crystal reflects 10 particles. Since some patients will receive 10 particles, how will less than 1 replication-competent particle in 10 be determined? Dr. Crystal agreed that this determination would have to be made on a cell line, not fresh human epithelial cells. Dr. Ginsberg noted that there are existing cell lines that are actually more sensitive that human epithelial cells.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Schaechter that Dr. Crystal's protocol be approved with following stipulations: (1) the title of the protocol and informed consent document should be changed as suggested by Dr. Walters, (2) the informed consent document should include a statement that there is no expected long-term clinical benefit from this treatment, and this statement should be included at the beginning of the benefit section, (3) individuals will not be excluded from participation in this protocol based on their fertility status, and (4) demonstrate that there is less than 1 replication-competent adenovirus helper particle in 20 ml of the highest vector concentration. The motion to approve the protoco

with the aforementioned stipulations was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

VI. MINOR MODIFICATION AND SUMMARY PRESENTATION OF THE PROTOCOL ENTITLED: *EX VIVO GENE THERAPY OF FAMILIAL HYPERCHOLESTEROLEMIA/DR. WILSON*

Presentation--Dr. Wilson

Dr. Murray called on Dr. James Wilson of the University of Michigan Medical Center, Ann Arbor, Michigan, to present an update on his approved human gene therapy protocol. Dr. Wilson explained that familial hypercholesterolemia (FH) is an autosomal dominant disease in which patients inherit two defective genes. One of these genes is the gene that encodes for the low-density lipoprotein (LDL) receptor; therefore, patients are either homozygous or compoundheterozygotes. The function of the LDL receptor is to metabolize LDL. A deficiency in this gene, results in the accumulation of LDL cholesterol levels between 500 and 1,000 milligrams per deciliter. For reasons that are unclear, FH patients exhibit diminished levels of high-density lipoprotein (HDL). These individuals have elevated LDL cholesterol levels from birth and eventually develop xanthomas and premature coronary artery disease. Patients who are completely void of the LDL receptor are called receptor negatives. This population has an average survival of approximately 10 years of age due to thesequelae of coronary artery disease. Patients who possess residual receptor function survive into their 20s.

The objective of this protocol is to genetically correct a limited number ofautologous hepatocytes and reinfuse these corrected cells back into the patient. On day 0, the left lateral segment of the liver is resected and a Pittman style catheter is inserted into the inferior mesenteric vein that feeds the portal circulation. Hepatocytes are isolated from the resected portion of the patient's liver and exposed to the recombinant retrovirus for 2 days. Within 72 hours following surgery, thesetransduced cells are reinfused into the catheter.

Dr. Wilson summarized the results of the baboon toxicity studies. Three large baboons underwent the aforementioned procedure with the same retrovirus vector proposed for the human trials. These animals have been followed for 1½ years. All 3 of these animals have a normal clinical profile. Serum chemistries and hematologies have also been performed and are normal except for a transient increase in liver enzyme tests at the time of resection.

In situ hybridization has been performed on liver sections from these animals to determine if the transduced cells and gene expression have persisted. Although there is some variability between biopsy specimens, results indicate that the promoter is functional for the duration of the animal experiment and that the transduced cells persist. Since these animals did not have any defect in their LDL receptor gene, the effect on serum cholesterol could not be monitored.

The human clinical protocol was approved for 3 patients with homozygous FH. Although the study was approved for patients of any age, the RAC suggested that an adult should be first patient to receive this treatment. This patient was required to have documented coronary artery disease, acceptable surgical risks, and no liver pathology. The first patient enrolled in this protocol was a 29 year old female from Quebec, Canada. The French Canadian population has an extremely high concentration of patients with FH, most of whose genotypes are known. This patient was a homozygote with two defective alleles, both of which had a tryptophane/glycine amino acid substitution at position 66 which resulted in a substantial binding defect. This patient had 2 brothers die from coronary artery disease at ages 20 and 26. The patient had a total serum cholesterol of 550 milligrams per deciliter due to an elevation ofLDL, a myocardial infarction at 16 years of age, and a coronary artery bypass. At the time of evaluation, one the

patient's bypasses had failed and there was a lesion in the left main coronary artery.

At the time of surgery, 250 grams of her left lateral liver lobe was resected. The liver was perfused according to the protocol and 3 x 10 viable cells were obtained. These cells were plated onto 800 10 centimeter dishes. After 48 hours, the cells were transduced and then harvested. A total of 2 x 10 cells were recovered and infused in 3 separate aliquots. Each aliquot was examined for gross contamination, assayed for the uptake and expression of the gene, and assayed for LDL receptor activity. LDL receptor activity was assessed by incubating the cells with fluorescent labelled LDL ligand and analyzing by fluorescent microscopy. A plate-to-plate variation of between 5 and 25% positive cells were observed.

Visualization procedures indicated an even distribution of the infused cells into the intrahepatic circulation. The patient demonstrated no evidence of clotting or intraluminal thrombus.

Three months following the treatment, the patient returned for clinical evaluation. Following a liver biopsy, the patient demonstrated no evidence of gross pathology. Her liver function tests were normal. Immediately following the cell infusion, the patient's LDL cholesterol decreased to approximately 180 milligrams per deciliter. Since the patient most likely had LDL receptors at this point, she was given medications to lower her cholesterol. Her LDL cholesterol levels continued to drop slightly; however, this may be due to fluctuations. The patient's HDL cholesterol level, which was originally very low, is increasing. The combination of increasing HDL and decreasing LDL has decreased her cardiac risk factor from 10 to 5.

Dr. Wilson described numerous patients who have been evaluated. A second patient from Costa Rica has been identified and evaluated. Recently, 3 additional patients have been identified who are eligible and would likely benefit from this treatment. However, due to the limitation on the number of patients, he requested a minor modification to his previously approved protocol. This minor modification would extend the number of eligible patients from 3 to 5. Increasing the number of patients would allow the investigators to treat 2 brothers from Cyprus ages 8 and 12, who have severe coronary artery disease and a 7 year old girl from the United States whose sister died at age 3 of severe coronary artery disease.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Ms. Buc to extend the patient enrollment for this protocol from 3 to 5 patients. The motion to approve the minor modification was approved by a vote of 16 in favor, 0 opposed, and no abstentions.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: GENE THERAPY OF CYSTIC FIBROSIS LUNG DISEASES USING E1 DELETED ADENOVIRUSES: A PHASE I TRIAL/DR. WILSON*

Review--Dr. DeLeon

Dr. Murray called on Dr. DeLeon to present her primary review of the protocol submitted by Dr. James Wilson of the University of Michigan Medical Center, Ann Arbor, Michigan. Dr. DeLeon stated that the significance and biological basis for adenovirus-mediated gene therapy of CF and the natural history of the disease have been extensively presented by Drs. Parkman and Crystal. Therefore, this review will be limited to comments on the procedures proposed in Dr. Wilson's protocol.

A total of 12 patients will be enrolled in this protocol. Patients will receive escalating doses of the vector, which will be administered by bronchoscopy. Pulmonary sampling will be performed on day 4, 6 weeks,

and 3 months following treatment. The endpoints of this study have been clearly defined. The investigators will determine gene transfer and expression via biological response and toxicity. Clinical efficacy is not a goal of this protocol. The proposal is straightforward and adequately addresses the issues of safety and vector classification. However, there is one remaining concern regarding the informed consent document. The consent form indicates that the patient's disease will be corrected. Since this protocol is a feasibility study and not an efficacy trial, only biological endpoints will be determined. This section of the informed consent document should be corrected.

In Dr. Haselkorn's absence, Dr. DeLeon noted several concerns which were outlined in his written review: (1) Why has the biopsy application section been omitted from the revised protocol? and (2) Why are the investigators administering such high titers of virus? Dr. DeLeon noted that the investigators have already responded to the viral titer question by decreasing the doses that will be administered to patients.

Review--Dr. Zallen

Dr. Zallen noted this protocol requires only 4 bronchoscopies; this requirement is in contrast to the number of procedures outlined in Dr. Crystal's protocol. The proposed bronchoscopies were originally going to be coupled with biopsies; however, the biopsies have been omitted from the revised protocol. This modification is satisfactory. Dr. Wilson needs to respond to Dr. Haselkorn's concern regarding the risk of lung collapse behind the balloon and associated hypoxia. Regarding the non-human primate model, Dr. Zallen asked when the animals were sacrificed following the infusion of the lac/Z adenovirus.

The exclusion criteria of fertile individuals was discussed previously during Dr. Crystal's presentation. The same guidelines are applicable for this protocol, namely, individuals should not be excluded based on their fertility status. Regarding the recruitment process, are there any concerns that these patients will feel an emotional obligation to participate in the protocol after they have received financial compensation? Dr. Zallen commended the investigators for other aspects of the recruitment process, i.e., visiting patients, their families, and private physicians during the initial screening process. Dr. Zallen said that she would like the opportunity to review the revised informed consent document.

The *Points to Consider* document is somewhat flawed because there are numerous references to the protocol. The document does not stand alone as an informational source. The RAC should establish whether this format is acceptable. The non-technical abstract is too brief; more information should be included regarding the experimental procedures.

Other Comments

Dr. Walters noted that the expected benefits section of the informed consent document clearly states that the patient will not benefit from this study; however, this assertion should be clearly stated in the purpose of the research section, which is in the first part of the document.

Dr. Parkman said that patients will be isolated in a negative pressure room for 10 days. If their cultures are negative, they will be released. How many cultures will be performed? If the patients continue to shed virus for longer than 10 days, how many times will they have to be negative before they can leave isolation? What are the exclusion criteria if a patient develops a viral infection in the period immediately prior to transduction? What are the exclusion criteria if a patient has E1a sequences in the bronchi at the time of treatment?

Presentation--Dr. Wilson

Dr. Wilson explained that an attempt was made to localize the product of the CFTR gene in the proximal airway using *in situ* hybridization and immunocytochemistry. The CFTR gene was difficult to detect in the surface epithelium of the proximal airways. However, CFTR is extensively expressed throughout the distal airway as well as the alveolar structures. These data suggest that the cellular targets of this therapy are quite numerous and diverse--starting with the proximal airway and submucosal areas and throughout the distal airway and airspace. An attempt will be made to selectively block a segment of the lung so that the efficiency of gene transfer can be determined in the bronchus, bronchioles, and the distal airway.

The animal model used to generate the *in vivo* data is the CF mouse, which was developed by Dr. John Engelhardt. This model takes advantage of the ability to grow human epithelial cells in a immune-deficient mouse. Bronchial epithelial cells are removed from a human CF lung and transplanted to the trachea of a rat that has been denuded of its epithelial cells. The human-seeded rat trachea is then grown in the nude mouse. The CF mouse model includes tubing that has been tied proximally and distally so that it imitates an airway, including mucus production. This airway must be irrigated several times a day in order to prevent occlusion. The advantage of this model is that it provides a setting for the introduction of genes into human airway epithelial cells.

He described *in vivo* experiments in which lac/Z and CFTR were injected into the lumen of the CF mouse. The human xenografts were subsequently removed and evaluated for gene expression. The epithelium generated on the surface of the xenograft is identical to that of the proximal airway, i.e., ciliated cells, goblet cells, and basal cells. Data demonstrates that 10 PFU injected into the lumen results in a graft transduction efficiency between 15 and 40%. Injection of 10 PFU resulted in transduction of 1 to 4% of the graft. When the human xenografts were left in the animals for 3 weeks following transduction, no diminution of gene expression was observed during this period of time.

Dr. Wilson presented data regarding the types of cells that are infected by the vector. Using immunofluorescent staining, he demonstrated that there is co-localization of the recombinant gene protein only in differentiated cells, not progenitor cells. Consequently, it may be necessary to administer subsequent vector challenges.

The human xenografts have been evaluated for adenovirus protein expression. Double immunofluorescence was used to determine which cells contain the recombinant adenovirus and if these cells express the adenovirus protein. Data suggests that there is immune localization to a 72kd early protein, E2a. Between 3 and 5% of the human cells express E2a. The same experiment was performed to determine expression of a late gene product using exon and fiber protein antibody. Expression of these late gene products were essentially undetectable. This result is consistent with other data regarding E1-deleted adenoviruses. Specifically, there is a block in the transition from early to late protein expression.

Dr. Wilson presented virus distribution experiments that were performed in the baboon animal model. Animals received 20 ml of lac/Z virus into the posterior segment of the left upper lobe. The lung was removed on day 3, perfused, and washed with X-gal to evaluate lac/Z expression *in situ*. Results indicate that the majority of gene expression remains in the posterior segment. The reason for localizing the infusion of the virus is to minimize the amount of lung at risk in the event that adverse reactions occur.

