U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
September 11-12, 1995

TABLE OF CONTENTS

I. Call to Order and Opening Remarks/Dr. Walters
II. Data Management/Dr. Walters
III. Minutes of the June 8-9, 1995, RAC Meeting
IV. Report on the *Ad Hoc* RAC Review Committee/Dr. Wivel
V. Report on the Panel to Assess NIH Investment in Gene Therapy Research/Dr. Wivel
VI. Addition to Appendix D of the *NIH Guidelines* Regarding a Human Gene Transfer Protocol Entitled: Gene Therapy for the Treatment of Advanced Prostate Cancer by In Vivo Transduction with Prostate-Targeted Retroviral Vectors Expressing Antisense c-myc RNA/Drs. Steiner and Holt
VII. Addition to Appendix D of the *NIH Guidelines* Regarding a Human Gene Transfer Protocol Entitled: A Phase I Study of Recombinant Adenovirus Vector-Mediated Delivery of an Anti-erbB-2 Single Chain (sFv) Antibody Gene for Previously Treated Ovarian and Extraovarian Cancer Patients/Drs. Curiel and Alvarez
VIII. Addition to Appendix D of the *NIH Guidelines* Regarding a Human Gene Transfer Protocol Entitled: A Phase I Study of Direct Administration of a Replication-Deficient Adenovirus Vector Containing the E. Coli Cytosine Deaminase Gene to Metastatic Colon Carcinoma of the Liver in Association with the Oral Administration of the Pro-Drug 5-Fluorocytosine/Drs. Crystal, Hershowitz, and Lieberman
IX. Addition to Appendix D of the *NIH Guidelines* Regarding a Human Gene Transfer Protocol Entitled: A Phase I Study of E1A Gene Therapy for Patients with Metastatic Breast or Ovarian Cancer that Overexpress HER-2/NEU/Drs. Hortobagyi, Lopez-Berestein, and Hung
X. Discussion Regarding Stipulations for Protocols #9406-079, Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wilt-Type P53 and Cisplatin, and 9412-096, Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wild-Type P53/Drs. Roth and Clayman
XI. Discussion Regarding the Deliberate Transfer of Recombinant DNA Molecules Encoding the Tumor Suppressor Gene, p53, to Humans
XII. Presentation on *In Utero* Cell and Gene Therapy/Dr. Golbus
XIII. Discussion of Containment Level for Murine Retrovirus Vectors/Drs. Anderson and McGarrity
XIV. Update Report from the Appendix B Subcommittee Regarding Classification of Commonly Used Animal Pathogens/Dr. Shih
XV. Chair Remarks/Dr. Walters
XVI. Future Meeting Date/Dr. Walters
XVII. Adjournment/Dr. Walters

DEPARTMENT OF HEALTH AND HUMAN SERVICES

NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
September 11-12, 1995

The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-third meeting at 9:00 a.m. on September 11, 1995, at the National Institutes of Health (NIH), Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on September 11 from 9 a.m. until 5 p.m. and September 12 from 9:00 a.m. until 4:00 p.m. The following were present for all or part of the meeting:

Committee Members:

Gary A. Chase, Georgetown University Medical Center
Patricia A. DeLeon, University of Delaware
Robert P. Erickson, University of Arizona
David Ginsburg, University of Michigan
Joseph C. Glorioso, University of Pittsburgh
Rochelle Hirschhorn, New York University School of Medicine
M. Therese Lysaught, University of Dayton
Kathleen M. McGraw, State University of New York at Stony Brook
Abbey S. Meyers, National Organization for Rare Disorders
Arno G. Motulsky, University of Washington
Robertson Parkman, Children's Hospital of Los Angeles
Gail S. Ross, Cornell University Medical Center
Karen Rothenberg, University of Maryland School of Law
Bratin K. Saha, Emory University
Marian G. Secundy, Howard University College of Medicine
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, Virginia Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health
A committee roster is attached (Attachment I).

Non-Voting Representative:

Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Charlotte Armstrong, OD
Bobbi Bennett, OD
Joe Gallelli, CC
Judith Greenberg, NIGMS
Christine Ireland, OD
Debra Knorr, OD
Becky Lawson, OD
Catherine McKeon, NIDDK

Jay Ramsey, NCHGR
Thomas Shih, OD
Sonia Skarlatos, NHLBI
Lana Skirboll, OD

Others:

Paul Aebersold, Food and Drug Administration
Ronald Alvarez, University of Alabama, Birmingham
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Dale Ando, Chiron Corporation
Elizabeth Austin, Glaxo Wellcome
Cameron Balzer, Genentech, Inc.
Bari Bialos, Cornell Medical Center
Mary Helen Binger, GeneMedicine, Inc.
Bridget Binko, Cell Genesys
Chris Bishop, Schering-Plough Research Institute
Arindam Bose, Pfizer Central Research
Laurent Bracco, GenCell
Andrew Braun, Harvard University
Channing Burke, Introgen Therapeutics, Inc.
Jeff Carey, Genetic Therapy, Inc.
Joy Cavagnaro, Food and Drug Administration
Jan Chappell, Genetic Therapy, Inc.
Yawen Chiang, Genetic Therapy, Inc.
Gary Clayman, MD Anderson Cancer Center
Larry Couture, Ribozyme Pharmaceuticals, Inc.
Kenneth Culver, Public
David Curiel, University of Alabama, Birmingham
Tom Dalonzo, GenVec
Robert DeJager, RGene Therapeutics, Inc.
Jessy Deshane, University of Alabama, Birmingham
Michelle Durand, The French Consolate
Suzanne Epstein, Food and Drug Administration
Paul Fischer, GenVec
Reinhard Fleer, Rhone-Poulenc Rorer
Joyce Frey, Food and Drug Administration
Martine George, GenCell
Richard Giles, MD Anderson Cancer Center
Mitchell Golbus, University of California, San Francisco
Angus Grant, Food and Drug Administration
Tina Grasso, GenVec
Lowell Harmison, Public
James Hawkins, Public
Douglas Hickman, T. Rowe Price Associates, Inc.
Edward Hirschowitz, Cornell Medical Center
Jeffrey Holt, Vanderbilt University
JoAnne Horowitz, Schering-Plough Research Institute
Mien-Chie Hung, MD Anderson Cancer Center

Jack Jaugstetter, Genentech, Inc.
Thomas Johnson, Georgetown University
Susan Jones, Public
Roger Jones, Food and Drug Administration
Connie Kirby, Canji, Inc.
Imre Kovesdi, GenVec
Steven Kradjian, Vical, Inc.
Bridget Laffler, FDC Reports, Inc.
Gloria Lee, Gencell
Michael Lieberman, Cornell Medical Center
Martin Lindenberg, RGene Therapeutics
Gabriel Lopez-Berestein, MD Anderson Cancer Center
Elizabeth Lovell, RGene Therapeutics
Daniel Maneval, Canji, Inc.
Tony Marcel, TMC Development
Gerard McGarrity, Genetic Therapy, Inc.
R. Scott McIvor, University of Minnesota
Stewart Mueller, Rhone-Poulenc Rorer
Andra Miller, Food and Drug Administration
Robert Moen, Baxter Healthcare Corporation
David Nance, Introgen Therapeutics, Inc.
Andrea Neuman, Technology Catalysts
Jeffrey Ostrove, Microbiological Associates, Inc.
John Parker, The Blue Sheet
Susan Peacock, University of North Carolina, Chapel Hill
Nicholas Pelliccione, Schering-Plough Research Institute
Thomas Porter, Enterprise Development Fund
M. Lynn Pritchard, Glaxo Wellcome
Raj Puri, Food and Drug Administration
Rex Rhein, Biotechnology Newswatch
Mark Roffmann, GenVec
Joseph Rokovich, Somatix Therapy Corporation
Jack Roth, MD Anderson Cancer Center
Michael Roy, Agracetus, Inc.
Patricia Ryan, Genetic Therapy, Inc.
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International, Inc.
Gene Siegal, University of Alabama, Birmingham
Mitchell Steiner, University of Tennessee Medical Group
Franck Sturtz, Progenitor, Inc.
Nevin Summers, Novation
Geoff Symonds, Johnson & Johnson
Bruno Tocque, GenCell
Mary Treuhaft, RGene Therapeutics, Inc.
Stanley Tucker, Aronex Pharmaceuticals, Inc.
Dominick Vacante, Magenta Corporation
Ann Vaffis, KPMG Peat Marwick
Katie Whartenby, Food and Drug Administration
Carolyn Wilson, Food and Drug Administration
Deborah Wilson, Introgen Therapeutics, Inc.

Lee Wrubel, GenVec
Teruhiko Yoshida, National Cancer Center Research Institute
Robert Zimmerman, Chiron Corporation
Louis Zumstein, Introgen Therapeutics, Inc.

I. CALL TO ORDER AND OPENING REMARKS/DR. WALTERS

Dr. Walters (Chair) called the meeting to order and stated that the notice of the meeting was published in the *Federal Register* on August 7, 1995 (60 FR 40186); and proposed actions to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* were published in the *Federal Register* on August 10, 1995, (60 FR 40984). He noted that a quorum was present and outlined the order in which speakers would be recognized: (1) primary reviewers, (2) other RAC members, (3) *ad hoc* experts, (4) responses from the principal investigators (PIs), (5) other NIH and Federal employees, (6) the public who have submitted written statements prior to the meeting, and (7) the public at large.

Dr. Walters welcomed Dr. Rochelle Hirschhorn, Professor of Medicine, New York University School of Medicine, New York, New York, as a new member of the RAC.

Dr. Walters noted the following items of interest: (1) A meeting on *Gene Therapy for Immune Deficiencies and Cancer* will be held on September 15, 1995, in Cleveland, Ohio, to highlight the fifth anniversary of the first human gene therapy experiment. (2) A special news report: *Gene Therapy's Growing Pains* (Science 269: 1050-1055, 1995) details the current status of human gene therapy clinical trials.

Dr. Walters summarized the current status of human gene transfer protocols submitted to the Office of Recombinant DNA Activities (ORDA) to date: (1) 122 human gene transfer protocols are currently being monitored by the ORDA for data management purposes (121 protocols have been submitted for review by NIH/ORDA and/or the RAC). (2) 15 amendments have been reported since the June 8-9, 1995, RAC meeting (Attachment II). (3) The PIs of 9 protocols have been notified of their exemption from RAC review (sole Food and Drug Administration (FDA) review) in accordance with Appendix M-VII, *Categories of Human Gene Transfer Experiments that May be Exempt from RAC Review*, of the *NIH Guidelines* (Attachment III). (4) 4 protocols were determined by ORDA to require RAC review and will be reviewed during this meeting.

Dr. Walters noted Ms. Debra Knorr's (ORDA) August 16, 1995, letter to the RAC indicating ORDA's intention to propose amendments to the *NIH Guidelines* that would change the current *semiannual* data reporting requirements to *annual* requirements. Ms. Knorr stated that the proposed amendments are in response to Dr. Gary Nabel's (University of Michigan) June 16, 1995, letter outlining the redundant and onerous reporting requirements of multiple Federal agencies and local institutions. Modifying the current requirements to accommodate **annual** data reporting will correspond with FDA and Institutional Review Board (IRB) requirements and greatly reduce the burden currently placed on PIs of human gene transfer protocols. Dr. Walters asked the RAC to consider the proposed amendments that will be published in the *Federal Register* for public comment prior to the December 4-5, 1995, RAC meeting.

II. DATA MANAGEMENT/DR. WALTERS

Dr. Walters inquired whether the RAC members had any comments or questions regarding the 15 amendments to human gene transfer protocols that occurred since the last RAC meeting. Dr. Parkman asked ORDA to contact Dr. Gary Nabel (Protocol #9306-049) regarding his July 18, 1995, amendment which eliminated the use of the antiviral drug, sCD4-PE40, to reduce viral burden during *ex vivo* selection and gene transfer due to lack of availability. Dr. Parkman noted that the safety data should be submitted demonstrating that the antiviral drug, Delavirdine, by itself sufficiently reduces viral burden. Dr. Parkman

was concerned about the possibility of activating human immunodeficiency virus (HIV) during *ex vivo* retroviral transduction of patient's lymphocytes. Dr. Noguchi (FDA) responded that FDA has reviewed Dr. Nabel's data in support of his amendment; FDA has determined that a single antiviral agent is effective in this case.

Ms. Meyers inquired about changes of the definition of Grades 3 and 4 toxicity in Dr. Albert Deisseroth's Protocols (#9306-044 and #9406-077). Dr. Walters noted that in a letter dated June 1, 1995, Dr. Deisseroth clarifies the amendment by stating that the changes would allow a temporary toxicity (myelosuppression) associated with the standard taxol therapy.

Dr. Walters noted that several adverse events were submitted to ORDA since the June 8-9, 1995, meeting. Upon receipt, ORDA transmitted these reports to the RAC Chair and Dr. Brian Smith, Chair, Subcommittee on Data Management. In all instances, these events were determined not to be directly related to gene transfer.

Dr. Walters summarized each of the 9 human gene transfer protocols that were submitted to ORDA and determined them to be exempt from RAC review (sole FDA review). He asked the RAC members for comments and questions regarding each protocol. Drs. Straus and Samulski reviewed Dr. Phyllis Gardner's protocol entitled: *A Phase I/II Study of tgAAV-CF for the Treatment of Chronic Sinusitis in Patients with Cystic Fibrosis* (#9507-114) Ms. Meyers stated that the protocol involves the transduction of maxillary sinus epithelial cells with an adeno-associated viral vector. Dr. Terrence Flotte's protocol entitled: *A Phase I Study of an Adeno-associated Virus-CFTR Gene Vector in Adult CF Patients with Mild Lung Disease* (#9409-083) was approved for a different target cell (airway epithelial cells). Ms. Meyers expressed concern that a change in target cell was an issue significant enough to be reviewed by the full RAC. The lesser severity of sinusitis versus lung infection poses an ethical question of gene transfer. Since no subjects had been entered on Dr. Flotte's study at the time of the June 8-9, 1995, Data Management Report, no data exist regarding the administration of an adeno-associated virus vector to humans. Dr. Parkman explained that the study of epithelial cells within the anatomically constricted space of the sinus is relevant in addressing scientific questions regarding the efficiency of gene transfer and correction of physiological abnormality of cystic fibrosis (CF). Dr. Hirschhorn inquired why eligible patients should be limited to those who have undergone bilateral antrostomies. Dr. Erickson responded that the procedure will provide an access for placing a catheter without further intervention. Dr. Noguchi said chronic sinusitis is a major concern for CF patients. Dr. Motulsky noted that the investigators stated in the scientific abstract that the study is limited to a subset of patients who have developed severe chronic sinusitis requiring bilateral antrostomies and monthly instillation of antibiotics in these sinuses. It is a perfectly acceptable subset of patients for the study. Dr. Walters asked ORDA to request copies of Dr. Gardner's local IRB and Institutional Biosafety Committee (IBC) approvals.

Dr. Walters noted Protocol #9508-115 by Drs. Gary Nabel and Alfred Chang is entitled *Phase II Study of Immunodeficiency of Metastatic Cancer by Direct Gene Transfer*. The protocol was reviewed by Drs. Motulsky and Erickson.

Dr. Walters noted Protocol #9508-116 by Drs. Michael Bozik, Mark Gilbert, and Michael Lotze is entitled *Gene Therapy of Malignant Gliomas: A Phase I Study of IL-4 Gene Modified Autologous Tumor to Elicit an Immune Response*. The protocol was reviewed by Drs. Smith, Chase, and Ross. Protocol #9508-117 by Dr. Joseph Rosenblatt entitled *A Phase I Trial of Autologous CD34+ Hematopoietic Progenitor Cells Transduced with an Anti-HIV-1 Ribozyme* that was reviewed by Drs. Glorioso, Lai, and Secundy. Several RAC members expressed concern regarding ORDA's decision to exempt Protocols #9508-116 and #9508-117 from RAC review. Dr. Zallen noted that FDA reviewers had requested that these protocols be reviewed by the RAC at its September 11-12, 1995, meeting. Dr. Wivel explained that Protocol #9508-116

involved the subcutaneous injection of *ex vivo* transduced viable tumor cells. A precedent was previously set by the RAC when it approved the injection of *ex vivo* transduced viable tumor cells for one of Dr. Steven Rosenberg's protocols. Dr. Wivel explained that Protocol #9508-117 utilized the LNL6-based retroviral vector, RRz2, which incorporates the hammerhead ribozyme targeted to HIV *tat*. A precedent was previously set by the RAC when it approved a similar vector for Drs. Wong-Staal and Poeschla's Protocol #9309-057. The previously approved vector, pMJT, encodes a hairpin ribozyme that cleaves HIV-1 ribonucleic acid (RNA) in the 5' leader sequence.

Dr. Walters noted that Protocol #9508-118 by Dr. Jeffrey Isner is entitled *Accelerated Re-endothelialization and Reduced Neointimal Thickening Following Catheter Transfer of phVEGF165*. The protocol was reviewed by Drs. Erickson and Hirschhorn and Ms. Meyers. Dr. Parkman inquired if the data from the ongoing trial on peripheral artery disease (Protocol #9409-088) was provided in the present proposal. Dr. Erickson responded that the investigators have data showing no toxicity in the ongoing study; the present study is a broader use of the same vector construct to accelerate re-endothelialization rather than neovascularization.

Dr. Walters noted that Protocol #9508-119 by Dr. Stanley Riddell is entitled *Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Autologous Unmodified and Genetically Modified CD8(+) HIV-Specific T Cells in HIV Seropositive Individuals*. The protocol was reviewed by Drs. Parkman, Saha, and Ross. Dr. Parkman noted that this protocol is for gene marking and is similar to the ongoing protocol (#9202-017) except using the neomycin resistance (*neo*) gene instead of the hygromycin resistance gene since the investigators have detected a host immune response that destroys the hygromycin resistance gene modified cells. Ms. Rothenberg asked if the investigators have responded to a concern raised by Ms. Meyers that the protocol excludes women who are of childbearing age. Dr. Motulsky remarked that scientifically there are few women of childbearing age who would be entering this study. Ms. Rothenberg stated that the eligibility criteria should not discriminate against women. Dr. Noguchi stated that FDA has recently published in the *Federal Register* a proposed regulation dealing with the clinical trial of products intended for the market. It requires the sponsors to demonstrate a specific medical reason why women, children, or other groups of human subjects would be excluded from the clinical trial. Ms. Meyers remarked that this protocol serves as an example of how local IRBs are not following Federal regulations which require that females should have equal access to protocols. IRBs should be educated about the pertinent Federal requirements. Ms. Rothenberg noted that NIH already has such a mandate for NIH funded projects and the NIH Office of Research on Women's Health is working with IRBs on this issue.

Drs. Ross and Zallen raised their concern regarding the failure of some investigators to respond to comments submitted by RAC members at the time a preliminary determination is made about whether a protocol is determined to be exempt from RAC review. In most instances, ORDA forwarded protocols to several RAC members to solicit comments regarding their qualification for exemption from RAC review. Although the majority of RAC members indicated that there were no significant issues that would preclude their exemption from RAC review, several reviewers submitted written comments and questions to PIs. Are investigators required to respond to RAC members' comments and questions prior to notification of exemption? Will PI responses be communicated to the RAC?

Dr. Walters noted that Protocol #9508-120 by Drs. Alfred Chang and Gary Nabel is entitled *Phase I Study of Tumor-infiltrating Lymphocytes Derived from In Vivo HLA-B7 Gene Modified Tumors in the Adoptive Immunotherapy of Melanoma*. The protocol was reviewed by Drs. Nelson Wivel and Thomas Shih (ORDA). Dr. Wivel noted that the protocol involves a modification unrelated to gene transfer (adoptive immunotherapy) of the ongoing study of Dr. Nabel's protocol (#9202-013) of intratumoral human leukocyte antigen (HLA)-B7 plasmid injection of melanoma patients.

