DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
December 4-5, 1995

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-fourth meeting at 9:00 a.m. on December 4, 1995, at the National Institutes of Health (NIH), Building 31, Conference Room 10, 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 December 4 from 9 a.m. until 5 p.m. and December 5 from 9:00 a.m. until 12:30 p.m. The following were present for all or par of the meeting:

Committee Members:

Gary A. Chase, Georgetown University Medical Center
Patricia A. DeLeon, University of Delaware
Robert P. Erickson, University of Arizona
Rochelle Hirschhorn, New York University School of Medicine

> Michael M. C. Lai, University of Southern California
> 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
Harriet L. Robinson, University of Massachusetts Medical Center
Gail S. Ross, Cornell University Medical Center
Karen Rothenberg, University of Maryland School of Law
Bratin K. Saha, Emory University
R. Jude Samulski, University of North Carolina, Chapel Hill
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University
Stephen E. Straus, National Institutes of Health
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.

Non-Voting Representative:

Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Lewellys Barker, NIAID
> Bobbi Bennett, OD
Shan Chu, NHLBI
Felix DelaCruz, NICHD
Judith Greenberg, NIGMS
Jay Greenblatt, NCI
Yutaka Hanazono, NHLBI
Christine Ireland, OD
Nobuhisa Iwata, NHLBI
Ruth Kirschstein, OD
Becky Lawson, OD
Catherine McKeon, NIDDK
Richard Morgan, NCHGR
Shinichi Muramatsu, NHLBI
Sun Rui, NCI
Thomas Shih, OD
Lana Skirboll, OD
Debra Wilson, OD
Tian Zitigang, NCI

Others:

Paul Aebersold, Food and Drug Administration

Estuardo Aguilar-Cordova, Texas Childrens Hospital
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Cathy Bacquet, Viagene, Inc.
Kameron Balzer, Genentech, Inc.
Mark Batshaw, Children's Seashore House
Mary Helen Binger, GeneMedicine, Inc.
Bridget Binko, Cell Genesys
John Bishop, Food and Drug Administration
Keith Black, University of California, Los Angeles
Arindam Bose, Pfizer Central Research
Andrew Braun, Massachusetts General Hospital
Francis Burrows, Chiron Corporation
Lynn Butler, The Wellcome Foundation
Rachel Carle, Genzyme Corporation
Ira Carmen, University of Illinois
Yung-Nien Chang, Genetic Therapy, Inc.
Yawen Chiang, Genetic Therapy, Inc.
Kenneth Culver, Oncorpharm, Inc.
Robert DeJager, RGene Therapeutics, Inc.
Jean-Sylvain Demelier, Rhone-Poulenc Rorer
Donald Drake, The Philadelphia Inquirer
Nathalie Dubois-Stringfellow, Chiron Corporation
Michelle Durand, The French Consolate
Julie Ely, Johnson & Johnson
Suzanne Epstein, Food and Drug Administration
John Fletcher, University of Virginia
Carolyn Gregory, Human Gene Therapy Research Institute
Lowell Harmison, Public
Donna Haseley, Inside Washington Publishing
James Hawkins, Public
Gabriel Hortobagyi, MD Anderson Cancer Center
Math Hukkelhoven, Sandoz Pharmaceuticals
Mien-Chie Hung, MD Anderson Cancer Center
Edie Irvine, Genetic Therapy, Inc.
Michael Kaleko, Genetic Therapy, Inc.
Ryuji Kawaguchi, SRL, Inc.
Dawn Kayda, Genetic Therapy, Inc.
Scott Kennedy, Pfizer Central Research
Connie Kirby, Canji, Inc.
Karen Kozarsky, University of Pennsylvania
Steven Kradjian, Vical, Inc.
Michael Langan, National Organization for Rare Disorders
Fred Ledley, GeneMedicine, Inc.
Gloria Lee, GenCell
Martin Lindenberg, RGene Therapeutics, Inc.
Charles Link, Human Gene Therapy Research Institute
Gabriel Lopez-Berestein, MD Anderson Cancer Center
Tony Marcel, TMC Development
Alan McClelland, Genetic Therapy, Inc.

R. Scott McIvor, University of Minnesota
Keith McRoberts, Human Gene Therapy Research Institute
Andra Miller, Food and Drug Administration
Susan Nemeth, Schering-Plough Research Institute
Naoyuki Nemoto, SRL, Inc.
Andrea Neuman, Technology Catalysts
Carol Ohmstede, Glaxo Wellcome, Inc.
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Microbiological Associates, Inc.
John Park, University of California, San Francisco
Toni Pitnam, Public
Doros Platika, Progenitor, Inc.
Jesus Prieto, University of Navarra, Spain
M. Lynn Pritchard, Glaxo Wellcome, Inc.
Raj Puri, Food and Drug Administration
Cheng Qian, University of Navarra, Spain
Joseph Rokovich, Somatix Therapy Corporation
Juan Ruiz, University of Connecticut Health Center
Patricia Ryan, Genetic Therapy, Inc.
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International, Inc.
Nevin Summers, Novation
Geoff Symonds, Johnson & Johnson
Mary Treuhaft, RGene Therapeutics, Inc.
Stanley Tucker, Aronex Pharmaceuticals, Inc.
Dominick Vacante, Magenta Corporation
Lisa White, The Blue Sheet
James Wilson, University of Pennsylvania
Robert Zimmerman, Chiron Corporation

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

Dr. LeRoy Walters (Chair) called the meeting to order and stated that due notice of the meeting and proposed actions to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* were published in the *Federal Register* on November 15, 1995 (60 FR 57528). 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 Harriet L. Robinson, Ph.D., Professor, Department of Pathology, University of Massachusetts Medical Center, Worcester, Massachusetts, as a new member of the RAC.

Dr. Walters noted that since the September 11-12, 1995, RAC meeting, 11 protocols have been exempted from RAC review (solely reviewed by the Food and Drug Administration (FDA)). He noted that the average turnaround time for the exempt decision by the Office of Recombinant DNA Activities (ORDA) is 14.9 calendar or 10.6 working days. It is well within the 15 working days

required by the *NIH Guidelines.*

One new and two resubmitted protocols will be reviewed at this RAC meeting. There will be a report from the *Ad Hoc* Review Committee (Chaired by Dr. Inder Verma), discussion of the RAC's future function, and a presentation on ethical issues associated with *in utero* gene therapy.

Dr. Walters noted several recent publications of clinical data from human gene transfer studies: *T Lymphocyte-Directed Gene Therapy for ADA(-) SCIDVPC: Initial Trial Results After 4 Years* by Dr. R. Michael Blaese and his colleagues (*Science*, Vol. 270, p. 475-480, 1995), *Gene Therapy in Peripheral Blood Lymphocytes and Bone Marrow for ADA(-) Immunodeficient Patients* by Dr. Claudio Bordignon and his colleagues (*Science*, Vol. 270, p. 470-475, 1995), and *Engraftment of Gene-Modified Umbilical Cord Blood Cells in Neonates with Adenosine Deaminase Deficiency* by Dr. Donald B. Kohn and his colleagues (*Nature Medicine*, Vol. 1, p. 1017-1023, 1995).

Dr. Walters reported that on October 3, 1995, President Clinton accepted a report of the Committee on Human Radiation Experiments which reviewed the experiments conducted by the U.S. Department of Energy and reviewed the conduct of human clinical research in general. On the same day, President Clinton issued an executive order to establish the National Bioethics Advisory Commission to introduce new policies regarding research in human biology and a review of ongoing government research projects.

Dr. Walters noted that a survey of Worldwide Gene Therapy Clinical Trial Enrollment Status has been conducted by Drs. Tony Marcel and J. David Grausz (TMC Development).

II. DATA MANAGEMENT/DR. WALTERS

Exempt Protocols

Dr. Walters summarized 11 protocols that were submitted to ORDA and determined to be exempt from RAC review (Attachment II-Sole FDA Review). The exempt protocols reviewed by the ORDA staff are as follows:

Protocol #9509-126 by A. P. Chen, *A Phase I Study of Recombinant Vaccinia Virus that Expresses Prostate Specific Antigen in Adult Patients with Adenocarcinoma of the Prostate*;

Protocol #9509-127 by Michael J. Welsh and Joseph Zabner, *Cationic Lipid Mediated Gene Transfer of CFTR: Safety of a Single Administration to the Nasal Epithelia*;

Protocol #9510-128 by David J. Cole, *Phase I Study of Recombinant CEA Vaccinia Virus Vaccine with Post Vaccination CEA Peptide Challenge*;

Protocol #9510-129 by Marie Roskrow et al., *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T-Lymphocytes as Therapy for Patients Receiving a Bone Marrow Transplant for Relapsed EBV-Positive Hodgkin's Disease*;

Protocol #9510-130 by Marie Roskrow et al., *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T-Lymphocytes to Patients with Relapsed EBV-Positive Hodgkin's Disease*;

Protocol #9510-131 by Elizabeth Connick and Steven G. Deeks, *A Randomized, Controlled, Phase II Study of the Activity and Safety ofAutologous CD4-Zeta Gene-Modified T Cells in HIV-Infected Patients*; and

Protocol #9510-132 by David Paulson and H. Kim Lyerly, *A Phase I Study of Autologous Human Interleukin-2 (IL-2) Gene Modified Tumor Cells in Patients with Locally Advanced or Metastatic Prostate Cancer.*

The following are exempt protocols which have been reviewed by one or more RAC members:

Protocol #9511-133 by Malcolm Brenner et al., *Phase I Study of Cytokine Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma Using an Adenoviral Vector* (reviewed by Drs. Parkman, Chase and Ms. Meyers);

Protocol #9511-134 by Mark J. Gilbert et al.,*Phase I Study to Evaluate the Safety and In Vivo Persistence of Adoptively Transferred Autologous CD4(+) T Cells Genetically Modified to Resist HIV Replication* (reviewed by Drs. Lai, Robinson, and Secundy);

Protocol #9511-135 by Ronald D. Alvarez and David T. Curiel, *A Phase I Study of Recombinant Adenovirus Vector-Mediated Intraperitoneal Delivery of Herpes Simplex Virus Thymidine Kinase (HSV-TK) Gene and Intravenous Ganciclovir for Previously Treated Ovarian and Extraovarian Cancer Patients* (reviewed by Drs. Smith, Glorioso, and Ross); and

Protocol #9511-136 by Cassian Yee and Philip D. Greenberg, *Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Autologous Unmodified and Genetically Modified CD8(+) Tyrosinase-Specific T Cells in Patients with Metastatic Melanoma* (reviewed by Drs. Saha, DeLeon, and Lysaught).

Dr. Walters noted that the NIH/FDA consolidated review procedure greatly facilitated the review process and allowed the RAC to focus on major issues surrounding gene transfer research.

Adverse Events

Dr. Walters noted that several adverse events have been reported by the investigators since the last RAC meeting. In Protocol #9303-037 (Van Gilder), a brain tumor protocol, the investigators reported a Grade 4 adverse event directly related to the administration of the PA317/G1Tk1SvNa.7 vector producer cells (VPC) into the Ommaya reservoir. Adverse events were reported in Protocol #9312-063 (Sznol), a melanoma protocol, and Protocol #9403-069 (Walker), a protocol studying HIV-infected identical twins. Dr. Smith commented that most of these adverse events were extensions of those that have been reported before and were due to the underlying diseases or complications of injections into the brain of VPC.

Dr. Ross inquired if the brain tumor protocols have been modified to avoid the continuous problems of adverse reactions. Ms. Debra Knorr (NIH) pointed out that in Protocol #9306-050 (Raffel) for brain tumors, the Ommaya reservoir has been eliminated from the surgical procedure, and patients will receive a single intra-operative injection of VPC (an amendment dated August 29, 1995). Ms. Meyers inquired if all other investigators are aware of the adverse events. Dr.Wivel responded that all investigators have been notified since this study is essentially the same protocol expanded to multiple sites. Dr. Marcel stated that he will include the protocol amendment in his newsletter to alert other investigators in Europe.

Amendments and Total of Protocols

Dr. Walters noted that a list of amendments to human gene transfer protocols (Attachment III) and an updated list of human gene transfer protocols (Attachment IV) are included in the meeting materials. A total of 136 protocols have been approved by the RAC or forwarded to the FDA, including 109 gene therapy and 27 gene marking protocols.

Discussion

Dr. Secundy inquired if the investigators submit copies of revised Informed Consent documents related to protocol amendments to ORDA or FDA. Drs. Nelson Wivel (NIH) and Philip Noguchi (FDA) responded that if the revision is minor, such amended documents usually are not submitted to the NIH or FDA. Dr. Secundy suggested that a statement should be included in the original Informed Consent document to inform the subjects that amendments might occur which would not necessarily be reflected in future revised documents. Ms.Knorr noted that "Modifications Related to Gene Transfer" is considered a category of experiments to be exempt from full RAC review in the *NIH Guidelines*. Dr. Walters noted that there should be a mechanism to inform the patients regarding substantial changes of the protocols, i.e., not using an Ommaya reservoir. Dr. Smith stated that the local Institutional Review Board (IRB) is charged with the primary responsibility for overseeing protocol amendments and for ensuring that the revised Informed Consent documents are presented to future participants. Dr. Zallen noted that Appendix M-III-B-2-b, *Long-Term Follow-Up*, of the *NIH Guidelines* includes a statement requiring subjects to be informed as part of the long-term follow-up of any changes in the experimental procedure or any adverse events. Dr. Noguchi noted that FDA regulation states that each patient is authorized to obtain any informed consent document revision

or any adverse event report related to his/her treatments.

Dr. Hirschhorn was concerned about the nature of the amendment to the protocols. Sufficient information is needed to determine if all these amendments are minor technical changes. Regarding the issue of disseminating the information of adverse events, Dr.Hirschhorn noted that the publication of adverse events in scientific journals such as the vector-induced inflammation encountered in the cystic fibrosis (CF) protocols serves as a good example to widely inform investigators in the field both in this country and abroad. Ms. Meyers said that it is important to have the information widely available throughout the world since scientific experiments go beyond the border of a single country.

Dr. Walters summarized two issues raised in the discussion: (1) a system for feedback on adverse events, and (2) a mechanism to handle major protocol amendments.

Regarding the issue of protocol amendment, Dr. Smith said the local IRB is entrusted to ensure that patients are properly informed about the changes; however, he insisted that the FDA has the responsibility to ensure that the amendment is scientifically and medically valid. Dr. Noguchi stated that public discussion of adverse events in CF studies has greatly facilitated the FDA in communicating with all the sponsors of the trials to adjust their protocols. There are now 35 patients being treated with similar adenovirus vectors, and none of the patients suffered any adverse effects since the first report.

Ms. Knorr noted that the *NIH Guidelines* can be amended to require mandatory reporting of adverse events in Appendix M-VIII, *Reporting Requirements-Human Gene Transfer Protocols*. Dr. Smith credited the late Dr. Leventhal for initiating the idea to create the current system of data management to widely disseminate information regarding the progress of gene transfer protocols which includes reporting of adverse events before any full publication of the studies in scientific journals. Ms. Meyers noted the public nature of RAC discussion is absolutely essential. Dr. Noguchi agreed that without the public disclosure of adverse events of CF protocols, FDA would not be able to readily persuade other investigators to adjust their adenovirus vector dosage in their studies. Alternatively, protocols would have been placed on clinical hold until the issue was resolved.

Dr. Ross asked how FDA would address the issue of the Ommaya reservoir in the brain tumor protocols. Dr. Noguchi responded that FDA is working constantly with the sponsor of the central facility to develop different approaches to avoid further adverse events, e.g., using tracer dye techniques to determine leakage of administered VPC and pretreatment with antiseizure medication to ameliorate the symptoms.

III. MINUTES OF THE SEPTEMBER 11-12, 1995, RAC MEETING/DRS. CHASE AND DELEON

The RAC approved a motion made by Dr. DeLeon and seconded by Dr. Chase to accept the September 11-12, 1995, RAC minutes (with the incorporation of minor editorial changes) by a vote of 16 in favor, 0 opposed, and no abstentions.