Dr. Wilson described an *in vivo* toxicity experiment in which a 33 kilogram baboon received the CFTR adenovirus into the posterior segment of the right upper lobe and lac/Z into the left upper lobe. Bronchial lavage was performed on both lobes on days 3, 14, and 21, in addition to brushings and biopsies. Although the data analysis is still in progress on the samples obtained from this animal, preliminary data indicates a variation in cell distribution ranging from neutrophils to macrophages. Transient expression of

both lac/Z and CFTR were observed on day 3 in the segments in which they were introduced. Studies have been initiated in the cotton rat model to evaluate toxicity and distribution of gene expression.

Revisions have been made to the clinical protocol regarding: (1) changes in vector dosages, and (2) elimination of the transbronchial biopsy procedure. Patients 1 through 3 will receive 10 PFU per ml, patients 4 through 6 will receive 10 PFU per ml, patients 7 through 9 will receive 10 PFU per ml, and patients 10 through 12 will receive 10 PFU per ml. The transbronchial biopsy has been eliminated as part of the evaluation because it is not critical in answering questions posed by the protocol. In addition, the procedure would probably contribute substantially to the morbidity of the bronchoscopy.

In response to Dr. D. Miller's concerns regarding helper virus contamination, Dr. Wilson noted that all of the vector seed lots have been evaluated by PCR analysis and biological amplification assays using the extended culture of the packaging cells. Biological assays, using HeLa cells, is the preferable method of analysis. HeLa cells can be diluted to the point that no cytopathic effects are observed. These cells can be propagated over a period of time. The important issue is what point should the recombinant stock be diluted to circumvent the cytopathic effects. Dr. Wilson proposed dividing the lot into 2 aliquots and evaluate one-half of the lot for replication-competent helper virus. One-half of a lot is equivalent to one-half of the maximum dose, i.e., 1 x 10PFU per 10 ml. Therefore, the limit of detection would be off by only 1 virus particle. Testing of one-half of a lot could be accomplished using approximately 200 10 centimeter plates.

Dr. D. Miller asked how long these plates will be cultured. Dr. Wilson answered that the plates will be grown for 28 days; these plates will be split 2 to 3 times during this incubation period. Dr. Post inquired whether the helper virus assay will be performed on the seed lot or the production lot of virus. Dr. Wilson stated that the production lot will be cultured. The seed lot will be evaluated for other adventitious viruses. A lysate will be made from the seed lot and analyzed for gross contamination.

Dr. Wilson agreed to incorporate all of the suggestions offered by the RAC members regarding the informed consent document. He stated that he was especially in agreement with the committee members about the issue of fertility. The likelihood of germ line transmission is extremely unlikely. The testes of the male primates have been extensively analyzed for recombinant protein and RNA expression. Rabbits have received large infusions of virus, and their gonads have been evaluated for gene expression. No evidence of germ line transfer has been observed in either the primate or rabbit models.

The issue of recruitment presents several difficulties. The majority of problems that have been encountered focus on the referring physician, who is known and trusted by these individuals. In certain circumstances, however, there are problems associated with misinformation on the part of the patient's physician. Despite the time that is taken to thoroughly explain the protocol to the primary physician, the information is not always relayed adequately to the patient. To avoid this situation, Dr. Wilson said that he or one of his co-investigators visits the referring physician when feasible. Travel is not always possible, e.g., the patient identified in Cyprus.

Dr. Wilson responded to the RAC's concerns regarding pre-screening for E1 sequences. Data suggests that E1 sequences can be found by PCR in pulmonary cells recovered from the lung. PCR analysis would provide misleading information and suggested that patients should be evaluated for any overt clinical infections through culture techniques.

In regard to the length of time patients will be required to stay in the negative pressure facility, Dr. Wilson stated that patients will be released after 3 consecutive negative cultures. Dr. Wilson called on his collaborator, Dr. Richard Simon, a pulmonologist, to respond to the issue of damage to the lung caused

by the proposed procedures.

Dr. Simon explained that only 5% of the patient's total airway will be occluded. This temporary loss of ventilation should not cause any significant problem to the patient. Any signs of hypoxia will be easily detectable because these patients will be monitored continuously for arterial saturation with a finger oximeter. The period of time that will be required for occlusion is 10 minutes in order to achieve excellent gene transfer.

Dr. Walters asked if the volume to be administered to the patient has been reduced from the 50 ml volume that was proposed originally. Dr. Wilson stated that the volume has been reduced to 20 ml. Dr. Walters asked Dr. Wilson if he would agree to move the expected benefit section of the informed consent as discussed earlier. Dr. Wilson agreed to move the statement to the front section of the document.

Dr. D. Miller noted that it is important for the RAC to remember that Dr. Crystal and Dr. Wilson are proposing vectors that have slightly different promoters. What is the level of CFTR expression from the proposed vector compared to the endogenous level? Dr. Wilson responded that the level of expression depends on the specific cell type. The level of expression in epithelial cells is greater than endogenous expression in most cases; however, probably not more that a 10-fold increase.

Committee Motion

A motion was made by Dr. Zallen and seconded by Dr. D. Miller to approve the protocol with the following stipulations: (1) delete the inclusion/exclusion criteria that limits the protocol to infertile individuals, (2) submit a revised informed consent document that incorporates the suggestions made by Drs. Zallen, DeLeon, and Walters, and (3) the production lots to be used in humans should be demonstrated to have less than 1 particle of replication-competent adenovirus per patient dose. The motion to approve the protocol passed by a vote of 16 in favor, 0 opposed and no abstentions.

VIII. DISCUSSION REGARDING PROPOSED RAC REVIEW OF EUROPEAN HUMAN GENE THERAPY PROTOCOLS/DR. BLAESE

Background--Dr. Wivel

In Dr. Blaese's absence, Dr. Murray called on Dr. Wivel provided background information regarding the European request for assistance in the review of human gene therapy protocols. Dr. Wivel explained that Dr. Blaese was contacted by the European scientific and medical community with a request that the RAC provide review for a protocol that has been proposed by European investigators.

The European community (EC) has not decided in a unified way how they plan to organize and legislate gene therapy. The EC is a multinational organization that has the responsibility for establishing policy on safety issues for biotechnology. The EC is requesting a one time review of a gene therapy protocol in order to assist them in the establishment of certain policies.

Dr. Parkman said that the EC has complemented the RAC by their request for assistance in the review process. It is appropriate for the RAC to undertake this task and review the proposed protocol with the same standards that have been exercised for previous protocols. Dr. Post said that the EC has already initiated several human gene therapy protocols, e.g., France and the Netherlands. Why has this request come at this point in time? Dr. Wivel responded that there is some concern about the degree of stringency that is employed in the review process, and that there is a great deal of variation in the process among countries. The EC is interested in establishing a set of uniform standards for review.

Dr. Anderson provided further background information on this issue. Approximately 2½ years ago a similar request was submitted to the Human Gene Therapy Subcommittee (HGTS). Given the political climate in Europe at the time, the Europeans decided that they did not want to rely on the advice of the Americans; therefore, they decided to establish their own review committee. Since that time, several European investigators have received approval to initiate human gene therapy protocols; however, the process was long and difficult. The EC has ascertained that a universal pattern of review is necessary. The EC has decided that if the RAC would be willing to provide review of a human gene therapy protocol, these standards would be adopted for the entire community.

Ms. Buc said that any individual from the EC is welcome to attend RAC meetings since they are open to the public. She expressed doubt that the review of a single protocol would provide enough meaningful information from which to base policy decisions. She stated that she was disinclined to provide the requested review.

Mr. Barton said that if a European decision-maker asked the RAC to provide advice in the same manner that the committee provides advice to the NIH Director, the RAC should agree to the request. However, if the request arises from a scientific community that mistrusts its regulators and wants the RAC's seal of approval in order to persuade officials, then the RAC should decline the request. Dr. Geiduschek agreed with the statements made by Mr. Barton. Unless this request has a particular origin and a particular status within the EC, then the RAC should probably not consider the request.

Dr. Parkman said that although the issues encompassing informed consent may present specific problems, the scientific issues should be universal. He encouraged the RAC to consider the EC request. Dr. Chase said that the RAC should not disaggregate the review of specific components of a protocol because the committee would not be providing a complete review. Conversely, the RAC has received this request from friendly nations; and perhaps individual members could volunteer to assist the EC in developing their own process. Dr. Chase said that the RAC should be cooperative; however, they should not serve as a substitute review body for other countries.

Ms. Meyers said that the RAC should consider all of the human beings in the world and that the goal of gene therapy is to alleviate the suffering of mankind. The RAC should do everything in its capacity to assist the EC in its efforts.

Mr. Pierre Lehn from Paris, France, commented on the issue of RAC review of a European human gene therapy protocol. He commended the United States for establishing a system of public review, noting that he has attended numerous RAC meetings. However, Mr. Lehn agreed with Ms. Buc that attendance at these public meetings should provide ample assistance to European investigators and regulators, and that the EC should be held accountable for establishing their own process of review.

Dr. Schaechter agreed with Dr. Chase's comments regarding the inappropriateness of review of this protocol by the entire RAC; however, there is sufficient expertise among the committee members that a volunteer committee could be established to provide the requested review.

Dr. Post suggested that the EC or any other body can consult any subgroup of the RAC and use any subgroup of the RAC as consultants, but that the RAC as an entity should not entertain foreign proposals.

Dr. Thierry Velu from Brussels University commented on the European request. He informed the RAC of an existing European human gene transfer committee which has recently been constituted. The first meeting of this committee was held several months ago. At the meeting, the committee discussed the

establishment of an advisory body that would be equivalent to the RAC. However, the recommendation was rejected by the committee members for several reasons. The committee agreed that any review body would be constituted with investigators who desire approval of their own human gene transfer protocols. Composition of the committee by such individuals would represent a clear conflict of interest. The committee decided that a set of guidelines should be established as the first step of this process, and that these guidelines should be equivalent to the *Points to Consider*.

Mr. Barton stated that an attempt to establish a resolution on this issue at this point may unintentionally draw lines and send signals that the RAC is not ready to send.

Dr. D. Miller said that a reply should be made that the RAC would like to assist the EC; however, subject to time limitations, the committee cannot review an unlimited number of protocols. He added that there is not enough information available to the RAC about this request to provide an adequate response at this time.

Dr. Murray stated that Mr. Barton and Dr. Schaechter have completed their terms as members of the RAC. Dr. Murray expressed the RAC's appreciation for their dedication and contributions to the committee and thanked them for their service. Dr. Murray stated that this is her last meeting as Chair of the RAC and thanked Dr. Wivel and the staff of the Office of Recombinant DNA Activities for their assistance and said that it was an honor to have served on the RAC. The members of the RAC commended Dr. Murray for her dedication and service while serving as both a member and as Chair of the RAC. Dr. Murray has executed her responsibilities in an exemplary manner and the committee is indebted to her.

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: CYSTIC FIBROSIS GENE THERAPY USING AN ADENOVIRUS VECTOR: IN VIVO SAFETY AND EFFICACY IN NASAL EPITHELIUM/DR. WELSH*

Review--Dr. Post

Dr. Murray called on Dr. Post to present his primary review of the protocol submitted by Dr. Michael J. Welsh of the Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City, Iowa. Dr. Post said that most of his original concerns have already been addressed and resolved during the presentation of Drs. Crystal and Wilson's protocols. Therefore, he said that he would keep his comments to those issues that are significantly different from the previously reviewed CF protocols.

The most significant difference about this CF protocol is that the virus will be applied to the nasal epithelium of the patient, not the lung. Nasal administration provides the added assurance that if untoward effects occur, e.g., an inflammatory response, the consequences will be less severe than effects that might occur in the lung. In regard to efficacy, the investigators will have more direct access to the transduced tissue.

The investigators propose to use a Type 2 adenovirus-based vector rather than a Type 5 adenovirus. Dr. Welsh has stated that the decision to use the Type 2 virus was arbitrary. The proposed vector has retained the E3 gene. Although the increased size of the vector has compromised yield, there are no longer concerns about the possibility of an inflammatory response to the vector that is greater than wild-type virus.

Due to the localized administration of this therapy, the dosages will be less than those proposed for the lung administration protocols. Patients will receive 3 doses that will escalate from 2 x 10 PFU to a maximum dose of 5 x 10 PFU.