Dr. Walters noted that Protocol #9508-121 by Dr. RobertFiglin is entitled *Phase I Study of HLA-B7 Plasmid DNA/DMRIE/DOPE Lipid Complex as an Immunotherapeutic Agent in Renal Cell Carcinoma by Direct Gene Transfer with Concurrent Low Dose Bolus IL-2 Protein Therapy.* The protocol was reviewed by Drs. Wivel and Shih of ORDA. This protocol is similar to Vogelzang's protocol (#9403-071) with the exception of focusing on a subset of patients who will receive interleukin (IL)-2 therapy as a part of their course of treatment. Dr.Wivel noted that IL-2 gene transfer has been previously approved for several protocols to treat renal cell carcinoma.

Dr. Walters said the last exempt protocol (#9508-121) by Drs. Michael Hawkins and John Marshall is entitled *A Study of Recombinant ALVAC Virus that Expresses Carcinoembryonic Antigen in Patients with Advanced Cancer.* The protocol was reviewed by Drs. Wivel and Shih. Dr. Wivel noted that ALVAC is a canarypox virus vector that has been classified as a Biosafety Level (BL) 1 agent by the RAC (June 7-8, 1993), and it is a safer vector than thevaccinia virus vector used in an ongoing vaccinia-carcinoembryonic antigen (CEA) study by Dr. J.Schlom and co-investigators of the National Cancer Institute.

Dr. Zallen raised several procedural questions regarding the NIH/FDA Consolidated Review process. First, several comments have been made by the reviewers on what should be the appropriate procedure to follow-up on the responses provided by the PIs. The second question is how to resolve the disagreements between NIH and FDA in their preliminary review regarding protocol exemption noting the examples of Protocols #9508-116 and #9508-117. A working group should be convened to establish an appropriate procedure.

Dr. Parkman asked Drs. Wivel and Noguchi to comment on the interagency decision-making process and how to respond to issues raised by reviewers in the final approval process at FDA. Dr. Noguchi responded that there is currently no mechanism for arbitration between the two agencies. FDA's recommendation regarding the need for RAC review of Protocols #9508-116 and #9508-117 involved only several technical questions such as using non-irradiated cells (#9508-116) and a new packaging cell line for the retroviral vector (#9508-117).

Dr. Parkman suggested that the FDA should forward PI(s) responses to the RAC as particular issues are resolved, e.g., additional data. Dr. Noguchi said that investigators' responses made to FDA regarding the issues raised in the RAC public forum could be made available to the RAC by its public disclosure mechanism. Ms. Meyers was concerned that issues not raised by the RAC in public will not be available to the public. She asked if ALVAC is a new vector for gene therapy (Protocol #9508-121). Dr.Wivel explained that the RAC has reclassified ALVAC from BL2 to BL1 physical containment since it is a safer agent than the vaccinia virus which is a BL2 agent. In addition, the RAC has previously approved a plasmid CEA protocol (Curiel #9406-073), and another vaccinia-CEA protocol is ongoing (initiated before the RAC amended the vaccine definition for exemption from RAC review). Taken together these precedents argue that the present protocol presents no new safety issues.

Dr. Hirschhorn asked if public RAC review is the only way that would allow FDA to publicly disclose the information regarding the particular protocol. Dr. Noguchi responded that public RAC review not only provides a broader perspective to the issues of gene transfer trials but provides FDA a mechanism to publicly disclose any adverse events as they occur as in the case of CF studies. Without public review, the adverse effects such as those which occurred in thefialuridine hepatitis drug trial were not publicly available in a timely fashion. Dr. Chase agreed that public review of almost all gene transfer protocols has allowed the public to have a whole view of the field. He was concerned that sole FDA review of many protocols in the future would preclude public access except for a public data reporting system. Dr. Walters

said RAC's Data Management Report will provide a comprehensive reporting on all adverse events at the regular RAC meetings. Dr. Parkman said the issue of public disclosure has been discussed by the *Ad Hoc* Review Committee, and a recommendation would be to request FDA to exempt the broad area of gene therapy from many of the proprietary restraints of ordinary products that come under FDA review.

Dr. Walters stated that NIH and FDA should develop an efficient mechanism of arbitration in the event that there is a disagreement between the agencies regarding the requirement for RAC review. He asked what should be a proper disposition of disagreements regarding Protocols #9508-116 and #9508-117. Dr. Zallen suggested a telephone conference call involving the RAC, ORDA, FDA, and investigators prior to the initiation of the studies. Dr. Parkman suggested having FDA follow-up on the issues raised in the review. Ms. Meyers said she would prefer the RAC to review the data on non-irradiated tumor cells. Dr. Wivel explained that a similar protocol using non-irradiated tumor cells was approved for Dr. Rosenberg (#8810-001), and there was no untoward effect. Dr. Parkman said immune response evoked by non-irradiated cells is greater than that of irradiated cells, and it is probably the reason for using such cells in the present study. Dr. Noguchi said non-irradiated cells have been used in many tumor vaccine protocols not involving gene transfer.
The consensus of the RAC was that the two protocols for which the FDA requested RAC review (#9508-116 and #9508-117) should be discussed with the FDA, ORDA, RAC reviewers, and PIs prior to the initiation of these clinical trials.

III. MINUTES OF THE JUNE 8-9, 1995, RAC MEETING

The RAC approved a motion made by Dr. Chase and seconded by Dr. Erickson to accept the June 8-9, 1995, RAC minutes (with the incorporation of minor editorial changes) by a vote of 14 in favor, 0 opposed, and no abstentions.

IV. REPORT ON THE *AD HOC RAC REVIEW COMMITTEE/DR. WIVEL*

Dr. Wivel stated that the most recent meeting of the NIH *Ad Hoc* Review Committee was held on August 28, 1995. This Committee (Chaired by Dr. Inder Verma) is charged with providing a comprehensive assessment of past and current RAC activities in an effort to develop recommendations regarding the future role of the RAC in the review of human gene transfer experiments. The final report will be discussed at the December NIH Director's Advisory Committee meeting in December. One of the recommendations is to request the FDA Commissioner to exempt gene transfer protocols from proprietary restraints for public disclosure. A similar exemption allows disclosure of information regarding clinical trials of acquired immunodeficiency syndrome related drugs and therapeutics. The FDA exemption would allow continued public access to data management reports and facilitate RAC's public discussion of gene transfer protocols.

Dr. Wivel asked Drs. Parkman and Zallen who serve as members of the committee to report on the committee findings. Dr. Parkman said that effective communication between NIH and FDA is essential for the success of the Consolidated Review process. Dr. Zallen mentioned another recommendation would be to ask the RAC to define the criteria and work out procedures for identifying specific protocols requiring public review. She said that the joint NIH/FDA working group which she suggested earlier, would be a useful forum to implement this recommendation.

Dr. Hirschhorn asked whether a protocol not reviewed by the RAC is subject to disclosure by FDA. Dr. Noguchi responded that the FDA does not have to make a disclosure if it is the sole reviewer; however, the current dual simultaneous submission to both the NIH and FDA would circumvent this problem. Dr. Walters added that adverse events will be reported at each RAC meeting, including those protocols that

have been exempted from RAC review. Dr. Ross inquired about the reporting status involving protocols that are not related to NIH funding. Dr. Wivel responded those protocols are not under the purview of the *NIH Guidelines*. Dr. Noguchi remarked that if the FDA Commissioner grants the exempt status for public disclosure regarding gene transfer products, it will apply to all studies funded by NIH and private sources. He mentioned a case involving the adverse event of the CF trials; the public disclosure of the vector-induced inflammatory response has prompted investigators of other CF protocols to adjust their vector doses and to modify the vectors to avoid such an adverse effect.

Ms. Meyers raised 3 concerns: (1) The secrecy issues should not become a public incident of disagreement between NIH and FDA; the public would perceive the secrecy issues as catering to some Wall Street interests; (2) The concern about any inadvertent germ line transduction has not been conclusively ruled out in all gene transfer protocols; and (3) An enhancement gene transfer protocol might proceed without adequate RAC review. Does FDA has any authority to refuse approval of an enhancement protocol if the vector has been used before and shown not to be dangerous? Dr. Noguchi stated that FDA has authority to bring to public discussion any issue that it deems to have impact on public health. FDA would urge the RAC to publicly discuss the enhancement issue; a study involving autologous fibroblasts transduced with the growth hormone gene is imminent. Dr. Noguchi noted that public interest in gene transfer would be a factor for the FDA Commissioner to consider the request for exempt status of proprietary restraints. Ms. Meyers stated that if the public forum of the RAC is eliminated all the decisions will be based on medical factors of safety with efficacy and the public is eliminated from the whole dialogue.

Drs. Lysaught and Ross stated that the PI responses to the comments raised by the reviewers in their preliminary review of the exempt protocols should be reported back to the RAC. Dr. Noguchi stated that he would forward to the RAC the responses received by FDA.

V. REPORT ON THE PANEL TO ASSESS NIH INVESTMENT IN GENE THERAPY RESEARCH/DR. WIVEL

Dr. Wivel requested that Dr. Judith Greenberg, Executive Secretary of the Panel to Assess NIH Investment in Gene Therapy Research, summarized the activities of this panel. Dr. Greenberg explained that the panel has been established by Dr. Varmus, NIH Director, to review how NIH has allocated resources and set priorities with regard to gene therapy research. The panel is Chaired by Drs. Arno Motulsky and Stuart Orkin. The panel has held three meetings. The first meeting was held for the purpose of evaluating the current status of NIH-funded gene therapy clinical trials, and the reports were provided by the NIH program staff. Subsequent meetings of the panel were held for the purpose of obtaining status reports from PIs conducting NIH-funded human gene therapy clinical trials. The final panel meeting will be held on October 5-6, 1995, and will be closed to the public. The purpose for closing the meeting is to allow the panel to deliberate its recommendations. The panel is currently preparing its recommendations which will be presented at the December 1995 NIH Director's Advisory Committee meeting. Dr. Motulsky added that the panel has covered a wide range of different issues of gene therapy research including the current state-of-the-art basic research, how to better optimize clinical research, intramural versus extramural NIH research, the involvement of NIH in gene therapy, and the problem of better training of both basic and clinical investigators.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED*: *GENE THERAPY FOR THE TREATMENT OF ADVANCED PROSTATE CANCER BY IN VIVO TRANSDUCTION WITH PROSTATE-TARGETED RETROVIRAL VECTORS EXPRESSING ANTISENSE c-myc RNA*/DRS. STEINER AND HOLT

Review--Dr. Saha

Dr. Walters called on Dr. Saha to present his primary review of the protocol submitted by Dr. Mitchell S. Steiner, University of Tennessee, Memphis, Tennessee, and Dr. Jeffrey T. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee. Dr. Saha stated that this protocol is for treating advanced (metastatic) prostate cancer using c-*myc* antisense RNA. The protooncogene, c-*myc*, is often overexpressed in prostate cancer; therefore, its downregulation could potentially be useful. A retroviral vector capable of expressing anti-*myc* mRNA will be delivered directly into the tumor site. 15 patients above the age of 35 who have failed standard therapy, but are likely to survive for 6-18 months, will be included in this study. The objective is to determine the maximum tolerated dose (MTD) of the virus. Clinical endpoints include: assessing uptake of vector, expression of c-*myc*, and cell death.

An important feature of the vector construct is the tissue specific murine mammary tumor virus (MMTV) promoter. Expression under the MMTV promoter appears to be confined to breast and prostate in transgenic mice. This feature should theoretically guard against any spread of the vector to other parts of the body. This vector, XM6:MMTV-antisense *c-myc*, has been previously approved by the RAC for a breast cancer trial (Protocol #9409-084). It is intriguing to note that the investigators are using an identical strategy to treat "cancer of the two sexes."

In cell culture, the MMTV promoter is inducible by dexamethasone. Initially, there was some confusion as to whether the patients would receive any hormone treatment (which might be contraindicated in these patients). In using a mouse model, the investigators demonstrated that the MMTV promoter was constitutively active *in vivo,* perhaps due to the presence of adequate glucocorticoid in the circulation; and that there was no enhancement of activity due to the addition of dexamethasone. Based on this finding, the investigators have now removed dexamethasone administration from the protocol. However, it is not apparent that the MMTV promoter will be constitutively active in humans, especially in patients with prostate cancer. What is the hormonal level in patients and will that be sufficient for induction of the MMTV promoter? Will the hormonal level be monitored before and during the treatment? Another point that needs to be clarified is the actual viral titer achievable in the investigators' laboratory and the viral dose to be administered.

The investigators have provided preclinical safety and efficacy data in support of their proposed trial. Their toxicity study indicates that the 8 nude mice were all healthy after 12 weeks of the viral administration. However, it is not apparent which viral stock was used, and how many virus particles were injected. If the stock was 7 x 10 particles/ml, then 0.5 ml/mouse (20 grams) will be translated into approximately 2.1 x 10 particles per 60 kilograms (kg) of human body weight. Thus, the murine dose appears to be on the border line of safety since a total of 4 administrations of 2 x 10 particles are being proposed for the patients. It was not clear why the mice were not injected for 4 successive days as proposed for the human trial?

Regarding efficacy, the investigators have demonstrated that anti-c-*myc* is quite effective in eradicating DU145 tumor cells in *ex vivo* and *in vivo* experiments. The fact that anti-c-*myc* was ineffective *in vitro* was puzzling, given the fact that transduced cells express antisense RNA, that c-*myc* is considerably downregulated, and that these events occurred in the absence of dexamethasone. These results suggest that a host factor is necessary for the killing of tumor cells. The issue of a bystander effect needed additional clarification with regard to the mechanism.

Dr. Saha stated that most of his concerns have been answered satisfactorily by the PIs in their written response. Dr. Saha mentioned a report just published by a Colorado group in the April 1995 issue of the *Proceedings of the National Academy of Sciences* regarding the antiproliferative activity of *c-myc*

antisense in vascular smooth muscle cells. In this paper, the authors found that RNA sequences unrelated to *c-myc* exhibit the same antiproliferative effect. Dr. Saha asked the investigators to comment on the mechanism of antisense *c-myc* inhibition.

Overall, Dr. Saha was satisfied with the protocol and would favor approval if the questions are answered satisfactorily.

Review--Dr. Motulsky

Dr. Motulsky stated that this protocol was submitted but not reviewed during the June 1995 RAC meeting until further studies and experiments became available. The reviewers requested additional experiments dealing with a variety of issues. The investigators have now shown the extent of transduction; however, they cannot account for the tumor shrinkage. Histologic evidence of inflammation is interpreted to be of immunologic origin and presumably explains the bystander effect of finding more tumor shrinkage than can be accounted for by the extent of transduction. The PIs have provided evidence for a c-*myc* antisense effect in DU145 tumor cells and showed that the MMTV promoter may be induced in prostate cells in the absence of androgen. The amount of retroviral vector needed to reduce tumor growth in mice has been established. The PIs have shown that dexamethasone is not required to enhance the anti-tumor effect of the viral construct. Current data have shown that the total amount of vector that can be constructed in thei facility is 2 x 10 virus particles per 4 ml. The MMTV promoter appeared to be specific *in vivo* and was limited to breast and prostate for its expression. No integration of the vector into other tissues were demonstrated. Mice treated with MMTV antisense c-*myc* showed no deleterious effects.

The *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects (Points to Consider)* have been addressed satisfactorily. Dr. Motulsky said that the investigators provided sufficient data to allow them to proceed with the human trial. The proposal is acceptable as written.

Review--Dr. McGraw

Dr. McGraw focused her review on the Informed Consent document. She stated that Mr. Capron's earlier review dated May 9, 1995, of the Informed Consent document was thorough and thoughtful, and the investigators have responded adequately to Mr. Capron's concerns. Dr. McGraw's remaining concern was the discrepancy in terms of the procedures described in the protocol and what was described in the Informed Consent document. In the protocol, it is stated that the patients would receive the vector once a day for 4 days. Conversely, the Informed Consent document mentioned 4 injections per day for 4 days, and it appeared to be a particularly invasive procedure. The investigators need to clarify the discrepancy. Dr. McGraw asked the investigators to clarify a statement in the nontechnical abstract that states the study "will allow patients to die with prostate cancer and not from prostate cancer." The PIs responded in writing that prostate cancer does have slow growing variants which are not life threatening. To be able to convert aggressive forms of prostate cancer to nonaggressive forms by gene therapy may allow the patients to remain alive until natural causes take their life rather than dying from complications of end stage prostate cancer. The abstract was reworded appropriately. Dr. McGraw recommended approval pending satisfactory responses to questions regarding the preclinical data.

Other Comments

Dr. McIvor stated that he has analyzed the deoxyribonucleic (DNA) sequences of the vector, and they are consistent with what is described in the protocol.

Dr. Parkman said that using an inducible promoter for a gene transfer vector is a very interesting aspect of the study. Although in animals the MMTV promoter appears to be constitutively active due to the presence of high enough circulating levels of glucocorticoid, he asked the investigators if the gene can be further turned on *in vivo* in humans with a dose of dexamethasone. Dr. Erickson noted that the written response to this question stated the *in vivo* human cortisol levels are 220-570 nM and the MMTV promoter is active in tissue culture at a dexamethasone concentration of 250 nM. Since the synthetic dexamethasone is 100-fold more potent than cortisol, will the normal level of human cortisol be enough to turn on the gene *in vivo*?
Dr. Ginsburg inquired if there was any data to show that MMTV promoter is specific to prostate and breast in humans; the specificity is demonstrated only in transgenic mice.

Dr. Zallen noted that the Informed Consent document does not mention the interest of the media and others in the research. Ms. Meyers said the follow-up should be lifelong. Dr. Lysaught asked how many biopsies will be performed during the 8 week period of follow-up mentioned in the protocol. In the event the trial shows clinical response, would the patients receive another course of the treatment? Dr. Ross asked if the patients would be checked for their levels of cortisol before treatment, and should the hormone level be part of the eligibility criteria.

Investigators Response--Drs. Holt and Steiner

Dr. Holt addressed the oncogene and molecular biology questions and Dr. Steiner, a urologic surgeon, answered the clinical questions. Responding to the question of dexamethasone, Dr. Holt said earlier animal studies were performed with dexamethasone administration with the hope that the drug would enhance MMTV promoter activity. In a previous review, Dr. Dusty Miller raised the question of whether the use of dexamethasone is necessary. A large study involving 20 animals was subsequently performed to investigate the question of the additional effect of dexamethasone *in vivo*. The mouse study showed that there was no difference with or without dexamethasone administration. This new finding is the basis for proposing the human trial without dexamethasone. Responding to Dr. Ginsburg's question about tissue specificity of the MMTV promoter, Dr. Holt said MMTV promoter clearly has tissue targeted elements that are independent of the response elements. There are data pertaining to prostate cancer cells in tissue culture, but tissue culture cells show promoter promiscuity. In the first breast cancer patient treated with a MMTV vector of an ongoing trial, there was no transgene expression outside of the breast tumor tissue. The question of promoter specificity will be studied in the present human trial. Dr. Parkman inquired if the tumor will be biopsied to examine for transgene expression without exogenous administration of steroid hormones and if no expression is detected will the patients receive dexamethasone administration. Dr. Holt said that the animal data does not indicate any effect of the exogenous steroid treatment. Dr. Parkman said that humans may be different from mice in that the endogenous levels of the hormone may not be high enough to turn on the gene. Dr. Holt responded that if there is no gene expression, such an exogenous dose of steroids will be acceptable. Dr. Saha asked if it were safe to give steroids to the prostate cancer patients. Dr. Holt responded that dexamethasone treatments are safe for these patients.

Responding to Dr. Saha's question of the nonspecific antisense effects, Dr. Holt said the Colorado group observed the effect in studies conducted with synthetic antisense oligonucleotides, and there has been a great deal of scientific debate regarding this observation. It has been pointed out by other investigators including Dr. Arthur Krieg (University of Iowa) that the nonspecific effect of the anti-*myc* oligonucleotides was due to the absence of a methylation site of the synthetic oligonucleotide. The CpG motif lacking the methyl group is common in bacterial DNA but not in mammalian DNA where most such nucleotides have an attached methyl group. The antisense oligonucleotides mimic bacterial DNA in triggering a potent response by mammalian immune cells activating B cells and natural killer cells. Dr. Holt said that the present study employs antisense RNA derived from transcription of the transduced gene, and no

antitumor effect has been observed with a vector construct containing the same *myc* sequence in the sense orientation. The effect appears to be specific to the anti-*myc* construct.