IV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I STUDY OF THE SAFETY OF INJECTING MALIGNANT GLIOMA PATIENTS WITH IRRADIATED TGF-+2 ANTISENSE GENE MODIFIED AUTOLOGOUS TUMOR CELLS*/DRS. BLACK AND FAKHRAI

Review--Dr. Samulski

Dr. Walters called on Dr. Samulski to present his primary review of the protocol submitted by Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California. Dr. Samulski stated that the protocol was reviewed and deferred at the March 6-7, 1995, RAC meeting due to lack of sufficient preclinical data. The RAC stipulated that the investigators and the primary reviewers would agree on a mutually acceptable experiment designed to address the scientific questions posed by the RAC reviewers. Once these studies had been conducted, the investigators were required to submit this data to the full RAC for review and approval. In a letter dated August 9, 1995, Dr. Fakhrai responded to the RAC review stating that the investigators have: (1) established 10 new human glioma cell lines; (2) constructed a new TGF-J2 antisense vector utilizing the human TGF-J2 sequence; (3) genetically modified 4 human glioma cell lines with the TGF-J2 vector; and (4) proposed an experimental design to demonstrate blocking of TGF-J2 production in gene-modified glioma cells persisting following irradiation. In a letter dated August 18, 1995, Dr. Ginsburg stated that he has reviewed the proposal, and that the general experimental design sounds reasonable and should address most of the issues raised in the initial RAC review.

Dr. Samulski stated that the overall strategy of the protocol is to inject gene-modified tumor cells into malignant glioma patients whose tumor cells overexpress TGF-J2. The objective is to use an antisense approach to modify these autologous tumor cells to reduce the amount of TGF-J2 production in expectation that the gene-modified tumor cells will elicit a more productive antitumor immune response. Dr. Samulski stated that there is no major safety concern regarding this protocol. Gene modification will be performed with an Epstein-Barr virus (EBV) based plasmid vector, pCEP-4/TGF-J2 antisense. After transduction, the autologous primary tumor cells will be monitored for TGF-J2 production. If the TGF-J2 production is reduced more than 50% after transduction, the transduced cells, after lethal irradiation, will be injected into patients during the vaccination procedure.

Dr. Samulski expressed his concern about the study rationale. The investigators plan to isolate the TGF-J2 negative cells from the primary tumor cell cultures and to use them only for blood studies. If the objective of this proposal is to genetically modify TGF-J2 positive cells to cells with reduced TGF-J2 expression for re-administration into the patient, why are the investigators discarding the naturally occurring TGF-J2 minus tumor cells? The identical clinical trial can be achieved using naturally isolated TGF-J2 negative cells in their vaccination program without any genetic modification. The investigators responded in writing that all of their preclinical data was generated with gene-modified cells, and they have no equivalent preclinical data to propose a clinical study using the naturally occurring TGF-J2 minus cells.

Dr. Samulski was concerned about using tumor cells exhibiting a variety of TGF-J2 levels in patient vaccination and the difficulty of interpreting such data.

Dr. Samulski asked the investigators to elaborate on the time frame of the experiment. How much time it would take to isolate the primary tumor cells from patient biopsies, totransduce the cells, to demonstrate 50% reduction of TGF-J2 production, to irradiate the cells, and to assay the cells for viability? Is the time frame compatible with the protocol flow chart to re-administer these cells to the patient? Are the number of cells produced in tissue culture at the end of the procedure adequate for administration to patients? The investigators responded in writing that in the interest of time and preservation of gene-modified cells, irradiation studies would be performed only on the non-modified cells. Dr. Samulski noted that sensitivity of non-modified cells to lethal irradiation may be different from that of gene-modified cells.

Dr. Samulski stated that the protocol does not present any major safety concerns, and he recommends approval with clarification of the questions raised.

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

Dr. Samulski stated that according to Dr. Ginsburg's written review, there are a number of improvements in the current protocol which address most of the issues raised by Dr. Ginsburg and other reviewers in the previous RAC discussion. However, several points would still benefit from additional detail:

1. The investigators have switched to a new TGF-J antisense vector containing human sequences in place of the simian cDNA of the original proposal. In addition, the investigators have added further controls to ensure the adequacy of the planned radiation dose in eliminating the potential for injection of viable cells. The investigators have addressed the criticisms of the interleukin (IL)-2 portion of their protocol by eliminating those experiments from the revised proposal and limiting their study to the use of theTGF-J antisense vector. This simplified design is a significant improvement in the protocol and is more consistent with the promising preliminary animal data.

2. The criteria for patient selection are still vague. The investigators stated that only patients whose tumors express TGF-J2 will be enrolled in the study and only modified tumor cell lines expressing less than 1 ng/106 cells/24 hours will be injected into the patient. What is the cutoff for a significant level of TGF-J2 in the initial patient plasma or tumor sample? What level of expression prior to transfection will be required to qualify a cell line for modification? Is there any point in transfecting cell lines with spontaneously reduced TGF-J2 expression? What degree of TGF-J2 suppression by the antisense transgene will be required before the modified tumor cells are injected?

3. The details of the investigators' previous success in establishing cell lines from patients and achieving effective antisense blockade of TGF-J2 expression are still only vaguely described. Dr. Ginsburg requested a straightforward and complete description. How many cell lines have been attempted and how many have been successful? What has been the average and range of time

spans from obtaining the initial biopsy until successful establishment of the cell line? What has been the magnitude of the antisense effect in these transfected cells lines (averages and ranges)?

Although the probability of clinical success with this approach is low, the preliminary animal data are reasonable and the human protocol presents minimal risk to the patient. Dr. Ginsburg would favor approval of the protocol if the investigators respond to the questions adequately.

Review--Ms. Meyers

Ms. Meyers stated that the Informed Consent document is acceptable. If the IL-2 arm of the study is to be deleted from the protocol, the statement regarding IL-2 treatment arm should be omitted from the Informed Consent document.

Other Comments

Dr. Smith asked the investigators to clarify the data presented in their written response regarding establishment of primary brain tumor cell culture from tumor resections. What is the success rate in establishing primary glioma cultures? What is the cell doubling time of the glioma cell culture? Will the cell cultures be expanded in a timely fashion to allow an adequate cell dose to be administered to patients? Regarding the question of the magnitude of the antisense effect in the transfected cell lines, the investigators stated in written response that they are able to block TGF-J2 expression up to 80% in some gene-modified cells. Dr. Smith asked about the average and the lowest levels of inhibition?

The investigators stated in written response that the lower limit of inclusion criteria for the transduced tumor cells is 200 pg TGF-J2/106 cells/24 hours. Dr. Saha remarked that the immunostaining technique to assay the expression level is inadequate for this purpose. Is there any alternative assay? Dr. Saha noted discrepancies between preclinical animal data and the proposed human trial pertaining to the degree of TGF-J2 suppression required to inhibit tumor growth (800 vs. 200 pg TGF-J2/106 cells/24 hours) and the cell dosage (106 vs. 105 cells without being adjusted for body weight differences). Dr. Samulski added that it is difficult to draw any definitive conclusion from the study that does not have a specific cutoff level of TGF-J; the 50% cutoff level of TGF-J suppression proposed by the investigators will result in inoculating patients with autologous tumor cells expressing different levels of TGF-J2 since their initial levels are different.

Investigator Response--Drs. Black and Fakhrai

In response to Dr. Samulski's question of why the investigators were not using the tumor cells expressing TGF-J2 to test the hypothesis in the present study, Dr. Black explained that this type of experiment has not been substantiated by a proper animal study. The investigators found that in the rat glioma model, vaccinating the animals bearing growing tumors with a tumor vaccine derived from a tumor cell of initially high level of TGF-J2 expression and subsequent suppression of its production with an antisense vector, one observed a significant antitumor effect. Such an

experiment has not been performed with a tumor cell that is not producing TGF-J2 initially. There are reasons to speculate that such TGF-J2 minus cells may not produce a similarantitumor effect since there are other mechanisms which would prevent them from being effective in immunization such as production of insulin-like growth factor and differences in the IL-2 receptor. Dr. Black stated that it is preferable to initiate a clinical study supported by preclinical data. The investigators are performing an ongoing experiment to compare the results from the animal model using tumor cells that are either high or low producers of TGF-J2.

Regarding the time frame issue of whether there is enough time to establish the cell culture, to transduce the cells, to obtain data of inhibition of TGF-J production, and then to sufficiently expand the cell culture to inject into patients, Dr. Black said these factors have been taken into consideration in designing the protocol. The investigators will select a patient population with median survival of about 9 months. Tissue specimens will be obtained at the time of surgery, and the total time required to establish the cell culture (4 weeks) and transduction (4 weeks) is 2 months. There is enough time to initiate immunization treatment following a standard 4 weeks of radiation therapy on these patients.

Dr. Black responded to other questions raised by RAC members: Irradiation does not affect the level of TGF-J2 inhibition by the antisense vector; the Informed Consent document will be modified in accordance with elimination of the IL-2 arm of the protocol; a bioassay rather than an immunostaining technique is to be used to determine the level of TGF-J2 production in the transduced cells.

Dr. Samulski remarked that the total pool oftransduced cells without clonal selection will be used for the treatment. Such population of cells will include cells exhibiting a high degree of TGF-J2 suppression, as well as cells showing little inhibition.

Responding to questions regarding the cutoff level (inclusion criteria) for the transduced cells, Dr. Black said that from his laboratory experience, it is difficult to inhibit TGF-J production lower than 200 pg TGF-J2/106 cells/24 hours. Dr. Black stated that the reason to select the criterion of 50% inhibition rather than an absolute number for TGF-J2 production is that there is variability of TGF-J production within human brain tumor cells. The ability of the vector to inhibit TGF-J production is a variable in different types of tumor cells. The hypothesis which the investigators are testing in the present protocol is based on preclinical studies of a beneficial antitumor effect found in experiments based on a percentage reduction of TGF-J2 production in transduced tumor cells.

Dr. Samulski noted that the study will be complicated by the fact that the transduced cell population is not homogeneous with respect to the level and degree of inhibition of TGF-J production. He suggested designing the experiment with a known mixture of cells with a definitive degree of inhibition, e.g., 10% of nonproducers with 90% of full producers. Dr. Black said the biological interaction of the system may be more complex than just the simple TGF-J phenomenon as suggested in his published studies. The tumor cells exhibiting high levels of TGF-J2 production may use different mechanisms to block the immune response from tumor cells with low expression levels.

Dr. Samulski asked what percentage of cells transduced by the square wave electropolation technique eventually are enriched in the cell culture. Dr. Fakhrai responded that the transfected cells are selected by hygromycin-B and all cells surviving in the culture will be gene-modified. Dr. Fakhrai explained that he chose not to use the TGF-J nonproducing cells because in the colorectal carcinoma model system in animals, the nonproducer CD26 cells do not protect the animals against subsequent tumor challenge.

Dr. Fakhrai presented data to address issues raised by RAC members. The standard curve for the TGF-J2 assay is nonlinear below the level of 200 ng/106 cell/24 hours and it cannot be used to accurately determine the factor amount; therefore, the lower limit is chosen at that level. He presented data on tumor cells isolated from 3 patients: (1) cells from Patient #1 produced 1ng TGF-J2/106 cells/24 hours, and its level is downregulated by the vector to more than 80%; (2) cells from Patient #2 produced 2 ng, and its level downregulated to 50%; (3) cells from Patient #3 produced only 200 pg with no appreciable inhibition produced by the antisense vector. Similarly, no inhibition was observed in another experiment using the antisense oligonucleotides in the low producer cells. Dr. Fakhrai stated that a similar strategy ofdownregulating TGF-J2 is effective in colorectal cancer. If the present human study ofglioma is successful, the same strategy can be extended to other types of cancers such as lung, ovary, prostate, and colon.

Dr. Black said that if the present strategy of using high TGF-J2 producers has proved to be effective, he will consider using lower or nonproducer tumors in future studies.

Dr. Samulski asked whether the lethal irradiation dose will be determined using nonmodified tumor cells. Will there be different radiation sensitivity for the transduced cells selected by hygromycin-B than the nonmodified cells? Dr. Black responded that the available data suggest that this issue is not a concern since the level of TGF-J2 production is not affected by irradiation.

Dr. Samulski noted that the protocol was initially recommended by FDA not requiring RAC review; the present RAC review has pointed out several insightful scientific questions and has suggested useful alterations of the protocol to the investigators. Would FDA provide similar suggestions to the investigators if the protocol was not reviewed by the RAC? Dr. Noguchi responded that the initial submission of the protocol is premature, and the types of questions raised by the RAC have improved the protocol. The FDA would have attempted to improve the quality of the protocol in the same manner.

Dr. Saha remarked that the investigators have not adequately responded to his questions regarding the proportionality of cell dosage between the rat and human experiments, and why the cutoff level of 200 ng was chosen. Dr. Fakhrai responded that in the initial immunization, the antigen dose is not critical as long as the antigen is recognizable by the immune system. The cell dose of 5 or 10 x 106 cells proposed for the human studies is sufficient to reactivate the sensitized T-cells.

Dr. Lai asked if the antisense inhibits TGF-J2 *in vivo* as well as that observed *in vitro* in tissue culture, and if the degree of suppression is stable *in vitro* as well as *in vivo.* The point is significant

since injecting patients with cells which resume high level TGF-J2 production is contrary to the purpose of the experiment. Dr. Black responded that TGF-J2 suppression has been observed to persist for months in tissue culture experiments. The question of *in vivo* TGF-J2 production is difficult to answer since it is technically difficult to measure the factor production of cells *in vivo*. Using an approximate method of immunohistochemical staining, it is estimated that similar inhibition is present *in vivo*. The matter is even more complicated since the TGF-J2 production levels of glioma cells in patients fluctuate in response to factors such as an immune stimulus.

Dr. Walters asked Dr. Samulski if he was satisfied with the answers provided by the investigators. Dr. Samulski stated that considering the variability of TGF-J2 levels in different patients, it is reasonable to select a cutoff point of 50% reduction rather than trying to set an arbitrary number of TGF-J level. The investigators' response regarding lethal irradiation is satisfactory. Most of the questions related to safety have been responded to by the investigators. The protocol attempts to answer an important scientific question of whether immunization with tumor cells with reduced TGF-J2 production will have an impact on the patient's immune response to a tumor. It will be interesting if a measure can be devised to determine whether vaccination with gene-modified tumor cells will affect the TGF-J2 production of nonmodified cells within the tumor mass. Dr. Black said it is possible to perform the TGF-J2 immunohistochemical assay on tumor specimens obtained from patients undergoing re-operation.

Dr. McGraw said it is useful to obtain such data on TGF-J2 levels of tumor cells during both pre- and post-treatment periods within each individual patient.

Dr. Black agreed to revise the protocol by including a cutoff requirement of a minimum of 50% inhibition of TGF-J2 expression by gene-modified autologous tumor cells and by including a study using the immunohistochemical assay to obtain information regarding TGF-J2 levels of cells in tumors during both pre- and post-immunization periods.

Committee Motion

Dr. Samulski made a motion to accept the protocol contingent on revising the protocol, including statements regarding the 50% cutoff and to acquire information of pre- and post-treatment levels of tumor cell TGF-J2 levels. Dr. Lysaught seconded the motion. Dr. Zallen made a friendly amendment to the motion to delete the statement regarding the IL-2 arm of the study from the Informed Consent document. Drs. Samulski and Lysaught accepted the friendly amendment.

The motion made by Dr. Samulski and seconded by Dr. Lysaught to accept the protocol submitted by Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California, was contingent on review and approval of the following by the primary RAC reviewers: (1) a revised protocol to include a cutoff requirement of a minimum of 50% inhibition of TGF-J secretion by gene-modified autologous tumor cells, (2) a revised protocol to include a study of pre- and post-treatment levels of TGF-J2 expression of cells in a tumor mass, and (3) an Informed Consent document deleting the statement regarding the IL-2 treatment arm of the study. The motion was approved by a vote of 16 in favor, 0 opposed, and no abstentions.

Protocol Summary

Drs. Keith L. Black and Habib Fakhrai of the University of California, Los Angeles, California, may conduct gene transfer experiments on 12 subjects (³18 years of age) with glioblastoma multiforme. An EBV based plasmid vector, pCEP-4/TGF-J2 antisense, encoding antisense RNA will be used to inhibit TGF-J2 production. Tumor samples obtained from the patients at the time of clinically indicated surgery will be grown in culture to establish a cell line for each patient. The patients' tumor cells will be genetically altered with the pCEP-4/TGF-J2 vector to inhibit their secretion of TGF-J. Following completion of the traditional post surgical radiation therapy, the first cohort of patients will receive, at 3 week intervals, 4 injections of 5 x 106 irradiated gene-modified autologous tumor cells. Subsequently, in dose escalation studies, the second cohort will receive 1 x 107 cells, and the third cohort, 2 x 107 cells. The results of this Phase I trial will be used to assess the safety of this form of gene therapy and may provide preliminary data to evaluate the potential utility of TGF-J2 antisense gene therapy in the management of gliomas.