Preclinical animal studies have been performed in monkeys, hamsters, and cotton rats.*In vivo* safety and efficacy experiments demonstrated efficient transduction of the nasal epithelium and only mild inflammation at doses that were significantly higher than those proposed for the human protocol. Virus detection in secretions and excretions was observed for only a few days.

Dr. Post noted that the DNA sequence of the entire vector was not provided. The DNA sequence that was provided by the investigators was that of the starting plasmid. Approval of the protocol should be contingent on the review of this sequence. Dr. Welsh has stated that he will submit the entire vector sequence when it becomes available.

Dr. Post stated that the detection of E1a sequences is probably easier in this protocol than for the other CF protocols because the total number of viral particles is significantly less. The PCR assay proposed by Dr. Welsh will detect 20 E1a positive adenovirus particles in a background of 10 particles. This level of sensitivity will detect 1 particle in
a single dose at the highest concentration. The investigators propose to perform HeLa cell assays in which 10 PFU will be plated onto 10 cells.

Dr. Post said that there is no requirement that the virus should be handled under sterile conditions during the purification process. The investigators responded that they will use sterile filters in the preparation and perform sterility testing. Perhaps the reason that sterility is not as critical as for the other protocols is that the vector will be administered to the nasal epithelium.

Dr. Post said that most of the pertinent issues have been dealt with effectively, and this protocol is complementary to the CF protocols reviewed earlier. He recommended that the protocol should be approved by the RAC.

Review--Dr. Hirano

Dr. Hirano expressed concern about the proposed sample size of the study. The investigators will treat a total of 3 patients, and each patient will receive a different vector dose, i.e., 2 x 10, 2 x 10, and 5 x 10 PFU. Will sufficient information be obtained to fulfill the stated objectives of this protocol; specifically, can safety and efficacy be demonstrated? What is the likelihood that patients will become ineligible for participation in future studies in which assessment of therapeutic benefits is a primary objective? What is the current status of the investigator's ability to produce production lots that have the required titer and absence of contaminating materials?

Review--Dr. Carmen

Dr. Carmen stated that he would like Dr. Welsh to discuss the implication of not incorporating the E3 deletion into the proposed vector. He noted that this protocol, along with the two CF protocols approved earlier, are important CF research being conducted at 3 separate universities focusing on the cutting edge of clinical investigation. This area of research needs as many qualified investigators working on therapies as possible. Dr. Carmen recommended approval of this protocol.

Other Comments

Ms. Meyers stated that the University of Iowa IRB expressed concern about the requirement that the patient will be held responsible for making arrangements for the payment of expenses related to treatment. Dr. Welsh's response states,*I agree with you and your committee's concern that a policy may*

need to be instituted to support the payment of any injury that could be incurred in the absence of negligence, but the present wording and regulations are ones with which I must comply. Ms. Meyers asked Dr. Welsh to discuss why he is required to comply with this policy since his own IRB has raised questions regarding the issue.

Ms. Meyers said that patients will be compensated for participation in this protocol. There are moral and ethical concerns regarding such compensation. Specifically, patients who are disabled and without income may be coerced unintentionally into participating in the study.

Dr. Post asked Dr. Ginsberg if the E1a minus virus in monkey cells is significantly different from the E1a minus virus in human cells. Dr. Ginsberg replied that in the consideration of safety, one should always consider results obtained in the animal model in which pathology is produced. Since pathology is not produced in the monkey, then it should not be used for comparison to the human situation. Dr. Doi asked for clarification regarding data on the MOI.

Dr. Parkman noted that the investigators will monitor virus secretion from the patient's nose. If a patient were to swallow some of the vector and it replicated in the gastrointestinal tract, there could be secretion from the stools. Therefore, a patient could have negative cultures from the nose but still have positive excretion for the stools. Why are stool cultures not being performed? If patients are still secreting or excreting virus after 1 month, they would be allowed to leave the negative pressure containment facility for humane reasons. What are the risks to other individuals of releasing a patient that is known to be actively secreting virus?

Presentation--Dr. Welsh

Dr. Welsh stated that he would limit his presentation to the issues of safety and efficacy. The safety profile in monkeys, cotton rats, hamsters, and cultured cells are very encouraging; however, there are specific questions about this therapy that only can be answered in the human. Since minor safety concerns still exist, a conservative approach to this therapy is being proposed. Information derived from this early study will provide critical data that will be used for the design of future clinical efficacy trials.

Dr. Welsh presented a brief outline of the protocol. Three patients will be entered into the study. In response to Dr. Hirano's concern about the limited number of patients enrolled in this study, Dr. Welsh stated that this number of individuals will provide sufficient information to proceed to trials in the lung. The dosages of vector that will be administered, between 2 x 10 and 5 x 10PFU, have been chosen to provide safety and efficacy data. Exclusion criteria and precautions have been outlined in the protocol.

Nasal epithelium has been chosen as a model system for the preliminary studies because the nasal and intrapulmonary epithelial cells have the same morphology, i.e., barrier properties and transport properties. Specifically, the nasal epithelial cells of these patients have the same chloride transport defect as the bronchial epithelia. The nasal epithelium has been used traditionally for the initial testing of therapies designed to treat CF. The nasal epithelium offers the following advantages for assessing safety and efficacy: (1) there is a defined area of application, (2) information can be derived regarding the relationship between the input of virus and the biological response in order to determine the optimal MOI, (3) nasal administration offers the advantage of frequent measurements because it is a non-invasive procedure, (4) daily measurements can be determined without risk to the patient, (5) surface fluid can be obtained for the purpose of culturing for virus, and cells can be monitored for possible inflammatory responses, (6) biopsies can be obtained from the nasal epithelium, which will be important for the assessment of cytopathic effects and the detection of subtle changes in the epithelium, (7) cell function

can be monitored by the measurement of trans-epithelial voltage across the nasal epithelium, and (8) nasal administration will allow for the determination of treatment-related versus procedure-related adverse consequences.

Dr. Welsh responded to Dr. Parkman's concern regarding patient cultures for the determination of virus excretion and secretion. Dr. Welsh stated that cultures will be performed on the nose, pharynx, blood, urine, and stools. Adenovirus antibody production will be monitored as well as cell analysis for the assessment of inflammation and cytopathic effects.

Dr. Welsh explained that the most important goal of the protocol will be to assess efficacy, i.e., will CFTR correct the chloride channel defect in these patients? Efficacy will be assessed by the trans-epithelial electrical potential differences observed across the nasal epithelium. This technique was originally developed by Drs. Michael Knowles and Richard Bouchet. Also, mRNA and protein will be monitored.

Patients will be greater than 18 years of age, male or female, genotypically defined, have mild or moderate lung disease, and be seropositive for adenovirus. Exclusion criteria include clinical instability, upper respiratory infection, and virus shedding.

The original protocol proposed that the nasal epithelium should be demonstrated to be positive for E1 genes. Considering the comments offered by Dr. Ginsberg during Drs. Crystal and Wilson's protocols and the sense of the RAC, Dr. Welsh asked that this requirement be deleted from the protocol.

The proposed vector is an E1 deleted Type 2 adenovirus that encodes for CFTR. This vector has an E1a promoter that retains the E3 region. This virus is impaired because of its substantial length.

Dr. Welsh described data derived from *in vitro* and *in vivo* model systems. Production of mRNA and protein has been demonstrated in monkeys that already have the wild-type CFTR function. The AdCFTR vector has been evaluated in the nasal epithelium of monkeys and the lungs of cotton rats. He described experiments in which human CF airway epithelial cells were cultured on a permeable support in order to create an epithelium. The apex side of these cells is at the air/liquid interface and the basal side of the cells is down. CFTR function can be easily demonstrated in this model support system. When the epithelium grown on these permeable supports is exposed to the adenovirus vector and adenosine 3':5'-cyclic phosphate (cyclic AMP) agonists, the chloride secretion defect is corrected. Significant responses were observed at doses as low as a 0.1 MOI. The promoter is not a high level promoter, but CFTR is not expressed at high levels in these cells.

Dr. Welsh described FACS analysis data regarding the percentage of cells that are corrected by this procedure. At 24 hours, little difference was observed between control and virus treated cells. At 48 hours the number of positive cells (fluorescent cells) increased dramatically. The number of positive cells continued to increase for 96 hours. Data indicates that at an MOI of 10, gene expression was observed in 60 to 70% of the cells. FACS analysis of transduced cells obtained from the bronchioli of cotton rats demonstrated that the majority of the vector resides in the apical cells; however, occasional staining was observed in the basal cells.

Dr. Welsh addressed the issue of safety. Experiments have been performed on a variety of tissue culture cell lines, primary cells, and *in vivo*. Gene transduction and protein expression have been observed in all instances. Viral mRNA synthesis was detected occasionally, suggesting that viral proteins can be made at a low level. Low level viral DNA synthesis was detected. There was minimal evidence of inflammation of cytopathic effects. The important issue is that in all of the *in vitro* and *in vivo* models tested, no evidence of virus replication was detected.

Dr. Welsh responded to Dr. Ginsberg's specific questions regarding the cotton rat model. A dose of 4 x 10 PFU of Ad2/CFTR-1 were administered to these animals. This dose is approximately 1,000 times larger than the highest dose proposed for human administration. Gene expression was observed in all animals. The inflammatory response was monitored in these animals. Bronchial/alveolar lavage demonstrated no increase in cell number over control animals.

Dr. Welsh responded to Dr. Post's comments regarding host cell protein shut-off and cytopathic effects. Host cell macromolecular synthesis is inhibited early in infection and cytopathic effects usually develop later. The host cell shut-off maps in part to E1b and cytopathic effects map in part to penton, which can be present in crude preparations. Host cell shut-off is not detected at an MOI of up to 200 with Ad2/CFTR-1 in HeLa cells. No cytopathic effects were detected at an MOI less than 100 with purified vector; however, effects were observed at an MOI of 1,000. In conclusion, at the doses proposed for this study, host cell shut-off and cytopathic effects should not be problematic.

In regard to the characterization of the production line, 7 preclinical production lots have been prepared. All of these lots meet the specified criteria. One of the 7 lots had 3 colony forming unit (CFU) of bacteria.

Dr. Welsh responded to Dr. D. Miller's concerns regarding wild-type virus production. Wild-type virus will be monitored by PCR and the HeLa cell infectivity assay. For the biological assay, 10 cells will be plated onto each of 10 plates. These cells will be subsequently infected with the vector at an MOI between 1 and 50. Using this criteria, 1 wild-type virus particle should be detectable in 5 x 10 Ad virus particles. Therefore, 1 wild-type virus particle should be detectable in the maximum administered dose.

Dr. Welsh noted that the original protocol stated that the rat-1 cellular transformation assay will be used. Since Dr. Ginsberg and the RAC have noted that this assay is not particularly sensitive, Dr. Welsh asked that the requirement for this assay be deleted from the protocol.

CF is a lethal genetic disease, and the proposed therapy could represent a major advance in the treatment of this disease. This protocol is designed to provide maximum safety to the participants by applying a small amount of virus to a defined region of the nasal epithelium. This study will provide critical data about biochemical efficacy and safety that allow for the design of new generations of vectors and the initiation of trials to assess clinical efficacy in the lung.

In response to Dr. Carmen's question regarding the proposed vector, Dr. Welsh explained that this vector is based on a Type 2 adenovirus; Drs. Crystal and Wilson are using Type 5 adenoviruses. There is substantial sequence similarity between the various vectors. All 3 vectors have the E1 region deleted; however, there are different promoter regions. This vector has an Ad-2 E1a promoter, which is a moderate strength promoter. The other 2 CF studies will use high level expression promoters, either the major late promoter or a cytomegalovirus (CMV) enhancer beta-actin promoter. The polyadenylation sites are different between the proposed vectors. The Ad2/CFTR-1 vector has an Ad-2 E1b polyadenylation site, the other 2 vectors will use SV40. The Ad2/CFTR-1 vector has retained the E3 region unlike the other vectors.

There has been some discussion regarding E3 positive versus E3 negative vectors. This E3 positive vector is larger than the wild-type virus, thus its growth is impaired even in the permissive 293 cells. The large size of this vector offers an additional safety advantage. The wild-type adenovirus out competes the E3 adenovirus vector. Mixing experiments indicate that even under the least optimal conditions the wild-type adenovirus rapidly outgrows the E3 negative adenovirus, In contrast, E3 positive adenoviruses out compete the wild-type virus. One of the products of the E3 positive virus, gp19, reduces the

expression of Class I MHC antigens on the cell surface. E3 negative viruses produce increased inflammatory responses as demonstrated in the lungs of the cotton rat.