With regard to the question of the mechanism of bystander effect, Dr. Holt explained that paracrine effects (host) contribute significantly to the antitumor effects seen following MMTV-antisense *myc* retrovirus treatment as the transduction rate does not equal the degree of tumor inhibition observed. These host effects are necessary for retroviral gene therapy to be successful since 100% transduction may not be achievable. The investigators have both molecular and cell biological data that suggest the mechanism involving deregulation of c-*myc* production by antisense c-*myc* is associated with BCL-2 gene downregulation, resulting in apoptosis. Apoptosis was not induced in prostate cancer cells in tissue culture, and it was presumed to be a host phenomenon.

Dr. Steiner stated that the Informed Consent document will be amended to include statements regarding media interest, lifelong follow-up, and confidentiality of the patient records. The statement of biopsy will be clarified. This Phase I protocol is a toxicity study, and patients will not be retreated. If there is efficacy, it will be evaluated with a Phase II or III trial. Dr. Walters asked how many patients will be treated? Dr. Steiner said that 15 patients will be studied initially. If there is any response, Dr. Steiner requested the option to expand the study to 50 patients to confirm the result. All patients will have a biopsy. At 8 weeks, patients who show a response from transrectal ultrasound scanning will have a repeat biopsy to confirm the clinical findings histopathologically. Gene expression will be evaluated from biopsies taken at 2 weeks and every day during the 4 day period of vector injections.

Dr. Ross asked what would be the rationale to expand the study to 50 patients. Dr. Steiner responded if any tumor shrinkage is observed, the study will be expanded. The protocol will be amended to allow patients to receive alternative treatment after 8 weeks.

Dr. Parkman asked if it would be agreeable to have an initial approval of 15 patients; and if there is any beneficial effect, the investigators could request a minor modification for an additional 35 patients. Dr. Steiner accepted Dr. Parkman's suggestion.

Committee Motion

A motion was made by Dr. Saha and seconded by Dr. Motulsky to accept the protocol submitted by Drs. Steiner and Holt. From the discussion of the data presented, it was unclear that steroids were essential to the activation of the MMTV promoter. However, if no evidence of gene expression is demonstrated, the investigators were asked to consider the addition of steroids and to define the appropriate criteria. The pertinent data should be analyzed by the primary reviewers of this protocol. The motion was approved by a vote of 14 in favor, 0 opposed, and no abstentions.

Protocol Summary: Dr. Mitchell S. Steiner, University of Tennessee, Memphis, Tennessee, and Dr. Jeffrey T. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee, may conduct gene transfer experiments on 15 male subjects (35 to 75 years of age) with metastatic prostate cancer. Malignant cells obtained from advanced prostate cancer subjects have been demonstrated to express high levels of the protooncogene c-*myc in vivo*. The mouse mammary tumor virus (MMTV) long terminal repeat is expressed at high levels in prostate tissue. Following removal of a biopsy core of malignant cells, subjects will receive a single transrectal ultrasound-guided intraprostate (biopsy site) quadrant injection of the retrovirus vector, XM6:MMTV-antisense c-*myc*, for 4 consecutive days. The objectives of this Phase I study are: (1) to quantitatively assess the uptake and expression of XM6:MMTV-antisense c-*myc* by prostate cancer cells *in vivo*, (2) to determine whether c-*myc* gene expression is prostate tumor-specific, (3) to assess safety of intraprostate injection of XM6:MMTV-antisense c-*myc*, and (4) to

assess biologic efficacy of antisense inhibition of tumor growth.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I STUDY OF RECOMBINANT ADENOVIRUS VECTOR-MEDIATED DELIVERY OF AN ANTI-erbB-2 SINGLE CHAIN (sFv) ANTIBODY GENE FOR PREVIOUSLY TREATED OVARIAN AND EXTRAOVARIAN CANCER PATIENTS/DRS. CURIEL AND ALVAREZ*

Review--Dr. Saha

Dr. Walters called on Dr. Saha to present his primary review of the protocol submitted by Drs. David T. Curiel and Ronald D. Alvarez, University of Alabama, Birmingham, Alabama. Dr. Saha stated that the protocol aims at treating ovarian and extraovarian cancer with an adenovirus capable of expressing a single-chain antibody against an oncogene, *erb*B-2, which is overexpressed in some instances of ovarian cancer. The interesting feature of this virus is that the expressed antibody will be localized in the endoplasmic reticulum of the infected cells and trap the newly synthesized *erb*B-2 prohibiting its expression on the cell surface. This leads to cellular apoptosis and destruction of the cancer cells. The virus will be administered intraperitoneally and a total of 18 patients above the age of 18 years old will be enrolled in this trial. The objective of this study is to determine the MTD of the virus by a single injection. The clinical endpoints are to ascertain the safety/toxicity and to evaluate the molecular response associated with the virus.

This protocol was submitted but was not reviewed during the June 1995 RAC meeting due to insufficient data. The reviewers requested that the investigators submit the complete DNA sequence of the vector construct and perform additional safety studies in support of their clinical trials. The investigators have now provided toxicity data in an immunocompetent murine model. In one study, the mice received 10 or 10 virus particles without any sign of toxicity. In another study, the mice were preimmunized with 10 virus particles, and then after 10 days challenged with 3 different doses (10, 10, and 10 particles, respectively). Again, no toxicity was observed in any of these doses. The second study is particularly interesting in the sense that it alludes to the safety of sequential administration of the virus. However, the investigators have abandoned the idea of repeat-dosing and want to focus on a single dose which will be escalated in successive cohorts. Dr. Saha stated that the data are encouraging. The maximum dose (10 particles) planned for the clinical trial are safe since it is several thousand-fold less than the highest tolerated dose in a mouse when calculated in terms of body weight.
The investigators need to clarify other issues. The strain of the mouse was not disclosed. The experiment was described in groups of animals but the number of animals in each group was not given. Is the study statistically significant? With the immunocompromised mice, a slightly higher dose of the virus (1.5 x 10 particles) caused death to 3 of the 10 animals injected (over a period of 30 days). What is the explanation? This observation raises several questions: (1) Was the toxicity study (with immunocompetent mice) carried out for a sufficiently long period (7 days in the first group and 17 days for the second group)? (2) If mice can tolerate 10 particles, why do primates die at the same dose (as reported by Dr. Savio Woo)? Is the explanation that the insert gene and the route of administration were different? What is the right animal model for adenovirus? Should it depend on the viral genes that are deleted, on the foreign genes that are inserted, the route of administration of the virus, the disease in question, and perhaps other considerations? Are immunocompetent mice the right model for this trial?

Dr. Saha had an additional question regarding the appropriate animal model for the adenovirus vector. In this protocol the investigators used the C57/Black-6 mice similar to that used in Dr. Crystal's protocol. In Dr. James Wilson's Protocol #9212-035, C3H mice were used. There is an enormous difference between these strains of mice in terms of Class 2 histocompatibility antigens, and these differences are important

to the issue of cellular immune responses to adenovirus vectors. Dr. Erickson recalled that Dr. Harold Ginsberg (Columbia University) stated in his previous review that the C57/Black-6 mouse is a better alternative if the cotton rat is not available. Dr. Saha asked the investigators or FDA officials to comment on the appropriate animal model.

Dr. Saha concluded that this protocol proposes a novel treatment for ovarian cancer, and he would recommend approval subject to a satisfactory reply of the issues raised.

Review--Dr. Motulsky

Dr. Motulsky was concerned with some of the murine toxicity studies initially using immunodeficient mice; however, immunocompetent mice are now being used. The studies were performed with a series of vector doses but the number of animals in each group was not mentioned. No studies were conducted in cotton rats. Dr. Motulsky asked the investigators to comment on why the cotton rat experiment was not conducted.

The investigators plan to administer the viral construct (which is now fully defined) to 3 patients at each of the 3 dose levels (10, 10, and 10 particles) if no toxicity occurs. If toxicity occurs in 1 patient at a given dose level, additional patients will be treated at that level. If 0/3 or 1/6 patient at a dose level experiences toxicity, dose escalation proceeds to the next level. If 2 patients at a given level experience toxicity, the study will be closed. Dr. Motulsky asked the investigators to provide the statistical reasoning for selecting the number of patients that will be studied under the outlined scenario.

Dr. Motulsky stated that the study appears worthwhile. Preclinical safety studies in immunocompetent mice have been conducted as requested by the reviewers, and the sequence of the vector and the transgene is now available. The investigators have responded to the various questions under the *Points to Consider.* Dr. Motulsky would recommend approval if the investigators satisfactorily addressed his concerns.

Review--Dr. McGraw

Dr. McGraw stated that the Informed Consent document has undergone a fair amount of scrutiny and revision since its last review. Dr. McGraw found the current version to be acceptable; however, she still had a few outstanding questions. The *Points to Consider* require that subjects be given information about the need for contraception during the active phase of the study. The investigators stated: "This patient population has invariably been surgically castrated and/or received substantial chemotherapy resulting in ablation of ovarian function" and "given the nature of the disease, reproductive potential is generally not an issue." Dr. McGraw did not understand either of these statements to mean that all women involved in the study would be unable to conceive. If any of the patients have reproductive potential, how do the investigators plan on advising them as to the importance of birth control?

Dr. McGraw asked the investigators to clarify what was meant by alternative therapy. It is unclear from the research protocol or the Informed Consent document whether currently ongoing alternative therapies may be continued during participation in this study or whether they must be discontinued. Dr. McGraw would recommend approval if the investigators adequately addressed her concerns.

Review--Dr. Parkman

Dr. Parkman said the investigators have responded in writing to some of his questions. Anti-adenovirus type 5 antibody titers will be measured. There is no statement about whether both antibody positive and

antibody negative patients will be enrolled. Will patients be stratified for the presence of pre-existing antibody to adenovirus? The investigators responded in writing that patients will be stratified based on the presence of pre-existing systemic antibody status.

In addition to the toxicity data, it is desirable that a Phase I study will yield basic biologic information that will be usable to other investigators. Dr. Parkman inquired about the transduction rate and the systems to assay transduction. He asked about the 2 parameters that are shown on the fluorescence activated cell sorter (FACS) histogram presented in the protocol? Where are the controls? The staining along the diagonal axis is suggestive of non-specific staining. It is not possible to evaluate the histogram without further information and the appropriate control. The investigators have provided additional data in their response. The two parameters evaluated were cell surface *erb*B-2 and *Lac*Z expression. Dr. Parkman asked the investigators to explain their detection system for gene expression. In the peritoneal washings there are a mixture of cells containing tumor cells and inflammatory cells. In order to determine transduction frequency, one should be able to differentiate tumor cells from inflammatory cells. The investigators have provided some data; however, it does not answer his concerns satisfactorily.

The investigators have provided data that demonstrate the differential transduction by adenovirus vector of human ovarian carcinoma cells but not mononuclear cells derived from bone marrow. Dr. Parkman said it is known that adenovirus vector is capable of transducing bone marrow cells, and the data may demonstrate a lack of gene expression in those cells.

Dr. Parkman was not satisfied that an adequate assay system is in place to address how frequently the tumor cells and normal cells are transduced? Dr. Parkman stated that the investigators have presented significant preclinical data to show that the injection of an anti-*erb*B-2 immunoglobulin containing adenoviral vector can result in the inhibition and death of human ovarian cancer cells. No significant animal toxicity has been observed; therefore, the proposed Phase I study in human patients would appear to be appropriate. He would recommend approval if the investigators adequately address his concerns.

Other Comments

Dr. McIvor stated that he has examined the DNA sequence data, and found it acceptable.

Dr. Glorioso commented that the study cannot distinguish if the antitumor effect is due to the inhibitory effect of the anti-*erb*B antibody gene or due to the immune response to the adenovirus vector. Dr. Parkman agreed that it is difficult to differentiate the effect after the first week since there is a cytotoxic T lymphocyte (CTL) response to all transduced cells. It is useful to stratify the patients for anti-adenovirus antibody status in order to interpret the data properly. In this Phase I study, the primary objective is to determine if there is any response. If there is response, further study should be designed to determine if the response is the result of the specific transgene.

Dr. Lysaught raised 2 questions related to the Informed Consent document: (1) The necessity and the discomfort level of placing the Tenckhoff catheter should be further explained to the patients. (2) Regarding the cost statement, it explicitly states that the anti-*erb*B-2 antibody gene, antiviral antibody titers, and peritoneal aspiration tests will be provided free of charge. Blood tests (including blood counts, chemistry, and HIV titers), and others that are not normally associated with the treatment of ovarian cancer? Dr. Lysaught inquired if the HIV test and a number of base line tests are considered covered by the protocol or are they part of the standard treatment for ovarian cancer so that the patients are responsible for their cost. She asked if the surgical procedure of catheter placement is covered by the protocol.

Ms. Meyers raised a concern about the overly optimistic statement of adenovirus gene transfer in the Informed Consent document. The wording should be changed to inform the patients that there has been severe side effects from adenovirus vectors in other gene transfer experiments (lung toxicity in CF protocols), and it should be made clear that this treatment is not without risk. She raised a concern about the cost statement of the Informed Consent document. It states that the patients are responsible for the costs if their insurance does not pay. She asked if patients have no insurance, will they be admitted to the protocol?

Dr. Parkman asked if the toxicity study in theimmunocompetent mice have been performed with mice preimmunized with adenovirus vector since over 80% of patients are expected to have antibodies to the vector and the toxicity to such people would be a concern.

Investigator Response--Drs. Curiel and Alvarez

Dr. Curiel responded to the scientific questions, and Dr. Alvarez, an gynecologic oncologist, would answer clinical questions.

In response to the question of an animal model for the adenovirus toxicity study, Dr.Curiel noted that the FDA official's presentation at the March 6-7, 1995, RAC meeting suggested that the utility of cotton rats is exclusively in the context of lung models. As a result of discussion with FDA officials, the investigators decided that the specific safety study would include testings in both *naive* and preimmunized C57/Bl-6 mice since this strain of mice has the largest database of toxicity studies. In terms of DrSavio Woo's toxicity study in primates of adenovirus with a *Herpes simplex* thymidine kinase gene, Dr. Curiel said that the study is not relevant to his protocol since it involves a different gene, different adenovirus vector, different animal, and will be administered to a different compartment (brain versus peritoneum).

Responding to Dr. Parkman's question of transduction and biological endpoints, Dr. Curiel said he has recognized that some of the assays initially planned may not be adequate. The investigators have undertaken studies with University of Alabama Gene Expression Core Laboratory to develop an *in situ* reverse transcriptase-polymerase chain reaction (RT-PCR) assay to address the concern of what percentage of cells is transduced. Dr. Parkman asked if the assay would differentiate gene expression in tumor cells versus other nonmalignant cells. Dr. Curiel responded that the *in situ* assay would differentiate those cells.

Responding to Dr. Glorioso's concern regarding gene specific antitumor, Dr. Curiel said a control vector with a *Lac*Z gene insert will be employed in the future study. In addition, CTL response in peripheral blood lymphocytes will be studied.

In response to Dr. Motulsky's question on the Phase I methodology, Dr. Alvarez stated that after consulting with a biostatistics consultant at his institution; a standard Phase I methodology of 3 patients in each dose group will be used. If 1 of the 3 patients develops toxicity, an additional 3 patients will be studied at that dose level. If 2 of those 6 patients experience Grade 3 or greater toxicity, that dose level will be defined as the MTD.

With regard to the questions raised with the Informed Consent document, Dr. Alvarez stated that alternative therapies cannot be used during the 8 week follow-up period; and if the disease progresses afterward, alternative treatments will be allowed. The Informed Consent document will be revised to explain to the patients that the placement of theTenckhoff catheter is a standard procedure for peritoneal dialysis or for administration of intraperitoneal therapies. Dr. Alvarez recognized that it is difficult to distinguish the experimental therapy from the standard therapy in terms of cost responsibility. The costs

that are normally associated with clinical monitoring of patients such as standard blood cell counts, chemistry tests for clinical toxicity, or efficacy will be the responsibility of patients. Ms. Meyers asked if the protocol will admit patients who do not have health insurance. Dr. Alvarez stated that he will not discriminate against any patients based on their inability to pay for their treatment. The investigators will follow the standard university guidelines in terms of taking care of indigent patients. Dr. Ross remarked that the RAC cannot stipulate the policy for the university, but all the items have to be defined carefully in the Informed Consent document so that the patients know in advance what is and what is not being paid for by the protocol.

Dr. Ginsburg stated that these cost issues should be dealt with by the localIRB, and these issues have nothing to do with recombinant DNA. Ms. Meyers said if gene transfer protocols are only accessible to those who have best health insurance policy, the study will not get the correct results. Dr. Ginsburg said that this issue concerns medical research in general and is not unique to gene therapy. Dr.Zallen said that the RAC should strive to establish the standards that are appropriate for all medical research. She asked if the Tenckhoff catheter is to be placed in a person who did not already have one, would the cost of such procedure be covered by the protocol? Dr. Alvarez explained that often patients on the protocol require a second laparotomy. If the patients, with persistent or recurrent disease, who are eligible for intraperitoneal therapies, a Tenckhoff catheter will be placed in that patient during laparotomy. The cost for this procedure in both occasions would be the patient's responsibility. If the patients with persistent or recurrent disease are not undergoing second laparotomy but a catheter is to be placed for any potential intraperitoneal therapy including gene therapy, such cost is expected to be the patient's responsibility.

Dr. Chase commented on a question raised by Dr. Motulsky regarding statistical considerations of the number of patients in the study design. Statistical inference refers to a formal argument with error rate and sample size. The whole area of science can fail to make progress if one ignores the fact that one is going to make a certain inference from an experiment that has only 3 patients in each cohort, because the number is not large enough to have a statistically significant P value. The accepted practice of choosing 3 patients for each cohort in the toxicity study is not a rule that is well justified for applying to all Phase I studies. Dr. Chase said this practice is common for most medical research; however, this concern should not be the basis for approval or disapproval of the present protocol. Dr. Alvarez said this Phase I study is not designed to make such a statistical inference, such a design will be the subject of the subsequent Phase II or III studies.

Dr. Parkman said local IRBs have the right to set rules about the issue of compensation as long as it is clearly stated in the Informed Consent document. However, he noted that his localIRB would not consider it acceptable to have patients pay for the cost of placing a catheter for an experimental procedure that may be used for some undefined future standard therapy. If the primary reason for placing the catheter is for the research purpose, it should be included in the research related cost. Dr. Alvarez stated that he would clarify this specific issue with his IRB.

Dr. Lysaught said the cost issue is an ethical problem. She asked if a specific dollar amount can be stated in the Informed Consent document so that patients would be able to make a decision based on the anticipated liability. Dr. Alvarez responded that he will inform the patients of the costs, but he has never seen such dollar figures in any Informed Consent document because sometimes these costs change. A list of tests and expected costs is routinely prepared for the patients, and he will assist the patients with their insurance carrier to determine what costs will be covered.

Dr. Alvarez agreed to revise the Informed Consent document to omit any reference to the fact that tumor cells can be easily transduced with adenoviruses and to delete the statement concerning the use of adenoviral vectors without any untoward effects.

Dr. Walters said that based on the previously approved protocols, it is a general rule that if the patient was not in a research protocol does not exist and the procedure would still be performed, then the patients are expected to pay for it. If the procedure is being performed for the purpose of the research protocol, then th institution will take care of the related costs.

Ms. Meyers noted that the RAC previously wrote a letter to Dr. Bernadine Healy, the former NIH Director, expressing concern about compensation of research related injuries. Dr. Walters said it is a different issue, and a staffperson in the NIH Director's office is planning on writing an essay for publication to raise this issue to the consciousness of the research community. Ms. Meyers asked the NIH to consider this a serious problem.