V. CHAIR REMARKS/DR. WALTERS

Dr. Walters noted that Drs. Tony Marcel and David Grausz have compiled a list which in entitled: *Worldwide Gene Therapy Clinical Trials Enrollment Status*. Human gene therapy trials have been conducted in 15 countries worldwide including the United Kingdom, Switzerland, China, Canada, Egypt, France, Germany, Israel, Italy, Japan, Holland, Spain, Sweden, and the United States. Dr. Marcel stated that there are formal review mechanisms for human gene therapy existing in the United Kingdom, France, Japan, and the United States. Only in the United States is there a public review forum, and Dr. Marcel attributed the rapid advance of the field in this country to the public review policy.

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

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. Gabriel Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung of the University of Texas, MD Anderson Cancer Center, Houston, Texas. The protocol was initially reviewed and deferred at the September 11-12, 1995, RAC meeting, 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.

Dr. Motulsky stated that patients with advanced metastatic breast and/or ovarian cancer who

overexpress the HER-2/neu oncoprotein will be treated by injection into the pleural or peritoneal cavity with a plasmid DNA/liposome complex containing the adenovirus E1A gene. The adenovirus E1A gene is a tumor suppressor that has been shown to inhibit expression of HER-2/neu oncogene in rodent and human breast cancer cells. No viral vectors will be used in the studies. Depending upon toxicity results, 15 to 30 patients will be investigated in 8 to 12 months. Dr.Motulsky commented on the following specific issues raised in the previous RAC review:

1. Clarification of experimental design. Patients with both breast and ovarian cancer who have pleural effusions and ascites will be treated. Three patients (presumably with either ovarian or breast cancer) will be treated at 4 different dosage levels (employing 100% increments) until drug-related toxicity of less than grade 2 is detected. Dose escalation at 25% increments will be conducted. Six patients will be studied at the maximum tolerated dose. Levels for the maximum biologically active dose and for the maximum tolerated dose will be determined for breast and ovarian cancers separately.

The exact titration of the dose and dose modification appears complex. If the FDA with their extensive experience of cancer Phase I protocols approves the dosing scheme proposal, Dr. Motulsky said he would agree to it. It is unclear why the investigators do not conduct studies for breast and ovarian cancer separately. Their cover letter of submission seems to indicate that the investigators intend to conduct the studies separately; however the protocol does not clearly make this distinction.

2. Assessment of *ex vivo* transduction efficiency. Cells from ascites fluid (10% tumor cells, 20% lymphocytes, and 70% endothelial cells) were transfected with beta galactosidase expressing plasmid using the delivery system planned for their studies. Five to 10% transduction efficiency for tumor cells was observed. On repeat exposure *in vivo* in the nude mice model with ovarian and breast cancer, transduction rates of 20 to 30% were observed. These results appear to be satisfactory.

3. Sensitivity of *in vitro* assays. A published paper (Zhang, et. al., *Oncogene, 10*, 1947-1954, 1995) documents that the proposed immunochemical methods are adequate to demonstrate expression of E1A, as well as suppression of the HER-2/neu gene expression.

4. Possible spread to gonadal tissues. Dr. Motulsky could not locate any data regarding gonadal assays in mice. Such data should be provided.

5. Informed Consent document. Dr.Motulsky would defer to Dr. Zallen in view of her earlier critique followed by extensive discussion on this issue during the previous RAC review.

Dr. Motulsky stated that during the last RAC review, the question of E1A being an oncogene rather than a tumor suppressor gene was raised. Following the RAC discussion, Dr. Motulsky was satisfied that E1A is *not* an oncogene. Dr. Motulsky would recommend approval of the protocol after the issues raised by the reviewers have been satisfactorily resolved.

Review--Dr. Zallen

Dr. Zallen stated that Dr. Motulsky has covered many points she raised in her written review. With regard to the Informed Consent document, there were extensive discussions during the last RAC meeting, and Dr. Zallen noted that the Informed Consent document submitted has been greatly improved. Dr. Zallen stated that the revised Informed Consent document from MD Anderson Cancer Center could become a model for future Informed Consent documents. Dr. Zallen made a few suggestions for the Informed Consent document, and these changes have been incorporated in the revised document. Dr. Zallen asked the investigators to address the issue of whether there is any permanent spread of the plasmid DNA sequences to the gonadal tissues.

Other Comments

Dr. Saha noted that the RAC has previously approved a protocol by Drs. Curiel and Alvarez (Protocol #9509-124) that targets the erbB-2 (HER-2/neu) oncogene of ovarian cancer using an anti-erbB-2 single chain antibody strategy. In this protocol, the investigators invoked restoration of apoptosis of tumor cells as the underlying mechanism for this treatment strategy. Dr. Saha asked if a similar mechanism would be operative in the present protocol.

Dr. Samulski asked if there is any effort to develop a vector with a tissue-specific promoter to restrict the expression of E1A to tumor cells. He was concerned about expression of E1A in normal cells. E1A interacts with the RB tumor suppressor gene, and genes for cellular p300 and p160 proteins in addition to downregulating the HER-2/neu oncogene. When the RAC approves a protocol, there is a tendency for other investigators to assume that permission has been granted to use this strategy for various other purposes. There are potential serious consequences in transducing the complicated adenoviral E1A gene to normal as well as tumor cells.

Dr. Robinson stated that E1A is a viral tumor antigen, and she asked if the issue has been discussed in the previous review. Dr. Motulsky responded that the issue has been raised by primary reviewers during the last RAC review, and it has been extensively discussed. He was satisfied that E1A acts as a tumor suppressor in the breast and ovarian cancers. Dr. Robinson was concerned that the complexity of this issue needs to be further discussed. E1A is the T antigen of a DNA tumor virus. It can interact with host cell tumor suppressor genes that regulate the cell cycle, and it can promote cell growth. It is a predisposing step toward prompting the cell to a malignant stage. Dr. Robinson emphasized that E1A is not simply a tumor suppressor gene.

Dr. Samulski agreed that Dr. Robinson has raised a serious concern. He suggested that the investigators consider constructing a vector with a tissue-specific promoter to express the E1A gene. E1A provides a first step in inducing the cell to enter the S-phase of cell division. He was concerned that if the treatment is to be combined with other chemotherapy or irradiation therapy that could induce other cellular mutational events, E1A could augment cell growth toward malignant transformation.

Review--Dr. Straus

Dr. Straus stated that he has reviewed the original and revised protocol, the correspondence between the RAC reviewers and the investigators, and the minutes of the last meeting. After lengthy dialogue with the investigators, the potential concerns of administering adenovirus E1A sequences into the peritoneal or pleural space of these patients is outweighed by the potential benefits. In their new submissions, the investigators have demonstrated their ability to document *ex vivo* and *in vivo* transduction rates. The revised experimental design includes separate escalations of treatment in patients with breast and ovarian cancer due to concerns voiced by the RAC regarding extrapolations to breast cancer of studies primarily conducted in ovarian cancer models. The Informed Consent document has been revised and improved. These modifications allay most of Dr. Straus' reservations about this protocol.

However, Dr. Straus stated that there are a few issues that he wishes resolved prior to approval of the protocol: (1) The exact number of subjects to be enrolled in the study needs to be clarified. Fifteen to 25 in the original protocol; 15 to 24 in the revised protocol; and then with separate tracks for breast and ovarian cancers, it goes up to 15 to 30. He was not certain that the number is concordant with the new study design. (2) In Dr. Straus' review of the original protocol, he requested the full preclinical safety data on the murine experiments. The investigators stated in their written response that the "primary safety data were submitted to the FDA as part of the IND." There were no such data in the RAC submission, and those data need to be provided. (3) Dr. Straus raised a question in his initial review regarding the transduction efficiency of the liposome system. While the investigators have shown additional data in their resubmission that the cells can be transfected *in vitro* and *in vivo*, Dr. Straus was unsatisfied regarding the response of the investigators stating, "*In vitro* transfection efficiency experiments were conducted in the following cell lines with results almost identical to SKOV-3." The data for these experiments have not been submitted. One of the cell lines is the human embryonal kidney 293 cell line, which has been already stably transformed with the E1A. It would seem impossible to document transfection of additional E1A in this cell line. (4) There are remaining concerns regarding certain details of the clinical protocol with regard to criteria for dose escalation and presumption of benefit. Specifically, the protocol indicates that a greater than 25% reduction in expression of HER-2/neu will be taken as evidence of successful transfection. While the investigators have shown that they could detect marked reduction in HER-2/neu synthesis, he does not expect that a 25% alteration inimmunohistochemical staining could be detected. Similarly, a greater than 50% reduction is required as the level for success in preventing further dose escalation. In both instances, Dr. Straus believes that these levels of alteration are too low to be meaningful or reproducible. He requested that more realistic levels of expression be incorporated into the protocol design or the investigators should demonstrate their ability to detect these levels of changes in a blinded study.

Dr. Straus was satisfied with the revised Informed Consent document. Dr. Walters indicated that the investigators have provided a written response dated November 20, 1995, addressing most of the questions raised by Dr. Straus. After reviewing this document, Dr. Straus stated that it addressed most of his questions.

Other Comments

Dr. Smith asked the investigators to elaborate on the E1A issue. He asked if there is any RAC policy concerning adventitious transduction of gonadal tissue. Dr. Wivel said that the RAC has consistently requested data from animal models to check for adventitious transduction of gonadal tissue. Dr. Anderson noted that in 1988 during the drafting of the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects* of the *NIH Guidelines*, the RAC decided that it would not consider germ line studies, but if there was an inadvertent transmission to the germ line, it would be considered as part of the risk/benefit analysis of a protocol. There was no definite evidence of persistent presence of vector DNA in the gonadal tissues of humans; Dr. Zanjani has presented his studies on sheep during his lecture on *in utero* gene therapy at the June 8-9, 1995, RAC meeting. Ms. Meyers remarked that no autopsies have been performed to address the issue of whether there is any adventitious transduction of reproductive organs. Dr. Zallen noted that transfection with a plasmid DNA is mostly episomal and unlikely to cause integration into the host cell chromosomes.

Dr. Lysaught asked the investigators to clarify a statement regarding the sponsor's responsibility regarding research related costs, what is included in this category of research related costs, and what is meant by the term "medical facilities."

Regarding germ line transduction, Dr. Motulsky noted that the main concern about the germinal contamination is the likelihood that transfected DNA would cause mutagenesis in the ovaries and the testicles. If you consider the issue that germinal spread might lead to mutations in ovaries, the investigators need to consider the advanced cancer stage of the patients and the length of time the material would persist in the gonadal tissue. This issue of gonadal spread raised a concern in the present protocol because the target cells of the treatment are the reproductive cells.

Dr. Noguchi commented on two issues: (1) for ovarian cancer patients, persistence of the transgene in the ovaries is probably an advantage; and (2) it has never been demonstrated that any human cell line can be transformed with the E1A gene. All of these factors need to be considered. In some cases, inadvertent germ line transmission will occur, and awareness of that risk factor is necessary.

Dr. Samulski noted that while it has never been demonstrated that E1A will transform a cell, it has been demonstrated that in combination with other genes, E1A can perform a critical role in inducing cell transformation. The E1A region of the adenovirus encodes two proteins and the conserved domains of these two proteins are involved in the binding to RB, p300, and other critical cellular proteins. Potentially, E1A mutants can be constructed that retain the property to downregulate HER-2/neu; however, they do not have the other cellular effects.

Dr. Walters asked about the function of p300. Dr. Samulski explained that the E1A protein, when in complex with the p300 protein, will activate a transcription factor called E2F and initiate the cell into the S-phase of the cell cycle. In the presence of other oncogenes such as *ras*, E1A can cause the cell to remain in the transformed state. These side effects can be minimized if the E1A gene is under the control of a tissue specific promoter or if E1A mutants are constructed by dissecting out the undesirable functions other than downregulation of HER-2/neu. Dr. Samulski was concerned that approval of the protocol could wrongly be perceived of setting a precedent to permit other gene

therapy experiments using E1A.

Dr. Robinson cautioned that E1A is a gene that affects transcription factors which affect DNA synthesis in cells, and it can inactivate a normal tumor suppressor gene. It is not the gene of choice to be used to treat a person who does not have advanced stages of cancer.

Dr. Straus indicated that E1A is a very pervasive viral gene present in most people. Adenoviruses types 1, 2, 5, and 6 carrying the E1A gene infect virtually 100% of the population; these viruses are persistent in circulating lymphocytes and are demonstrated to be present in human placenta. The study can be restricted to women who are no longer of childbearing potential if there is a serious concern about reproductive cell transduction.

Dr. Robinson noted that E1A will be transduced outside the context of the whole adenovirus genome. Other mechanisms that can counter the effect of E1A are absent, such as the immune response to cells infected with the adenovirus.

Investigator Response--Drs. Hortobagyi and Hung

Dr. Hortobagyi addressed the questions raised regarding the study design. He would prefer to place the study in the context of developing new agents or therapeutic approaches in oncology. More than 95 to 98% of Phase I trials in oncology are broad studies that include patients of various tumor types, simply because there is no tumor specific agent developed to date. Therefore, it is more a proof of concept rather than developing a tumor specific treatment. In the present protocol, HER-2/neu overexpression is not tumor specific; therefore, the approach to include both breast and ovarian cancer is based on the concept that this gene is overexpressed in these two tumor types.

The investigators have revised the protocol to include 2 parallel-dose escalation trials on patients with ovarian and breast cancers. Each trial will include sufficient numbers of patients at each dose cohort. Since the dose escalation schemes are exactly the same for both trials, there is no reason to separate them into 2 protocols. Establishing the safety or efficacy of this agent in breast cancer is not a necessary condition for starting the trial in ovarian cancer and vice versa.

The dose escalation scheme is similar to other Phase I trials in oncology. Each dose level will have 3 patients. Before proceeding to the next higher dose level, at least one of the 3 patients should have completed a full course of therapy. If there is a significant biological effect, e.g., substantial downregulation of HER-2/neu overexpression, the dose group will be expanded to 6 patients before proceeding to fully evaluating both the tolerance and efficacy at that level.

Responding to questions regarding the patient number, Dr. Hortobagyi explained that if the first dose level is the maximum biologically active dose (MBAD) or the maximum tolerated dose (MTD), the study will be completed with 6 patients in each tumor type (total of 12). It is possible that the study will go through all 4 dose levels without reaching MBAD or MTD, and then the total number of

patients will be 24. At that point it has to be decided if the study will continue beyond the proposed highest dose level.

Dr. Ross asked if the 2 trials will be kept completely separate from each other. In other words, adverse effects observed in breast cancer will have no impact on the ovarian cancer study and vice versa. Dr. Hortobagyi responded that the expected toxicity is likely to be associated with the anatomical sites. For example, there will be different concentrations of the agent because of the volume differences between these 2 sites. Since these 2 trials are part of the same protocol with the same investigators, all of the information obtained from each trial will be evaluated in planning the other trial.

Dr. Chase illustrated the problem of not completely responding to RAC's suggestion to separate the studies into 2 completely separate experiments. For example, the agent could appear to be perfectly safe in one dose level but would have serious toxicity in the next higher level, and that knowledge might not be available from the other side of the study. These two experiments should be performed in sequence rather than at the same time as proposed. Dr. Zallen remarked that the present design appears to be reasonable since the two trials are conducted separately. Dr. Chase said these two studies should not be approved as a single protocol. One experiment should be conducted at a time. Since these 2 trials are superficially similar and are combined into a single protocol, toxicity information from one trial does not justify the continuation of dose escalation in the other trial.

Dr. Lysaught remarked that the toxicity studies in mice do not follow the same dose escalation scheme proposed for human trials.

Dr. Noguchi stated that FDA reviewers are confronted by the same kind of questions on a daily basis. If both trials are conducted simultaneously, the chances that one would be able to transpose the data would be augmented by the present design rather than by conducting the trials in sequence. The toxicology data are adequate for this particular proposal. There are 4 CF trials using essentially the same adenovirus vectors to treat the patients in the nose and in the lung. When all the data are obtained, indicate that effects in the lung are replicated in the nose even though the investigators are dealing with anatomically different spaces.

Dr. Straus noted that the preclinical toxicity profile provided by the investigators is not complete, e.g. it is lacking blood counts. Dr. Hortobagyi responded that those studies have been performed; however, the data are not included in the written response.

Dr. DeLeon remarked that these 2 studies would be enhanced by conducting them simultaneously rather than sequentially. Dr. Hortobagyi stated that the investigators and research related personnel will meet weekly to assess the data as they evolve. It will be beneficial to the investigators to conduct these 2 trials at the same time.