Dr. Welsh explained that the patient compensation concerns noted by Ms. Meyers were addressed by the Institutional Biosafety Committee, not the IRB. Although the exclusion of compensation for non-negligent injury is standard practice (both at the national and university level), there is a moral obligation to make every attempt to care for the patient and minimize any potential financial consequences in the event of untoward research-related consequences.

Dr. Welsh said that although the possibility of a patient excreting or secreting virus after 4 weeks is remote, precautions will be taken to avoid the spread of virus to other individuals and the environment in the case that a patient was positive after this period of time.

Dr. Gregory addressed the issue of stability of the DNA and the copy number per cell, specifically, the increase in viral molecules from an MOI of 50 to 550. E1 deleted viruses can replicate and DNA synthesis can occur at high MOIs. The observed increase most likely results from the E2 transcription unit of the virus not being completely quiescent in the absence of E1a. The E2 transcription unit encodes DNA polymerase and other associated proteins needed for viral DNA replication. The major issue is that DNA replication is not necessarily related to virus replication. No evidence of recombinant virus has ever been observed in these cells. There is a low level of DNA synthesis, which is in the order of 1,000 to 10,000-fold less than would occur with the wild-type virus.

In response to Ms. Meyers concern about financial compensation for participation in this protocol, Dr. Welsh said that the amount of compensation should not be a persuasive factor. Patients will receive a minor reimbursement, $75 per day for the inconvenience they incur as a result of their 10 day to 3 week hospitalization.

Discussion

Ms. Meyers asked Dr. Welsh about the course of action that would be taken in the event that one of these patients were to develop a very unusual type of pneumonia following treatment and their insurance company refused to compensate for treatment after their participation in this experimental protocol. Dr. Welsh said that since these patients already have mild to moderate lung disease, it is possible that they may develop a complication of their disease during the course of this study that is unrelated to the treatment. In such an instance, the patient would be cared for, but the usual mechanisms of cost-reimbursement would be applied. Ms. Meyers asked if the pneumonia were to persist for a long period of time and the patient was not able to pay for medical treatment, would he/she be turned away? Dr. Welsh explained that this question is very difficult to answer, and that he has discussed it with the director of clinical research, university lawyers, and the local IRB. He said that he could not be any more specific than to state that every attempt will be made to minimize any financial burden in the event of adverse consequences.

Dr. Walters noted that both the title and the introduction of the informed consent document do not clearly explain that this is a preliminary study to test what happens in the nasal epithelium, and that there will be no direct benefit as a result of participation in this study. The use of the words *gene therapy* and *efficacy* is a concern. In addition, the introduction explains the ultimate purpose of the research, and patients may misunderstand the expected benefit of this particular protocol. Dr. Welsh offered to incorporate a statement in the introduction that states that this treatment will not be efficacious to the patient. However, the patient needs to be made aware that he/she will derive no therapeutic benefit by the fact that this procedure will be performed on the nasal epithelium, not the lung. Dr. Walters suggested including the

statement, *It is unlikely that participation in this study will directly benefit you*, should be inserted into the introduction section of the informed consent document. Dr. Welsh agreed to include this statement.

Dr. Ginsberg stated that it should be recognized in terms of safety, the results obtained regarding inflammation in the nasal epithelium may not necessarily translate to those effects that may be observed in the lung. However, he acknowledged that the nasal epithelium is a good site for the initial administration. Individuals who possess antibodies may have a dormant adenovirus infection in the lymph nodes and lymphatic cells. Even in the most severe adenovirus pneumonias, viremia has never bee demonstrated; so the problem of viremia is not an important issue. Approximately 1 in 10 lymphoid cell antibody positive individuals contains adenovirus DNA.

Dr. Michel Perricaudet of the National Center for Scientific Research, Paris, asked if the expression of proteins has been studied in the absence of E1a. Dr. Welsh responded that these experiments have not been performed.

Dr. Murray asked Dr. Ginsberg if he could anticipate any difficulties that could arise in future protocols regarding recombination and aerosol administration. Dr. Ginsberg said that because Type 5 and Type 2 adenoviruses do not produce severe disease in adults, there will probably not be any serious problems associated with the aerosol
administration of these viruses. However, the possible clinical result of a recombinant virus is unknown.

Ms. Meyers provided a list of changes that should be incorporated into the informed consent document: (1) the phrase, *CF symptoms* should read *CF lung symptoms*; (2) paragraph 1(b) should be modified such that the patient is informed that he/she will be monitored closely for 8 weeks following the procedure; however, there will be long-term follow up for many years; (3) the patient should be informed that data derived from animal experiments does not guarantee that the virus will produce the same effects in humans; (4) the statement, *To learn whether the experimental virus can correct cystic fibrosis abnormalities...*, should be expanded to read, *To learn whether the experimental virus can correct cystic fibrosis airway abnormalities*, (5) the following sentence should be added, *You will not be allowed to participate if you are planning to become pregnant*; (6) the patient should be clearly informed that if their insurance company does not provide payment for costs, the individual will be expected to pay all costs; (7) the sentence that describes the request for autopsy should be modified to read *In the unlikely event of death, an autopsy would be expected*; (8) a statement regarding protection from the media should be included in the confidentiality section; and (9) the disclosure of information section should be expanded to include NIH among the list of parties that may have access to the patient's records. Dr. Welsh deferred the RAC regarding the proposed changes in the consent form. Dr. Murray suggested that the requirement to exclude women if they are planning to become pregnant should be changed to a *suggestion* that they should not become pregnant. Dr. Parkman said that the expectation of researchers involved in human gene therapy is that they will be following patients long term. Long-term follow-up should be indicated without any specific definition about the time period. Dr. Walters complemented the investigators on their detailed responses to the *Points to Consider*.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Carmen to approve the protocol with the following stipulations: (1) removal of the requirement for the E1a negative assay, (2) removal of the requirement for the rat-1 transformation assay, (3) incorporation of the informed consent changes suggested by Dr. Walters and Ms. Meyers, and (4) submission of a 3½ inch diskette with the entire vector sequence in ASCII format.

Dr. D. Miller said that he wanted to make the statement, for the record, that he does not think that there wi be any untoward consequences as a result of the release of CFTR adenovirus into the environment. D Ginsberg agreed with the statement made by Dr. D. Miller and added that is extremely unlikely that if a recombinant occurred, that it will contain the CFTR gene. Data indicates that the inserted gene is alwa lost from recombinants. The motion to approve the protocol passed by a vote of 16 in favor, 0 opposed, and no abstentions.

X. AMENDMENT TO THE *POINTS TO CONSIDER REGARDING REPORTING REQUIREMENTS FOR HUMAN GENE TRANSFER/THERAPY PROTOCOLS*

Presentation--Dr. Leventha

Dr. Murray called on Dr. Leventhal to give a presentation on investigator's submitted progress reports. the RAC meeting of September 14-15, 1992, the committee recommended that a letter should be forwarded to investigators of approved protocols regarding the progress of their studies. The letter suggested that the approval of their protocols might be reconsidered in the case of non-compliance. Essentially, responses were received from all investigators who have initiated their trials.

Dr. Leventhal distributed a summary table of the information that was submitted by the respondin investigators. The table included the following information: (1) investigator's name, (2) protocol title, (3) starting date, (4) target number of patients, (5) number of patients treated, (6) report of toxic effects, and (results. The results column described experiments that have demonstrated the transfer of the gene or demonstration of function resulting from the gene transfer. She provided a brief summary of the data that was submitted by the investigators in response to the letter.

Dr. Leventhal stated that the RAC should develop a uniform data reporting format, similar to the on represented in the table. She requested input from other RAC members regarding the development of a proposed format.

Dr. Parkman said that the proposed format is acceptable. However, the response column should include investigator responses in the same context that was originally proposed. In other words, if an investigator proposed three objectives for a protocol, he/she should submit responses according to how the data relates to each specific objective. For example, if gene marking of peripheral blood and tumor regression have been stated as goals, then the investigator should provide gene marking and tumor regression data. Dr. Leventhal stated that her only concern regarding data reporting on tumor regression is that the RA should not usurp more of the investigator's information than they are required to present in a public forum. Dr. Parkman said that the investigators should be required to provide data that relates to the specific aims of the protocol. Dr. D. Miller suggested that the form should include a question that will allow the investigator to report any problems that were encountered in pursuing the protocol.

Dr. Blaese stated that he had concerns about the request for detailed information because of the publi forum in which the RAC meetings are held. One of the original manuscripts describing the adenosine deaminase (ADA) deficiency protocol was rejected from a prominent journal because the results ha been reported to the public.

Dr. Leventhal said that the goals of the protocol can be separated from clinical observations in terms o gene transfer. Investigators should be required to submit only data regarding the endpoints that relate to gene transfer; therapeutic benefits do not have to be reported in this format.

Dr. Post emphasized that investigators should submit copies of published articles and abstracts that have

been presented to the RAC. One of the reasons for this data reporting exercise is to assist the RAC with future protocol approvals, but the other reason is to identify categories of protocols that might qualify for a different level of review based on experience. He asked Dr. Leventhal if she has developed an suggestions for future directions.

Dr. Leventhal said that a large number of patients have not been entered into the protocols; approxima 40 patients to date. Data indicates that patients who have received either the neo gene and the LNL6 vector have not experienced any problems. Only 12 of these 40 patients have actually been shown to possess gene-marked cells. Dr. Post inquired if these 12 patients represented sufficient information with regard to safety and efficacy of gene transfer. Specifically, could the RAC recommend that neo and LNL6 marking experiments no longer require RAC review? Would the Food and Drug Administration (FDA) and IRB approval be sufficient? Dr. Leventhal said that although she could not represent the entire RAC believed that these particular marker protocols could be approved solely by the FDA and the IRB if th vector was prepared by the same manufacturer in a standard way. If the RAC can be confident that the FDA will proceed carefully with the approval of new vector preparations, then the committee is certainly approaching the point where these markers will be considered safe.

Drs. Leventhal and Post recommended that publications that were submitted by the investigators i response to the data reporting letter should be distributed to all of the members of the RAC.

Dr. Murray suggested that the reporting format should include vector information. Dr. Secundy support the development of a standard data reporting format. Dr. Dronamraju stated that investigators shoul submit positive data as well as negative data and include information regarding the socio-economic status of these patients. Dr. Leventhal explained that the RAC would have to wait for a longer period o time to receive positive results in the form of publications and abstracts. This delay would preclude obvious concerns regarding confidentiality.

Larry Thompson, a science writer for *Science* magazine, urged the RAC to request all pertinent information and to demand this data if necessary. Representatives from both the *New England Journal of Medicine* and *Science* have provided public statements that any government body that requests information from a scientist will not prejudice the magazine against the results being published in that magazine. Dr. Leventhal acknowledged that these statements have been made in a public forum however, investigators have still experienced problems having their data published after it has been presented in public. Dr. D. Miller said that he was not convinced that *Science* would publish an investigators data after the information had already been presented.

Dr. D. Miller suggested that the data reporting form should include any safety issue regarding the patient, e.g., did the patient die? What was the cause of death? The form should include information regarding any problems that were encountered with implementation, i.e., was the investigator able to establish a lower limit of helper virus contamination?

Dr. Leventhal said that she would revise the data reporting form incorporating the suggestions offered the other RAC members. She asked for assistance with regard to establishing scales for socio-economic status. Dr. Secundy stated that she would assist Dr. Leventhal in the development of these standa

Ms. Meyers inquired if the RAC could hold executive meetings, which are closed to the public, for the purpose of reviewing confidential information. Dr. Wivel responded that closed sessions can be held or for the discussion of proprietary information. Ms. Meyers asked if prepublication data is considered proprietary information. Dr. Leventhal asked if this information is considered intellectual property. Mr Barton said that the definition of *trade secret* involves the potential for commercial value. The key issue is

probably whether the prepublication information comes from an academic research institution or a private company.

Dr. D. Miller said that he did not want access to an investigator's results before publication. RAC members should take into account that data could be presented that could influence the research of particular committee members. The RAC should not put itself in a position to review this prepublication data. In addition, access to this preliminary information could influence the RAC's ability to judge future propose trials because a bias may have already been introduced. Dr. Leventhal stated that she feels very stron about this issue. Some patients, particularly cancer patients, will respond to any therapy that is administered. If tumor regression is considered too seriously at this stage in human gene therapy, the RAC may draw inaccurate conclusions about efficacy if the natural history of each particular tumor is unknown. Major difficulties will arise if the RAC attempts to evaluate the therapeutic efficacy of any protocol that has been approved.