Ms. Rothenberg stated that it is unethical to require the patients to pay for a particular procedure that is being conducted for the experiment. She would disagree with Dr. Ginsburg and stated that the cost issue should be a RAC concern. Ms. Rothenberg said that it would be useful to all parties concerned if the RAC can formulate a standard statement for the Informed Consent document. If a procedure is performed for th purpose of the experiment and is a necessary requirement for the experiment that would not otherwise be conducted with conventional therapies, it is not the responsibility of the patients to pay for it. Clearly in a Phase I study in which there is no expected benefit to the patients, it is not ethical to ask patients to pay fo the cost of the procedure.

Dr. Zallen agreed with Ms. Rothenberg's position on the cost issue related to the present protocol. She recalled that in all RAC approved protocols, the research related costs have been assumed by the investigators or their sponsors and not of the subjects themselves.

Dr. Saha asked the investigators to respond to Dr. Glorioso's question about including a control group with vector alone or with a *Lac*Z gene. Dr. Curiel stated that such a control group will not be part of the Phase I study. Dr. Glorioso's suggestion of a construct with a reporter gene would have utility if the *in situ* RT-PCR assay is not adequate for detecting transduced cells. Dr. Curiel would accept a stipulation to submit data to demonstrate quantitation of gene expression in human ovarian tumor cells. Dr. Parkman said he would like to review the documentation that the biological effects could be quantified.

Committee Motion

A motion was made by Dr. Saha and seconded by Dr. Erickson to approve the protocol with a contingency to submit data to demonstrate quantitation of transduced tumor cells by the *in situ* RT-PCR assay.

Dr. Zallen made a friendly amendment to the motion to revise the Informed Consent document regarding the cost issue. The Informed Consent document should outline what costs would be to the patients, what would be covered by the investigators or sponsors, and what would be the standard therapy to be covered by the insurance. Dr. Alvarez said it is not appropriate to have such a list with a specific dollar amounts in the Informed Consent document. Dr. Ginsburg suggested a simple statement that states any costs directly related to the research protocol should not be the responsibility of the patients. Dr. Lysaught preferred to have an explicit list of costs. Dr. Alvarez said he would provide such a list to the patients in a separate document. Ms. Rothenberg agreed with Dr. Ginsburg's suggestion to include a statement in the Informed Consent document that all costs directly related to the research will be assumed by the institution. Dr. Parkman said such a revision will be subject to the approval of the IRB. Dr. Glorioso asked for a clarification about a peritoneal infection resulting from placing a catheter. Will the cost of treatment be related to experimental study? Dr. Parkman said if the catheter is placed solely for the purpose of

administering the vector, then the cost is clearly related to the experimental study. But if an infection occurs several years later, it will be a more difficult problem to determine who is responsible for costs.

A motion was made by Dr. Saha and seconded by Dr. Erickson to accept the protocol submitted by Drs. Curiel and Alvarez contingent on review and approval of the following by the primary RAC reviewers: (1) the data derived from *in situ* RT-PCR assays demonstrating quantitative gene expression in human ovarian tumor cells, and (2) a revised IRB-approved Informed Consent document which includes a statement that costs associated with the research aspects of this clinical trial will not be the responsibility of the patient; all such costs will be the responsibility of the institution. The motion was approved by a vote of 14 in favor, 1 opposed, and no abstentions.

Protocol Summary: Drs. David T. Curiel and Ronald D. Alvarez, University of Alabama at Birmingham, Birmingham, Alabama, may conduct gene transfer experiments on 18 subjects (³18 years of age) with persistent or recurrent ovarian or extraovarian cancer whose tumors express the *erb*B-2 gene. In this Phase I dose-escalation study, subjects will receive a single intraperitoneal bolus injection of the adenovirus vector, AdCMV-sFv. This recombinant adenovirus vector encodes the single-chain antibody, sFv, directed against the extracellular domain of the human *erb*B-2 oncoprotein. The objectives of this study are to: (1) determine the maximum tolerated dose of AdCMV-sFv, and (2) quantitatively assess the tumor-specific activity at the molecular level.

VIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I STUDY OF DIRECT ADMINISTRATION OF A REPLICATION-DEFICIENT ADENOVIRUS VECTOR CONTAINING THE E. COLI CYTOSINE DEAMINASE GENE TO METASTATIC COLON CARCINOMA OF THE LIVER IN ASSOCIATION WITH THE ORAL ADMINISTRATION OF THE PRO-DRUG 5-FLUOROCYTOSINE/DRS. CRYSTAL, HERSHOWITZ, AND LIEBERMAN*

Review--Dr. Ginsburg

Dr. Walters called on Dr. Ginsburg to present his primary review of the protocol submitted by Drs. Ronald G. Crystal, Edward Hershowitz, and Michael Lieberman, New York Hospital-Cornell Medical Center, New York, New York. Dr. Ginsburg stated that this protocol is aimed at the treatment of colon cancer liver metastasis by the direct injection of an adenovirus encoding the *E. coli* enzyme, cytosine deaminase (CD), followed by treatment with systemic 5-fluorocytosine (5FC). 5FC is converted by the CD enzyme to 5FU, a mainstay chemotherapy drug for colon cancer. Thus, tumor cells infected by the injected adenovirus should express CD, resulting in high local concentrations of 5FU converted from systemically administered 5FC. Preliminary studies demonstrate the safety of significantly higher doses of the recombinant adenovirus and 5FC in mice and tumor cell killing both *in vitro* and in a nude murine model.

The protocol is straightforward and clearly written. The rationale is well described and is similar to the *Herpes simplex* thymidine kinase gene strategy used in previously approved RAC protocols to confer susceptibility to ganciclovir (GCV) in injected tumors. A similar adenovirus vector was used by these same investigators in a previous RAC approved protocol for the treatment of CF, differing only in the substitution of the CD coding sequence for cystic fibrosis transmembrane conductance regulator (CFTR) gene. Thus, no significant new questions of public safety are raised. In fact, the risk of transmission to health care workers would appear to be less than the CF protocol with this injected virus than from virus administered to the airway.

Dr. Ginsburg raised several specific questions: Is there any risk of unexpected large-scale conversion of 5FC to 5FU resulting in toxicity? What is the maximal potential level of 5FU that might be achieved and

what toxicity would be expected at this level? Should blood levels of 5FU be monitored in the patients to document any systemically significant conversion of 5FC? If unexpected high 5FU levels are detected, how rapid will the turnaround time be, and how quickly can the therapy be halted? The investigators responded in writing that they will be monitoring the plasma levels of 5FU. Dr. Ginsburg asked the investigators to elaborate on the timing of the serum level changes. Will they be able to get 5FU levels back to normal fast enough if the levels are too high and the treatment with 5FC has to be stopped? Dr. Ginsburg said it is extremely unlikely that there will be systemically toxic levels of 5FU. A similar point has been raised in Dr. Samulski's review that in the context of virus dissemination beyond the injected local tumor nodules.

The adenoviral vector to be used in the human studies (AdGVCD.10) differs from the vector used in the animal studies, AdCMV.CD, in the orientation of the CD expression cassette. Though this simple reversal of orientation would not be expected to result in major differences in the level of gene expression, do the investigators have any data to support this assumption? In the unlikely event that the AdGVCD.10 vector produces a significantly higher level of CD expression, could this conceivably result in considerably greater toxicity in the human studies? The investigators responded that in their experience there have not been any significant differences in gene expression among adenovirus vectors between left to right and right to left orientation of the expression cassette. The investigators provided no data on direct comparison of these two viruses.

All of the investigators on this study appear to be either surgeons orpulmonologists. Should the team include an experienced oncologist who is familiar with the standard administration of 5FU chemotherapy for liver metastasis of colon cancer and the associated toxicities? Do any of these investigators have clinical experience in administering standard 5FU or other chemotherapy to cancer patients? The investigators have agreed to invite an experienced oncologist as co-investigator of this study. In this regard, Roy L. Silverstein, M.D., Professor of Medicine, Chief, Hematology and Medical Oncology at the New York Hospital-Cornell Medical Center, will be the co-investigator.

There is nothing tumor specific in the current experimental design, i.e., all cells in the area of the injection should be susceptible to killing. In this way, this approach does not differ significantly from any other local ablative therapy such as radiation, intrahepatic artery infusion of chemotherapy, or even surgery. Do the investigators intend to develop this method solely for the local individual treatment of a single metastatic lesion or will this eventually lead to a systemic therapy? If the goal is the latter, would a potentially more tumor specific promoter, rather than the broadCMV promoter, achieve a greater degree of tumor selectivity? The investigators responded that tumor specific promoter is a logical next step of the research if they observe any relevant biologic changes in the present study.

Dr. Ginsburg stated that in summary, this protocol is straightforward, does not appear to pose any new risks from the recombinant DNA point of view, and may provide useful information. Assuming the investigators can address the above points, Dr. Ginsburg would recommend that this protocol be approved.

Review--Dr. Samulski (presented by Dr. Ginsburg)

Dr. Ginsburg stated that Dr.Samulski had a number of points that should be addressed by the investigators before approval can be recommended. In these experiments, the viral vector utilizes aCMV promoter which will hopefully express the enzyme at high levels. It is the use of this promoter that raises concern. Dissemination of the vector to any other tissue will result in local expression of CD and conversion of 5FC to the toxic 5FU product. This conversion would in turn generate unwanted localized damages in that tissue. The toxicology data provided by the investigators do not address this toxicity.

Where else does the vector go? Does it express CD activity in any other tissues? Is CD expression toxic to the organ in question? One way to eliminate this risk factor is to use a tissue specific promoter in this study. It is highly recommended that the investigators consider using a tissue specific promoter for their study.

For evaluation of a lesion that is surgically removed, there are no data supplied as to how valid the assays are. It should be noted that regarding the lesions to be removed, the only meaningful data will be PCR or enzymatic assays for CD since there will be such localized trauma of 8 separate injections of 800 ml into the localized tumor sites.

A major concern with the clinical protocol is 200 mg/kg body weight per day of systemic 5FC administration for 10 consecutive days. In the best case scenario, if gene transfer and expression is very efficient, then lethal amounts of 5FU will be produced during this period. Because of this potential, it is recommended that after 1 day of administration of theprodrug, 5FU levels should be measured in the serum.

While this is a Phase I study, the patients will not have their tumors removed but will undergo further treatment. It appears that it will be difficult to separate effects from gene transfer and other treatment effects, such as the standard 5FU therapy. Can the investigators comment on this issue and how they might separate efficacy if possible.

Could the investigators comment on potential immunological responses to CD enzyme present in the gut flora?

The investigators have responded to Dr. Samulski's questions in writing, and he stated that his overall concerns have been addressed. The protocol should be approved unless his clinical colleagues have specific objections.
Review--Ms. Rothenberg

Ms. Rothenberg has raised several questions regarding the Informed Consent document, and most of the questions have answered by the investigators: (1) The actual consent form is only a few pages and basically serves as a summary with rather extensive attachments. Thus, to read the form, the prospective participant has to go back and forth in order to put the summary into context. Furthermore, the participant is instructed that the details related to the study will follow, but they are not listed in page order. Ms. Rothenberg suggested that if there is going to be a summary consent form with attachments, it would be less confusing if the form tracks carefully with subheadings the order of the topics and attachment that will follow. The investigators responded that the study is to be conducted at 2 clinical sites, thus requiring two separate summary forms. (2) The Informed Consent document states that all costs associated with the surgery will be "the responsibility of your usual health care coverage." Does this mean that the uninsured are not eligible? The investigators responded that patients will not be denied eligibility due to their insurance coverage. (3) The protocol requires an HIV test, but does not explain why. It states that "if the test proves positive, you will receive the appropriate counseling from the investigators as detailed in these forms." It needs to state, however, that the participant should receive pre-test counseling prior to consenting to HIV testing, and that a positive test result will mean that he/she will not be eligible for participation in the study. The investigators have responded by adding a statement regarding the exclusion of HIV positive patients. (4) The Informed Consent document should state how long the patient will have to decide whether to participate. The timing issue, in fact, raises a basic problem to the consent procedure. It appears that the patient conditionally accepts to participate in the study, subject to a confirming needle biopsy, and then the patient immediately has the first administration of the virus. Is this a good ethical process? Patients need sufficient time to consider their options. If the risks and discomfort

of the needle biopsy justify this process, then the patient needs to be instructed of the requirement of needle biopsy explicitly prior to enrolling in the protocol. Ms. Rothenberg asked the investigators to address the rationale for the informed consent process. The investigators responded that all possible measures will be taken to ensure that the patients understand their options.

Ms. Rothenberg asked a couple of additional questions. She asked the investigators to clarify the difference between conventional and experimental treatments. In the *Overview of Protocol* section of the Informed Consent document, there is a confusing statement that reads: "the present study adds to experimental therapy to be carried out in these 2 weeks, and then to be followed after the surgery." This statement is unclear. The Informed Consent document needs to clarify whether the individual obtains other therapy besides the scheduled abdominal surgery and participation in this protocol. It should be clarified in terms of benefits of the study. The protocol states that the patient: "may create some benefit in the slowing of tumor growth, it is unlikely that you will receive significant benefit..." Does this statement mean that slowing tumor growth will have little positive benefit on survival or that the slowing of tumor growth is just a possibility? Ms. Rothenberg asked the investigators to clarify the needle biopsy procedure. The Informed Consent document states that "it is possible that an individual will not be able to continue with the protocol because of adverse effects, although all possibilities will be explored to prevent removal from the study, as long as the welfare of the study participants is ensured." Ms. Rothenberg stated that withdrawal from the study should be a decision of the patients, not the investigators. She said that the statement regarding permission for autopsy is excellent.
Other Comments

Dr. McIvor stated that the submitted DNA sequence is satisfactory. He raised a concern of possible diffusion out of the cell of the prodrug metabolites. The present prodrug activation scheme is inherently different from the HSV-TK activation of thymidine; the phosphorylated thymidine product is confined within the cell while the 5FU converted from 5FC is freely diffusible to outside of the cell. This phenomenon can be utilized for a potent bystander effect, but Dr. McIvor was concerned with systemic accumulation of 5FU and its toxic effects. What will be the limiting toxicity level of the plasma 5FU? Dr. Ginsburg agreed that the investigators should address this important problem.

Dr. Zallen asked for a clarification of the informed consent process. How and who is going to obtain informed consent from the patients? Is there any conflict of interest?

Dr. Lysaught stated it is legitimate for individuals who are scheduled for Part A of the protocol to pay the cost of their surgery as a part of their normal treatment. She asked if the gene transfer procedure will increase the cost of such a surgery. Dr.Lysaught asked the investigators to clarify if the second round of injections will be on Day 6 or Day 7 since it is stated differently in the protocol and the Informed Consent document. The usage of the terms "I" or "you" should be consistent in all statements of the Informed Consent document. A request for autopsy should be asked not only for patients who died during the course of the study but should include those who died during long-term follow-up.

Dr. Parkman asked why cell killing is not complete if tumor cells are 100% transduced? Dr. Motulsky remarked that the general autopsy will yield little information; the important point is to determine if there is any vector transfer to germ cells. Such instruction should be included in the autopsy section of the Points to
Consider.

Investigator Response--Dr. Crystal

Responding to the autopsy question, Dr. Crystal agreed that it is important to determine the adventitious

transduction of germ cells. The technical problems of timing and recovering useful specimens during the autopsy will be considered. Autopsy will be requested from patients who died during the lifelong follow-up.

Regarding Dr. Parkman's question about the *in vitro* experiment, Dr. Crystal said the observation has not been extended beyond 48 hours; eventually all transduced cells should be killed.

Responding to the question of monitoring the serum levels of 5FU, Dr. Crystal agreed to perform such assays within 24 hour of prodrug administration. In most clinical practice, such assays have not been conducted because the serum level has not been found to correlate with the adverse effects. The half life of plasma 5FU is 11 to 16 minutes. If 0.1mM concentration of 5FU is detected in the blood, the 5FC administration will be stopped immediately. There is another study with fungal cytosine deaminase suggesting that conversion of the prodrug by the fungal enzyme does not produce 5FU systemic toxicity. The study involves normal people who have a large burden of the fungus with cytosine deaminase in their bodies. Dr. Crystal said 5FC is a fairly safe drug; it can be taken in large doses.

With regard to the question of reversing the orientation of gene insert in present vector construct, Dr. Crystal said it increases gene expression by about 20% over the original orientation from left to right; the enhanced gene expression is the reason to choose this construct.

Accepting a recommendation by Dr. Ginsburg, Dr. Crystal said an oncologist will be invited as a co-investigator of the protocol.

Regarding the suggestion by Dr. Samulski about using a vector with tissue specific promoter, Dr. Crystal explained that within a population of neoplastic cells, there is lack of uniform expression with such specific promoters. It is the reason for not using the specific promoter in this initial study; it will be considered in future studies.

Responding to Ms. Rothenberg's question of conventional versus experimental treatments, Dr. Crystal explained that the present gene transfer treatment will superimpose the patients conventional therapy a 2 week period. In Part A of the protocol, the conventional treatment is surgery; and in Part B, it is chemotherapy. Dr. Crystal stressed that the protocol will not delay or interfere with any conventional treatment. In terms of the informed consent process, Dr. Crystal said the patients will initially be referred to the surgeons; and if they are not candidates for surgery, the patients will be treated with chemotherapy. These decisions will be made independent of the gene transfer treatment. The Informed Consent document will be revised to use the term "experimental study" in referring to the gene transfer procedure superimposed on other treatments. Dr. Crystal said there is not an exclusion criterion for anyone who is getting 5FU for a period of time; however, during the study period, the patients will not be on 5FU. Patients who are taking 5FC for fungal infections will not be included in the study. In terms of the liability issue, Dr. Crystal would accept the standard practice of RAC approved protocols. The needle biopsy procedure will take 45 to 60 minutes. With regard to management of potential side effects, Dr. Crystal said the study will be limited to a 2 week period, and it will not affect the surgery or other treatments. Dr. Crystal will clarify the Informed Consent document so that the patients can make their own decision about whether to continue participating in the study.

Responding to Dr. McIvor's question of diffusion of 5FU out of the cell, Dr. Crystal said that it is part of the strategy to have a bystander effect to kill those untransduced tumor cells.

Dr. Crystal agreed to revise the Informed Consent document according to Appendix M-III of the *NIH Guidelines* as suggested by Dr. Zallen. The staff members, nurses, and investigators will discuss the

protocol with the patients. It requires usually a period of several days to allow the patients to make a bette informed consent.

Responding to Dr. Lysaught's questions, Dr. Crystal said the protocol will not incur additional cost to the patients. The Informed Consent document will be revised in terms of the points raised by Dr.Lysaught. Dr. Crystal explained part of the reason for the inconsistent use of the terms "I" and "you;" different forms are used for the 2 study sites.

Committee Motion

A motion was made by Dr. Ginsburg and seconded by Dr.Lysaught to accept the protocol submitted by Drs. Crystal, Hershowitz, and Lieberman. The motion passed by a vote of 14 in favor, 0 opposed, and 2 abstentions.

Protocol Summary: Drs. Ronald G. Crystal, Edward Hershowitz, and Michael Lieberman, New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 18 subjects (18 to 70 years of age) with metastatic colon carcinoma with liver metastases. In this Phase I dose-escalation study, subjects will receive computed tomography-guided intratumoral injections of the adenovirus vector, AdGVCD.10, into the same hepatic metastasis in 4 equal volumes (100mliters), each with a separate entry into the liver. This dosage schedule will be performed on Days 1 and 7. 5-Fluorocytosine (200 milligrams/kilogram/24 hours) will be administered orally in 4 equal doses starting on day 2 and continuing through the time of laparotomy. The objectives of this study are to: (1) determine the dose-dependent toxicity of direct administration of AdGVCD.10 to hepatic metastases combined with oral administration of 5-fluorocytosine, (2) quantitatively assess transfer and expression of the cytosine deaminase gene in target cells, and (3) determine the biologic effects of direct ADGVCD.10 administration to hepatic metastases.