Responding to the issue of gonadal transduction, Dr. Hortobagyi stated that the median age for patients with breast cancer is 63 and for ovarian cancer is mid-60's, and they are all beyond their

reproductive age. For ovarian cancer patients, the initial treatment requires removal of both sides of the ovaries. In general, patients enrolled onto the protocol have been already treated withcytotoxic chemotherapy that for all practical purposes produces a chemical ovarian ablation. The patients are required to use contraception during the gene transfer study. The life expectancy of the advanced cancer patients is too limited to allow for childbearing. Taking into consideration of all these aspects of patient selection, there will be a minimal possibility of transferring the gene through the germ line.

Dr. Hortobagyi stated that in the preclinical safety studies,transfected DNA sequences were detected by a highly sensitive polymerase chain reaction (PCR) assay in many organs including the gonads. It is unknown if the DNA sequences are integrated into the host cell DNA. Studies are ongoing to explore this issue. The investigators will conduct an autopsy in some of the patients to resolve this important issue of gonadal transduction. The issue will become even more important if this protocol is successful, and the treatment is then extended to aprognostically more favorable group of patients.

Dr. Hortobagyi concluded that in view of the preclinical data that indicate antitumor efficacy and with all the patient characteristics, a potential for benefit is substantially greater than the potential for risk. Since the study employs a plasmid DNA/liposome complex and there are no viral agents involved in the protocol, it is highly unlikely that health care workers or patient's families will be exposed to the agent.

Dr. Smith asked the investigators to clarify if thetransfected DNA persisted beyond 14 days in any tissue. Dr. Hortobagyi responded that the study is being conducted.

Responding to the question of research-related costs, Dr.Hortobagyi stated that they have made a commitment that all research-related costs will be covered by the sponsor. The costs include all the tests necessary to screen for eligible patients; all costs needed to evaluate the patient before, during, and at the conclusion of their participation in this study; all costs related to preparation and administration of the agent; and the diagnosis, evaluation, and treatment of complications of this treatment, if they should arise.

Ms. Meyers inquired why the investigators are so resistant to the idea of splitting the proposal into 2 separate protocols. Dr. Hortobagyi responded that there are 2 major reasons: maximum information will be obtained from the present study design while placing the smallest number of patients at risk; and it is more costly to conduct the study with 2 separate administrative structures for 2 trials.

Dr. Hung addressed the issue of E1A stating that E1A is not a transformingoncogene; in his opinion, E1A is a tumor suppressor gene.

Dr. Hung summarized the scientific developments that led to the earlier concept that adenovirus E1A gene was classified as an immortalization oncogene as follows: (1) The adenovirus E1B or *ras* oncogene is capable of transforming established cell lines and has been classified as a *transforming oncogene*. (2) The E1B or *ras* oncogene cannot transform a primary culture cells such

as primary rat baby kidney cells or primary human embryo retinal cells. (3) To transform primary embryo cells, *ras* or E1B oncogene would require cooperation with E1A. (4) A major difference between established cell lines and primary culture cells is that established cell lines have passed the crisis stage of cell culture and have been "immortalized." For this reason, the E1A gene was classified as an *Immortalization Oncogene*. (5) The E1A gene alone cannot transform either primary cells or established cell lines. Expression of E1A in a rodent embryo fibroblast cell line does not cause cell transformation such as growth in soft agar or tumor growth in nude mice. (6) Interaction between E1A and retinoblastoma protein, RB, may contribute to the immortalization function of E1A. The active RB protein causes growth arrest of cells in the late G1-phase of the cell cycle. Binding of E1A to RB will inactivate the growth arrest function of RB and allow cells to enter the S-phase of the cell cycle. (7) Although the E1A gene which is associated with the immortalization function can immortalize a primary cell to become a cell line, the E1A gene alone cannot transform the cells; therefore, the E1A gene is *not* a *Transforming Oncogene*.

Dr. Samulski remarked that E1A alone is not sufficient to be anoncogene; however, it is capable of transforming cells by cooperation with other genes; it provides the initial immortalization step within a cell toward full transformation. Dr. Hung explained that in allowing E1A to function as an immortalization gene, the E1A gene should be expressed continuously in the primary cell culture to allow the cells to be established as cell lines. The protocol proposes a transient gene transduction system that allows only transient expression of E1A in target cells, and thus decreases the potential to induce tumors.

Dr. Hung reiterated that adenovirus-5 E1A is a tumor suppressor gene, citing the following research findings: (1) E1A inhibits HER-2/neu expression and suppresses tumorigenicity and transformation induced by HER-2/neu overexpression; therefore, E1A exhibits a tumor suppression function for the HER-2/neu overexpressing cancer cells. (2) E1A reduces the metastatic potential of the *ras*-transformed rat embryo fibroblast cell line. (3) Stable expression of the E1A gene reduces anchorage-independent growth and tumorigenic potential in many human tumor cell lines in which HER-2/neu is not overexpressed. These cell lines include HT1080 fibrosarcoma, A2058 melanoma, NCI-H23 non-small cell lung carcinoma, and HeLa cells. (4) E1A is capable of inducing apoptosis.

Dr. Straus inquired how a gene that induces apoptosis (programmed cell death) can immortalize a cell, two seemingly opposed cellular phenomena. Dr. Hung responded that Dr. Straus had raised a good question; however, he does not have a satisfactory explanation. Dr. Hung emphasized that E1A is not a transforming oncogene, in his view, the benefit to patients with advanced breast and ovarian cancers outweighs its risk.

Dr. Samulski asked if the E1A induces a tumor suppressor effect by the same mechanism as that of anti-erbB-2 single chain antibody used in another protocol (Protocol #9509-124 by Curiel and Alvarez). Dr. Hung explained that the antibody causes abnormal localization of the erbB-2/HER-2/neu protein while E1A downregulates the gene expression. Dr. Hung was unsure if these two genes induce apoptosis by the same mechanism since it has been reported that E1A induces apoptosis not only by a *p53*-dependent (similar to erbB-2) but by a *p53*-independent pathway.

Responding to the question of using a tissue specific promoter to express the E1A gene, Dr. Hung

stated that since his data suggest that E1A is not anoncogene; this this step is unnecessary. He emphasized that the transient expression system, unlike a retrovirus vector, does not integrate the vector sequences into host cell DNA. If injection is ceased after a few months, the effect will be eliminated. Regarding the question of which proteins encoded by the E1A gene inhibit HER-2/neu overexpression, Dr. Hung said both proteins acting alone will be sufficient for this function. He agreed with Dr. Samulski's suggestion that eventually E1A mutants could be constructed that will downregulate HER-2/neu but will not bind to other cellular proteins.

Dr. Lai stated that Dr. Hung has convinced him that E1A is a tumor suppressor gene and that E1A by itself cannot cause transformation. The fact remains that under certain conditions, E1A together with oncogenes such as E1B or *ras*, does cause transformation. It is a double-edged sword, and the RAC has been struggling with this issue. In some conditions, E1A induces apoptosis; yet under other conditions it potentially can cause transformation. Dr. Hung agreed that E1A could promote transformation if a cell already harbors an activating mutation of a*ras* protooncogene. But he considered this issue to be less critical if a transient expression system is used for gene transduction.

Dr. Lai asked if the RAC has ever approved a protocol using an oncogene for human gene transfer experiments; if not, guidelines regarding the use of vectors carryingoncogenes are needed.

Dr. Hortobagyi indicated that 1% of patients who receive curative treatment for breast cancer with adjuvant chemotherapy will develop acute leukemia, and more than 10% of patients with Hodgkin's and non-Hodgkin's lymphoma treated with chemotherapy will develop secondary cancers. It is important to consider all of the risk/benefit ratios of the protocol as opposed to the absolute answer of whether E1A is capable of inducing transformation.

Dr. Walters noted that his only recollection regarding Dr. Lai's question regarding previous RAC approval was that there is a theoretical concern ofinsertional mutagenesis caused by retroviral vectors.

Dr. Hirschhorn stated that risk/benefit evaluation should be considered in approval of the protocol. It is acceptable when dealing with a lethal disorder for which there is no current therapy, and it should be made clear that such an approval is not a license to use E1A for other less life-threatening disorders. Dr. Hirschhorn asked if it is procedurally possible to draw the line. Dr. Wivel responded that the RAC does not need to be bound by precedent, and the RAC has reserved the right to review each case on its own merits. Dr. Ross would like to have a proviso regarding the spread of vector to the gonads. Women should be past childbearing years. Some of the discussion of E1A is quite similar to the *p53* problem.

Committee Motion

Dr. Motulsky stated that major issues raised by the RAC, i.e., study design,gonadal spread, and E1A as an oncogene, have been addressed by the investigators. Dr. Motulsky made a motion to

approve the protocol as it is proposed. The motion was seconded by Dr. Smith.

Dr. Zallen urged the investigators to make a serious commitment to request autopsy; the subjects and their families should be informed in advance that it is absolutely critical to gain information regarding gonadal spread of the vector DNA. Dr. Motulsky said that it should be a specific autopsy to address this question. Dr. DeLeon noted that unlike the case with breast cancer, ovaries are the target organ of ovarian cancer treatment. Ms. Meyers asked if autopsy should be part of the stipulation. Dr. Straus noted that an autopsy request is already included in the Informed Consent document.

The motion made by Dr. Motulsky and seconded by Dr. Smith to accept the protocol submitted by Drs. Gabriel Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung of the University of Texas, MD Anderson Cancer Center, Houston, Texas, was approved by a vote of 11 in favor, 5 opposed, and no abstentions. The RAC strongly urged the investigators to encourage the patients' families to consent to an autopsy addressing the issue of adventitious transduction ofgonadal tissue.

Protocol Summary

Drs. Gabriel Hortobagyi, Gabriel Lopez-Berestein, and Mien-Chie Hung, of the University of Texas, MD Anderson Cancer Center, Houston, Texas, may conduct gene transfer experiments on a maximum of 24 adult patients (12 for each cancer) withmetastatic breast or ovarian carcinoma. Overexpression of the HER-2/*neu* oncogene occurs in 30% of ovarian and breast cancers, and it is associated with enhanced metastatic potential, drug resistance, and poor survival. The E1A gene of the adenovirus type 5 functions as a tumor suppressor gene whentransfected into cancer cells which overexpress the HER-2/*neu* oncogene. E1A expression induces downregulation of the level of the HER-2/*neu* oncoprotein by a transcriptional control mechanism. A plasmid, pE1A, encoding the adenovirus E1A gene with its own promoter will be administered as a DNA/lipid complex via the intraperitoneal or intrapleural route. The objectives of the study are: (1) to determine E1A gene transduction into malignant cells after the administration of E1A/lipid complex by intrapleural or intraperitoneal administration, (2) to determine whether E1A gene therapy candownregulate HER-2/*neu* expression after intrapleural or intraperitoneal administration, (3) to determine theMBAD or the MTD of the E1A/lipid complex, (4) to determine the toxicity and tolerance of E1A/lipid complex administered into the pleural or peritoneal space, and to assess the reversibility of such toxicity, and (5) to evaluate tumor response.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* **REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED:** *A PHASE I STUDY OF ADENOVIRAL VECTOR MEDIATED GENE TRANSFER TO LIVER IN ADULTS WITH PARTIAL ORNITHINE TRANSCARBAMYLASE DEFICIENCY* **/DR. BATSHAW**

Review--Dr. Erickson

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

Mark Batshaw of the Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania. Dr. Erickson stated that this protocol proposes to evaluate the safety of treating patients with partial ornithine transcarbamylase (OTC) deficiency using an E1-deleted, E2A-temperature-sensitive adenovirus vector expressing the human OTC enzyme. OTC is an urea cycle enzyme and complete deficiency of this enzyme leads to early neonatal death despite any treatment. OTC deficiency is an X-linked disorder; the patients are eitherhemizygous males with mutations which leave them with about 20% or more of the enzyme activity or heterozygous females who, because of "lionization," have similar levels of enzyme activity to normal individuals.

This protocol proposes to use an adenovirus which has a limited length of expression to be delivered to the liver by placing a catheter into the hepatic artery. The investigators are to be commended for their effort to improve the adenovirus vectors. The investigators presented extensive data on the toxicity of the standard adenoviral vectors and described the modification, a temperature-sensitive mutation in the E2A gene, that makes the vector much less toxic. The investigators have extensively studied vectors carrying the OTC gene in an animal model, the *sparse fur* mouse which has a deficiency in OTC. Findings indicated that while the human gene was not expressed for significant lengths of time, relatively long-term expression could be achieved when the autologous mouse OTC gene was used.

Dr. Erickson raised a concern regarding the target population. The males who have OTC gene mutations that leave them with partial enzyme activities and females who because of "lionization" could possibly have a mutation, which when passed to their sons, could be lethal. Females with partial enzyme activity could develop symptoms following a high protein meal. Usually, OTC patients benefit from the therapy that Dr.Batshaw has developed, i.e., limited protein intake supplemented with sodium benzoate and sodium phenylacetate/sodium phenylbutyrate. The chemicals in essence will eliminate excess urea and excess precursors of ammonia. It is unlikely that these individuals will have crises as implied in the protocol. Not only are these patients usually in good health, but the proposal is to treat patients who have never had symptoms. Dr. Erickson found that the proposal using this asymptomatic patient population is not justified.

Dr. Erickson was concerned that the protocol uses a very invasive procedure. The protocol describes the delivery of a virus to half of the liver by placing a catheter in the hepatic artery. It is an invasive procedure even though the investigators are planning to use a vector of reduced toxicity. The temperature-sensitive mutation of the E2A gene of the second generation vector renders the gene ineffective at the body temperature.

The patient population consists of adults who can give an informed consent. Dr. Erickson questioned if the protocol will produce any significant efficacy, since the vector is incapable of long-term expression of the OTC gene.

Dr. Erickson stated that the investigators have indicated that based on a pilot study in the animal model, the vector can be delivered via the intravenous route instead of via a hepatic artery catheter in human patients. Dr. Erickson suggested that the intravenous route of vector administration should be considered for the present protocol.

Dr. Erickson stated that the potential of vector-induced liver inflammation will aggravate the metabolic status of the patients with respect to nitrogen catabolism. Dr. Erickson was also concerned about the hepatotoxicity noted in the baboon experiment. There is a biphasic increase in the release of liver enzymes from damaged cells of the liver into the circulation. In the second phase, there is a 12-fold increase of the liver enzyme levels. If similar toxicity occurred in human liver, it would be sufficient to induce a crisis in these patients with partial OTC deficiency since the liver is the sole source of OTC synthesis.

The investigators have provided the data obtained from the *sparse fur* murine study that demonstrates the benefit of the treatment. Dr. Erickson remarked that it is difficult to extrapolate murine data to the human study. The regeneration rate of the damaged liver cells may be different between mice and humans.

Dr. Erickson was concerned about the following issues: (1) the treatment is potentially toxic; (2) adenovirus cannot be repeatedly administered to patients to achieve a long-term effect; and (3) the treatment is to be given to a target population who are nearly asymptomatic. The patients are adults and most likely would give informed consent to participate in this study; however, Dr. Erickson does not consider it to be a sufficient justification for the protocol. Dr. Erickson would consider it to be more acceptable if the vector can be repeatedly delivered by the less invasive intravenous route, and the treatment is given to affected children with life threatening OTC deficiency. The investigators responded to Dr. Erickson's concerns in writing, but he was not completely satisfied with the responses.

Review--Dr. Hirschhorn

Dr. Hirschhorn stated that OTC is an enzyme of the urea synthesis pathway, and its deficiency results in episodic life threatening hyperammonemia, coma, and brain damage. In this proposal, the gene product is primarily localized to the liver with a minor contribution from the intestine. The gene is on the X chromosome, and the disorder has been classified both as an X-linked recessive (only males affected) and as an X-linked dominant mutation because of the high percentage (5-10%) of heterozygous female carriers who exhibit disease. Symptomatic heterozygous female carriers presumably have skewed X chromosome inactivation in hepatic tissue with the mutant X chromosome being active in a majority of hepatocytes. As in other inherited disorders, there is variation in severity of disease with neonatal onset cases associated with undetectable enzyme activity and milder cases with mutations resulting in substantial (1-20%) residual enzyme activity ("partial" OTC deficiency). Patients with milder disease are at risk for early death during crisis. The patient population will consist of adult males with "partial" OTC deficiency, symptomatic female carriers, and included in the definition of "partial" deficiency, asymptomatic female carriers. Approximately 80% of asymptomatic females will never have symptomatology.

Therapies utilizing metabolic manipulation and hepatic transplantation have been utilized for this disorder. The metabolic manipulation is cumbersome, unpleasant, and only partially therapeutic; frequently a liver transplant is required. Liver transplantation has high morbidity and mortality; however, the effectiveness of liver transplantation provides the supporting rationale for an attempt at

gene therapy directed to the liver. The occasional prevention of mental retardation by metabolic manipulation provides hope for potential efficacy of maneuvers correcting metabolic abnormalities.