Dr. Walters said that he was in complete agreement with the statements made by Drs. D. Miller and Leventhal . When a committee is charged with data monitoring, it is extremely important that it not dra premature conclusions. These human gene therapy studies have been performed on a very limited number of patients.

Dr. Leventhal stated that the important issue is whether there are implementation problems with th protocol. If an approved vector is not working under any circumstance, then the investigator should not be allowed to treat another 10 patients. These are the types of issues that the RAC should be considering.

Dr. Leventhal asked if she had the authorization of the committee members to pursue specific questior with investigators. Specifically, could she contact Dr. Rosenberg about the tumor necrosis factor/tumor infiltrating lymphocyte trial. More data needs to be provided regarding the fate of the transduced cells a they were readministered to the patients. The request for additional information would be limited to dat regarding the efficacy of the gene transfer procedure. The RAC members agreed that Dr. Leventhal should pursue necessary requests for additional information.

Dr. Leventhal proposed that investigators will be requested to submit a copy of the patient death report that are sent to the FDA. The RAC cannot request this information from the FDA, but it can request a copy from the investigator. She proposed to develop a data reporting format that the investigator will be required to complete for each protocol. The *Points to Consider* requires that investigators should file a report every 6 months; however, she suggested that once a year will probably be often enough if patient enrollment is small. Perhaps investigators should be required to file a report once a year or after every 10 patients, whichever comes first. Any published results of trials that are received by the Office of Recombinant DNA Activities (ORDA) should be circulated to the entire RA

XI. AMENDMENT TO THE *POINTS TO CONSIDER REGARDING SAFETY OF DELIVERY/EXPRESSION SYSTEMS AND REPORT OF MURINE REPLICATION-COMPETEN RETROVIRUS (RCR) ASSAYS/DR. ANDERS*

Review--Dr. D. Miller

Dr. Murray called on Dr. D. Miller to give his primary review of the report submitted by Dr. Anderson regarding RCR data. Dr. D. Miller stated that the report is a very comprehensive summary. However, th RAC should recognize the fact that a procedure must be developed for the standard testing of RCR tha will apply universally to retrovirus vectors. It is clear that these standards cannot be extrapolated from the history of the production of a particular vector by one company. The development of standards for the

testing of retrovirus vector preparations should be balanced between the safety to the patient and the ability of the vector supplier to perform the helper virus assays. There are physical and financial limitations to RCR testing, e.g., if the supplier is required to culture 20,000 dishes of cells, this requirem will introduce a practical impossibility to the testing process. The question is where do you draw the line? Risk versus benefit has to be weighed in establishing RCR testing criteri

Review--Dr. Geidusche

Dr. Geiduschek stated that it was his expectation that this report would outline specific propose improvements in the testing for RCR . However, the report was a redefinition of the problems associate with these assays. The certification of retrovirus vector lots can be greatly improved, and these improvements are necessary. With improved testing, currently generated materials can be demonstrated to be free of RCR contamination in ways not possible by current methods. It is preferable to refine thes analytical methods rather than to relax the testing criteria. The RCR report should be deferred fo revisions, and it could be reconsidered at the next RAC meeting.

Dr. Geiduschek noted that the report makes a specific claim about the margin of safety that is based o argument that is not correct. The calculations are probably off by several logs. In addition, he was not in agreement with the reported number of RCR that are detectable by current assays. The report suggest that the conventional SL assay has an efficiency considerably less than 1, probably less than 0.1, when performed in the presence of a large quantity of RCR . Therefore, the standard SL assay should b abandoned in favor of the one of the amplification assays. He stated that he was disappointed that no new data was accumulated in the last 6 months with regard to the development of improved assays. This issue was originally discussed at the June 1992 RAC meeting. In particular, co-cultivation assays could have been brought to the evaluation stage based on the data that was presented at the June meeting.

Dr. Geiduschek said that he was discouraged that there has been no incentive to generate a mor absolute measure of quality assurance. The report was presented in terms of the global experience of one vector supplier. How would this data generate quality assurance for a new provider? The report states tha the material supplied by this one company has had no evidence of RCR contamination in 147 lots. Sin this report was submitted, RCR contamination has occurred. However, there is reassurance in the fac that the contamination was detected by current testing methods.

Review-- Mr . Bart

Mr. Barton stated that most of his comments had been covered thoroughly in Dr. Geiduschek's review. reminded the RAC that they should consider the importance of maintaining consistency in risk and safety assessment among different kinds of vectors as more experience is gained. Ultimately, statistical analysis will be the key factor. The RAC must define the probability that there will be no greater that*n* RCR particles in a total of 1 x 10 particles. Establishing this *n* may imply that further analysis must be performed on significant dose levels. In addition, patients should be informed that there is always going to be a finite risk of RCR , even though that risk is extremely smal

Presentation--Dr. Anderson

Dr. Anderson stated that he appreciated the RAC's efforts in the review of this report, and that thei comments have been extraordinarily helpful. Dr. Anderson explained that the majority of the reviewers questions will be addressed in the presentation.

Dr. Anderson acknowledged that the report did not define the number of RCR particles required t

produce pathology. He presented *in vivo* data derived from experiments involving 19 monkeys. Four of the monkeys were part of a bone marrow transplantation protocol that was performed over 5 years ago; the animals were severely immune compromised. Five of the monkeys were part of a safety study in which they received cyclosporin and prednisone; these animals were moderately immune compromise Ten of the monkeys were part of a study conducted by Dr. Neinhuis , 3 of which developed lymphomas

How many viral particles did each animal actually get? The 5 monkeys that were moderately immunocompromised received 3 x 10 PFU . None of these 5 animals had any evidence of patholog 4 severely immune compromised monkeys received 1.2 x 10 PFU of RCR , not retrovirus vector par None of these 4 animals had pathology. The 10 animals in Dr. Nienhuis ' study were immun compromised due to lethal irradiation, i.e., the monkeys were T cell depleted. In addition, these animals received either 5-fluorouracil (5-FU) or stem cell growth factor. Subsequently, their CD34(+) progenitor cells were exposed to 80 to 86 hours of continuous virus exposure by 4 separate additions of virus. These animals were exposed to 2 x 10 PFU . Three out of 10 of these monkeys developed lymphoma. This re demonstrated that there is a 30% chance of getting lymphoma in a severely immune compromised animal in which their cells were exposed for 80 to 86 hours at an exposure rate of 2 x 10 PFU . The actual num of vector particles was 2 x 10. Severely immune compromised patients have a 10% chance of getting lymphoma from the suppression itself.

How do these results translate into safety factors for clinical protocols? Basically, there are 4 barriers. First, there has to be breakout. There was no instance of breakout in the first 50 production runs performed by Genetic Therapy, Inc. (GTI). Each run produces 3 lots of vector. Since that time, a break has occurred. The second barrier is that an RCR would have to be missed. Under the current testin conditions, the level of detection is 1 RCR per ml. How are these assays performed? Approximately 10 ml of a production lot (5 liters per lot) are added to cells. Any RCR that is present will be amplified Standard assays will yield positive or negative RCR results. The problem is that the level of detection is RCR particle per 100 ml, not in the 5 liter production lot
What are the chances that there are zero RCR in the 100 ml sample, but there are still viral particles in remaining 4,900 ml? Statistically, there is the probability that between 5 and 20 undetected RCR partic could be present in the untested portion of the production lot. Knowing that approximately 1 x 10 RCR required to produce pathology, do 5-10 undetectable particles represent a risk?

Dr. Anderson explained that Dr. Geiduschek's concern about inaccurate calculations regarding th monkey data was due to poor wording in the report. The number of viral particles produced in the monkeys that resulted in a clonal event (at the time of the clonal event, not at the end) was approxi 10 particles. In other words, an inoculating dose of 2 x 10 PFU is sufficient to give a 30% chance o getting 10 PFU and a clonal event. An additional safety margin exists in that patients can be tested viremia . If a viremia occurs, a patient could be treated with antibodies against the murine retrov therefore, the patient would presumably never reach the 10 particle level.

Dr. Anderson stated that it is important for the RAC to remember that sooner or later a patient will probabl develop a leukemia from a retroviral insertion. It is bound to happen. Will it take 10 patients? The number of patients is unknown. The point is that the present RCR assays are adequate and have to b maintained conscientiously. It is certainly rational to discuss the inclusion of better assays as long as they are reasonable.

Dr. Anderson said that Dr. Geiduschek has suggested the requirement for an assay that would detec breakout. Breakout can be detected by adding a defined number of virus-producing cells to a defined number of production cells. These cells are then co-incubated for a period of time, e.g., several weeks. If no breakout is detected in the aliquot that has been co-cultured, then the frozen production lot is assumed

to be safe. This assay is reasonable and straightforward and should be performed for all retrovirus vector production lots.

Discussion

Dr. D. Miller said that culturing the production run cells and splitting them at the appropriate rate to preserve any helper virus is an enormous undertaking. Dr. Anderson stated that the entire production run would not be tested, only a portion of the cells. Dr. D. Miller expressed concern that a representative sampling might miss a breakout. Dr. Anderson agreed that there is a statistical risk; however, these are severely immune compromised patients. These patients already have a 10% risk of developing lymphoma. If this risk is increased by .0000001%, is it worth spending tens of thousands of dollars and expending many person years to expand the testing criteria? The lower-limit of risk in non-severely immune compromised patients is unknown. Dr. D. Miller said that he was in complete agreement with Dr. Anderson's conclusions. Testing of an entire production run could cost as much as $100,000, which would be prohibitive.

Dr. D. Miller explained that the same concerns exist with adenoviruses. The investigators who received approval for the use of adenovirus vectors indicated that they could detect less than 1 particle per patient dose. If these investigators find that they cannot achieve this level of sensitivity, what criteria will the RAC establish? Perhaps the normal exposure to adenovirus in the environment should be considered.

Dr. Geiduschek stated that what needs to be determined is the slope of the line that gives the rise of th breakout in terms of how fast that slope increases per day, then decide how many days rise is needed in order to detect a break out. In turn, the production run should be cultured for twice that number of days. The number of days or weeks is unknown because these reconstruction experiments have not been performed. It is unlikely that a non-productive extension in the incubation period will increase costs by a factor of 10.

Dr. Murray suggested that the FDA should comment on the criteria they have established for testing RCl Dr. Henry Miller stated that the agenda for this meeting was moved up, and that the FDA representative regarding this issue is not present. Dr. Murray agreed to postpone the discussion until the FDA representative arrived.

XII. DISCUSSION REGARDING COSTS ASSOCIATED WITH TREATMENT OF NON-NEGLIGENT RESEARCH-RELATED INJURY/(CONTINUED)

Dr. Murray recalled the RAC's attention to the revised letter to the NIH Director regarding costs ass with the treatment of non-negligent research-related injury. This letter was revised by Drs. Walters, Zalle Geiduschek , and Ms. Meyers; and distributed to the committee for comment

Dr. Parkman stated that he still has great difficulty with this issue. He said that he would only support a position that did not focus solely on gene therapy or research funded by the NIH . He would endorse letter to the NIH Director that would recommend that a proposal should be sent to the administratio recommending that this issue be considered in the formulation of a reformed health care policy. The recommendation of the RAC should be very broad.

Dr. Chase expressed concern regarding the revised letter because of the implication that gene therapy is inherently more dangerous than any other type of experiment. He objected to the recommendation that the NIH Director should convene a panel to discuss this issue. If the RAC decides that patients shoul receive compensation for non-negligent research-related injury, then they should make a specific

recommendation. What is the necessity for creating a panel? Dr. Chase said that he does not support the revised letter.

Dr. Geiduschek supported Dr. Chase's comments and suggested that the RAC recommend a course o action, not a panel to study the issue. However, Dr. Geiduschek disagreed that the letter suggests tha there is an inherent danger associated with gene therapy, which is greater than any other experimental treatment.

Dr. Zallen suggested the inclusion of the following sentence in the first paragraph: *This situation occurs in most areas of medical research*. Dr. Walters suggested removal of the paragraph that specifically recommends the formation of a panel. If the RAC wants to bring this matter to the attention of the NIH Director, perhaps the letter should simply state that the RAC has encountered this problem and is asking that the Director find a way to respond to the problem.