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I STUDY OF E1A GENE THERAPY FOR PATIENTS WITH METASTATIC BREAST OR OVARIAN CANCER THAT OVEREXPRESS HER-2/NEU/DRS. HORTOBAGYI, LOPEZ-BERESTEIN, AND HUNG*

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

Dr. Walters called on Dr. Zallen to present the primary review by Dr. Straus of the protocol submitted by Dr. Gabriel Hortobagyi, MD Anderson Cancer Center, Houston, Texas. Dr.Zallen stated that this protocol is a Phase I study to be conducted in patients with metastatic breast or ovarian cancer. There are novel aspects of the protocol which warrant consideration by the entire RAC.

The proposal is to administer liposome containing adenovirus E1A sequences into the peritoneal or pleural spaces of 15 to 25 patients with ovarian or breast cancer metastatic to those spaces. Only those individuals whose tumors overexpress the HER-2/neu oncogene will be eligible for the treatment. Administration will be repeated in 3 weekly cycles followed by a week without treatment. Treatments will be repeated until disease progression or excessive toxicity is detected.

There is no "live" vector involved in this protocol. The plasmid vector has been approved and used previously. The adenovirus E1A gene is novel to RAC protocols. This E1A gene has traditionally been considered a critical adenovirus transactivating element and a component of the virus transformation mechanism. Recent data summarized by the investigators suggest that E1A functions as a tumor suppressor and may down-regulate expression of the HER-2/neu oncogene. *In vitro* studies described by

the investigators showed that infection of human ovarian cancer cells in culture with an adenovirus expressing E1A diminishes the ability of the cells to grow in soft agar. The authors describe and provide a reprint of studies in which nude mice were seeded intraperitoneally with human ovarian cancer cells overexpressing HER-2/neu. Five days later, at a time when the tumor could be directly detected throughout the mesentery, the mice were injected with liposomes expressing E1A. E1A gene transfer led to a significantly prolonged survival of the mice.

On the basis of the above findings, the authors propose delivering E1A expressing plasmid DNA via liposomes to patients in whom metastatic ovarian or breast cancer has seeded their pleural or peritoneal spaces. The protocol has been approved by both the IRB and IBC of the MD Anderson Cancer Center. Dr. Straus wrote that the proposal is interesting and novel; however, a number of concerns need to be addressed prior to voting.

The first concern was the selection of subjects for the study. The protocol requires individuals to have metastatic tumors that are overexpressing HER-2/neu. There is no definition in the protocol of what constitutes the expected level of expression of HER-2/neu and what the strict criteria are for overexpression. Is overexpression a uniform feature of a patient's tumor or can there be various levels of expression at different sites of tumor metastasis?

The investigators described a series of experiments by which they optimized liposome-mediated transfection. What efforts will the investigators undertake to verify the extent of transfection in patients so that optimal liposome/DNA ratios are being used? The investigators conducted studies with 1 ovarian cell line *in vitro*. Is it expected that similar optima will be achieved with other cell lines or patient cancers?

The documents describe hemorrhage and congestion of the lungs in mice and that "at the highest dose (120 mg) these changes were more pronounced in the lungs and also found in the liver." What is the presumed mechanism of these toxicities? How will they be specifically sought in study subjects?

At various points the protocol indicates that liposome treatments will be repeated for 1 year; at other points it indicated that it will be repeated in cycles until the patient shows progressive disease. Which of these is correct?

The protocol requests permission for studying 15 to 25 subjects. Assuming 3 patients at each dose level are studied and if no serious toxicity is detected, the protocol would require 12 subjects. With toxicity observed at repeated dose levels, it is still difficult to understand from the protocol why 25 patients will be needed for this study. Dr. Straus asked the investigators to clarify the patient number.

Under Section 10.1 of the protocol, it states that patients will be removed from the study for progressive disease after 2 courses of therapy at doses "sufficient to produce some evidence of toxicity or other biologic effect." In the absence of any toxicity or of measurable biological effect, patients with progressive disease would remain on the study. What is the rationale for continuing treatment when it has already failed?

Finally, the reprint of the paper submitted by the investigators includes no toxicity data (*Liposome-mediated in vivo E1A gene transfer suppressed dissemination of ovarian cancer cells that overexpress HER-2/neu*, *Oncogene,* Vol. 11, 1995, in press). Where is the primary safety data for this work? In this paper, the single study in which the E1A-expressing liposome is shown to prolong mouse survival involves a very small number of animals. The smaller tumor sizes were observed in the animals treated with liposomes only. There are data indicating that animals treated with liposomes only showed invasion of tumor into blood vessels, but not into more vital tissues. This leads to speculation about the

specificity of the therapeutic effect assumed to be due to the liposome expressing E1A. Given the small number of animals, the fact that the study was performed only one time with the variability of the results, Dr. Straus would prefer to review additional data confirming these observations.

Review--Dr. Hearing (presented by Dr. Zallen)

Dr. Zallen said that Dr. Hearing raised several concerns in his written review. He stated that it is well established that the adenovirus E1A can cooperate with oncogenes such as activated *ras* to augment the production of tumors in animals. The applicants noted these results, but discussed other circumstances where E1A expression reduced the oncogenicity of transformed cells. An important concern is that E1A expression may augment, rather than reduce, tumor progression in humans. While there appears to be no oncogenic capacity of adenoviruses in humans, this observation is in the context of intact adenovirus where multiple viral gene products are expressed and where some in fact may counteract certain effects of E1A expression. A specific example is the E1B encoded 19K protein which inhibits apoptosis induced by E1A expression. In the proposed studies, there is a concern that E1A expression in cancer cells that already are expressing an activated *ras* oncogene in addition to increased HER-2/neu may increase rather than decrease the oncogenic potential of cancer cells. It is unclear from the data if the state of many other protooncogenes has been evaluated in human breast and ovarian tumor samples from multiple sources. The model under discussion in the proposal appears valid for transformed NIH3T3 cells and SKOV-3 cells, two clonal transformed lines, but what of the general population of non-clonal human tumors?

It is well established that E1A expression alone leads to apoptosis in cells expressing this product. Do the results in these assays in the nu/nu mice reflect the introduction of E1A into newly introduced SKOV-3 cells and the subsequent induction of apoptosis to reduce tumor progression? Is this approach justified for use in humans in comparison to more traditional approaches of antiproliferative chemotherapy taking into consideration the aforementioned risks of possible E1A augmentation of tumor development?

Would the inhibition of intraperitoneal progression of tumor development in humans be expected to provide benefit to a patient with disseminated tumors in comparison to the mouse nu/nu model where the only tumor load is the intraperitoneal injection of cells?

Review--Dr. Zallen

Dr. Zallen commented on many aspects of the study in her written review and some of which have been satisfactorily responded to by the investigators.

All *in vivo* data presented relate to ovarian cancer. Have the *in vivo* studies, analogous to the SKOV-3/nude mouse assays, been conducted using breast cancer cells? On what basis do the investigators justify their intent to include breast cancer patients in the study? The investigators provided additional data in their written response, and Dr. Zallen asked the investigators to explain the data.

Dr. Zallen asked if it would be more fruitful to concentrate on either the ovarian or breast cancer rather than both since this is a Phase I study looking primarily at toxicity and utilizing a small number of patients.

Dr. Zallen inquired if there are any data pertaining to the question of E1A sequences in the gonadal tissues. With regard to the question of oncogenicity of E1A gene, are there data to demonstrate that E1A does not transform normal human cells other than the provided data demonstrating that E1A-transduced NIH3T3 cells do not differ from the untransduced NIH3T3 cells? Regarding experimental procedures, are there data demonstrating transduction efficiency on the freshly isolated tumor cells? Is there any plan for

post mortem studies?

Dr. Zallen had serious concerns regarding the Informed Consent document, most of which have not been addressed by the investigators. Many items of specific requirements of gene transfer studies described in Appendix M-III of the *Points to Consider* are omitted including long term follow-up, media interest, patient confidentiality, and request for autopsy.

Dr. Zallen stated that in Item 11 of the Informed Consent document, it has an inappropriate institutional "boilerplate" statement that reads: "I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost. Should the investigationa agent become commercially available during the course of the study, I understand that I may be required to cover the cost of the subsequent doses. Costs related to my medical care, including expensive drugs, tests or procedures that may be specifically required by this clinical research study, shall be my responsibility unless the sponsor or other agencies contribute toward said costs." The main purpose of the consent process and the Informed Consent document is to offer every possible protection to the subject, not to protect the institution. Subjects are entitled to have the conditions of their participation in the experiment explicitly stated in the Informed Consent document. They should not have to investigate what the risks are, including any financial risks. This statement is especially onerous if subjects are being asked to pay for the initial testing to determine their HER-2/neu status, only to find out they do not meet the eligibility criteria. The investigators have indicated elsewhere that the subjects will not have to pay for participation. Dr. Zallen asked why there cannot be a statement in the Informed Consent document indicating clearly to the subjects that they will not incur costs for experimental procedures of the protocol.

Dr. Zallen found that Item 12 of the Informed Consent document stated that the subjects cannot expect to receive any compensation or benefit from the subsequent use of information developed through their participation in the project is unreasonable for subjects. Any subject who does not pay for participation in an experiment has, *de facto*, become a sponsor of this research. If other sponsors are able to reap the financial benefits of such research, why is it that the "subject-sponsors" are expressly excluded according to the terms of the Informed Consent document?

Other Comments

Dr. Parkman noted that the animal model ofintraperitoneal tumors for ovarian cancer has a reasonable parallelism with the clinical disease; such an appropriate model is not available for breast cancer. The preclinical data are not sufficient to justify inclusion of breast cancer patients in this study. Dr. Parkman noted that there is no data demonstrating theimmunohistochemistry assay of transduced tumor cells. The question of the transduction of tumor cells and its differentiation from inflammatory cells is very important for this Phase I study.

Dr. McIvor stated he has checked the DNA sequence, and it is consistent with the proposal. He raised a concern of using the E1A oncogene in the human gene transfer trial; the tumor causing possibility of transducing normal cells should be addressed. Dr. Ginsburg emphasized his agreement with Dr. McIvor regarding the question of E1A. There is no safety issue in using the liposome complex with a plasmid DNA; what is really novel is the use of E1A, which has not been previously approved as part of a RAC protocol other than in an adenovirus. This issue deserves special attention.

Dr. Chase agreed with Dr. Zallen that many members of the RAC and the public would not consider it reasonable for individuals to take the risks with unknown treatments and to pay for the cost of participatior to the trial. The subjects should be indemnified. Dr. Ginsburg said it is reasonable to have subjects paying

for routine costs for treating their diseases; it is unreasonable to ask the patients to pay for costs of the experiment and the drugs when they become available. Ms. Rothenberg stated that Item 11 of the Informed Consent document regarding the cost issue is not acceptable. The presumption has to be reversed, i.e., instead of asking the patient to pay for the cost, the sponsors should bear that responsibility Dr. Parkman remarked that such a blatant statement of patient's cost was not in any previously approved protocols from the MD Anderson Cancer Center.

Dr. Lysaught noted that the *Points to Consider* only requires the investigators to declare the costs to the participants. Dr. Parkman said the RAC can only recommend acceptable cost statements to the investigators; the local IRB has the final authority in approving the Informed Consent document. Dr.Zallen said the investigators agreed that there are no cost related to the research and it should be stated clearly in the Informed Consent document. Dr. Chase said the RAC does not have the regulatory authority to enforce the Informed Consent document; however, it can use moral persuasion to sensitize people to these issues. Dr. Lysaught said the RAC can provide researchers with a tool to take back to persuade their institutions.

Ms. Meyers said she would vote against the protocol because the Informed Consent document is objectionable. Ms. Rothenberg stated that it is not that the Informed Consent document is wrong; it only spells out the policy of the institution. The problem is that the institutional policy is wrong. It may be correc legally, but the policy itself is unethical. The RAC should be able to address this problem. Dr. Wivel said NIH legal counsel has previously offered an opinion that it is beyond the purview of the RAC to dictate to a local IRB with regard to what must be in the Informed Consent document. The RAC can only act as an advisory body. Dr. Secundy recalled that the RAC has revisited the same issue many times. Despite the opinion of NIH legal counsel, the RAC still needs to address this cost issue. There were many acceptable Informed Consent documents from MD Anderson protocols previously approved by the RAC, so it is not an institutional problem. Dr. Zallen said the cost should be weighed against the benefits, so it is a benificence issue.

Dr. Motulsky said he would like to get back to the substantive safety issue raised by 3 members of the IRB. The protocol involves use of an oncogene that could cause cancer rather than inhibit cancer, and he would like to have more discussion of this safety issue. Dr. Ginsburg said given the potential danger of the E1A gene, he asked the investigators to address any likelihood of germ line transmission when this DNA is administered to the peritoneum. Dr. Parkman asked about the transforming potential of E1A gene in the primary tissue culture cells rather than cells from an established cell line. Drs. McIvor andGlorioso noted that the RAC has an ongoing discussion of a similar problem with the *p53* gene. Dr. Noguchi remarked that human cell studies by Dr. Maurice Green of the St. Louis University suggested that the actual danger of adenovirus oncogenicity *per se* appears to be rather minimal.

Investigators Response--Drs. Lopez-Berestein and Hung

Dr. Lopez-Berestein said Dr. Hung would address the issue related to the E1A gene; Dr. Hung cloned the HER-2/neu oncogene and demonstrated that E1A is a tumor suppressor gene.

Dr. Hung stated that E1A as an oncogene is a mistaken notion and E1A does not produce any tumor at all. The E1A gene was first classified as an immortalization oncogene in the 1980's because it is required for cooperation with *ras* oncogene or adenovirus E1B oncogene to transform primary rat baby kidney cells and primary human embryo retinal cells. Although E1B or *ras* oncogene alone can transform established cell lines, neither alone can transform primary embryo cells. To transform primary embryo cells,*ras* or E1B oncogene requires cooperation with the E1A gene. However, E1A alone cannot transform either primary cells or established cell lines. One major difference between established cell lines and primary

culture cells is that the established cells lines have "passed the crisis;" therefore, they are "immortalized." Because of this observation, the E1A gene has been historically classified as an "immortalization oncogene" and E1B and *ras* oncogenes as "transforming oncogenes." E1A gene by itself does not exhibit transforming phenotype.

Dr. Hung emphasized that in order to allow E1A to function as an "immortalization" gene, it needs to be permanently integrated into the host cell genome and persistently expressed. The present transduction is based on a transient expression system of transfection with a plasmid DNA/liposome complex. Dr. Hung said his published studies demonstrated the tumor suppressor activity of the E1A gene. E1A gene induces apoptosis similar to that observed with the *p53* tumor suppressor gene. His studies show that E1A gene products act as the transformation suppressors of the HER-2/neu oncogene.

Regarding the question of whether E1A is oncogenic to normal human cells, Dr. Hung said there is no direct data pertaining to normal human cells, but E1A is not tumorigenic to mouse cells. When E1A is introduced into human cancer cell lines, it suppresses the tumorigenicity of human cancer cells in nude mice.

Addressing Dr. Hearing's question of the mechanism of apoptosis induced by E1A, Dr. Hung said that apoptosis induced by E1A requires the presence of wild-type p53 protein, and the ovarian cancer cell model he studied has a mutated *p53* gene.

Responding to the question of preclinical studies of breast cancer, Dr. Hung provided data of a mouse model of breast cancer treated with E1A. These data shows that E1A suppressed tumor growth in breast cancer cells overexpressing HER-2/neu oncogene. With regard to the entrance criteria of immunohistochemistry assay of HER-2/neu expression in tumor specimens, Dr. Hung said the Pathology Department of his institution has routinely performed such assays.

Dr. Parkman asked how the intralesional injection used in the breast cancer model is relevant to the intrapleural administration of the liposome complex in the clinical protocol? Dr. Lopez-Berestein responded that there is no appropriate animal model for intrapleural breast cancer. The intralesional injection of the E1A plasmid demonstrated its activity against breast cancer cells. Dr. Ross inquired why include breast cancer in this study given the small number of patients?

Regarding questions of the Informed Consent document, Dr. Lopez-Berestein said that most of the elements of the Informed Consent document are a standard form approved by the IRB of the MD Anderson Cancer Center. He agreed to improve the statement regarding long term follow-up, media interest, and to discuss the amendments with his IRB. Dr. Lopez-Berestein said the cost issue is a difficult one for him; his IRB believes the cost statements are consonant with all the rules and regulations regarding the Informed Consent document.
Dr. Lopez-Berestein briefly responded to many questions asked by Dr. Zallen including transduction efficiency, the number of patients, and E1A in gonadal tissues without giving detailed information. In terms of autopsy, he said his IRB does not allow him to make any statement about the autopsy or necropsy in the Informed Consent document.

Dr. Parkman noted there is no data demonstrating the level of sensitivity of the immunohistochemistry assay of HER-2/neu expression of patient samples. Dr. Lopez-Berestein said he would provide the data and the standard table used by his pathologists.

Responding to Dr. Ross' question of including breast cancer in the study, Dr. Lopez-Berestein said that in the Phase I study it is advantageous to cover as many tumor types as possible. Dr. Chase disagreed with

this strategy. A fundamental principle of biological investigation is that if there are very few patients in the experiment and so many unknown factors, such patients should be a group that is as homogeneous as possible. Dr. DeLeon remarked that these 2 cancer types, ovarian and breast, have the same characteristics of HER-2/neu gene overexpression. Dr. Parkman said if the investigators have equivalent preclinical data to treat these 2 tumor types, then the inclusion of both tumors in the same study is justified, but that is not the case here. Dr. Noguchi remarked that FDA considers the rationale of the protocol acceptable and has no particular safety concern particularly when the treatment is for advanced cancer patients. Any minuscule amount of hope for such patients is a very important therapeutic aspect. Ms. Rothenberg said even as a consumer she would prefer results of this Phase I protocol are obtained if all 3 patients treated have the same kind of cancer. Dr.Hirschhorn shared the concern with the investigators that if this Phase I study included only ovarian cancer, one might miss the opportunity to explore whether the treatment might work with other tumor types which share the same mechanism. Dr. Chase stated that the current study design will not produce any useful information.

Ms. Meyers disagreed with Dr. Noguchi's rationale of offering cancer patients some hope, and she asked if there is any danger in giving patients an oncogene. Dr. Noguchi said in terms of expense, the use of a plasmid DNA/liposome is much less expensive than any viral based vector for cancer patients. The types of tumors with fluid accumulation in the peritoneal or pleural space have very little alternative treatments. E1A has not been shown to be oncogenic in human cells. Overall, FDA's evaluation of this protocol is that it seems to be a relatively safe procedure.

Ms. Meyers stated that the RAC has reviewed several protocols from MD Anderson Cancer Center, and none of them had an Informed Consent document as poorly written as the present study. Dr. Lopez-Berestein noted that all the Informed Consent documents have the same kind of statements. Dr. Zallen said the previous investigators all agreed to consult with their IRB on the RAC's recommendations. Dr. Secundy said she has reviewed several Informed Consent documents from this same institution. Unlike the other Informed Consent documents from the same institution, the quality of this document and the investigators response is
unacceptable.

Dr. Parkman remarked that it is not unusual for a Phase I cancer protocol to involve several tumor types particularly if they have a common mechanism of disease. Dr.Zallen said the protocol involves 2 different anatomical sites which may have different toxicity. Dr. Erickson agreed the toxicity may be different between the pleura and peritoneum. Dr. Chase reiterated his objection to the proposal stating that splitting resources among heterogeneous groups of patients is a poor experimental design. Dr. Ginsburg said the study design issue does not involve recombinant DNA; it has been the standard practice of experimentation in chemotherapy for malignancies.