From knowledge of the disease in humans, there is an excellent scientific rationale for attempting liver specific gene transfer for OTC deficiency, both with respect to the lack of an existing effective therapy and the likelihood of efficacy. The choice of male adults with partial deficiency and symptomatic female carriers as subjects represents an appropriate choice of a target population in order to detect any possible efficacy as defined by the metabolic responses; however, the rationale and justification are unclear for including asymptomatic female carriers, particularly when such individuals are defined only on the basis of having had two affected children rather than by a metabolic study that would indicate risk for symptomatology. Such a correlation of results of metabolic studies with risk for symptomatology has not been defined. Since many mothers with one affected child and even with 2 affected children may not be asymptomatic carriers, Dr. Hirschhorn asked whether there are any data confirming the ability of the allopurinol-loading test to identify and to exclude such individuals. Will any attempt be made to define the particular mutations present in the subjects? The investigators have responded in writing that mutations will be determined to identify the carriers, and they provided interesting preliminary results with regard to the 15N-urea assay to identify asymptomatic female carriers.

Dr. Hirschhorn raised several additional issues in her written review: (1) asking the investigators to provide more detailed description of the preclinical data; (2) defining better the rationale for the proposed dosing schedule; (3) the possible excretion of the vector; and (4) if the alternative pathway therapy will affect the 15NH4Cl study. The investigators have provided a detailed written response.

Regarding the vector risk, Dr. Hirschhorn stated that the present study uses a second generation adenoviral vector which contains a temperature-sensitive E2A mutation. The temperature-sensitive mutation probably is leaky in humans since the body temperature (370C) is not at the inactivating 390C. Although there is diminished liver necrosis by this second generation vector, it is not the final vector in the development process. Treating patients with this vector would interfere with future treatment of the same patients with improved vectors, due to immune response. Dr Hirschhorn suggested that it is more promising to pursue further preclinical animal model studies using the new strategy and new vectors before attempting the human trial at the present stage with so many deficiencies.

Review--Dr. McGraw

Dr. McGraw raised three major concerns:

1. The first concerns the proposed ratio of 1 male to 2 female patients in conjunction with the dose escalation plan. At each dose, 3 subjects will be tested, and if no toxicity is evident, 3 more subjects will be tested at the next dosage level. If no toxicity occurs, 18 subjects (6 males and 12 females) would be enrolled in the study. Dr. McGraw's concern is not with the 1:2 ratio *per se* that is justified by the fewer number of adult males with the disorder, but with the possibility that the etiology and manifestation of OTC deficiency differs for male and female patients. It is important for design and

ethical reasons to "yoke" the male and female participants so that 2 females and 1 male receive each dosage level. The investigators want to avoid the possibility of enrolling 6 females at the first two dosage levels and then enrolling the first male at the 3rd dosage level if there is any reason to hypothesize that the biological responses of males and females will differ.

2. The second issue concerns the method of solicitation. The study will be "advertised" in the newsletter of the National Urea Cycle Disorder Foundation (NUCDF), and letters will be sent to all 100 members with partial OTC deficiency. With regard to the direct letter solicitation, Dr. McGraw asked if members of the NUCDF have previously agreed to the release of their names and addresses for research purposes. The study is being endorsed by the co-presidents of the NUCDF by a letter of support included in this protocol. Do the investigators intend to have this endorsement accompany the newsletter solicitation? This support must be used judiciously. A recommendation from an "insider" or prominent group members will always boost volunteer rates in any kind of research study targeting special groups; however, the initial solicitation must accurately describe that this Phase I study may not benefit those who choose to participate. The investigators need to avoid conveying misleading optimistic information about the proposed study, because interested patients may psychologically commit to participation, then find it difficult to rationally assess the many risks and limited benefits once the protocol has been fully explained. Dr. McGraw does not argue that this method of solicitation is improper, and indeed thinks that organizational support is a positive factor; however, she is concerned that one should not "oversell" the study at the initial stages of solicitation. She asked if the IRB has reviewed this aspect of patient solicitation.

3. Dr. McGraw made several specific suggestions regarding the Informed Consent document and most of the suggestions have been taken into consideration in the revision of this document. Dr. McGraw was satisfied with the revised Informed Consent document.

Other Comments

Dr. Ross noted many repetitive statements within the Informed Consent document and revisions are necessary. Dr. Zallen asked if family members and health care workers will be screened for vector excretion.

Investigators Response--Drs. Batshaw and Wilson

Responding to the question of patient recruitment and the issue of women with partial OTC deficiency, Dr. Batshaw stated that 10-15% of females with OTC deficiency will develop clinical symptoms during their lifetimes, and there is no reliable test to predict when the symptoms will occur and in which patient. Frequently, the first time the episode happens is the last time because the patients die. The 15N-ammonia test is being developed to predict which of these patients are most likely to have fatal episodes. In this test, asymptomatic OTC patients are given a small amount of ammonia labeled with the stable isotope, 15N, and their urea synthetic capacity is examined (an *in vivo* way of examining how much of their OTC pathway is open). Dr. Batshaw provided an example of a 28-year-old woman who has had 2 male newborns die of OTC deficiency. Her mother had died at 42 years of age, with recurrent hyperammonemia having developed at age 30. These are the

types of patients who would like to participate in this study. Ethically, it is preferable to begin the study with the group of patients who are adult and who can give informed consent.

Dr. Batshaw stated that the protocol is a Phase I study in which safety is the primary objective. It will cease with the occurrence of toxicity or with observation of efficacy.

Responding to Dr. McGraw's question of patient solicitation, Dr. Batshaw stated that the investigators are not planning to use the endorsement letter byNUCDF officials for patient solicitation. The investigators will inform the physicians, rather than the patients, regarding the availability of the protocol.

Responding to the question of the route of vector administration, Dr.Batshaw stated that hepatic artery catheterization was originally chosen because of the safety consideration to limit exposure to one lobe of liver. Dr. Batshaw agreed to modify the protocol to employ a less invasive procedure, i.e., intravenous route of vector delivery.

Dr. Wilson stated that the investigators have conducted a very extensive safety study regarding the second generation of adenovirus vectors, especially when the vectors are to be utilized by*in vivo* systemic administration in patients who are not lethally ill.

Dr. Wilson presented his toxicity studies of the vectors. As a worst case scenario, the toxicity studies were first conducted with the much more dangerous E1-deleted adenovirus vectors in primates. The investigators observed that the liver function tests measuring the presence of liver enzymes in blood circulation resulting from liver damage are the most sensitive indices of liver toxicity of adenovirus. Dr. Wilson showed the alanine serum transaminase (AST) data of the Rhesus monkey experiment. Rapid rise of serum AST was observed in an animal injected with a maximum dose of the first generation vector; the animal developed extensive hepatocellular damage and died in 2 days. Less hepatic damage was observed in both adult and newborn monkeys using the second generation vector proposed for this protocol.

Dr. Wilson commented on the issue of vector-induced immune response to either the vector or the transduced cells. Most studies were conducted in conjunction with the CF protocols. Dr. Wilson stated that immune responses ultimately will be an issue with virtually any *in vivo* delivery of adenovirus vectors. It is unclear whether humoral immunity has been elicited in the CF studies and whether there is any therapeutic effect. Complex protocols are being developed to investigate whether there are any rate-limiting immune response that could be overcome by immune suppression medications. Extensive preclinical studies in mice indicate that a combination of adenovirus with immune suppressive drugs is a promising approach to overcome immune system elimination of adenovirus vectors.

Dr. Wilson presented the data on studies performed with the*sparse fur* mouse model. *Sparse fur* mice are deficient in OTC. To simulate a clinical crisis, the mice were injected with ammonia and scored for clinical phenotypes, i.e., ataxia and seizures. Normal mice tolerated the

hyperammonemia challenge well, while the *sparse fur* mice developed serious complications or died. The efficacy of OTC gene therapy is evaluated in these mice following several days after vector administration. It was noted that 7 days after vector administration, most animals were protected from the ammonia challenge. In this disorder, there is a clinically meaningful endpoint in the animal model.

In terms of the route of vector delivery, Dr. Wilson stated that the proposal to administer the vector through a catheter to the hepatic artery is intended to limit the vector exposure to a portion of the liver, an approach analogous to the bronchoscopic delivery to the lung with the vector in the CF protocols. Dr. Wilson stated that toxicity studies have been performed with the present vector in primates either through the hepatic artery or a peripheral vein, with no difference in toxicity. If the RAC prefers the intravenous route of vector delivery, there are toxicity data to support this kind of approach.

Dr. Erickson asked if the investigators are ready to administer this vector every 3 months since transduction only lasts 60 to 70 days with each administration. Dr. Wilson responded that if the study demonstrates that the vector is safe for these type of patients, he envisions the progression of the study to treat patients with an impending crisis. Within 24 hours of treatment, a patient can obtain a clinically meaningful gene expression. Dr. Wilson stated that in this disorder, there is a clinical niche, especially in a severe crisis, irrespective of how long the vector persists in the patients. The investigators are developing strategies to overcome immune responses to repeat vector administration, e.g., co-administration with humanized antibody to CD4 or use of the immunosuppressive drug, cyclophosphamide, to block the activation of the immune system.

Dr. Ross inquired whether the vector is able to target one-half of the liver if it is administered via the intravenous route. Dr. Wilson responded that it will target the entire liver.

Dr. Saha stated that about one third of the mothers who have OTC deficient children are asymptomatic, and he asked if there are procedures to screen for these women. DrBatshaw responded that these women will be screened for OTC mutations. An assay using 15N-ammonium chloride to assess the urea synthetic capacity has been developed to screen for asymptomatic women. There are three metabolic assays to obtain clinical endpoints of efficacy: the allopurinol-loading test, 15N-urea, and 15N-glutamine assays.

Dr. Smith asked if the investigators are ready to extend the enrollment to include newborns. Dr. Batshaw responded that the Phase I study will involve only adults. OTC deficiency is a very rare disease; there are about 100 women who have clear OTC deficiency, and they will be the initial target population. Responding to the question of symptomatic vs. asymptomatic patients, Dr. Batshaw said that it is difficult to distinguish these two populations since an individual who is asymptomatic may have severe symptoms in the future. It is possible to use the 15N-urea assay as a screen for an individual. If a patient's urea synthesis is absolutely normal, it is likely that individual will not develop clinical symptoms.

Dr. Erickson noted that women who develop preexisting immunity will no longer be accessible to

future therapy with the adenovirus vectors. Dr. Wilson stated that there is hope as suggested by his primate studies. Development of vector immunity is dose-related and the immunity drops substantially within 6 months; after passing beyond this window, the patients may become accessible again. The human study is important to see if the primate results can be extrapolated to humans. To choose to treat asymptomatic adults rather than newborns is an attempt at obtaining maximum information from this Phase I safety study.

Dr. Straus noted that liver damage appears to be more severe in adults than in neonatal primates, as indicated by the higher elevation of serum aminotransferase levels in adult primates. He inquired if the preexisting immunity in adult primates is higher than that of the newborns. Dr. Wilson responded that he does not know the answer. Dr. Straus noted that human adenoviruses are fairly restricted from replicating in primate cells in general and questioned whether the primate studies with a human virus constitute a relevant experiment. Dr. Wilson said that the liver is an exception; human adenovirus type 5 is quite permissive in mouse liver; however, he does not have the primate data.

Dr. Erickson asked about the basis for the biphasic elevation of hepatic enzyme levels in serum noted in the baboon experiments. Dr. Wilson said that the biphasic rise is consistent with the notion of the first acute direct virus insult followed by a second, antigen-specific cellular immune response. Dr. Straus asked if the existence of prior immunity to adenovirus in adult patients aggravates the hepatocyte injury. Dr. Wilson stated that preexisting immunity in animal experiments has been associated with less toxicity and with less gene transfer. He stated that he waited until the second generation of safer adenovirus vectors were developed before proposing the human protocol. The study will start with a dose level much lower than the animal dosage that demonstrates any toxicity.

Dr. Hirschhorn asked the investigators to clarify if the lesser hepatic toxicity noted for the second generation vector in animal experiments is due to a lower degree of expression. Dr. Wilson responded that the vector used in these experiments carries a reporter gene, and the toxicity is due to the adenovirus vector backbone.

Dr. Hirschhorn shared the same concern with Dr. Erickson that after repeat administration there will be much less OTC gene expression due to an immune response to the adenovirus vector. Dr. Wilson stated that the murine experiments have been conducted to distinguish the mechanisms of cellular versus humoral immunity in rejecting the second dose of adenovirus vectors. The results show that the primary mechanism is the development of antibodies that would block the readministration of the virus. Dr. Hirschhorn inquired if a vector which does not elicit this humoral immune response will be developed in the near future. Dr. Wilson responded that the antibody response is induced by the capsid proteins of the adenovirus, and it is an intrinsic problem of the viral vectors. Dr. Hirschhorn asked if this is a complement dependent antibody response; it is difficult to envision that a humoral response would produce severe liver necrosis in such a short period in the murine experiments. Dr. Wilson stated that most of the pathology observed in the first phase of liver damage is not antigen specific; a high dose of virus proteins will produce cytotoxicity or induce non-antigen specific natural killer cell activity.

Ms. Meyers asked if the treatment will cause deterioration of the clinical condition of asymptomatic

carrier women. Dr. Batshaw responded that if an acute episode is triggered by hepatitis during the trial, the clinical condition can be stabilized with an effective treatment of intravenous injection of sodium benzoate or sodium phenylacetate, a treatment Dr. Batshaw developed.

Dr. Wilson agreed to revise the protocol to administer the adenoviral vector via the intravenous route; he would agree to screen the asymptomatic carriers with the 15N-ammonia assay.

Committee Motion

Dr. Erickson stated that the RAC discussion and the investigators' response have allayed most of his initial concerns about the protocol. He moved for approval with an intravenous route of vector administration and a stipulation of a clear description of the exclusion criteria based on the 15N-ammonia metabolic study of asymptomatic carriers. Ms. Meyers seconded the motion. Dr. McGraw stated that the risk section of the Informed Consent document should be revised in accordance with the change of the vector administration route. Dr. Hirschhorn made a friendly amendment for surveillance of virus excretion from the nose and pharynx. Ms. Meyers made a friendly amendment to revise the Informed Consent document to include statements of autopsy, lifetime follow-up, and use of barrier contraception for males and females.

Dr. Erickson and Ms. Meyers accepted the friendly amendments.

Dr. Erickson requested that the inclusion criteria in the 15N-ammonia study should be reviewed by the primary reviewers. The criteria should clearly describe what level of abnormality will be required; carriers who have completely normal 15N-ammonia handling capacity should not be included in the study.

Dr. Straus asked if it is correct to state that some individuals who have suboptimal 15N-ammonia handling capacity may live a normal life and never have life threatening crisis. Dr. Batshaw responded that it is a correct statement. Dr. Straus stated that the protocol is a precedent for treating patients with a nonfatal disease by gene transfer with a live virus vector.

Dr. Lysaught asked if the change of the vector administration route from intrahepatic artery to intravenous would change the potential for liver toxicity. Dr. Erickson said that aside from reducing the complication of putting a catheter in a hepatic artery, liver toxicity from circulating adenovirus should be about the same since most of the virus delivered by the hepatic artery route will pass through the liver and enter into the general blood circulation. Dr. Straus was concerned about damaging the liver, the organ that produces the missing OTC enzyme in these patients.

Responding to Dr. Straus' question of setting a precedent for treating a nonfatal disease with a live virus vector, Dr. Erickson said that, considering the seriousness of OTC deficiency in children and affected males, OTC deficiency is a disorder justified for gene therapy. The investigators have conducted extensive preclinical studies, and there is a possibility of developing a repeat treatment strategy by combination with immunosuppression medications co-administered with the adenovirus

vector. Dr. Erickson stated that he would favor approval of the protocol. Dr. Chase stated that he agreed with the investigators' assessment that it is not necessarily the best strategy to treat the people with the most serious disease in order to obtain the most significant scientific information from the study; the relatively healthy patients who volunteer for this experiment should be recognized for their participation.

Dr. DeLeon asked about who would pay the cost of the alternative chemical therapy if asymptomatic mothers should develop symptoms during the trial. Dr. Wilson responded that his hospital has budgeted such payment for the chemical therapy if it is needed.

Ms. Meyers stated that the protocol is an innovative study; if it is successful, it will benefit many people with a wide variety of liver diseases.