Dr. H. Miller stated that it is the position of the FDA that the current requirements of the Federal regulations, namely that, *...an explanation as to whether any medical treatments are available if injury occurs, and if so what they consist of..*, is adequate. RAC members who feel strongly about this issue should submit their views to the NIH Director outside of the RAC since the issue does not specificall involve recombinant DNA or gene therapy.

Dr. Parkman said that the sentence that describes a divergence in policy is inflammatory. The reality is that policies have been very consistent; namely, that the compensation for non-negligent research is not the responsibility of the research institution. He quoted the following statement from the letter: *If the benefit of this is to society as a whole, then the responsibility for payment is of the society as a whole. It is not the responsibility of the investigator or the institution in which they are found.* He said that this statement confirms the necessity that compensation for non-negligent injury should be addressed as part of the administration's revised health care plan. The RAC should propose that the NIH Director recommend t it is not the right of an insurance company to deny payment to an individual because of the fact of non-negligent involvement in a research protocol. The ongoing concern is that insurance companies are going to deny payment because research is involved. Compensation should be applicable to research that has been approved by any legally constituted IRB , not just NIH funded resea

Mr. Barton proposed the deletion the last sentence of the second paragraph because it offers only one approach of a number of alternative options. The letter should explain that it is unfair to expect individuals to absorb unpredictable and potentially substantial medical costs. This statement would inform the NIH Director that there is a problem, but leave flexibility in the form of a solution. He agreed with Dr. Parkman that this issue will undoubtedly arise in the development of a national health care program. This recommendation will probably be appreciated by the NIH Director and other people within the researc community who want to emphasize that the future of biomedical research has to be taken into account during the early phases of discussion.

Dr. Murray reiterated the comments made by Ms. Buc earlier in the meeting; namely, if people ar informed enough to sign the consent document, then they should be considered informed enough to understand their financial obligations.

Dr. Parkman stated that if society believes that they will benefit from biomedical research as a whole, then the financial responsibility is to society. To say that an institution is responsible for coverage is inappropriate.

Dr. Secundy stated that it is not incumbent on the RAC to resolve this issue, but to decide whether to t

a position relative to the acknowledgement that this issue is a matter of human concern and fairness. The RAC should not attempt to resolve this problem; the committee should simply bring this matter to the attention of the NIH Directo

Dr. Parkman stated that the draft letter could be edited. The question is whether the RAC wants to take a position of any kind on this issue relative to the NIH Directo

Committee Motion

A motion was made by Mr. Barton and seconded by Dr. Secundy to approve the letter with the followin changes: (1) add the following sentence to the end of the first paragraph,*This situation occurs in most areas of medical research*, (2) delete the last sentence of the second paragraph starting,*In my judgement*, and (3) change the word*might* to *should* and delete the phrase *NIH fund*from the third paragraph.

Dr. Walters asked Dr. Parkman if there were any changes that could be introduced into the letter that would persuade him to endorse this letter. Dr. Parkman said that even with all these modifications, he will vote against the letter because the statements about divergence of policy, etc., are not part of the issue. The issue is the concern that people who encounter non-negligent injury will be excluded from reimbursement of medical costs by the primary payer, either state or public, because the injury was generated as part of a research protocol.

Dr. Carmen stated that he is in support of the letter; however, the deletion of the phrase *NIH fundi*s inappropriate. The NIH Director speaks only for NIH , which has certain funding obligations. The RA an NIH committee. He proposed the following substitute language*but all types of research carried out at institutions receiving NIH suppo*Mr. Barton accepted Dr. Carmen's revision as a friendly amendment. The letter reads as follows:

> In reviewing protocols for human gene therapy, we as RAC members have become increasingly concerned about the divergence in policy among various research institutions on the issue of providing medical care to subjects who may suffer injury in the course of their participation in research. This situation occurs in most areas of medical research. In some cases, the institution promises to cover the costs of immediate care but hedges on long-term care arrangements. In other cases, the costs of medical care for such injuries are, according to the consent forms, to be paid by the subjects themselves, or by their insurance companies if the subjects are insured and the insurance companies will pay.
>
> In any area of biomedical research, the consequences of the research for subjects are not fully known. In our view, it is unfair to expect individuals, their families, or their insurers to absorb unpredictable and potentially substantial medical costs arising out of these individuals' participation as research subjects.
>
> We as RAC members, have reviewed this matter at considerable length. On the basis of our deliberations, we recommend that you create a panel to study the question of how the medical costs of research-related non-negligent injuries should be covered. Such a panel should make policy recommendations that would apply, not only to gene therapy research, but to all types of research involving human subjects. We believe that a uniform policy on this question would be welcomed by investigators, who are concerned about the welfare of the patients whom they invite to participate in research, as well as by the research subjects themselves.

A motion to call the question was made by Dr. Secundy and seconded. The motion to call the questio passed by a vote of 9 in favor, 0 opposed, and 2 abstentions.

The motion to approve the letter with the aforementioned changes was approved by a vote of 9 in favor, 4 opposed, and no abstentions.

XIII. AMENDMENT TO THE *POINTS TO CONSIDER REGARDING SAFETY OF DELIVERY/EXPRESSION SYSTEMS AND REPORT OF MURINE REPLICATION-COMPETEN RETROVIRUS (RCR) ASSAYS (CONTINUE*

Presentation--Dr. Noguchi

Dr. Murray called on Dr. Philip Noguchi to start the continued discussion on RCR assays. Dr. Noguch commented on the FDA's standard for RCR testing. He explained that the important issue is the reaso why these assays are performed. The FDA does not really expect that every investigator will be able to detect the absolute number of RCR in a defined volume, but the sensitivity of the assay does infer measure of safety as demonstrated by current events.

Experiments performed in Dr. Nienhuis ' laboratory provided the first evidence that RCR have the ca to cause disease. Recently, 3 consecutive lots of vector from a supplier demonstrated the presence of a recombinational event. These 2 incidents have assisted the FDA in quantifying the amount RCR tha produce pathology. Dr. Noguchi commended the report submitted by Dr. Anderson and stated that the report supplies the kind of information that the FDA welcomes.

In regard to the supplier that produced 49 consecutive RCR -negative lots prior to experiencing a RCR -positive lot, Dr. Noguchi said that this occurrence indicated that the detection of RCR is no cumulative experience, but a stochastic event. Therefore, every production lot must be viewed separately. Hazards are not always incompatible with treating patients. The critical issue is what are the risks associated with the administration of RCR into a patien

Dr. Noguchi explained two concepts employed at the FDA. First, there should be a master cell bank that is used as the *gold standard* for the testing of complex biological products. The assumption is that all of the cells within the master cell bank are identical. Second, the production bank, or the working cell bank, is obtained from cells that are cultured from the master cell bank. Testing of the working cell bank is different from the testing performed on the master cell bank. The master cell bank is assayed only once.

Dr. Noguchi introduced Dr. Arifa Kahn of the FDA to present additional information regarding RCR t requirements.

Presentation--Dr. Kahn

Dr. Kahn stated that given the present knowledge regarding the generation of RCR , one cannot rely o the theoretical probability of safety of any packaging cell system. Each system must be rigorously tested for the presence of RCR using the most sensitive assays, and must be demonstrated to be saf

Dr. Kahn distributed a draft of the FDA's proposed recommendations for the testing of gene therapy products. These recommendations were formulated based on the FDA's scientific and regulatory

knowledge and on the advice of experts in the field of retrovirology

The FDA recommendations are as follows: (1) testing of the master cell bank and the final production cells by an initial co-cultivation step with cells that are susceptible to a wide variety of murine retrovirus namely *Mus dun* cells, and subsequent passage of these cells for 4 weeks; (2) testing of the supernatan generated from the co-cultivation procedure for the presence of RCR ; (3) testing of each individua production lot, since it is unknown at what passage RCR are generated; (4) testing of the final producti lot supernatant by amplification in *Mus dun* cells, because this cell line is susceptible to a wide range of murine retroviruses, except Moloney murine leukemia virus (MMLV); (5) testing for MMLV on NIH 3T3 cells; (6) testing the supernatants generated from the amplification procedures for RCR testing of the transduced target cells by an initial co-cultivation wit*Mus dun* cells followed by immunofluorescence or PCR assay; (8) testing of the supernatant generated from the transduced the SL assay. Dr. Kahn stated that the aforementioned assays are those that the FDA recommends as currently being the most sensitive assays for the actual detection of RCR

The viable immunofluorescence assay can be performed on infecte*Mus dun* cells using a broadly reactive monoclonal antibody that will detect the envelope products of all 4 host range classes of retroviruses, ecotropic , xenotropic , amphotropic , and mink cell focus-inducing (MCF)

The FDA recommends that the feline SL assay should be performed with the PG4 cell line for the detection of xenotropic and amphotropic viruses and the mouse SL should be performed using D56 for the detection of ecotropic viruses. DNA from infecte*Mus dun* cells and transduced target cells ca be analyzed by PCR using murine leukemia virus (MuLV)-specific pr

Dr. Kahn described 2 assays that could be performed in addition to the aforementioned retrovirus assays; however, they are not as sensitive as the other assays. Transmission electron microscopy (TEM) can be used to examine transduced human cells, and the RT assay can be performed on supernatants ge from these cells. TEM and RT may be useful for the detection of potential human retroviruses o recombination in the transduced human cells, because there are no cell lines or assays that can b recommended at this point due to their unknown host range.

Dr. Kahn stated that these recommendations are subject to modification with the development of more sensitive assays for the detection of RCR

Discussion

Dr. Parkman said that the long-term culture of production lines, which was proposed by Drs. D. Miller and Geiduschek , should be the most appropriate and simple assay for the detection of RCR . How long the production line have to be fed and passaged ? What would the additional cost be to an investigator long-term culture were required? Dr. D. Miller stated that the production line would have to be passaged every 3 days; therefore, a 4-week culture would require 10 passages.

Dr. D. Miller explained that there are probably as many as 100 different murine viruses that could b assayed. The reconstruction assays have to be designed to yield optimal information. Does the FDA want investigators to assay for all 100 possible murine viruses? These viruses all have different tropisms. W about the number and types of helper viruses? He proposed the following scenario: what if 1 weakly-replicating virus is present but it takes 3 months to obtain a positive readout. Does the FDA want investigators to aim at this target as well? The testing process is not as simple as it appears. Tests can be performed for the detection of amphotropic viruses, i.e., the culture could be spiked with amphotropic and cultured for 3 weeks. RCR should be readily detectable after this period of time

Dr. Parkman noted that an amphotropic virus was the agent that produced the monkey lymphomas Therefore, amphotropic viruses represent definable risks as opposed to the theoretical risks imposed b other viruses. Testing should focus on those viruses that have the potential to become RCR . There i more confidence in assaying the entire production lot for potentially hazardous agents, amphotropic viruses, than to perform multiple tests on an aliquot of the production lot as proposed by the FDA.

Dr. Post said that the degree of confidence imposed by testing an aliquot of the production lot depends primarily on the volume of that aliquot. Dr. Kahn responded that the FDA is proposing that the amount of the production lot that should be tested should be approximately 5% of the total lot. This testing volume fo a 5 liter lot would be approximately 100 ml. Based on the FDA's calculations, the co-cultivation assay is approximately 10 times more sensitive than testing the supernatant directly; therefore, 0.5% of the total pool sample is recommended.

Dr. D. Miller asked if the FDA has tested the co-cultivation technique, i.e., have reconstruction experiments been performed in which *Mus dc* cells have been co-cultivated with packaging cell lines. Dr. Kahn said that the actual reconstruction experiments have not been performed by the FDA; however, co-cultivation has generally been proven to be a more sensitive assay than other methods in retrovirolog Similar data has been derived from experiments with the human immunodeficiency virus (HIV). Dr. D. Miller said that HIV is a very unique virus and should not be compared to this setting. Dr. Kahn stated that the FDA has been made aware of data indicating that co-cultivation of cells detects RCR , whereas dire testing of the supernatant does not detect RCR . Dr. Anderson inquired about the source of this data. D Kahn said that she could not discuss the co-cultivation data. Dr. Kahn added that Dr. Janet Hartley has data in her notebook that she is willing to share if necessary. Dr. Anderson was concerned that the FDA is proposing a regulatory assay based on preliminary data that is still in someone's notebook. Dr. D. Miller said that the RAC should have access to this data, because it is central to this discussion.