Dr. Glorioso inquired if there is a particular safety issue that is specific to either the pleural or the peritoneal space. Dr. Parkman said there is a safety concern in the present study design. The scenario to be concerned is that if patients to be treated at first 2 dose levels are all intraperitoneal ovarian cancer patients, it will provide no guidance for the first intrapleural breast cancer patient to be entered onto the third dose level cohort. Dr. Noguchi agreed that it is a good point. Dr. Parkman said instead of focusing on the question of tumor types, anatomic sites is more of a concern. The pleura space has more potential risk to the patients. Dose escalation may occur by mixing tumor types but not anatomic sites. Dr. Lopez-Berestein agreed to accept a compromise scheme to have 2 peritoneal and 2 pleural patients in each cohort. Dr. Noguchi suggested escalating the dose in each patient instead of performing a dose-escalation cohort study.

Committee Motion 1

A motion was made by Ms. Meyers and seconded by Ms. Rothenberg to disapprove the protocol. The investigators would have to submit a new protocol responding to all the concerns raised by the RAC, especially regarding the Informed Consent document.

Dr. Parkman said he would vote against the motion. There is a basic idea in this protocol that has promise, and he would support the approval if an acceptable study design is agreed upon. Dr. Ross indicated she would agree with Dr. Parkman's motion for approval of the protocol. Dr.Glorioso urged the RAC to vote against disapproval.

Dr. Zallen indicated she would vote for disapproval since there are too many outstanding issues including *in vitro* transduction efficiency, gene transfer to gonads, sensitivity of theimmunohistochemical assay, and the Informed Consent document. She has just spoken to and has been assured by the Chair of MD Anderson IRB that all costs to the patients can be covered. In addition, the Informed Consent document should be amended with regard to media interest and long term follow-up.

Dr. Noguchi said that the FDA will make sure the investigators address the concerns raised by the RAC. With regard to the issue of the study design, it is important to be as consistent as possible with all other oncology submissions to FDA. Dr. Ginsburg stated he would vote for approval of the protocol with a stipulation to amend the Informed Consent document.

Dr. Secundy was concerned about the statistical issue raised by Dr. Chase that the study will not be able to draw any significant inferences from the data, and her understanding of the RAC policy is that no approval can be given if the necessary information is not included in the submission. Dr. McGraw said the protocol can be considered after resubmission. Dr. Wivel stated that most often the RAC votes for deferral rather than disapproval with the knowledge that investigators would return. Ms. Meyers indicated that the investigators need to return to theirIRB to reconsider the issues in the Informed Consent document. Dr. Parkman noted there is distinction between disapproval and deferral. Dr. Anderson stated that the RAC has disapproved a single protocol, and the present one does not belong to that category. Dr. Parkman added that disapproval implies that the protocol has no scientific merit.

Ms. Rothenberg stated that deferral makes sense for this protocol, and she inquired if the investigators would revise the study design regarding the anatomic sites. Dr. Lopez-Berestein responded that he would agree to concentrate on ovarian cancer in this protocol. Regarding the informed consent issues, he said he is the Vice Chair of the IRB; and he would ask the IRB to reconsider the issues raised by the RAC. Dr. Noguchi stated that there are proper agencies overseeing IRBs across the country, and IRBs have been delegated with the authority for final approval of the Informed Consent documents. Dr. Parkman called the question for a vote.

Dr. Walters called Ms. Meyers' motion of disapproval for a vote. The motion to disapprove the protocol (absence of relevant scientific data supporting the proposed study) failed by a vote of 4 in favor, 9 opposed, and 2 abstentions.

Committee Motion 2

Dr. Parkman made a motion to defer the protocol contingent on review and approval of several outstanding issues. There is no serious recombinant DNA issue associated with the introduction of the E1A gene. The stipulations involve revision of the Informed Consent document, restructuring of the basic study design regarding the anatomic sites, providing data on *ex vivo* transduction rates and sensitivity of the immunohistochemical assay of HER-2/neu gene expression. The issue of gonadal transmission

should be addressed. Dr. Ross seconded the motion. Ms. Rothenberg made a friendly amendment that the revised protocol should be reviewed by the full RAC. The amendment was accepted by Drs. Parkman and Ross.

The amended motion was to defer the protocol contingent on full RAC review of: (1) a revised experimental design (particularly relating to specific anatomical sites), (2) quantitative assessment of *ex vivo* transduction rate, (3) data demonstrating the level of sensitivity of the immunohistochemical assays of HER-2/neu expression, and (4) a revised Informed Consent document. The motion passed by a vote of 13 in favor, 1 opposed, and 1 abstention.

X. DISCUSSION REGARDING STIPULATIONS FOR PROTOCOLS #9406-079, *CLINICAL PROTOCOL FOR MODIFICATION OF TUMOR SUPPRESSOR GENE EXPRESSION AND INDUCTION OF APOPTOSIS IN NON-SMALL CELL LUNG CANCER (NSCLC) WITH AN ADENOVIRUS VECTOR EXPRESSING WILD-TYPE P53 AND CISPLATIN, AND #9412-096, CLINICAL PROTOCOL FOR MODIFICATION OF TUMOR SUPPRESSOR GENE EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) WITH AN ADENOVIRUS VECTOR EXPRESSING WILD-TYPE P53*.

PIs: Drs. Jack A. Roth (#9406-079) and Gary Clayman (#9412-096), MD Anderson Cancer Center, Houston, Texas

In a letter dated April 12, 1994, Dr. Jack Roth of M.D. Anderson Cancer Center, Houston, Texas, submitted the human gene transfer protocol entitled: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression and Induction of Apoptosis in Non-Small Cell Lung Cancer (NSCLC) with an Adenovirus Vector Expressing Wild-type p53 and Cisplatin* to the Recombinant DNA Advisory Committee for formal review and approval. During the June 9-10, 1994, Recombinant DNA Advisory Committee meeting, the committee approved Dr. Roth's protocol by a vote of 11 in favor, 1 opposed, and abstentions. Approval was contingent on the review and approval of the following: (1) intra-pleural administration of the adenovirus vector will be eliminated from the protocol; therefore, a revised protocol and Informed Consent document are required; (2) the protocol will be revised to include patient sputum titration assays on 293 cells (for both the wild-type and mutant vector) to be conducted until virus is no longer detectable (patients will be isolated for a period of 1 week). The RAC deferred the intra-pleural administration portion of the protocol until the investigator returns to the full RAC with a revised protocol that includes toxicity data demonstrating the effect of the adenovirus vector and cisplatin in an appropriate animal model. The RAC *strongly* recommended the cotton rat as the most appropriate model.

In a letter dated October 5, 1994, Dr. Gary Clayman of the MD Anderson Cancer Center, Houston, Texas, submitted a human gene transfer protocol entitled: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wild-type p53* to the Recombinant DNA Advisory Committee for formal review and approval. During the December 1-2, 1994, Recombinant DNA Advisory Committee meeting, the committee approved Dr. Clayman's protocol by a vote of 16 in favor, 1 opposed, and 1 abstentions. Approval was contingent on the review and approval of the following: (1) development of sensitive screening assay (preferably biologic, such as a human papilloma virus assay), to detect the presence of p53 mutants in the adenoviral vector stocks. In the event that a mutant is identified, that stock will be discarded; (2) the specific p53 mutation will be characterized for all subjects undergoing gene transfer; (3) submission of a detailed description of the analyses that will be conducted on available post-mortem tissue. The motion for Dr. Clayman's approval included the provision that these same stipulations would apply to Dr. Roth's protocol that had been provisionally approved on June 9-10, 1994.

In a letter dated December 20, 1994, Dr. Nelson Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, informed Dr. Roth of the discussion of the December 1994 Recombinant

DNA Advisory Committee meeting and the additional stipulations on his protocol.

Over the next several months, Drs. Roth and Clayman submitted materials to address the stipulations. These materials were forwarded to reviewers. One reviewer had a concern with the mutagenicity assay for p53. An *ad hoc* consultant reviewed the materials submitted by Drs. Roth and Clayman and proposed a monoclonal antibody assay. Drs. Roth and Clayman requested that the Recombinant DNA Advisory Committee review the materials submitted during the June 1994 meeting to determine if they met the stipulations of the committee. In response to the request of the RAC reviewers and the *ad hoc* consultant, Drs. Roth and Clayman attempted to develop an assay for p53 mutants using the PAb240 monoclonal antibody from Oncogene Sciences. From the data they presented, two different cell lines containing wild-type p53 showed positive fluorescence as well as the cell line H322 which was known to contain mutant p53. Despite marked reduction in the concentration of the PAb240 antibody, fluorescent staining o both wild-type p53 and mutant p53 occurred. As a result of this outcome, Drs. Roth and Clayman concluded that their SAOS osteosarcoma cell assay, using apoptosis as the measurable endpoint, had much greater levels of specificity.

Dr. Walters called on Dr. Erickson to present the outstanding issues regarding these 2 protocols. Dr. Erickson stated that the protocols used an adenovirus vector expressing the wild-type p53 tumor suppressor gene to treat patients with NSCLC and HNSCC. A major concern was oncogenicity of p53 mutants which might accumulate within the adenovirus vector stocks. P53 mutants could allow the virus to grow to a higher titer. In a normal adenovirus infection, the role of the E1B gene of the virus is to bind the cellular p53 in an effort to promote viral growth. This mechanism of inactivation of the cellular p53 suggests that viral growth has evolved to inactivate p53 for optimal replication. The selection of adenovirus recombinants that are defective for p53 expression or function will have a growth advantage and accumulate in a stock. An assay should be developed to detect any such mutations within the vector stocks before they are administered to patients.

Dr. Roth proposed in a letter dated June 23, 1995, a functional assay for mutant or biologically inactive p53 using the SAOS osteosarcoma cells. This biologic assay compares the activity of a standard stock of adeno-p53 vector to the activity of newly produced stocks. The standard stock of adeno-p53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an multiplicity of infection (MOI) of 50:1 (titer > 5x10) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) adeno-p53 vector will be determined by adding increasing amounts of adeno-luc (luciferase gene insert) to the adeno-p53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by adeno-p53. The test lot of adeno-p53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive vector.

Dr. Samulski has consulted with Dr. Arnold Levine of Princeton University who is an expert on tumor virology and p53 tumor suppressor gene. Based on the advice from Dr. Levine, an approach could be devised that could start to measure mutant forms of p53 recombinants in a vector preparation by using adenovirus infected cells, the monoclonal antibody (Pab240 from Oncogene Sciences, Long Island, New York), and cell sorting.

Dr. Roth responded in a letter dated June 23, 1995, stating: (1) The assay does not measure changes in biologic function resulting from the p53 mutation; (2) The antibody that Dr. Levine proposed for use in the assay is not specific for mutant p53; (3) The antibody assay will not detect some of the common p53 mutations; (4) The antibody assay will not detect p53 deletions; and (5) The antibody assay cannot be standardized nor can its sensitivity be accurately determined. Dr. Roth provided additional data showing

that his SAOS cell assay is a reproducible and sensitive bioassay for inactive vector.

Dr. Samulski stated in a letter dated August 23, 1995, that it has been well established that any one of a number of missense mutant p53 proteins can participate in the transformation of cells to an oncogenic state. Clear evidence from a plethora of studies demonstrates that one mechanism by which transformation may occur is through dominant inactivation of wild-type p53. Certain mutant forms of p53 have oncogenic properties even in the absence of wild-type p53. The first concern is not simply that the wild-type stock would be "diluted" with the generation of viruses encoding mutant p53, but that a potentially oncogenic virus will be created and delivered to normal as well as abnormal cells, thereby promoting rather than suppressing the oncogenic process. Dr. Samulski found the "dilution" assay proposed by Dr. Roth to be inadequate to detect oncogenic mutations.

Dr. Erickson said that the issue has not been totally resolved. In addition, some of the tests would be very expensive to perform and are not practical to assay the vector stocks batch by batch.

Dr. Ginsburg noted several issues concerning the controversy of adeno-p53. Consistency of RAC approval is an issue since some adeno-p53 protocols have been approved before the issue of p53 mutations became a major RAC concern. There is less concern about inserting a normal CFTR gene into an adenovirus vector than inserting an oncogene into this vector. There is a possibility of dissemination to normal individuals. His major concerns are the issues regarding accumulation of mutant p53 in the vector stocks and the potential dominant effect of the mutant gene when introduced into a normal cell. These are important issues and are worth obtaining expert opinions on them. Dr. Ginsburg considered the risks to be very small; however, there is a theoretical risk and a public safety issue involved. Dr. Walters noted that Dr. Samulski has suggested Drs. Steve Friend, Arnold Levine, Terry Van Dyke, and Bert Vogelstein as possible experts to be invited to discuss the p53 issues.

Dr. Parkman stated that the original intention of the stipulation is to require a biologic assay to detect the transdominant effect of p53 mutations. Dr. Levine's monoclonal antibody assay would not address this point. Other chemical assays to detect the hot spots of p53 mutations are reasonable; however, it does no address the biological endpoints. Dr. Parkman preferred to have consultants explore the available biologic assays; and if there is no such assays, then he would accept the other surrogate assays.

Dr. Ginsburg said the consultants should be asked to address a broader question. Dr. Levine was specifically asked to develop an assay for p53 mutations. Dr. Ginsburg would prefer to solicit Dr. Levine's opinion on a more general question in terms of what is the potential risk of this gene transfer approach. If the consultants concluded that there is a significant danger either to the patients or to the public, then the issue of an effective screening assay should be considered.

Dr. Chase was concerned that the RAC has previously approved protocols using the adeno-p53 vectors. Dr. Parkman noted that in addition to Dr. Roth's protocols (#9406-079 and #9412-096), there is another protocol (#9412-097) by Drs. Alan Venook and Robert Warren to treat liver cancer using the adeno-p53 vector. As a point of clarification, Dr. Wivel explained that a similar stipulation requiring a biologic assay for p53 mutations has been retroactively applied to the previously approved protocols. The RAC preferred a biologic rather than a PCR assay for p53 mutations. Dr. Chase agreed that a panel of consultants should be invited to address this serious issue; however, Dr. Roth's delay to proceed with his protocols should be minimized.

Dr. Erickson indicated that the data provided by the investigators showing "dilution" of the inhibitory effect on SAOS cell growth by mixing adeno-p53 with a vector carrying the *Luc* gene is inadequate. The loss of the inhibitory effect could be due to a variety of reasons other than p53 mutations.

Dr. Noguchi asked Dr. Bob Anderson of the FDA to explain FDA's evaluation of the p53 assays for these protocols. Dr. Anderson said FDA approaches this question from a perspective of developing pharmaceuticals or biologics. The type of assay adequate for adeno-p53 protocols is still in a developmental stage and no standard of validation has been provided to the investigators. Dr. Parkman remarked that the RAC mandates an assay to measure the biologic endpoint ofoncogenicity, but it is the investigators' responsibility to develop the assay and to determine the level of sensitivity that could be evaluated by the RAC reviewers as in the development of the SL assay for the retrovirus vector.

Dr. Noguchi said that FDA has already approved both Roth (#9406-079) and Clayman (#9412-096) protocols. Dr. Ginsburg inquired if the safety issue regarding the p53 gene has been addressed. Dr. Noguchi responded that such consideration has been taken into account in granting approval. Dr. Parkman said if the FDA information is provided to the RAC, it would facilitate the discussion.

Investigators Response--Drs. Roth and Clayman

Dr. Roth stated that p53 mutations is an extremely important issue. He presented data to demonstrate tha adeno-p53 is safe for administration to cancer patients specified in the protocols. Based on the available information, adeno-p53 poses no safety risk for other health care workers.

Dr. Roth stated that theSAOS cell assay of p53 mutations has been approved by the FDA. He objected to convening an expert panel, since this research area is constantly evolving and no one other than the investigators is an expert in this area of developing the assay.

Dr. Roth said his protocol was provisionally approved by the RAC during the June 1994 meeting. There was considerable discussion regarding the possibility of p53 mutations in the viral stocks. He concluded that the safety considerations were met since adeno-p53 expression is transient, the vector is replication incompetent, and safety precautions have been employed in the production of viral stocks. He had not received the final NIH Director approval of his protocol at the time Dr.Clayman's protocol was discussed during the December 1994 RAC meeting. Dr. Clayman's protocol was approved with a stipulation to develop a sensitive screening assay, preferably biologic, to detect the presence of p53 mutations. The SAOS cell assay was then developed by Dr. Roth's laboratory; however, the reviewers suggested another monoclonal antibody assay that compounded the issue.

Dr. Roth briefly described the function of the p53 tumor suppressor gene. Transgenic animals lacking this gene can survive but are predisposed to tumor development. The gene functions as a guardian of the genome by protecting against excessive DNA damage. There are two fundamental types of p53 mutations. Most mutations result in the loss of thetransactivating function of the gene product, and the second type of mutation involve "gain of function." This "gain of function" is manifested by a weak transforming activity in mouse cells by cooperating with other oncogenes, and there has not been any transformation of primary human cells by mutant p53. Mutations such as thecodon 248 mutation, which exists in a lung cancer cell line, actually retain the tumor suppressor function.

Regarding safety considerations for the patients, Dr. Roth said that patients in both protocols have a very limited life expectancy of 3 to 6 months, and there will be no opportunity for the development of second tumors in these patients. P53 mutations, if existing, would be overcome by the excess of wild-type p53 carried by the vector. There is no toxicity detected in the ongoing protocol (#9403-031) using a retrovirus vector expressing the wild-type p53 gene.

Regarding safety considerations of health care workers and other individuals exposed to the virus, Dr.

Roth explained that emergence of a replication competent adenovirus carrying the mutant p53 cannot occur since the insertion of the p53 gene to the virus genome will exceed its packaging capacity. Even if there is an infection, the adenovirus causes only a transient gene transduction. The mutant p53 would have to be inserted into a cell with a number of preexisting genetic changes to have anyoncogenic effect. Such a probability of tumorigenic effect is very low. Furthermore, co-transduction with the wild-type p53 and the bystander effects of p53 would eliminate anyoncogenic effects of the mutant p53 since the wild-type is dominant over the mutant.

Regarding the possibility that a mutant p53 could become dominant in the viral stocks, Dr. Roth said he has assayed 4 viral stocks with the SAOS assay and detected no evidence of the presence of p53 mutants. All viral stocks maintain the same biological activity of the wild-type p53. Dr. Glorioso inquired if the assay has been validated by a reconstruction experiment involving the mutant p53 vector. Dr. Roth responded he has been specifically discouraged by the RAC to construct any such mutant p53 adenovirus. The reconstruction experiment has been performed with a surrogate vector carrying the *Luc* gene.

In terms of other biochemical assays for p53 mutations, Dr. Roth gave several examples. Direct DNA sequencing is extremely laborious and there are numerous possible mutation sites within the p53 gene. PCR amplification of a particular mutation site is feasible, but it has a large error rate that could yield a false positive result. Single strand conformation polymorphism assay to detect mutations by a gel migration assay of PCR amplified DNA has a low sensitivity. Recently, a yeast assay has been developed to detect the lack of the transacting activity of mutant p53; the assay has an unacceptable error rate due to the PCR step. Other assays have been explored including the binding assay by DNA repair proteins, and they are all unsatisfactory.

The PAb240 monoclonal antibody proposed by Dr. Levine has many shortcomings. Dr. Roth presented data obtained by using this assay. The PAb240 antibody cannot accurately distinguish between mutant and wild-type p53 conformations in the FACS assay. Thus, the use of this antibody would not be appropriate for developing an assay to detect expression of mutant p53 by an adenovirus vector.

Dr. Roth presented his data using the SAOS biologic assay. He said the assay can detect the gain of transforming function as well as the loss of wild-type p53 function by their loss of the ability to suppress the growth of the SAOS human tumor cells. The investigators have searched for cell lines whose growth can be accelerated by the presence of the mutant p53, but no such cell lines have been found to develop other assays. At the present time, the SAOS assay is the only practical assay for p53 mutations.