Dr. Zallen asked if there is any risk of virus spread to health care workers and family members. Dr. Wilson responded that in the ongoing CF protocols, patients are kept in isolation until the assays demonstrate negative virus shedding. Dr. Straus said the prior precedent is to assay the adenovirus with the 293 cell system and to isolate the patients until negative virus shedding is observed. Dr. Smith added that in terms of the safety issue, the OTC gene is more similar to the Cystic Fibrosis Transmembrane Conductance Regulation (CFTR) gene used in the CF studies, than the *p53* gene used in several other RAC approved protocols; the *p53* gene has the potential for oncogenic mutations. He stated that it would be reasonable to keep the patients in isolation for a few days until the virus assay is completed. If the virus is assayed at 48 hours, the patient isolation would be approximately 5 days. Dr. Wilson agreed to the stipulation to perform an assay at 48 hours and to isolate the patients until negative virus shedding is observed. Dr. Erickson and Ms. Meyers accepted the friendly amendment.

Dr. Straus stated that he is still concerned about the potential of hepatic injury. The adenovirus vector is not a minimally toxic agent, and it is difficult to justify conducting a trial with patients who are relatively healthy.

Dr. Erickson made a friendly amendment to exclude patients infected with hepatitis B and C viruses. Ms. Meyers accepted the friendly amendment. Dr. Wilson agreed to the stipulation.

Dr. Lysaught stated that from an ethical perspective there are two major risks for conducting this gene transfer study on healthy volunteers: (1) immune response to adenovirus that will prevent future treatment with similar vectors, and (2) the question of liver damage. Both issues have been responded to by the investigators. Dr. Lysaught considered this protocol to be the first step in developing gene therapy for neonates. The relatively healthy adults have other options for their diseases; they can give informed consent without the coercion of their illness to influence their decisions. She was satisfied with this proposal.

Responding to Dr. Lysaught's comment on giving informed consent without coercion, Dr. Hirschhorn remarked that having a sick child is more coercion than the mother herself being ill. Dr. Hirschhorn was uncertain that the intravenous vector administration is less of a risk than the intrahepatic route

since the latter route is more selective. Dr. Wilson noted that according to his primate studies, there is no qualitative difference in the bio-distribution of vectors by these two routes.

Dr. McGraw made a friendly amendment to revise the Informed Consent document to reflect the risks of using the intravenous vector administration. Dr. Erickson and Ms. Meyers accepted. Dr. Wilson agreed to the stipulation.

The motion made by Dr. Erickson and seconded by Ms. Meyers to accept the protocol submitted by Dr. Mark Batshaw of the Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, was approved contingent on review and approval of the following by the primary RAC reviewers: (1) a revised protocol to administer the adenoviral vector via the intravenous route; (2) a clear description of the exclusion criteria based on the 15N-ammonia metabolic study of the asymptomatic carriers; (3) patient isolation until the assays demonstrate negative virus shedding; (4) the exclusion of patients infected with hepatitis B and C viruses; and (5) a revised Informed Consent document including statements regarding autopsy, lifetime follow-up, use of barrier contraception, and the risk associated with intravenous administration of the adenovirus vector. The motion passed by a vote of 12 in favor, 1 opposed, and 4 abstentions.

Protocol Summary

Dr. Mark Batshaw of the Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct gene transfer experiments on 18 subjects (³ 18 years of age) with partial OTC deficiency. A recombinant adenovirus type 5 vector, H5.110CBhOTC, containing a temperature-sensitive mutation in the E2A region will be used to express the human OTC gene. The study will evaluate the toxicity and efficacy of *in vivo* gene transfer by selective intravenous infusion of the recombinant adenovirus vector. The study will focus on the immune response to the vector and to the genetically modified cells, as well as on evaluation of metabolic correction after gene transfer. The primary goal is to establish a viral dose that will achieve effective gene transfer without toxicity.

VIII. DISCUSSION REGARDING THE REPORT FROM THE *AD HOC* REVIEW COMMITTEE/DR. WALTERS

Dr. Walters called on Dr. Lana Skirboll, NIH Associate Director for Science Policy, to discuss the report of the *Ad Hoc* Review Committee on the RAC review process. Dr. Skirboll's office oversees ORDA which administers the RAC.

Presentation--Dr. Skirboll

Dr. Lana Skirboll provided background information regarding the NIH Director's decision to reevaluate NIH oversight of human gene transfer experiments. She stated that Dr Zallen, a member of the committee, would report on the *Ad Hoc* Review Committee since Dr. Inder Verma, Chair of the

committee, was unable to attend this RAC meeting due to a previous commitment.

Dr. Skirboll noted that the RAC has come under increasing scrutiny and has been portrayed as: (1) delaying new therapies and (2) duplicating the regulatory efforts of the FDA. Although currentNIH policy regarding gene therapy overview should be optimized, public accountability and scientific quality remain a paramount concern to Dr. Harold Varmus, NIH Director. There is an increasing public perception that gene therapy holds great therapeutic promise; however, there is negligible scientific evidence of clinical efficacy. Clearly the RAC remains critically important with regard to its public deliberation about the potential risks associated with novel therapies and applications relevant to gene therapy.

Dr. Skirboll stated that Dr. Varmus has established two committees to review the field of gene therapy research. The *Panel to Assess the NIH Investment in Research on Gene Therapy* has been co-chaired by Drs. Stuart H. Orkin and Arno G. Motulsky. This panel is charged with assessing the current status and promise of gene therapy and to provide recommendations regarding future NIH-sponsored research in this area. The committee will report to theNIH Director's Advisory Committee on December 7, 1995.

The *Ad Hoc* Review Committee was to review the activities of the RAC, and Dr. Verma will report to the NIH Director's Advisory Committee on December 7, 1995. The *Ad Hoc* Review Committee's deliberations have been completed, and its recommendations were published in the *Federal Register* for public comment on November 15, 1995 (60 FR 57528).

Dr. Skirboll stated that the NIH Director has several options regarding RAC purview:

1. The NIH Director can terminate the RAC;

2. The NIH Director can maintain the RAC's *status quo*, i.e, FDA and NIH Consolidated Review; or

3. Modify the RAC's roles and responsibilities such that the RAC maintains public accountability, i.e, data management, but RAC and NIH Director approval of individual experiments is eliminated.

Option #3 could be implemented by the following processes: (1) promulgate amendments to the*NIH Guidelines* such that approvals of human gene transfer protocols are terminated while data reporting and adverse event reporting requirements remain unchanged; or (2) promulgation of amendments to the *NIH Guidelines* such that all human gene therapy-related requirements are eliminated subsequent to implementation of the FDA proprietary exemption referenced in recommendation #5 of the *Ad Hoc* Review Committee recommendations.

Dr. Skirboll noted that Dr. Ruth Kirschstein, the NIH Deputy Director, was present at the meeting; she welcomes RAC comments regarding the recommendations made by the *Ad Hoc* Review

Committee. Dr. Varmus is expected to make his decision shortly.

Ad Hoc **Recommendations and Comments/Drs. Doris Zallen and Estuardo Aguilar-Cordova**

Dr. Zallen, *Ad Hoc* Review Committee member, summarized the deliberations of the *Ad Hoc* Review Committee during the course of its five meetings. Dr. Zallen recalled that Dr. Varmus' initial concern about the issue of the scientific quality of some of the gene therapy protocols prompted him to establish the review committee. The initial charge to the committee was to establish the scientific criteria for RAC review of human gene transfer protocols. During the course of their five meetings, the committee focused on a broad issue of RAC functions. At the September 8, 1995, meeting, the committee had finished its task and summarized its conclusions in the document entitled: *Executive Summary of Findings and Recommendations, Ad Hoc Review Committee, Recombinant DNA Advisory Committee*.

Dr. Zallen stated the two major conclusions of the *Ad Hoc* Review Committee:

"1. Gene therapy represents a special development in medical research because of its potential for modification of the human genome and for creation and dissemination of novel transmissible pathogenic vectors. In addition, there is the possibility of controversial extensions of this work, such as modification of the germ line or use of gene transfer for enhancement purposes. Thus, gene transfer differs in major ways from other clinical technologies in use or under development and is, therefore, deserving of continued public scrutiny.

"2. The RAC has served -- and is continuing to serve -- several important purposes for the scientific community, patients, and the general public. In particular, by focusing its attention on the emerging field of gene therapy research and helping to set appropriate scientific, safety, and informed consent guidelines for investigators. As a public forum of discussion, RAC has provided an enormous service not only to the general public, researchers at academic and similar institutions, and within the biotechnology industry, but also to officials at the FDA. In addition, RAC continues to be a credible forum for airing a wide range of public concerns about this emerging field of medical research."

Dr. Zallen noted the 5 specific recommendations of the *Ad Hoc* Review Committee:

"1. To avoid duplication of effort and unnecessary delay, RAC should no longer carry out case by case review of every clinical gene transfer protocol. This function is carried out by the FDA, which is required by statute to review all such protocols before approval.

2. Review of protocols by the RAC in an open public forum should continue in several areas of concern in which a particular protocol or new technology represents a significant degree of departure from familiar practices. Such departures include, but are not limited to, the use of novel vectors, particularly in cases in which modified human pathogens (such as herpes viruses or

lentiviruses) are being evaluated; gene transfer *in utero*, potential germ line modification, and other similar manipulations; and gene transfer in normal volunteers. In addition, review of protocols by the RAC is warranted in other situations which could lead to the formulation of significant new policy.

3. The RAC should define the criteria and work out procedures for identifying specific protocols requiring public review.

4. The RAC should continue to provide advice on policy matters revolving around gene therapy and other recombinant DNA issues to the NIH Director, individual members of the research community, institutional review boards, and the public. Moreover, that critical function should be extended, enabling RAC explicitly to provide advice and recommendations on policy matters to FDA; however, the committee recommended against reconstituting RAC or a comparable advisory body within the FDA, pointing out that several important policy functions of RAC are outside the mission of that agency.

5. A mechanism should be devised to enable ORDA, NIH and the RAC to continue to be provided with the data needed for monitoring clinical gene transfer protocols. Hence, the committee recommends that the NIH Director urge the FDA Commissioner to exempt the broad area of gene therapy from many of the proprietary restraints reserved for ordinary therapeutic drug products and biologics that come under FDA review. Such a broad exemption, similar to the one now in place for products being developed for the treatment of individuals infected with HIV, would greatly expedite efforts to monitor and evaluate gene transfer protocols and, ultimately, would accelerate progress in the clinical application of gene therapy."

Dr. Zallen noted that the RAC has continuously reexamined its role and has responded in a timely fashion to modify its role as knowledge has been gained. She commented that there may be a significant risk if the NIH Director chooses an option other than *status quo*. Specifically, investigators may avoid compliance with data reporting requirements in the event that the NIH Director relinquishes "control" of specific studies via the NIH approval process.

Dr. Estuardo Aguilar-Cordova, *Ad Hoc* Review Committee member, emphasized the importance of the RAC with regard to discussion of broader issues prior to actual protocol consideration, e.g., *in utero* gene therapy. The RAC would serve an extremely important service relevant to information gathering far in advance of the review of novel applications with possible societal implications.

Dr. Walters stated that written comments regarding the *Ad Hoc* Review Committee's deliberations were submitted by Alan Goldhammer, Ph.D., Director of Technical Affairs, Biotechnology Industry Organization in a letter dated December 1, 1995. On behalf of the private sector, Dr. Goldhammer expressed concern regarding the following *Ad Hoc* Review Committee recommendation:

"A mechanism should be devised to enable the ORDA, NIH, and the RAC, to continue to be provided with the data needed for monitoring clinical gene transfer protocols. Hence, the committee recommends that the NIH Director urge the FDA Commissioner to exempt the broad area of gene

therapy from many of the proprietary restraints reserved for biologics that come under FDA review. Such a broad exemption, similar to the one now in place for products being developed for the treatment of individuals infected with HIV, would greatly expedite efforts to monitor and evaluate gene transfer protocols and ultimately, would accelerate progress in the clinical application of gene therapy."

RAC Comments

Ms. Rothenberg asked about the benefits and risks of choosing any of the three options mentioned by Dr. Skirboll. Dr. Zallen stated that Option #2, the *status quo* of the NIH/FDA consolidated review process, is evolving to meet the needs of the new types of scientific questions posed by new protocols, and public deliberation about human gene transfer issues involving participation of the private sector and patient advocates. This option allows the NIH Director to keep in close contact with evolving gene therapy research. Option #3 eliminates RAC and NIH Director approval of individual protocols; it is very amorphous and cannot assure public accountability of gene therapy research. Dr. Zallen considered that the *status quo* option will provide the most benefit for the scientific community, regulatory agencies, and the public.

Ms. Rothenberg commented that if the NIH Director chooses Option #3, both the NIH and the FDA would make a public commitment to implement the necessary procedures. In the absence of such a commitment from each agency, there is a significant risk of losing public accountability for gene therapy. Dr. Skirboll responded that in the event that the NIH Director chooses to relinquish approval in cooperation with the FDA, all interagency negotiations would focus on the issue of public access to information. Clearly, the RAC cannot function in the abstract; information is a critical component to gene therapy discussions.

Dr. Skirboll noted that one of Dr. Varmus' key considerations is that some protocols reviewed by the RAC are lacking in scientific validity. When the Director of a research agency "approves" a proposal, there is the appearance that the "science" is being approved. This issue will probably be a primary consideration in Dr. Varmus' decision. There is a concern that NIH's "research" mission is overlapping the "regulatory" authority of the FDA. Ms. Meyers noted that the RAC continuously struggles with the issue of safety versus quality.

Dr. Chase suggested that the approval criteria should be more clearly defined. If the NIH relinquishes its approval authority, the RAC will be dependent on the FDA for the provision of data. There should be some safeguards in such a proposal to ensure access to data. Since both agencies are under the Department of Health and Human Services, the DHHS Secretary could provide such an assurance.

Dr. Straus inquired whether the RAC is the only NIH component to exert such "regulatory" author Dr. Skirboll responded that the RAC is the only NIH body that currently exerts such authority. Kirschstein explained that the NIH (previously Laboratory of Hygiene) was the first agency regulate drugs, vaccines, etc. In 1972, the NIH regulatory component moved to the FDA an became the Center for Biologics Evaluation and Research. Until 1972, the NIH maintained bot regulatory authority and research. Subsequently, the NIH relinquished its regulatory function to th

FDA.

Dr. Straus noted that the current pressures placed on the RAC and the NIH Director are driven b the proprietary interests of industry. In actuality, the RAC has not retarded the development of this technology. The RAC's open forum for gene therapy review has facilitated the development of ge therapy for the world. Dr. Straus stated that he could not participate in continued discussion of gene therapy unless there was an open public forum of review, "a forum which has some teeth in it." The RAC cannot exist in the context of broad philosophical issues alone.

Dr. Erickson stated that RAC members are both emotionally and intellectually dedicated to RAC review. In the absence of approval/disapproval, he stated that he would resign as a committee member.

Dr. Samulski stated that it would be impossible for RAC members to adequately review future no therapies without the personal experience that can only be accomplished through individual protocol review. The RAC would eventually be distanced from data and issues as protocols are maturing. Without clinical information, the RAC would cease to function because knowledge could not be gained to make informed decisions.

Ms. Meyers noted that NIH purview of gene therapy initially involved preliminary review by th Human Gene Therapy Subcommittee. This committee voted itself out of existence. The facts demonstrate that the NIH has quickly responded and is continuously reevaluating its roles an responsibilities. Industry assertions that the RAC has impeded progress in this field are simply untrue. She expressed concern regarding Dr. Goldhammer's written comments regarding publi access to clinical data. Is it in the public's best interest to identify such information as proprietary at some future time? This question is particularly pertinent since such information has previously been publicly accessible with almost negligible challenges to public accessibility. She noted that eliminating public access to gene therapy data could drive industry to conduct research solely on "profitable" diseases with little or no interest in the orphan diseases. Enhancement gene transfer could become a real possibility in the absence of publicly available data.

Dr. Ross said that the RAC resonates as a model of the*Ad Hoc* Committee's recommendations with the exception of the recommendation regarding the FDA provision of data. The NIH has to ensur guaranteed access to data and public accountability. Dr. Smith stated that, in the absence of approval, there would not be the same level of commitment; therefore, he would not feel that he is serving a useful purpose on the committee.

Dr. Smith inquired about the human immunodeficiency virus (HIV) data exemption referenced in recommendation #5. Dr. Skirboll noted that the specific precedent identified in recommendation # signifies an example of an FDA approach to public data access via the 1988 Health Omnibus Extensions Act (Hope Act). The Hope Act is a specific authorization to FDA for HIV/acquired immunodeficiency syndrome (AIDS) clinical trials. The Hope Act cannot be interpreted as relevant to the other NIH research areas; however, other mechanisms for proprietary exemptions may b available. Dr. Noguchi noted that the majority of funding for the HIV/AIDS database is provided by the NIH . This database is a model for clinical trial information. Anyone can dial an 800 telephon

number to obtain information about any ongoing HIV clinical trials regarding where the trial is being conducted and detailed information regarding the protocols.