Dr. Kurt Gunter of the FDA responded that the FDA is currently in the process of conducting the suggested reconstruction experiments. These experiments will be compared to other testing methods and validated. Once the FDA has solid information (solid data on the relative sensitivities and the relative usefulness of co-cultivation), a rational decision will be made about the preferable method of testing. However, it is reasonable that co-cultivation assays should be performed if there is no undue burden placed on the investigator. Dr. Gunter explained that the FDA recommendations distributed by Dr. Kahn are for discussion purposes. These are procedures that are being considered by the FDA.

Dr. Anderson stated that it is very important that the RAC continue to work with the FDA on this issue. However, he expressed concern about the FDA's proposed recommendations. Although the FDA has proposed to perform RCR testing in a responsible manner, Dr. Anderson said that he would like to offe several counter-suggestions. The margin of safety imposed by present assays must be considered. Is it necessary to establish more sensitive assays? There is no question that sensitive assays must exist, and that investigators must be conscientious. There is no evidence, however, that the present assays need to be improved. In response to the FDA's comments regarding the preliminary nature of these proposals, Dr. Anderson reminded the RAC that the FDA had proposed a clinical hold on all gene therapy protocols several months ago until new standards could be developed. Subsequently, the FDA withdrew the proposed clinical hold. Although the FDA has not delayed any human gene therapy protocols, the threat still remains.

Dr. Anderson explained that the assays proposed by the FDA are reasonable assays. In fact, they have included every assay that could possibly be conceived to improve the level of sensitivity. However, these are not regulatory assays. The proposed testing will cost investigators hundreds of thousands of dollars

and an enormous amount of time. The FDA could bring the entire field of biotechnology to its knees, and then the U.S. might have to buy its recombinant products from another country such as Japan. It is imperative that the RAC and FDA ask the fundamental question. Do we need new, more sensitive assays? Dr. Anderson stated that he is of the opinion that new, more sensitive assays are not necessary.

Dr. D. Miller asked Dr. Anderson about the current cost of certifying a vector for human use? Dr. Anderson asked Dr. Gerard McGarrity of GTI to respond to Dr. D. Miller's question. Dr. McGarrity stated th entire cost is approximately $100,000. If the cost of vector certification increases, these expenditures would be prohibitive to small start-up companies.

Dr. Post asked Dr. Anderson to describe the assay(s) that were performed that detected the breakout t occurred in one of the vector lots? Dr. Anderson responded that the breakout was detected by the standard SL assay and confirmed with NIH 3T3 amplificatio

Dr. Noguchi reminded Dr. Anderson that there is data that is available, only to the FDA, that indicates that it is reasonable to consider additional testing. Dr. Anderson asked if these findings were demonstrated on a supernatant assay. Dr. Noguchi stated that he could not discuss the data. Dr. Anderson said that these data were derived from supernatant assays.

Dr. Anderson said that if co-cultivation proves to be twice a sensitive as current methods, but costs 10 times as much, what is the end result? Dr. Noguchi said that it is reasonable to discuss this issue, and tha the FDA will work with investigators to develop reasonable recommendations.

Dr. Parkman said that there is a basic problem with the FDA's proposal to test an aliquot, because there will always be the possibility that your aliquot does not contain a vector-producing cell. There will always be a degree of uncertainty that you have missed by selection up front, the agent you are trying to detect. Dr. D. Miller reminded Dr. Parkman that testing an entire lot assures that there is zero contamination. Is it really necessary to ensure zero contamination? Culturing the entire production lot for a month is unworkable in terms of cost and unreasonable in terms of risk. At some point, the RAC and FDA have to decide on an acceptable level of contamination.

Dr. D. Miller said that at this point, decisions can only be made based on current data. If more informative data is verified, then present criteria may have to be modified. Current data, however, suggests that the established testing criteria are sufficient.

Dr. Kahn stated that the proposed testing procedures developed by the FDA were established for the purpose of simplification, as well as adding a sensitivity factor. The draft proposal is not intended to complicate testing procedures or increase costs. As a point of clarification, investigators would not be required to perform all of the assays that are listed for the detection of RCR . Any one of these assays i sufficient to detect retrovirus. GTI is already performing SL and PCR assays. The immunofluoresc assay is suggested as an alternative, because it can detect all four classes of retroviruses. In no way is the FDA stating that they will require that all of these assays should be performed for the detection of retrovirus. In addition, the TEM assay is only offered as a suggestion. TEM offers the additional measure that unknown retrovirus particles can be detected.

Dr. Kahn responded to Dr. Anderson's question regarding the need for more sensitive assays. Gene therapy products are unique because of the fact that there are no inactivation procedures as with other biological products, e.g., monoclonal antibodies. The FDA's safety concerns are greatly heightened due to the recent results regarding the generation of RCR . Therefore, there is a need for the development o new, more sensitive assays. If more sensitive assays are established, there will be an obligation to use

these testing methods as they become available.

Dr. D. Miller said that the distinctions drawn between monoclonal antibody production and vector production are not as clear as Dr. Kahn suggests. The FDA limits the amount of contaminating DNA that can be present in monoclonal antibody producer cells. DNA could be transferred for chinese hamste ovary (CHO) cells during the production of recombinant products that could in turn be taken up by othe cells. Dr. D. Miller stated that retroviruses are not any more unique than monoclonal antibodies. Viruses can be inactivated from these biological products, but the DNA remains; and the FDA is concerned about this level of contamination.

Dr. D. Miller said that the experiment proposed by Dr. Parkman is ideal and should be performed to determine how rapidly the virus spreads through a culture and the feasibility of performing this assay on the entire production lot. If the additional procedure costs less than $5,000, than it is probably reasonable to perform. However, if the proposed testing were to cost $100,000, this cost would be prohibitive. The RAC is charged with developing testing procedures within reason that will provide the greatest safety possible. Dr. D. Miller stated that it is unreasonable for the FDA to pursue more sensitive assays in the absence of any observable risk.

Dr. Geiduschek said that he is satisfied to know that this issue will be pursued vigorously in the nea future. He asked about the fraction of cost for gene therapy that resides in vector production. Dr. Anderson responded that the cost of producing the vector is probably 50% of the entire cost of the gene therapy procedure. The cost of producing the vector is substantial.

Dr. McGarrity responded to the issue of cost. When current RCR testing procedures were compare standards used prior to the summer of 1992, GTI estimated an increase of about 7-fold in the staff tim required for quality assurance assays based on the inclusion of co-cultivations and amplifications. If this increase is beneficial and cost effective, this money is well spent. He questioned Dr. Kahn on the 0.5% estimate she provided regarding the proposed testing aliquot. Dr. McGarrity noted that his calculation indicated that 2% of the lot would be tested. Dr. Kahn said that 0.5% refers to the number of cells, not the volume of supernatant.

Dr. McGarrity said that there has been a lot of discussion regarding the lack of data regardin co-cultivation and amplification. He would like to include the viral immunofluorescence in this list o non-validated tests. Dr. Kahn said that the immunofluorescence test was proposed as an additional tes but the FDA is not requiring investigators to perform this assay; it is one of 4 tests that can be performed t detect retroviruses. Dr. McGarrity added that TEM, even in the mouse producer cell lines, is a ver inefficient test. Dr. Wivel agreed with Dr. McGarrity's assessment of the TEM assay. Dr. Wivel n the level of sensitivity is extremely low, and that this technique will not detect viral replication unless there is budding. Dr. McGarrity said that GTI is in the process of trying to validate a number of new assa however, the data is not conclusive at this stage. Additional data will be forthcoming.

Dr. Murray stated that since there is no further discussion, Dr. Anderson will revise the report and submit i to the RAC at a future date.

XIV. DISCUSSION REGARDING PROPOSED REVIEW OF EUROPEAN HUMAN GENE THERAPY PROTOCOL (CONTINUED)

Presentation--Dr. Blaes

Dr. Murray called on Dr. Blaese to start the continued discussion regarding the review of a Europea

human gene therapy protocol. Dr. Blaese provided additional background information regarding th European request for RAC review of a human gene therapy protocol. A group of European scientists have inquired about the possibility that the RAC would assist them in establishing an acceptable review format. These scientists are concerned that if individual European countries develop their own guidelines and legislation, than cooperation between international boundaries will be impossible. The EC is considering the adoption of the RAC model of review.

In an effort to establish a set of standards that is similar to the RAC, The EC has proposed the dual review of several protocols. The European committee may actually establish a working group to participate in the RAC review of these proposals. This process will provide structure and legitimacy to European scientists' proposal that the EC establish a multinational review committee. This request has come from the president of this organization.

Dr. Dronamraju asked Dr. Blaese to be more specific regarding the origin of this request. Dr. Blae that this committee, the Working Group for Gene Therapy and Gene Transfer, is comprised of 40 individuals from Spain, France, Belgium, Holland, Germany, Italy, the United Kingdom, and Sweden. The first organizational meeting of this working group was held in October 1992; at that time, the president, officers, and board members were elected.

Dr. D. Miller stated that a large body of information regarding the review process can be obtained from the *Points to Consider* and the RAC meeting minutes. Earlier discussion suggested that the request should not be honored unless it came from a regulatory authority. Dr. Blaese explained that an official reques would not be made until the organizational structure has been established and the European members have attended RAC meetings.

Dr. Carmen stated that as a political scientist, he sees a great deal of merit in acceding to this request. The RAC is advisory to the NIH Director. NIH is part of a Federal agency and it is our responsibility offer expert views on recombinant DNA research, particularly human gene therapy. The RAC has been asked to provide advice and counsel the formulation of high policy in an area of great concern to the EC. Providing assistance would be a great step forward in cooperation between the U.S. and the EC. If the RAC establishes that this request has come from a duly constituted representative of the EC, then the RAC should provide its assistance.

Dr. Seth Pauker of the FDA said that the EC has a committee that reviews proprietary medicinal produ This directive was established in 1987, and the committee has been responsible for the review of all biotechnology produced pharmaceuticals. This committee has a biotechnology working group that has established guidelines similar to the FDA's points to consider. Dr. Pauker said that is reasonable t suspect that the members of this committee have given this issue considerable thought.

Dr. Dronamraju asked Dr. Wivel about NIH's policy regarding such issues. Dr. Wivel respond is nothing in the *NIH Guidelin*that would provide the RAC with this particular purview. Dr. Doi aske Dr. Blaese if the EC is interested in scientific feedback alone or the social and ethical input that the RA members could provide. Dr. Blaese stated that it is his understanding that this committee wants advice more than just the scientific aspects of these protocols. The European investigators involved are certainly capable of providing scientific review; they are interested in the broader analysis.

Ms. Meyers said that she does not understand why this request has developed into such a contentious issue. Clearly, the RAC should cooperate with the rest of the world to obtain the global objectives of treating and curing disease.

Dr. Blaese said that the European committee is not asking the RAC for a commitment, only for assuran that they will provide assistance. If the EC can be confident that the RAC will be receptive to this request, then they will be able to focus their strategy.

Dr. Schaechter stated that there is a serious question about the valuable information that would b obtained from this exercise. However, the RAC should not consider the efficacy of this request. The RAC should take the position that if the EC believes that useful information will be derived from the exercise, then the RAC should support them. Dr. Murray added that if the request were to come from an appropriate official body, this request would be an important consideration.

Committee Motion

A motion was made by Dr. D. Miller and seconded by Dr. DeLeon to provide a positive response to thi request. The motion passed by a vote of 13 in favor, 0 opposed, and 1 abstentions.

Dr. Murray noted that in the interest of time the next agenda item regarding the separation of the gene marking informed consent document from the therapeutic informed consent document will be postponed to a future RAC meeting.

XV. DISCUSSION REGARDING COMPASSIONATE PLEA EXEMPTIONS TO RAC REVIEW/DR. WALTERS AND MR. BARTON

Presentation--Dr. Walters

Dr. Murray called on Dr. Walters to give his primary review on compassionate plea exemptions. Dr. Walters explained that a request has been made to the NIH Director regarding the treatment of a singl brain tumor patient on a human gene therapy protocol that has not been reviewed or approved by the RAC. He explained that he is sympathetic with the plight of the patient but suggested that the RAC should not become involved in advocating the compassionate treatment of individual patients in protocols that have not been formally reviewed and recommended for approval by the committee.

He proposed an alternative to the compassionate use policy for protocols; namely, the approval of minor variations to approved protocols, e.g., relaxation of the inclusion/exclusion criteria of a particular study. If a patient was presented who was otherwise untreatable and failed on 2 of 3 inclusion criteria, there ought to be a mechanism to respond to that particular patient without waiting until the next scheduled RAC meeting. The RAC should establish a mechanism for approving minor deviations to previously approved protocols.