Dr. Roth explained the SAOS assay. SAOS is a human osteosarcoma cell line with a homozygous p53 deletion. If one adds a wild-type p53 to SAOS cells, it suppresses their growth dramatically. If a mutant p53 is present, it would reduce the ability of the wild-type p53 to suppress the growth of these SAOS cells. Dr. Roth presented data obtained by this assay of a reconstitution experiment by mixing the wild-type p53 vector with a vector carrying a surrogate *Luc* gene. Loss of the SAOS cells growth suppression can be detected by mixing the wild-type p53 virus stocks with 1%, 5%, and 10% of *Luc* vector. The result is highly reproducible, and it is the state-of-the-art assay for monitoring the biological activity of the p53 gene.

Dr. Parkman inquired if the same SAOS cell is co-transduced by the wild-type p53, the presence of mutant p53 would not be detected since wild-type is dominant. Dr. Roth responded that the same situation will happen in patients infected by the virus and the assay mimics this clinical setting. There is a dilemma in developing this assay in that the MOI can be set too low to avoid cotransduction, then the background of the assay becomes very high.

Dr. Parkman said the level of sensitivity of the SAOS assay is about 2%. Dr. Roth said the other assays have a much higher degree of uncertainty, and he agreed that this is an excellent area for research to improve the test. Dr. Erickson noted that his initial concern was that the mutant p53 might accumulate to a high level (30 or 40%) of the viral stocks. The data provided by Dr. Roth showed that as little as 3% of p53 mutants would be detected by his assay. Dr. Erickson would accept the SAOS assay as an adequate biological test at this stage of the Phase I study.

Dr. Parkman asked if there is any plan to continue the virus culture and monitoring for p53 mutations after the time of administration to the patients in order to make sure that there is no further accumulation of p53 mutations. Dr. Noguchi said that retrovirus precedent may be not appropriate for p53 mutations since the retrovirus assays are to ensure that there is no presence of replication competent virus in the vector stocks. Dr. Glorioso remarked that Dr. Parkman's suggestion makes sense.

Dr. Debra Wilson (Introgen Therapeutics, Inc.) stated that she is collaborating with Dr. Roth on production of adenovirus vectors. She explained the difference between producing a retrovirus vector and an adenovirus vector. Retrovirus is continuously produced from infected cells for a long period of time. A batch of adenovirus is produced considerably different than the manner in which the retrovirus is produced. Each batch of adenovirus is produced by a *de novo* infection; the virus is harvested from the cells within 48 hours after infection. The infected cells do not continue to grow for an extended period of time. The follow-up assay suggested by Dr. Parkman is not feasible for adenovirus. Dr.Glorioso explained that the point made by Dr. Parkman is different. The test is to take the batch of adenovirus that will be used for patients, and to amplify the virus to determine if it accumulates more p53 mutations. Dr. Roth said that the experiment has been conducted since the virus stock has been expanded several times from a seed batch.

Dr. Parkman stated that the investigators have responded to the RAC stipulation and have come up with a biologic assay. The expert panel could provide the RAC with guidance if any assay with a sensitivity better than 2% is scientifically necessary. Dr. Noguchi endorsed Dr. Parkman's statement, and Dr. Noguchi noted the assay can be improved if necessary in the future.

Dr. Frank Sturtz, Progenitor, Inc., proposed to add a suicide gene such as the HSV-TK gene to the adenovirus vector; and if there is any inadvertent infection of a health care worker, the infected cells can be eliminated by administration of GCV. He agreed with Dr. Roth that the risk to advanced cancer patients is very small.

Dr. Ginsburg reiterated his suggestion to convene an expert panel to address the overall philosophical issue of putting p53 gene in an adenovirus vector. The panel will be asked to address first the global issue of public health safety concern; and if there is significant concern, an acceptable assay for p53 mutations should be recommended. Dr. Roth said experts has been consulted, such as Dr. Levine. As a point of clarification, Dr. Ginsburg said if the expert panel finds no potential public health risk, then he has no disagreement about the assay. However, if there is a major risk, then the question has to be revisited. Dr. Noguchi said that similar public health concerns have been addressed during the discussion of CF protocols, particularly the protocol dealing with aerosol administration. In his opinion, the concept of whether an adenovirus can spread to other individuals besides the patients has been addressed; there is no need for an expert panel. Dr. Ginsburg stated that there is a fundamental scientific difference between inserting p53 into an adenovirus and inserting a CFTR gene. The latter does not have the concern of oncogenicity. Dr. Noguchi said that all the considerations taken regarding these particular protocols with these particular patients, led to the conclusion that there were no serious safety concerns from the FDA's point of view.

Dr. Ginsburg said the nature of the gene insert in the adenovirus constructs is an important factor concerning its public health risk. He would insist on having an expert panel to address the global issue.

Dr. Parkman noted that the present 2 protocols involve local bronchial and subcutaneous injection. The injected patients will be isolated for 2 days. He asked if the 2 day isolation is sufficient to prevent spreading of the virus. Dr. Noguchi said that in the CF protocols, most virus shedding occurs within the first 24 hours. Dr. Parkman said if the duration of isolation of the patients encompasses the longest duration of virus shedding, the potential risk to normal individuals would be minimized.

Dr. Parkman stated a decision regarding these 2 protocols should be made before an expert panel can be convened in the future. Dr. Chase proposed the solution of approving the two protocols while convening an expert panel. If the expert panel has serious safety concerns, future adeno-p53 protocols should not be approved. Both Drs. Erickson and Glorioso found this solution acceptable. Dr. Clayman's protocol involves direct injection into head and neck tumors, and it presents very little risk of virus leaking. Dr. Parkman said he is more concerned about the Roth protocol. Since the CF protocols revealed that adenovirus shedding has been detected up to 4 days, Dr. Parkman would accept the Roth protocol if the duration of isolation is extended to 4 days instead of 2 days.

Committee Motion

Dr. Parkman made a motion to accept the protocols with a stipulation to extend the duration of patient isolation from 2 days to 4 days for the Roth protocol until data is available to demonstrate that there is no virus shedding. Dr. Erickson seconded the motion.

Dr. Walters asked if the SAOS assay is acceptable for the adeno-p53 virus used in the liver cancer protocols by Drs. Venook and Warren (#9412-097). Drs. Parkman and Noguchi responded that a similar assay can be used. In terms of duration of isolation, it is similar to the Clayman protocol since it involves intraarterial injection. Ms. Connie Kirby (Canji Inc.) said her company is the sponsor of the liver cancer protocol. She confirmed that her protocol was approved with the same contingency as the Roth and Clayman protocols.
The motion made by Dr. Parkman and seconded by Dr. Erickson to accept the protocols submitted by Drs Roth (#9406-079) and Clayman (#9412-096) contingent on the following: (1) the period of patient isolation following intratumoral Ad5CMV-p53 injection will be extended from 2 days to 4 days for Dr. Roth's protocol (#9406-079) until the endpoint of vector shedding can be determined; and (2) adenovirus vector stocks (Protocols #9406-079 and 9412-096) will be screened for p53 mutations using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. The motion passed by a vote of 10 in favor, 1 opposed, and 2 abstentions.

Protocol Summary (#9406-079): Dr. Jack A. Roth, MD Anderson Cancer Center, may conduct gene transfer experiments on 42 subjects (³18 years of age) with refractory non-small cell lung cancer (NSCLC). Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, AD5CMV-p53, to deliver the normal human p53 tumor suppressor gene. The E1 region AD5CMV-p53 has been replaced with a p53 expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) 21 subjects will receive Ad5CMV-p53 alone, and (2) 21 subjects will receive Ad5CMV-p53 in combination with cisplatin. Following vector administration, subjects will be isolated for 96 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of this study are to determine: (1) the maximum tolerated dose of Ad5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutations using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer ñ 5x10) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc (control adenovirus vector containing the luciferase gene) to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9406-079)

Protocol Summary (#9412-096): Dr. Gary Clayman, MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on 21 subjects (³ 18 years of age) with refractory squamous cell carcinoma of the head and neck. Subjects will receive direct intratumoral injection of a replication-defective type 5 adenovirus vector, Ad5CMV-p53, to deliver the normal human p53 tumor suppressor gene. The E1 region of Ad5CMV-p53 has been replaced with a p53 expression cassette containing the human cytomegalovirus promoter (CMV). Subjects will be divided into 2 treatment groups: (1) those with non-resectable tumors, and (2) those with surgically accessible tumors. Subjects will receive multiple injections of vector in each dose-escalation cohort. Following vector administration, subjects will be isolated for 48 hours during which time, assays will be conducted to demonstrate the lack of shedding of adenovirus vector. The objectives of the study are to determine: (1) the maximum tolerated dose of Ad5CMV-p53, (2) qualitative and quantitative toxicity related to vector administration, and (3) biologic activity.

Prior to administration, adenovirus vector stocks will be screened for p53 mutations using the SAOS osteosarcoma cell assay that was submitted by Dr. Roth on June 23, 1995. This biologic assay compares the activity of a standard stock of Adp53 vector to the activity of newly produced stocks. The standard stock of Adp53 will be defined as mediating cell death in 100% of SAOS cells (human osteosarcoma cell line with homozygous p53 deletion) at an MOI of 50:1 (titer ñ 5x10) on day 5 of culture. The sensitivity of the assay for detecting inactive (presumed mutant) Adp53 vector will be determined by adding increasing amounts of Adluc to the Adp53 stock to determine the percentage of inactive vector required to decrease growth inhibition of SAOS cells mediated by Adp53. The test lot of Adp53 will be tested for its ability to inhibit SAOS in a 5 day assay. Significant loss of inhibitory activity compared with the standard would indicate an unacceptable level of inactive (presumed mutant) vector. (Protocol #9412-096)

XI. DISCUSSION REGARDING THE DELIBERATE TRANSFER OF RECOMBINANT DNA MOLECULES ENCODING THE TUMOR SUPPRESSOR GENE, p53, TO HUMANS

The RAC continued its discussion regarding the scientific and ethical parameters for approving human gene transfer protocols involving the tumor suppressor gene, p53.

A motion was made by Dr. Chase and seconded by Dr. Parkman to recommend that ORDA staff invite consultants identified in Dr. Samulski's August 23, 1995, letter to ORDA. They are Steve Friend, M.D., Fred Hutchinson Cancer Research Center, Seattle, Washington; Arnold Levine, Ph.D., Princeton, University, Princeton, New Jersey; Terry Van Dyke, Ph.D., University of North Carolina, Chapel Hill, North Carolina; and Bert Vogelstein, M.D., Johns Hopkins University, Baltimore, Maryland.

Before the vote, Dr. David Nance (Introgen Therapeutics, Inc.) inquired if NIH has a policy in place governing the disclosure by those experts of their commercial affiliation or any vested interest they may

have. Dr. Wivel responded that NIH has very strict rules on conflict of interest of members for advisory committees. Such information is not required for the *ad hoc* consultants since they are non-voting members. Dr. Nance said the expert opinions could affect the votes of the committee, and the disclosure of conflict of interest is relevant.

Dr. Noguchi said there are many experts in the broad area of the safety issue of p53 and adenovirus, but there are very few experts in adeno-p53 vectors. Dr.Glorioso stated that the motion is too narrow and the problem is a much larger one. The issues is not limited to p53 and adenovirus. Dr.Hirschhorn said there are some very real concerns raised by the RAC, and the suggested experts are mature scientists, and they should be able to address the issues within the limitation of their expertise.

Dr. Walters made a friendly amendment to state that the motion simply stating a representative group of experts rather than a certain number of them. Drs. Chase and Parkman accepted the amendment.

Ms. Meyers asked if these two protocols are accepted before convening the expert panel. Dr. Chase responded affirmatively stating that although the RAC has approved these two protocols, it does have significant concerns about the use of adeno-p53 vectors.

The motion made by Dr. Chase and seconded by Dr. Parkman recommended that the ORDA staff invite a representative group of *ad hoc* experts to discuss the potential safety concerns of administering genes encoding p53 to humans. ORDA staff should give primary consideration to the following consultants identified in Dr. Samulski's August 23, 1995, letter to ORDA: (1) Steve Friend, M.D., Fred Hutchinson Cancer Research Center, Seattle, Washington; (2) Arnold Levine, Ph.D., Princeton, University, Princeton, New Jersey; (3) Terry Van Dyke, Ph.D., University of North Carolina, Chapel Hill, North Carolina; and (4) Bert Vogelstein, M.D., Johns Hopkins University, Baltimore, Maryland. The motion passed by a vote of 13 in favor, 0 opposed, and no abstentions.

XII. PRESENTATION ON *IN UTERO CELL AND GENE THERAPY/DR. GOLBUS*

Dr. Mitchell Golbus, Professor Emeritus of Obstetrics and Gynecology and Reproductive Sciences, University of California, San Francisco, California, presented data derived from *in utero* bone marrow transplantation experiments in response to a recommendation made by the RAC at its March 3-4, 1995, meeting. Dr. Golbus is the second speaker in a series of *ad hoc* experts invited to address the RAC for the purpose of providing educational information relevant to *in utero* gene therapy. In a telephone conference call held on February 14, 1995, the *In Utero* Gene Therapy Subcommittee recommended that the RAC should carefully consider the scientific, safety, ethical, and legal considerations of such studies in a public forum. The consensus of the RAC was that such issues should be discussed extensively in a public forum prior to the consideration of an *in utero* gene therapy proposal.

Presentation--Dr. Golbus

Dr. Golbus stated that his work in the last 3 decades involved studies of the fetus, its genetic constitution, and the burgeoning field of prenatal diagnosis. In the last decade, more and more studies have directed toward medical therapies of the fetus *in utero* including surgical therapy. Dr. Golbus focused his presentation on treating single gene disorders by stem cell transplantation or gene therapy.

The concept of hematopoietic stem cell transplantation is straight forward. If a gene is missing, a stem cell that will make the appropriate gene products can be easily transplanted to the fetus via the intraperitoneal route. The subdiaphragmatic lymphatic system will transport the transplanted stem cells. The technique of direct access to the circulation has been improved, and most work is now performed by intravascular

injection.
There are many reasons to prefer *in utero* rather than post natal transplantations: (1) There are about 20% of families who do not have a marrow match, and the patient's condition deteriorates before matching marrows are available. In the extreme case such as a-thalassemia, it is lethal *in utero* without treatment. Complications from ablating the marrow, rejection, and graft-versus-host disease (GVHD) can be avoided. There is a window of immunologic tolerance during fetal development. The outer limit of this window is not well defined in the humans although it is known in several experimental animal models. (2) The fetus can be treated early prior to clinical disease manifestation and deterioration of the patient's condition. (3) There is a natural marrow seeding from the fetal hematopoietic liver cells; the transplanted cells can join this natural migration of cells without the need to ablate the fetal marrow.

Dr. Golbus described murine experiments involving the transplantation of donor fetal liver as the source of hematopoietic stem cells to fetal recipients in order to treat a stem cell deficiency. C57/Black mice with W and V alleles are deficient in their bone marrow. The experiment is to study stem cell transplantation across the H2 barrier. C3H mice were chosen as donors since they are allogeneic at the H2 locus. The donors and the recipient blood cells can be distinguished by slightly different hemoglobins, which can be separated by isoelectric focusing. Dr. Golbus presented data on an experiment that all 3 recipient C57/Black mice engrafted the C3H donor cells. Close to 100% chimerism was achieved in the murine experiments. In these experiments the donor cells have a biologic advantage over the deficient hematopoietic cells of the recipient mice.

Similar experiments have been performed in different animal species. Dr. Golbus presented data on chatihakashi cats which demonstrate chimerism in the offspring, and the degree of chimerism depends on the biologic disadvantage/advantage ratio of the recipient and donor cells.

Dr. Golbus described sheep experiments conducted by Dr. Zanjani, University of Nevada, Reno, Nevada, in which donor fetal liver cells resulted in donor chimerism in fetal recipients. These experiments suggest that this chimeric shift of the hemoglobin marker requires a minimum of 7 weeks after the transplant. It has not been determined whether this minimum period is the time required for the donor cells to get into the circulation or is a result of the birth process with changing physiology during the 7 week period.

Dr. Golbus described rhesus monkey experiments conducted at the University of California, San Francisco, California. A series of experiments were performed in which fetal liver hematopoietic stem cells were transplanted into 40 to 100 day old fetuses (165 day gestation period). Since monkeys are random bred animals, the animal's sex chromosomes was used as the marker to trace the donor cells. Chromosome analysis of cells obtained by chorionic villi sampling was used to determine the sex. 7 to 10% chimerism was achieved in the monkey experiments, and the chimerism is stable for 2 years. The level of chimerism is constant despite attempts to improve engraftment. In the monkey experiments, both the donor and recipient cells are normal, and there is no growth advantage of the donor cells in the recipient animals. Only in the WV murine experiments in which the donor is normal while the recipient is deficient, has a nearly 100% chimerism has been attained. The level of chimerism of 7 to 10% is quite consistent across a number of species in the animal model studies.

Dr. Golbus described his human studies conducted at the University of California at San Francisco. The first study involved a woman with a-thalassemia. The first baby was delivered by Caesarean section and died of a-thalassemia. Both the 2nd and 3rd pregnancies had prenatal diagnosis, and the woman elected termination of pregnancy. During the 4th pregnancy, a prenatal diagnosis was conducted by a fetal DNA blot analysis. Dr. Golbus presented data that confirmed the deletion of the 10kb DNA fragment of the a-globin gene. Another analysis of the globin gene products of the fetal cells indicated that this fetus was making mostly g, some b, but not aglobin. These testings indicated an affected fetus, and the woman

agreed to participate in the experimental study of *in utero* transplantation.

The maternal marrow cells were used as the donor. 10 cells were removed from hip marrow, and after T cell depletion, 6.3 x 10 cells were infused intraperitoneally to the fetus at about 18 and 19 menstrual weeks. 5 weeks later the woman returned for a fetal blood test. The fetal globin analysis indicated no sign of engraftment (no a-globin).

The first human experiment was an incomplete study since the timing of post-transplant globin analysis was too early to confirm the engraftment as judged by the data from Dr Zanjani's sheep studies. The woman elected termination of pregnancy after the diagnosis. The aborted fetus was examined by a pathologist. Surprisingly, the pathological studies of many organs of the fetus indicated signs of engraftment. There was extramedullary hematopoiesis in the liver and the pancreas, which was confirmed by the presence of female sex chromosomes in the donor cells found in the male fetus.

The second patient involved severe combined immunodeficiency (SCID). The first child was diagnosed to have SCID and died at 2 years of age. During the next pregnancy, a prenatal diagnosis for the X-linked disease was performed. Since it was a female fetus, the pregnancy was carried to term. Unfortunately, the infant was an affected individual indicating it had an autosomal recessive form of SCID. The infant died at the age of 1 year. The woman delivered a healthy boy during her 3rd pregnancy.

During the 4th pregnancy, the prenatal blood sampling indicated an immunodeficient fetus. *In utero* transplantation of the maternal marrow cells was performed by the same procedure used in the first subject. The cells were infused both intravenously and intraperitoneally to the fetus. 5 weeks after transplantation, the woman requested that if there has been no success of transplantation to terminate the pregnancy. The fetus was aborted and no sign of any microengraftment was observed in the pathological studies of the aborted fetus.

The 3rd experiment involved a Chedish-Higashi syndrome family. The 1st and the 3rd children were normal and the 2nd child was affected. During the 4th pregnancy, fetal blood sampling indicated an affected fetus. *In utero* maternal marrow transplantation was performed. The pregnancy was carried to term. Unfortunately, there was no evidence of any engraftment, and the infant was treated by post-natal transplantation. The patient is healthy.

Dr. Golbus said that all these transplants were performed in the 18 to 20 menstrual weeks window. There was a concern that the timing had missed the immunologic window. Dr. Golbus considered it might be too late for the a-thalassemia case but not for the SCID fetus.

Dr. Golbus summarized the human experiments that have been performed worldwide. The first experiment was conducted in the United Kingdom involving a fetus with Rh blood type incompatibility. The fetus was transplanted at 18 weeks using the maternal bone marrow as the donor cells of Rh negative hematopoietic stem cells. There was no evidence of engraftment from this experiment.