Ms. Meyers inquired about the status of the gene therapy database that was established by appropriations initiated by Senator Hatfield. She noted Dr. Wivel's previous statement that previo NIH /FDA gene therapy database negotiations have been discontinued. Dr. Noguchi responded th funding for the database mandated by Senator Hatfield was never appropriated; therefore, the database is operated by the FDA under funds that are available. Dr. Noguchi stated that the database has been downsized but has not been discontinued.

Dr. Skirboll reminded the RAC that they should separate the issues of public data access an protocol approval. She solicited comments regarding relinquishing approval. This scenario would only be considered in the event that public access can be assured. Ms. Rothenberg expressed concern regarding the RAC's role in the event that a specific area demonstrates statisticall significant negative data. If "regulatory" purview is relinquished, what will the RAC's role be in suc a scenario?

Dr. Walters noted that in the 1970's, NIH made a commitment to Congress to oversee gene thera in response to preliminary legislation that established specific regulatory oversight of gene therapy, i.e., a separate Federal agency comparable to the Nuclear Regulatory Commission.

Dr. Noguchi noted that the RAC has been extraordinary successful in its efforts to maintain public access to gene therapy information. The RAC has "extended" the authority of FDA "informally" via providing a mechanism for public discussion, particularly of adverse events. Industry has benefitted tremendously from public access to data. This access has provided the private sector with timely data that has facilitated informed decisions regarding the validity of pursuing certain areas of research. Negative data are extremely important to industry. Significant savings are realized by eliminating duplication of efforts relating to negative data. Dr. Noguchi stated that the RAC should consider the "specifics" that it could provide that would be complementary to the FDA.

Dr. Erickson reiterated his statement that he would resign from the RAC if the RAC cannot have meaningful deliberations and an approving role of gene transfer protocols especially those that deemed to represent novel approaches. Dr. Ross agreed with Dr. Erickson's statement that the RAC would lose its credibility in terms of the public's view if it were not able to approve these categories of protocols.

Dr. Motulsky made a brief historical account of events that led to the creation of the RAC with it initial charge of guarding the safety aspect of recombinant DNA research. As RAC evolves to oversee human gene transfer experiments, the RAC becomes a hybrid of members, i.e., scientists and public members. The RAC does not currently have a sufficient number of scientific members to review diverse approaches of gene therapy research. The public members are concerned about the informed consent issues that are the purview of the Office of Protection from Research Risks and IRBs . Dr. Motulsky stated that the RAC should be disbanded. Dr. Kirschstein noted that th strength is its moral fortitude. The RAC provided the forum by which the NIH convinced Congres

not to establish a regulatory agency.

Dr. Straus stressed that the RAC has it special niche for its role, and it should exist whether under the aegis of NIH or FDA. The strength of the RAC is its openness and the expertise that add breadth and depth to deliberation about human gene transfer experiments that complements FDA's regulatory function. Most of the FDA's public advisory committees are involved with approval recommendations at the final stages of new drug development--a role that is different from the RAC's oversight of new technology development at its very early stag

Ms. Meyers noted that discussion of informed consent issues has been extraordinarily important to the patient community. The RAC represents a microcosm of a society where not only scientists but public members can deliberate on all issues of societal concern.

Dr. Lai inquired about the guidelines under which the RAC operates. The RAC continues to deliberate its role relevant to safety aspects of gene therapy research. With the three options outlined for the RAC, Dr. Lai asked if there is a proper body to review the scientific quality and merit of gene therapy proposals. Dr. Wivel explained that NIH has initial review groups such as st sections to review the scientific merit of NIH funded programs. The RAC does not have authority assign funding priority to a particular gene transfer proposal. Instead, the RAC functions with a threshold model, and the common denominator frequently becomes safety rather than scientific quality. Dr. Skirboll noted that Dr. Varmus has established a panel to assess NIH investme gene therapy research that will make recommendations on how to raise the scientific quality. There are many protocols that come across the RAC that do not have NIH funding. The RAC has t perform a complex balancing act between the issues of safety and scientific quality. The stamp of NIH approval of a particular protocol sometimes is misinterpreted as NIH approval of its scien merit. Dr. Robinson stated that when the RAC approves a protocol, there should be a real consideration of its merit. Dr. DeLeon suggested including RAC approval of a proposal in the N granting process, i.e., requirement of RAC approval before NIH awards a grant to an investigator Both safety and scientific issues should be addressed.

Dr. Hirschhorn noted that the RAC has served to "balance" the hype and publicity of gene therap portrayed by the media with deliberation of the general safety issues based on the protocols submitted to the RAC. Dr. Straus noted that it is not entirely correct to state that the RAC is concerned only with the safety issue. There have been numerous occasions of intense discussion of scientific merit of protocols. The investigators are asked to provide data to demonstrate transduction efficiency, the rationale and scientific justification of a clinical trial, risk/benefit assessment, and other questions with regard to the scientific merit of a proposal. Drs. Erickson and Smith concurred with Dr. Straus' statement.

Comments from the Public

Dr. Walters invited comments from the audience regarding the*Ad Hoc* Review Committee report.

Dr. Fred Ledley (Gene Medicine, Inc.) made two remarks: (1) The risks associated with somati gene therapy protocols are not different from many other types of medical applicationd researc conducted by academia or industry. The issues of informed consent and reimbursement are important; they have been dealt with in other types of medical research as well. (2) Dr. Ledley sta that he first proposed to the RAC in 1991 to establish a patient registry of gene therapy trials. The registry was intended to protect the patients, to facilitate long-term follow-up, and to track the patients in the event of adverse effects. However, his original proposal was not funded by NIH . T data management system currently being contemplated by the RAC is not adequate for long-term assessment of gene therapy; it is not rigorous, and it is not a controlled study. Regarding sole FDA review of gene therapy protocols, Dr. Ledley noted that FDA review of gene therapy protocols i being held to standards of clinical and statistical rigor.

Dr. Mary Treuhaft (Gene Therapeutics, Inc.) suggested to move the RAC to FDA as its advisor committee. Serious adverse events are required by FDA regulation to be reported immediately; there is immediate dialogue between FDA officials and investigators, and a real time assessment of the safety data is made possible.

Dr. Doros Platika (Progenitor, Inc.) stated that the quality of protocols is reviewed by other review groups; the RAC is mainly to address the safety issues concerning the use of recombinant DNA technology for the treatment of human diseases. The proper expertise exists within the FDA, and the FDA is capable of reviewing human gene transfer protocols, which are not fundamentally different from the development of drugs, biologics, and other medical treatments.

Dr. Michael Langan (National Organization for Rare Disorders) stated that NIH should take i consideration various legislative reform proposals concerning FDA now pending at Congress.

Mr. Andrew Braun (Massachusetts General Hospital) stated that the RAC's present purview of ge therapy is confined to a very narrow segment of clinical studies. It should extend its expertise to address the wider issues of clinical studies in general.

Dr. Tony Marcel (TMC Development) noted that the RAC has worldwide impact. The United State has the largest number of patients enrolled in gene therapy trials (890 vs. 134 in the rest of the world); Dr. Marcel attributed this success to the public forum of the RAC. The RAC is the key learning place for all those individuals interested in gene therapy worldwide.

Dr. Tomiko Shimada (Ambience Awareness International, Inc.) stated that the RAC has expedite government decision-making on gene therapy in Japan. It took only 9 months from the inception of the Gene Therapy Committee to the allocation of research funding for gene therapy by the Ministry of Health and Welfare of Japan. She attributed this prompt government response to the wealth of high-caliber information openly available through the RAC. She suggested that the RAC continue to concentrate on science review and the FDA on the safety aspects of the protocols.

Dr. Joseph Rokovich (Somatix Therapy Corporation) found that the open forum of the RAC

been helpful in developing programs and safety guidelines for his company. At the same time, the broader mandate of FDA and its availability on a daily basis is an important aspect of gene therapy regulation.

Committee Motions

Dr. Erickson stated that the RAC should continue to review gene transfer protocols on a selective basis, and he stressed that NIH approval authority is essential for this review process. Ms. Meyer asked if Dr. Erickson's recommendation is in keeping with the *Ad Hoc* Review Committee recommendation. Regarding the issue of NIH Director approval of protocols, Drs. Zallen Aguilar-Cordova stated that the *Ad Hoc* Committee has discussed this issue at length. The intention of the committee is to retain the current policy of approval by the RAC and the NIH Director o selective gene transfer protocols; otherwise, there are no "teeth" in the review process. Dr. Erickson was satisfied with the present selective review of novel gene transfer protocols.

Ms. Knorr noted that the only language that is currently in Section III-A, *Major Actions under the NIH Guidelines*, states that the NIH Director's approval of a recombinant DNA experiment is base mainly on safety concerns. The additional criteria stated in the Appendix M, *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* are not directly tied to Section III-A in terms of NIH Director's responsibility to approve human gene transfer protocols.

Dr. Walters noted that the *NIH Guidelin* reflect their history where the main concern has been biohazard.

Dr. Chase remarked that the *NIH Guidelin* should be amended to clearly state that the scientific merit of a gene transfer proposal is one of the major criteria for RAC approval. Dr. Chase suggested that the strength and expertise of RAC members with diversified backgrounds can be employed by the NIH Director to address general issues of clinical research, such as financing and patients rights, which are not specific to gene therapy.

Dr. Motulsky noted that Dr. Varmus has recently established a clinical research panel headed David Nathan to examine the general issues of translational types of medical research, and Dr. Motulsky suggested that the RAC can coordinate with this panel to work on these broader issues Dr. Motulsky stated that somatic gene therapy research should be part of the mainstream clinica investigation; it is different from germ line gene alteration.

Committee Motion 1

A motion was made by Dr. Erickson and seconded by Dr. Ross that the RAC should make the following recommendation to the NIH Director regarding RAC oversight of human gene therapy: T RAC should continue to function under *status quo*, i.e., selective review and approval of novel

human gene transfer protocols. The motion passed by a vote of 16 in favor, 0 opposed, with no abstentions.

Committee Motion 2

Dr. Walters asked the RAC for a vote to ratify the recommendations of the *Ad Hoc* Review Committee. Ms. Rothenberg stated that the #5 recommendation should be emphasized since it is particularly critical from the standpoint of political, regulatory, and legislative aspects. The #5 recommendation requests that "the NIH Director urge the FDA Commissioner to exempt the broa area of gene therapy from many of proprietary restraints reserved for ordinary therapeutic drug products and biologics that come under FDA review."

Dr. Lysaught noted that under the current NIH /FDA consolidated review process all data can submitted to NIH without an FDA special exemption for gene therapy data. Dr. Walters noted tha the #5 recommendation is theoretically a concern if a complete public record will be maintained, including protocols funded totally outside NIH funding mechanism

Dr. Smith stated that if the RAC continues to exist, it should stress the review of the scientific merit o protocols; alternatively, the RAC can be replaced with another mechanism that provides in depth review of selective protocols.

A motion was made by Ms. Rothenberg and seconded by Dr. Erickson to endorse all recommendations of the *Ad Hoc* Review Committee report with a special emphasis on the #5 recommendation to urge the FDA Commissioner to exempt gene therapy data from customary proprietary restraints. The motion passed by a vote of 14 in favor, 0 opposed, and 1 abstention.

Dr. Zallen abstained from voting due to a conflict of interest based on her being a member of th *Ad Hoc* Review Committee.

IX. DISCUSSION REGARDING FULFILLMENT OF RAC STIPULATIONS FOR PROTOCOL ENTITLED: *PHASE I STUDY OF ADENOVIRAL VECTOR DELIVERY OF THE HSV-TK GEN AND THE INTRAVENOUS ADMINISTRATION OF GANCICLOVIR IN ADULTS WIT MALIGNANT TUMORS OF THE CENTRAL NERVOUS SYSTEM* **/DRS. GROSSMAN AND WOO**

Review--Drs. Samulski , Smith, and Zal

The protocol entitled: *Phase I Study of Adenoviral Vector Delivery of the HSV-TK Gene and th Intravenous Administration of Ganciclovir in Adults with Malignant Tumors of the Central Nervou System* by Drs. Robert Grossman and Savio Woo of Baylor College of Medicine, Houston, Texas was provisionally approved by the RAC at its December 2, 1994, meeting. The approval was contingent on the review and approval of the following: (1) data derived from ongoing dose-escalation toxicology studies in non-immune cotton rats in which animals undergo intranasal

immunization with the adenovirus vector followed by brain administration with and without the administration of ganciclovir) up to a dose that is greater than the dose that was lethal for baboon (2) data derived from preclinical cotton rat studies (including histological analysis) in which pre-immunized animals receive direct injection of the adenovirus vector into the brain (to determine the effect on the central nervous system); and (3) revision of the Informed Consent document to include a statement that informs potential participants of the adverse events (toxicity) that have been reported for a similar Phase I human gene therapy protocol involving an HSV-TK /retrovirus gen delivery system. On January 19, 1995, Drs. Grossman and Woo submitted the proposed experimental design for the preclinical toxicity experiments. A subcommittee of the RAC (Drs. Samulski , Smith, and Zallen) concurred with the proposed experiments. On July 12, 1995, the derived from these cotton rat toxicity studies were submitted for RAC review. Drs. Samulski an Smith requested further evaluation of this preclinical data by an *ad hoc* expert, Neil W. Kowall , M. Associate Professor of Neurology and Pathology, Boston University School of Medicine, Boston, Massachusetts.

Dr. Samulski stated that Dr. Kowall's evaluation of the pathological slides from the toxicity st concluded that neuropathological changes of ventriculitis with choroidal , ependym subependymal inflammation were observed in most animals. Dr. Kowall concluded that given terrible prognosis and lack of any curative treatment for malignant brain tumors, his clinical opinion would favor proceeding with human trials.

Drs. Samulski , Smith, and Zallen concurred with the expert opinion that toxicity was observed cotton rats at high vector dosage, but that the risk/benefit ratio of the adenoviral vector for malignant brain tumor patients is acceptable. These data fulfill the RAC stipulations for the protocol.

X. AMENDMENT TO THE NIH GUIDELINES REGARDING ANNUAL DATA REPORTING/MS KNOR

Dr. Walters called on Ms. Knorr to report on her proposed amendments to th*NIH Guidelin*, which would allow the semiannual data reporting requirement to be amended to an annual reporting requirement (letter dated November 2, 1995). Ms. Knorr stated that in a letter dated June 16, 199 Dr. Gary Nabel outlined the redundant and onerous reporting requirements of multiple Federa agencies and local institutions. Ms. Knorr stated that at a minimum, amending th*NIH Guidelin*to accommodate annual data reporting requirements should greatly reduce the burden currently placed on PIs of human gene transfer protocols.

Dr. Walters stated that the proposal is straight forward. Dr. Smith stated that he would made a motion to approve the amendment, and Dr. Motulsky indicated he would second the motio

Ms. Meyers raised a concern about changing the reporting requirements from a semiannual to an annual basis. One of the major roles of the RAC is to monitor the progress of gene therapy, and annual reporting may not be able to flag significant events in a timely manner. Dr. Wivel explaine that serious adverse events or unanticipated events are required to be reported immediately, and it is independent of the regular annual report of protocols. The RAC would not be deprived of the most

critical types of information by this amendment.

Dr. Hirschhorn noted that Dr. Nabel's main concern is the multiple reporting forms he needs complete and file with many agencies. A single form simultaneously reporting to all agencies is most beneficiary.

Ms. Knorr noted that the amendment will not affect the information currently available at eac quarterly meeting, including the updated protocol list, amendments to protocols, information regarding the exempt protocols, and adverse event reporting. Ms. Knorr proposed to establish a interagency working group to optimize the reporting system. The working group should consist of representatives from FDA, ORDA , and OPRR which oversees the local

Dr. Smith stated that an annual review would allow a more comprehensive review of the data. Dr. Ross would favor annual data reporting since it takes a long time before patients are enrolled, studied, and the results analyzed. Annual reporting would be adequate.

Dr. Zallen was concerned that timely information regarding the scientific progress of the protocol would not be available if data are reported on a yearly basis. Ms. Knorr noted that some of th information regarding protocol progression can be obtained from the investigators' requests for protocol amendments.

Dr. Robinson favored a single common form for reporting to all agencies. Dr. Noguchi stated that a unified format would benefit everyone.