In regard to this specific request, the RAC would lose any semblance of quality control in the field of gene therapy if it were to respond to compassionate use pleas that were not in conjunction with a protocol that had been reviewed by the committee in its final form.

Presentation-- Mr . Bart

Mr. Barton stated that he reviewed the compassionate plea request from a legal perspective. Currently, the RAC cannot approve a request that has not been reviewed. Unless the RAC has reviewed a protocol that has received due public notice in the *Federal Register*, then the RAC should not approve the request. There is a possibility that the investigator would receive a court injunction if the public has not received due notice and been allowed to comment on the proposed action.

The RAC ultimately considers the safety to the subject. Compassionate plea exemptions are an issue for the FDA to consider. In general, the FDA approval process does not apply to the Phase I testing; however, some investigators have applied prior to their investigational new drug (IND) submission. T date, the RAC has not considered any protocols that are beyond Phase I; therefore, adverse affects of the therapy must be considered.

Unlike other biologic therapeutics, gene therapy presents additional concerns regarding third-party transmission. For example, the RAC has just approved several protocols for the treatment of CF using adenovirus vectors. Data suggests that these patients will continue to shed recombinant virus. What are the implications for the public? These safety concerns are reflective of the RAC's role, namely, reassur the public that this technology is going to be used in a way that will be safe. The RAC must consider to what extent future patients might be deprived of the benefits of a solid efficacy analysis based on the first generation of patients.

Discussion

Dr. Parkman said that he is basically in support of the relaxation of inclusion/exclusion criteria as outlined by Dr. Walters. A mechanism is already in place for the approval of minor modifications. The *Points to Consider* states that requests for minor modifications can be approved by the Chair of the RAC and that the Chair may consult with other committee members if necessary. This procedure is endorsed by IRBs is perfectly appropriate for an investigator to request a minor modification of an approved protocol.

Ms. Meyers asked for clarification regarding the proposed request. Dr. Wivel stated that the reques originated from a physician who wants to perform gene therapy on one patient. This request was submitted directly to the NIH Director

Ms. Meyers asked for clarification regarding the investigator's obligation to obtain RAC review. Dr. Murray explained that research conducted at an NIH sponsored institution is subject to th *NIH Guidelin* Therefore, if an investigator does not receive NIH funding, the RAC has no purview over the propose experiment. For the record, Dr. Murray reminded the RAC that they have reviewed a number of protocols that were submitted voluntarily by non- NIH funded institutions. Dr. Murray said that she is not aware if requesting institution receives NIH funding

Dr. Ivor Royston, Scientific Director of the San Diego Regional Cancer Center, responded to the RA members' comments. The San Diego Cancer Center is an NIH grantee institution; therefore, this resea is subject to the *NIH Guidelin* and RAC review. He noted that he had submitted a human gene therapy protocol to the HGTS for review in November 1991. The original protocol involving cytokine gene thera with fibroblasts was deferred by the HGTS . He said that he will return with a formal request in 199

Dr. Royston explained that this compassionate plea request is for an individual patient who is the wife of the chairman of the board of the San Diego Cancer Center. Therefore, the patient is aware of the research that is being conducted and has asked the investigators if gene therapy could provide any therapeutic benefit. The patient has a Stage IV glioblastoma . This brain tumor is incurable. The patient's cells hav been established in culture and have been transduced with the IL-2 gene

Dr. Royston stated that prior to the patient's request for gene therapy, brain tumor research was never contemplated. The patient's cells have been transduced with a vector that has not been approved by th RAC and a vector from Dr. Bernd Gansbacher that has received RAC approval. Dr. Royston stated tha the FDA has been very responsive to his request, but the NIH does not have a similar mechanism fo providing guidance regarding the treatment of individual patients who could die prior to the next regularly

scheduled RAC meeting. Dr. Royston said that the recourse for proceeding without the NIH Director' approval could be the withdrawal of all NIH grants from the San Diego Cancer Center. He stated that h did not want to see this happen. If FDA approval is not received, then he would not seek RAC approval. However, if the FDA approves the single patient IND exemption, he would request that someone from RAC or NIH authorize the treatment of this individua

Ms. Meyers stated that there are approximately 20,000 to 30,000 glioblastoma patients in the U.S. Wh do you say to these individuals? Dr. Royston stated that he is not optimistic that this therapy will work for this patient. He is merely a scientist who has been requested to provide this therapy. This patient has presented her case strongly to numerous doctors throughout this country and to Federal government officials. Dr. Royston said that he encouraged the patient to enroll in Dr. Edward Oldfield's NIH app glioblastoma protocol, but she was informed that the protocol is not ready for her at this tim

Dr. Royston stated that he would be happy to send the FDA single patient IND exemption material t anyone who wishes to review it. Dr. D. Miller noted that the FDA material had not been included as part of the information that was forwarded to the NIH Director. Dr. Royston stated that he was not asking for R approval today, but to urge the RAC to develop a mechanism in which he can communicate with the RAC.

Dr. D. Miller said that the NIH Director would have to decide whether she would allow a change in th procedures that are in place for the evaluation of a request that is not within the RAC's purview. Dr Royston noted that the request for this discussion originated from the NIH Director. Dr. Wivel stated the NIH Director can request that the RAC modify or amend th*NIH Guidelin*regarding this issue. The RAC could make a specific recommendation regarding the *NIH Guidelin*, which the Director can either accept or reject.

Dr. Post asked Dr. Royston how this situation developed; namely, that the investigators are in a position where their protocol is far enough along that it can be submitted to the FDA, but has not been formally submitted to the RAC. Dr. Royston said that he was not aware that the RAC would entertain a single patient protocol. Dr. Royston said that he had consulted the Director's office on this issue, and that he had not been advised to submit a single patient protocol. Dr. Royston said that it may not be appropriate for the entire RAC to entertain a single patient request. Dr. Murray reminded Dr. Royston that the RAC has approved protocols with as few as 3 patients. There are no limitations to patient enrollment. Dr. Royston stated this issue goes beyond this one patient.

Dr. Chase said that he was troubled by the fact that the original request came from a U.S. Senator to the NIH Director. The RAC is an advisory committee. The NIH Director is free to accept or reject the advice. The RAC has been put in the position of assuming an executive authority that it does not possess. This request is completely inappropriate for an advisory body.

Dr. Parkman stated that it is his understanding that no substantive actions can be taken by the RAC that are not part of a public meeting. Therefore, it is appropriate to make minor modifications to approved protocols outside of the public forum. There is no mechanism, based on the *NIH Guidelin*, for the RAC to review a protocol outside of a public meeting. In order for the RAC to review protocols between meetings, an amendment would have to be made to the *NIH Guidelin*.

Dr. Carmen asked Dr. Royston if this compassionate plea request would be for submission to the protocol that was deferred by the HGTS in November 1991. Dr. Royston said that he could not give an exac answer to the question because the 1991 protocol proposed the administration of transduced autolog fibroblasts in combination with irradiated tumor cells. The compassionate use IND submitted to the FD

proposes the administration of transduced autologous fibroblasts and/or transduced autologou cells.

Dr. Anderson stated that the Federal Advisory Committee Act, as interpreted in part by the *NIH Guidelines*, states that the NIH Director gives approval and is required to seek the advice of the adviso committee (RAC). Does the discussion of this issue at this meeting satisfy this criterion? If the current discussion can be interpreted as *seeking the advice of the RAC*, then the final decision could be made by the NIH Director

Mr. Barton said that he had a problem with Dr. Anderson's interpretation of the *NIH Guidelin* The *NIH Guidelines* clearly state that the RAC cannot approve any formal action without giving the appropriate *Federal Register* notice. If there is an individual who has reason to argue that the proposed experiment is particularly dangerous, he/she would have been deprived of the opportunity to try to bring that issue to the attention of the RAC. One of the major reasons that the RAC exists is to ensure that those arguments can be presented before any final actions are taken. The *NIH Guidelin* state that the NIH Director will no make a decision until she has a recommendation from the RAC. The public is entitled to rely upon that fact. Technically, any recommendation made at this meeting would not fulfill the requirement to *seek the advice of the RAC* because a recommendation made by the RAC without *Federal Register* notice is arguably no recommendation at all.

Ms. Meyers stated that society is still very afraid that gene therapy and recombinant DNA will cause the escape of viruses that will create *two-headed monsters*. Therefore, there is still a great need to review all proposals in a public forum. She noted that Dr. Royston has acknowledged that there is no expectation that this protocol will provide any therapeutic benefit to the patient. Ms. Meyers explained that there is nothing worse than raising false hope in a dying patient. Ms. Meyers asked Dr. Royston why he had not submitted his request before the required 15 day *Federal Register* notice. Dr. Royston said that he was not aware that this procedure was an option. As a point of clarification, Dr. Wivel stated that the RAC h approved the adoption of a protocol submission plan that allows for adequate review by the RAC members and investigator responses. The RAC requires that protocols must be submitted 8 weeks in advance of a regularly scheduled meeting. Dr. Post noted that the letter to the NIH Director from Senat Harkin was dated October 8, 1992. There was obviously sufficient time for the investigators to submit this request according the RAC's submission criteria. Dr
Royston said that he did not have a written protocol at the time Senator Harkin's letter was sent to the NI Director.

Dr. Walters proposed a two-part solution. First, the RAC will entertain single patient protocols and is prepared to act expeditiously in the review of such protocols. Second, the RAC will approve minor modifications to approved protocols in order to accommodate a particular patient. Dr. D. Miller explained that relaxation of the inclusion/exclusion criteria would allow Dr. Royston's patient to be enrolled in the approved glioblastoma protocol conducted by Dr. Edward Oldfield . Dr. Royston said that Dr. Oldfi protocol has not been approved by the FDA; therefore, patients are not being accepted into the study. Dr. Anderson said that Dr. Oldfield's protocol has been approved by the FDA. Dr. Royston said that he wo try to convince his patient to consider Dr. Oldfield's protoco

Dr. McGarrity reminded the RAC that there are thousands of glioblastoma patients in the U.S. T disease is incurable. Dr. Oldfield and GTI have already received over 650 requests for entry into t protocol; many of these requests have been on a compassionate use basis.

Dr. Royston said that he will follow any mechanism that the RAC adopts; but it has to be expeditious, not on a 3 month basis. Dr. Murray explained that there are many individuals who do not believe that gene

therapy has reached a stage that it can be granted on a compassionate use basis, especially when the request comes from a laboratory that has not received protocol approval.

Mr. Barton stated that in his view the only option is the submission of a protocol in the current time frame (January 4) for review at the March 1-2, 1993, meeting. Dr. Royston encouraged the RAC to establish a rapid mechanism of review that can accommodate the RAC and NIH Director. Dr. Murray stated that it unclear that the RAC is ready for establishing such a mechanism nor is the public ready for expedited review.

Dr. Carmen recommended that a working group should be established to propose recommendations with specific language that could be presented at the March 1-2, 1993, RAC meeting. Dr. Murray appointed Dr. Walters to chair the working group.

Dr. Royston said that it is his understanding that he RAC will entertain a single patient protocol if submitted by January 4, 1993. He said that he would be happy to submit this proposal by the deadline. However, he stated that his expectation is that he will submit a request to initiate this treatment prior to the March meeting. He will submit the protocol, and deal with the government authorities as needed.

Dr. D. Miller said that he is entirely uncomfortable proceeding with this protocol. There has been no review of efficacy. There has been no review of the possible dangers. Although Dr. Royston proposes to use a certified vector, nothing is known about the cell line, e.g., is replication competent retrovirus being produced? Dr. Royston stated that there must be a rapid review mechanism. Dr. D. Miller explained that there is no evidence that the proposed therapy will provide a therapeutic effect. Dr. Royston said that the constitutional rights of this patient to receive the therapy that she wants is the real issue.

Dr. Zallen asked if Dr. Royston's protocol has been approved by his IRB ? Dr. Royston stated that th protocol has not been approved by the IRB , and he will be subject to that approva

XVI. FUTURE MEETING DATE OF THE RECOMBINANT DNA ADVISORY COMMITTEE

Dr. Murray noted that the next meeting of the RAC will be March 1-2, 1993.

XVII. ADJOURNMENT

Dr. Murray adjourned the meeting at 3:17 p.m. on December 4, 1992.

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.

Date: 3/1/92

Barbara E. Murray, M.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health