There were 3 attempts in Israel, and all without any indication of engraftment. The transplants were all conducted quite late. Dr. Golbus explained that in the monkey experiments, the successful window was 60 to 80 days of pregnancy; at 100 days, all donor cells were rejected since the immunological tolerance period had already passed.

The most successful experiments were performed in Lyon, France. Fetal liver cells were used as the source of hematopoietic cells. The first experiment was to treat bare lymphocyte syndrome. The transplant was done at 28 weeks. In a report in the journal *Lancet*, the investigators reported 10 to 25% donor human

leukocyte antigen type cells identified in the infant. Since the child received 25 more post natal transplants, it is impossible to evaluate the contribution of *in utero* treatment to the health of the patient. A total of 5 patients were treated for this disease; 4 were born alive and there was 1 miscarriage. 3 fetuses tested positive using a PCR technique for the engraftment, and 1 fetus was producing small amounts of adult hemoglobin.

Dr. Golbus said there were a couple of human studies that were not reported in the medical literature. One involved a couple in Sacramento with Hurler syndrome. There is some studies being conducted in Scandinavia.

Dr. Golbus stated that one critical issue for successful transplantation is the timing. The animal model suggests a window of opportunity for a transplant, and the right window for humans is probably before the 18-19 week period used in most experiments. The time frame for chorionic villus sampling must be optimized so that prenatal diagnosis can be determined early.

The second critical issue is the source of cells used for transplant. In the murine study, fetal liver is preferred over adult marrow cells as the donor cell source. If fetal tissue is allowed for transplant, human studies should be performed with fetal liver cells obtained from a fetus less than 13-14 weeks. The specimen should be bacteriologically clean and can be thawed frozen cells. There is a need to establish a bank of frozen fetal liver cells that have been characterized bacteriologically, virologically, and genetically.

Since most animal models suggest a low percentage of chimerism, a research goal in this field should be to make the animal more tolerant to the transplant. Dr. Golbus conducted a mouse study using the normal instead of the immunodeficient WV mice, although the degree of chimerism was very low in normal mice. The study is to investigate tolerance involving skin transplants from C3H to C57/Black across the H2 barrier, and Balb/c to C57/Black as a control for an unrelated H2 locus. Specific tolerance to the H2 locus can be induced by exposure to the transplant cells *in utero* allowing chimerism to occur in a postnatal skin graft. Dr. Golbus said that the concept of introducing the tolerance that will allow a postnatal boost may be a more appropriate way to try in the future rather than trying to get a whole cure on an *in utero* approach.

The *in utero* transplant can be modified by an *ex vivo* gene transduction step to introduce chimerism to the transduced gene. Dr. Golbus described 3 possible avenues for *in utero* gene therapy: (1) Autologous fetal hematopoietic stem cells obtained from fetal blood or fetal liver biopsy would be a possible source for transduction. The amount of blood to be obtained from the fetus is limited (less than 1 cc). One technical challenge is to expand the stem cells *ex vivo* without inducing commitment. Fetal cells can be transduced with a retrovirus vector more efficiently than adult cells. The transduced cells can be injected either intraperitoneally or intravascularly. T cell depletion will reduce the chance of GVHD but reduce the percentage of chimerism as well. There is more chance of unintended transduction of target cells than in post natal gene transduction. (2) Fetal hepatic cells are a possible source for transduction. A technique has been developed to obtain fetal liver tissue by biopsy rather than surgery. It is easier to expand the fetal hepatocytes in tissue culture than adult liver cells. The expanded liver cells can be transduced and reinfused to the fetal blood vessels under ultrasound guidance. (3) Direct delivery of genes into fetal organs such as the liver is feasible. The DNA can be coupled to a protein that targets to a cell receptor or the DNA can be coated on particles to be delivered by a gene gun. Animal experiments should be conducted to explore if long term expression can be achieved by these methods. One future approach is the injection of adenovirus or adeno-associated virus vectors into the amniotic fluid. Since the fetus swallows amniotic fluid, it may be possible that gene transfer could occur through this process.

Dr. Golbus concluded that a great deal of preclinical animal work should be performed before more

experimental trials are conducted on human fetuses.

Other Comments

Dr. Saha inquired if the better success rate with the French study is due to the wider window between 12 and 28 weeks used by these investigators. Dr. Golbus responded that these studies of bare lymphocyte syndrome and SCID are all involving immunologic disorders, transplant can take place at any time of gestation. Dr. Golbus stressed that the real significant factor for the higher success rate is the use of fetal liver cells.

Dr. Erickson inquired if research is being conducted for the purpose of isolating human embryonic stem cells as an universal donor. Dr. Golbus responded that some investigators are conducting such studies but no success has been reported to date. Dr.Glorioso said there are interesting studies being conducted with embryonic stem cells in animals. The stem cells were induced to differentiate into muscle or brain cells by particular cytokines; and after transplantation into fetuses, those cells were able to grow and integrate into a normal differentiated tissue.

Ms. Rothenberg asked Dr. Golbus to comment on the aspect of the human element of his study (mother's desire to terminate pregnancy), and the risks associated with the *in utero* transplant procedure to the fetus and to the mother. Dr.Golbus responded that the risk is not an issue; a similar procedure has been performed with 3rd party cells for the intrauterine transfusion for Rh disease in hundreds of thousands of patients. The issue of counseling the mother should be handled carefully. Multiple counselors are involved so that the patients are counseled independently by different people. Ms. Rothenberg was particularly concerned about the mother's decision for intentional terminations following prenatal diagnosis. Dr. Golbus remarked that if a woman is determined to go to term, there is no justification to do another fetal blood sampling in order to get information for the study.

Dr. Parkman noted that the process of maturation from hematopoietic stem cell to mature T cell requires approximately 12 weeks. If fetal transplantation is optimal between 18 and 20 weeks, no information would be available until the time of birth regarding engraftment of donor cells.

Dr. Chase remarked that dealing with women who volunteer to be part of this kind of study which for them had only a marginal hope of benefit, should be informed about the pioneer status of *in utero* transplantation. Dr. Golbus agreed that the patients should understand that there is very little chance of success. Dr. Ginsburg said the risk of partial success is significant. Most of the diseases to be treated are normally fatal within the first few years of life; partial success would convert the disease into a lifelong disability at tremendous expense that is crippling to the family. Dr.Golbus noted an example from his patients that *in utero* correction of a lethal urinary tract obstruction ended up with a lifelong chronic renal problem for the patient.

Dr. DeLeon inquired if there is any risk of germ line chimerism resulting from the *in utero* transplant procedure. Dr. Golbus responded that the chance of germ line chimerism is small for hematopoietic cell transplant; however, it could be an issue for the embryonic stem cell transplant.

Dr. Ginsburg remarked that another option to offer the parents is an early prenatal diagnosis of the recessive genetic disease, preferably at the 8-cell stage of the preimplantation embryo; and if the fetus is normal, the parents can decide to carry the pregnancy to term.

Dr. DeLeon inquired if there is any risk of blood cell chimerism in terms of responding to hormones. Dr. Parkman noted there was a woman who had totally male hematopoiesis, but she remained a

phenotypically normal female.

Dr. Chase remarked that considering the risk of long term disability of an infant born with the partial correction of its disease, society should consider sharing the cost of long term care with the family for their participation in these studies. Dr. Golbus noted there is no coordinated national strategy to deal with this kind of problem. One way to avoid the problem of partial correction is to choose the proper disease for *in utero* therapies; a neurological disease would not be the proper candidate.

Dr. Lysaught remarked that she agreed with Dr. Chase's concern; the research subjects should be considered as in a partnership with the investigators in the research project and are entitled to share with the researchers any reward resulting from the study. Dr.Hirschhorn noted that most patients participating in the experimental studies are altruistic; they are aware of the risks and will do it without compensation. Ms. Meyers said the world has changed, and the patients who are not to benefit from the experiment are being asked to pay for the costs. She said the Informed Consent document should describe the pertinent information for the patients.

XIII. DISCUSSION OF CONTAINMENT LEVEL FOR MURINE RETROVIRUS VECTORS/DRS. ANDERSON AND McGARRITY

Dr. Walters stated in a letter dated July 31, 1995, Drs. Gerard JMcGarrity (Genetic Therapy, Inc.) and W. French Anderson (University of Southern California) have requested the RAC to designate a BL1 physical containment as appropriate for handling of murine retroviral vectors. He noted that this item is a *minor action*; therefore does not need to be published in the *Federal Register* as a *major action*. A *minor action* can be handled by ORDA. Ms. Rothenberg inquired if it is appropriate for the RAC to discuss this item without due notice to the public. Ms.Knorr noted that under Section IV-C-1-b-(2)-(b) of the *NIH Guidelines*, assigning containment levels for experiments not explicitly considered in the *NIH Guidelines* can be adopted as a minor action.

Presentation--Dr. Anderson

Dr. Anderson said their request is simply to clarify a bookkeeping issue regarding handling of retroviral vectors of the *NIH Guidelines*. There are 3 categories of experiments concerning retroviral vectors within the *NIH Guidelines*: (1) For tissue culture experiments, they are exempt experiments specified under Appendix C-I, *Recombinant DNA in Tissue Culture*, since the retroviral vectors contain less than one half of an eukaryotic viral genome. The experiments can be performed under BL1 containment and registration with the IBC is not required. (2) For whole animal experiments, they can be conducted under BL1 or BL1-N containment under Section III-C-4, *Experiments Involving Whole Animals*, since the retroviral vectors contain less than one third of an eukaryotic viral genome. (3) For human gene transfer experiments, the containment level is not specified. Dr. Anderson stated that for bookkeeping purposes, it should be explicitly stated that human experiments with the murine retroviral vectors, which have more than 50% deletion of the virus genome, can be conducted under BL1 containment. As the number of countries with clinical trials using retroviral vectors increases, there is a need for a clearly stated policy regarding the level of biohazard containment appropriate for these agents. Hopefully, such a policy will result in a uniform regulatory policy regarding the packaging, shipping, importation, laboratory, and clinical use of retroviral vectors. Certain foreign regulatory authorities already have designated BL2 containment for murine retroviral vectors. Such a designation places a severe and unnecessary burden on the packaging and shipping of these agents as well as their receipt, quarantine, and handling at foreign clinical sites.

Dr. Wivel stated that historically the shipping of recombinant DNA reagents is not a minor issue. The RAC

finally got many restrictions removed, including the one that treated all recombinant DNA as pathogenic.

Ms. Rothenberg inquired how the unilateral change of the *NIH Guidelines* affect the countries adopting a BL2 classification. Dr. McGarrity explained that theoretically, the PIs can ship the recombinants from the United States as a BL1 agent, but Switzerland, Germany, and Sweden will not accept them. A clear statement within the *NIH Guidelines* would help convince the foreign countries to accept the same containment recommendation. In addition, a clear statement will assist local IBCs assigning a proper containment for these vectors.

Dr. Lysaught asked if changing the *NIH Guidelines* affects handling of these vectors at Good Manufacturing Practice (GMP) facilities. Dr. McGarrity explained it will not. GMP is required by FDA for manufacturing of the vectors for clinical use.

Dr. Hirschhorn questioned the appropriateness of including the statement concerning administration of the vectors under BL1 containment to the patients. Dr. Parkman said it is already a *de facto* practice to perform human gene transfer experiments using retroviral vectors under BL1 containment.

Dr. Walters said the volume of the vector preparations should be less than 10 liters; otherwise it will trigger the need for Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules.

Dr. Zallen said the aerosolized vectors would pose more safety concerns. Dr. McGarrity said the *NIH Guidelines* require aerosolized experiments to increase their containment level by one step, and the IBC is authorized to designate the appropriate containment for a particular experiment.

Committee Motion

Dr. Parkman made a motion to accept the statement proposed by Drs. McGarrity and Anderson for handling of retroviral vectors and to include the statement in the appropriate section of the *NIH Guidelines*. Dr. Erickson seconded the motion.

Ms. Rothenberg stated that the amendment of the *NIH Guidelines* will not solve the problem of packaging and shipping of these vectors to foreign countries that designate the vectors as BL2 agents. Dr. Parkman said the explicit statement of the *NIH Guidelines* will help persuade other countries to change their containment requirement. Ms. Rothenberg inquired why other agencies designate retroviral vectors as BL2 agents. Dr. McGarrity explained from his experience with IBC, that if there is no defined statement in the *NIH Guidelines*, the IBCs have a tendency to upgrade the containment requirement. The primary objective is to clarify the language of the *NIH Guidelines* and then to work for a uniform standard for IBCs in this country and regulatory agencies of other foreign countries.

The motion made by Dr. Parkman and seconded by Dr. Erickson to accept the following amendment to the *NIH Guidelines* in the form of a recommendation to ORDA:

"Murine retroviral vectors to be used for human gene transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under Biosafety Level 1 (BL1) containment."

The motion includes the recommendation that this amendment be inserted in the appropriate section of the *NIH Guidelines* by ORDA. The motion passed by a vote of 15 in favor, 0 opposed, and no abstentions

XIV. UPDATE REPORT FROM THE APPENDIX B SUBCOMMITTEE REGARDING CLASSIFICATION OF COMMONLY USED ANIMAL PATHOGENS/DR. SHIH

Presentation--Dr. Shih

In the absence of Dr. Straus (Chair of the Appendix B Subcommittee), Dr. Walters requested Dr. Shih (Executive Secretary of the Subcommittee) to present an update report regarding classification of the commonly used animal pathogens. At its June 9, 1995, meeting, the RAC recommended approval of the proposed amendments to Appendix B, *Classification of Etiologic Agents on the Basis of Hazard*, of the *NIH Guidelines* contingent on the development of a recommended list of animal etiologic agents that are commonly used in the laboratory or for human studies.

Dr. Shih stated that the original Appendix B is a 20 year old outdated document which classifies the etiologic agents according to the containment classes. The new Appendix B classifies the organisms into different Risk Groups based on the pathogenicity of the agents to healthy human adults, i.e., seriousness of the disease they cause and whether there are available preventive or therapeutic interventions. This Risk Group classification serves as an initial guidance to assign an appropriate containment level for a particular experiment by the IBC and the investigators. Since the new Appendix B is primarily concerned with human pathogenicity, it addresses only the human etiologic agents and leaves out all animal agents. This omission creates a problem because some of the animal agents, particularly the group of viruses known as oncogenic viruses, are frequently used as vectors for gene transfer in the laboratories or in human studies. The RAC recommended that this list of animal etiologic agents be identified as Appendix B-V, *Animal Viral Etiologic Agents in Common Use.* Most of these agents were previously listed as Class 2 oncogenic viruses in 2 separate categories of low and moderate risk agents in the original Appendix B. Since none of these animal etiologic agents are associated with disease in healthy human adults, one version of Appendix B-V is to list these agents as a single group recommended for BL1 containment and another version is to list them in a two-tier system for either BL1 or BL2 containment. Dr. Shih requested recommendation as to which version of the Appendix B-V was acceptable to the members of the Appendix B Subcommittee in a letter dated June 13, 1995. Drs. Andrew Braun, Donald Blair, and Dusty Miller preferred a single listing, while Dr. Stephen Straus, Dr. Diane Fleming, Ms. Linda Wolfe, and Ms. Gwladys Caspar opted to have a two-tier listing. Subsequent discussion with the members of the Subcommittee concluded that while there is no reason to have a separate group of "moderate" risk agents in this list, it is prudent to recommend conducting experiments under a BL2 containment with several agents that are capable of infecting human cells, e.g.,amphotropic and xenotropic murine leukemia virus. The completed Appendix B-V reads as follows:

Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy human adults; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses

Herpesvirus ateles
Herpesvirus saimiri
Marek's disease virus
Murine cytomegalovirus

Papovaviruses
Bovine papilloma virus
Polyoma virus
Shope papilloma virus
Simian virus 40 (SV40)

Retroviruses
Avian leukosis virus
Avian sarcoma virus
Bovine leukemia virus
Feline leukemia virus
Feline sarcoma virus
Gibbon leukemia virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Rat leukemia virus

Other Comments

Dr. Glorioso observed that some viruses in the moderate risk group can infect human cells but their replication used restricted to their animal hosts. Some viruses with oncogenes such as SV40 have been treated more cautiously than viruses without oncogenes. He would prefer a two-tier list. Dr. Wivel explained that listing a group of animal viruses as "moderate risk" agents introduces an inconsistency into Appendix B. Some strains of these viruses, although capable of infecting human cells, have not been shown to be associated with any disease in healthy human adults. They fall within the definition of Risk Group 1 agents, i.e., agents that are not associated with disease in healthy adult humans.

Drs. Chase and Parkman inquired why several viruses in the original Appendix B are not listed in the new version. Dr. Shih explained that several rarely used viruses such as chick embryo lethal orphan virus are deleted from the new list. The list includes commonly used organisms and it is not intended to be inclusive since most of other animal agents are not listed.

Dr. Parkman noted that infectivity toward human cells does not equate causing a clinical disease in humans. Dr. Ross remarked that description of some viruses as being a moderate risk implies that there is some risk involved, and it is not an accurate description. Dr. Wivel explained that description dates back to a 1974 classification of oncogenic viruses by the National Cancer Institute. At that time, the oncogenic potential of those agents was not well understood, and it was a cautious attitude. Dr. Ross asked for a verification of the statement that although a virus is capable of infecting human cells, it may not necessarily cause a human disease. Dr. Wivel said the statement is correct; these viruses are not human pathogens. Dr. Parkman noted studies demonstrating that people who have cats with leukemia (caused by feline leukemia virus) in their household have no increased incidence of leukemia in such families. Dr. Glorioso stated he would continue to recommend as a precaution, handling those viruses capable of infecting human cells under BL2 containment.

Dr. Walters stated that the consensus of the RAC is to accept the list of animal viruses in Appendix B-V as a reasonable modification of the Appendix B.

XV. CHAIR REMARKS

Dr. Walters stated that his term as RAC Chair has expired. Prior to his membership on the RAC, Dr. Walters was Chair of the RAC's Human Gene Therapy Subcommittee. He first attended a meeting as a member of the RAC in December 1992. It was during this meeting that the first 3 CF gene transfer protocols were reviewed by the RAC. At that time, the RAC had reviewed a total of 36 human gene transfer protocols.
January 1993 was the first RAC meeting at which Dr. Walters acted as Chair. It was during this meeting that Dr. Bernadine Healy (former NIH Director) addressed the RAC regarding her decision to grant approval on the single-patient expedited review protocol submitted by Drs. Sobol and Royston on a compassionate use basis. Dr. Healy approved Drs. Sobol and Roystons' protocol subsequent to RAC disapproval of the proposal.

Dr. Walters stated that the RAC has come a long way in a very brief period of time. It continues to examine its role and incorporate procedures to expedite protocol review and function more efficiently. Currently, 122 human gene transfer protocols have been submitted to the NIH. The NIH and FDA have recently adopted a consolidated review process which now allows certain protocols to be exempted from RAC review (monitored for adverse events and data management).

Dr. Walters thanked the ORDA staff for their dedication and the members of the RAC for the extraordinary time spent reviewing material for RAC meetings, and particularly the significant amount of time members spend between meetings providing expert advice. He stated that, "ORDA and the RAC exemplify the true spirit of public service."

Dr. Walters concluded his remarks by stating: "It is my hope that the early years of gene therapy will be held up as a model of how biomedical research should be conducted; there has been accountability every step of the way."

XVI. FUTURE MEETING DATE/DR. WALTERS

The next meeting of the RAC will be December 4-5, 1995, NIH, Building 31C, Conference Room 10.

XVII. ADJOURNMENT/DR. WALTERS

Dr. Walters adjourned the meeting at 12:00 p.m. on September 12, 1995.

Nelson A. Wivel, M.D.
Executive Secretary

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

Date: September 12, 1995

LeRoy B. Walters, Ph.D.

Chair
Recombinant DNA Advisory Committee
National Institutes of Health