Dr. Hirschhorn made a friendly amendment to the motion to include an unified format for annual c reporting. Ms. Knorr stated that the data reporting form the RAC has developed contains detaile and specific questions; it could be used as the basis for the interagency working group discussion. Dr. Hirschhorn added that the same form should be used for reporting to IRBs . Drs. Smith Motulsky accepted the amendmen

Dr. Ross inquired if the reporting requirements include all investigators and institutions under the purview of the *NIH Guidelin* Ms. Knorr responded yes; it includes the protocols reviewed by th RAC and the protocols exempt from RAC review and solely reviewed by FDA.

Dr. Robinson stated that in addition to a common reporting form, it should have a common reporting date, i.e., based on a calendar date. Dr. Walters stated that while most reporting forms are required on anniversary date, the RAC would desire to have calendar year reporting. Dr. Noguchi noted that FDA's reporting is based on the anniversary date of protocol approval, and stated that it would be impossible to amend the FDA procedures just for one specific area of therapeutics.

Committee Motion

A motion was made by Dr. Smith and seconded by Dr. Motulsky to approve the amendment to th *NIH Guidelin* for annual data reporting. A working group consisting of representatives from NIH FDA, OPRR , IRB and Institutional Biosafety Committees (IBCs) should be formed to estab common reporting form (i.e., the current RAC semiannual data reporting form) and a common reporting date. The motion passed by a vote of 14 in favor, 0 opposed, and no abstentions.

XI. PRESENTATION ON ETHICAL ISSUES ASSOCIATED WITH *IN UTER* GENE THERAPY/DR. FLETCHER

Dr. Walters called on Dr. John C. Fletcher, Kornfeld Professor and Director of the Center fo Biomedical Ethics, University of Virginia, Charlottesville, Virginia, to discuss the ethical issues associated with *in uter* gene therapy, in response to an invitation made by the RAC at its March 3-4, 1995, meeting. Dr. Fletcher is the third speaker in a series of *ad hoc* experts invited to address the RAC for the purpose of providing educational presentations relevant to *in uter* gene therapy. These lectures are to provide information in a public forum to address the scientific, safety, ethical, and legal issues prior to the consideration of an *in uter* gene therapy proposal.

Dr. Walters noted that Dr. Fletcher has been a pioneer in the new discipline of biomedical ethics. Dr. Fletcher published a seminal paper on human experimentation ethics in the consent situation in 1967 in the journal *Law and Contemporary Problems*. He was the Chief of the Bioethics Program at the NIH Clinical Center from 1977 to 1987 before he moved to the University of Virginia. Dr. Fletc has published several classic papers regarding the ethical issues of human gene therapy: a paper co-authored by Dr. French Anderson published in the *New England Journal of Medicine* in 1980 that established criteria for when it would be considered ethically and scientifically appropriate to proceed in studies involving human subjects; a paper published in 1983 in the *Virginia Law Review* that discusses the ethical issues involved with germ line gene therapy; and papers dealing with ethical issues in *in uter* surgery.

Presentation--Dr. Fletcher

Dr. Fletcher emphasized that the RAC has a place in public bioethics. It is important to have a historical continuity in RAC deliberation about bioethical issues. Dr. Fletcher raised two major issues regarding experimental *in uter* gene therapy: Is it ethically acceptable? Is it a morally praiseworthy goal?

Is it ethically acceptable? In a paper published in 1990, Dr. Eric Juengst gave five considerations experimental human gene therapy: (1) clinical benefits and risks to subjects, (2) voluntary and informed consent, (3) fair selection of subjects, (4) harm to germ line cells, and (5) public oversight. Dr. Fletcher stated that he would address these five issues as they are related to *in uter* gene therapy later in his presentation.

Is it a morally praiseworthy goal? When President Truman was asked by a colleague whether to pursue a particular idea, President Truman responded, "Well, step back and look 5 to 10 years

down the road. Think about what you could have done to foresee where you would be 5 to 10 years down the road, once you embark on this particular idea." Dr. Fletcher paraphrased President Truman's remark in considering the following five questions:

1. The promise made in prenatal diagnosis. Due to the emergence of amniocentesis and later chorionic villi sampling techniques, several meetings were held at NIH in the late 1960's a 1970's to address the issues of the promise made in prenatal diagnosis. Dr. Fletcher noted that a "promise" is not an absolute duty; it is a hope and an intention to do certain things ethically. The meetings were to examine whether the promise of prenatal diagnosis can be legitimately kept. One of the concerns raised in the discussion was selective abortion of unwanted fetuses.

2. Preimplantation embryo diagnosis. Is it a better choice for couples who are at high risk to hav offspring with a Mendelian genetic disorder? In Dr. Fletcher's opinion preimplantation emb diagnosis is a safer and more efficient procedure than *in uter* gene therapy at the present time, although it is still a very expensive procedure that does not serve a large number of people. Any responsible physician or counselor should bring up in the discussion with couples the proven alternative approach of prenatal diagnosis followed by a strategy of selective abortion.

3. Alternative to selective abortion. For people whose moral view of the status of the embryo would prevent them from taking the strategy of selective abortion, *in uter* gene therapy offers an alternative.

4. Technical and moral responses to experimental *in uter* gene therapy. Dr. Fletcher stated that as a lay person he is still skeptical about the effectiveness of homologous recombination and site-directed gene replacement. Is the current technology adequate to replace a defective gene of a fetus with a good one at precisely the right place on a chromosome?

Dr. Anderson agreed that it is still not possible to perform homologous recombination for a human embryo. Dr. Hirschhorn noted that even preimplantation diagnosis is still technically unsatisfa Dr. Walters noted that the technology of homologous recombination is more critical to germ line genetic intervention with preimplantation embryos tha *in uter* gene therapy which is a form of early somatic cell gene therapy. Dr. Noguchi noted that it is not a giant step in terms of technological advance to proceed from *in uter* stem cell transplantation to *in uter* gene therapy. The question is whether a society is ready for this type of treatment. Dr. Samulski remarked that the technology f preimplantation gene therapy is similar to that used in transgenic animal studies in the last 10 to years. The technique involves microinjection of the DNA into the embryo at the first-cell stage; the transgene requires a proper transcriptional regulation for its expression; however, it does not nee to be located to a specific site for its function. Dr. Hirschhorn noted that it is only in an ideal form gene therapy, which is not yet perfected, that a transgene could be inserted into an exact locus o chromosome in order to prevent insertional mutagenesi

Dr. Chase inquired if there will be any impact on the fitness of species that results from reducing genetic variation. Dr. Fletcher responded that it may affect the species in the long-term. Dr. Motuls noted that the same argument has been raised about modern medicine and the changing of our

gene pool; infants with infantile diarrhea have been kept alive permitting them to transmit their genes to the next generation. Dr. Hirschhorn noted that in a recessive genetic disorder most of th deleterious genes are present in heterozygous carriers; treating the small number of homozygous fetuses will have little impact on the gene pool. Dr. McIvor noted that the imminent *in uter* gene therapy proposal will be based on gene addition rather than homologous recombination. The rationale is to treat the disease in an early stage *in uter* with somatic cell gene therapy.

Ms. Rothenberg noted that there is a continuum from the preimplantation embryo to birth. scientifically significant question is whether treating patients earlier is more advantageous. Dr. Anderson responded that the *in uter* gene therapy technique will not be used except in those cases where there is irreversible damage before birth or proof of principle that *in uter* gene therapy could be successfully performed.

Dr. Fletcher continued his presentation regarding the technical and moral responses to experimental *in uter* gene therapy. He noted that there are certainly higher social and economic priorities in our society than genetic services or human gene therapy. There is a great need to deliver basic health care to the general populace in this country; genetic services ought to be part of basic health care. There are concerns about harming germ line cells and the gradual progression toward germ line gene therapy. *In uter* gene therapy would be a another step toward these concerns.

5. *In uter* gene therapy: would it be appropriate in very rare cases? The first case of *in uter* gene therapy might be an unplanned pregnancy where it is too late to have preimplantation diagnosis; a number of reasons, the couple would consent to this kind of experiment.

Is Experimental In Utero Therapy Ethically Acceptabl

1. Clinical benefits and risks to subjects (pregnant woman and fetus).

The risks to the fetus are the risks of the long-lasting effect of the procedure especially if there is a failure. The survivor, the treated fetus, has never consented to the procedure. It needs lifelong follow-up to determine the long-term consequences. There is a potential psychological impact to a person's life once he/she has been treated as a fetus. Dr. Fletcher stated that the issue of the effects of prenatal diagnosis both on the survivors and other children in the family should be studied.

The risk to the pregnant woman is the procedure itself, and there is a measurable risk of failure. Can a homologous site-specific transduction vector be developed to minimize the chance of insertional mutagenesis?

2. Voluntary and informed consent by the pregnant woman.

Dr. Fletcher stated that the pregnant woman is the focus of the informed consent process, and her participation is central and morally non-negotiable. She must be a capable decision maker. Informed consent is a process. The quality of the process is important; it involves more than the Informed Consent document. The mother's physician should be involved in this process to assist her in understanding the risks and benefits of the procedure. The father of the future infant must be involved if it is reasonable to involve him. If there is a conflict between a father and a mother, the mother's consent would be morally overriding. In the first few cases, an impartial physician to serve as the fetus's advocate might be sensible. It is useful to have a genetic counselor to explain the procedure to the family, but it is not absolutely necessary. Documentation of the informed consent process is vital. There should be an option of the protection of privacy; however, it cannot keep a family from going public if the family wants to publicize its involvement in an experiment.

3. Fair selection of subjects.

The fair selection of subjects should begin with the question of whether other alternative therapies are available. Morally, the first treatments should be directed toward diseases in which the damage develops prenatally , e.g., Lesch-Nyhan or Tay -Sachs disease. However, one would be wil make a pragmatic choice of candidate diseases that appear to be the safest and the most promising for effectiveness of the treatment, e.g., severe combined immunodeficiency (SCID) due to adeno deaminase deficiency (ADA) or purine nucleoside phosphorylase deficiency. Will the experiments be attempted on couples who are morally opposed to abortion or couples who have a window of opportunity between prenatal diagnosis and the abortion decision? Dr. Fletcher would not make a couple's attitude toward abortion an absolute moral fulcrum for the couple. Would twins be subjects if one twin is not affected? Dr. Fletcher stated that it is not moral to conduct *in utero* gene therapy in a situation of multiple gestations if some fetuses are healthy. One should avoid potential harm to a healthy future infant.

4. Harm to germ line cells.

Thorough animal studies, including studies on primates, are needed to ascertain that there is no damage to the germ line by *in utero* gene therapy. There will be no absolute guarantee that germ line cells will not be harmed.

5. Public oversight.

Public oversight involves partnership among the IRB , IBC, and the RAC. Dr. Fletcher strongly fav the continuation of the RAC, a public forum where deliberation is based on solid data, not on ideology and strong precommitments . A democracy cannot survive without a forum like the RAC. Fletcher is looking forward to the establishment of the National Bioethics Advisory Commission whose mandate is to provide a public forum to debate the societal concerns of human genetics. The Ethical, Legal, and Social Implications Program of the NIH National Center for Human Genom Research is a national resource for human genetic research.

Other Comments

Ms. Meyers raised a concern that in the future, if *in utero* gene therapy demonstrates promise, there may be commercial entities entering the field. She is particularly concerned with attempts at gene enhancement therapy. If the therapy does not involve NIH funding, there will be no federal oversi other than the FDA. Dr. Wivel responded that FDA has the responsibility for all biologic product including *in utero* gene therapy products. Dr. Noguchi added that the RAC provides a public forum to raise any issues that concern the society at large. Dr. Lysaught stated that initial attempts will b made with patients who have severe diseases. A larger issue is to examine the social and public impact of the new technology, not necessarily in terms of public health considerations.

Dr. Smith remarked that the enhancement issue is complex. There are types of dwarfism where patients live a normal life span; gene therapy, if it is justifiable, would make living more pleasant. Many of the issues of *in utero* gene therapy are not unique; they are common issues with other types of therapy, such as *in utero* stem cell transplantation, which are not under the purview of the RAC. One of the issues that is unique to *in utero* gene therapy is the adventitious transduction of germ line cells. Dr. Fletcher noted that the questions that are unique involve the technology of gene transfer and its impact on the fetus and the pregnant woman. Dr. Smith noted that most of the issues deliberated at the RAC have broader implications for general clinical research.

Dr. Hirschhorn remarked that there are generic clinical research issues that have special importa for gene therapy. There is a need for an unbiased presentation of alternative therapies to the patients from a genetic counselor, a need for long-term follow-up (individuals who have received successful bone marrow transplantation have been followed for a period of over 20 years), and a need to conduct studies of the psychological impact on treated children.

Dr. Fletcher said it is difficult to foresee the actuality of the consent process in the future. Based on his recent experience with the first fetal therapy experiment with a mother of a fetus with ambiguous genitalia, Dr. Fletcher stated that the best method is to have good rehearsals of the anticipated scenarios. The RAC has had the experience of preparing itself for the first gene therapy experiment. There is an underlying drama when certain investigators become entrusted with the role of initiating a new type of human experimentation. Responding to Ms. Meyers' concern about commercialization, Dr. Fletcher stated that in an open society such as the United States, one cannot guarantee, beyond the early stages of the human experimentation, what will be marketed. Dr. Hirschhorn noted that an unbiased counselor is important if an investigator has a vested interes

Ms. Rothenberg recalled instances in earlier cases of *in utero* stem cell therapy attempts in which women did not keep their promises of not aborting their treated fetuses. She inquired if there should be an inclusion criterion of a strong commitment to not having an abortion. Ms. Rothenberg noted that another potential conflict could occur between the mother and the father, particularly when the father is the carrier of the genetic disease.

With regard to the abortion criterion, Dr. Fletcher stated that one ought to be as neutral as possible with respect to the abortion issue and should have a fair procedure for selecting subjects. The

decisions of the participants should be respected. He would prefer to have the fetal experiments conducted in the post-abortion decision window, after the couple has decided on this issue.

Dr. Chase inquired how the bioethical community responded to the situation where the investigators and their sponsors frequently make unqualified claims of success for their gene therapy experiment. Sometimes the experiments have been described as if they were far more effective than they in fact are. Dr. Fletcher noted that the bioethical community is a very incipient interdisciplinary field, and there is insufficient literature directly bearing on this commercialization issue.

Responding to Ms. Rothenberg's question of women not keeping their word to carry the treated fetuses to term, Dr. Noguchi stated that these women often choose to have an abortion after the medical tests showed that there was no evidence of successful stem cell engraftment. Ironically, in one of the aborted fetuses it was later shown by post-abortion pathological study that engraftment had indeed occurred. The timing of the fetal testing for engraftment in this case might have been performed too early. Dr. Noguchi noted that the woman's commitment to not having an abortion is a very complex issue. Dr. Hirschhorn remarked that the mother and father should be provided wit accurate information, and it is their decision to make. Dr. Fletcher noted that the woman has the legal and the moral right to withdraw from the experiment at any time.

Dr. Anderson announced that he and Dr. Esmail Zanjani are ready to submit a preclinical d document for an *in uter* gene therapy protocol to the March RAC meeting, so that the RAC will have a definite case, including all of the data for critical review. This proposal will not be submitted for approval of a human trial; however, it will include all the preclinical data. Dr. Walters commented that Dr. Anderson's suggestion was an excellent idea. He recalled that in 1987, Dr. Anderson and his colleagues had submitted a proposal of the first human gene transfer experiment almost a year in advance of the actual clinical protocol.

Dr. Ross welcomed Dr. Anderson's proposal. She asked if it is technically possible to avoid any chance of adventitious transduction of germ line cells. Dr. Anderson stated that in the sheep experiments, there was one animal which appeared at first to have gene transfer to the sperm; further analysis concluded that the adventitious DNA was not in the germ line cells *per se*. The germ line cells in sheep develop in the first trimester; gene transfer in these experiments has been performed during the second trimester at a time when the germ cells have already established. A more thorough study involving 24 sheep is ongoing.

Dr. Lysaught commented about the importance of a specific protocol for RAC deliberation. The R is the only forum where a particular scientific protocol can be reviewed in the entire context of various aspects, including its scientific merit for advancing the field, the informed consent process and document, the public impact, and its stewardship of resources. Good ethics is important for good science. Dr. Hirschhorn stated that specific philosophical and ethical issues would be raise by considering a specific protocol with all of the scientific details.

Dr. Walters thanked Dr. Fletcher for his presentation.

XII. GENERAL COMMENTS REGARDING INFORMED CONSENT ISSUE/DR. WALTERS

Dr. Samulski asked if the Informed Consent document is considered when a protocol is exempte the NIH /FDA consolidated review process. Dr. Wivel explained that it is considered as a pack with the rest of the protocol in the exempt decision. If the reviewers make specific comments, the investigators are asked to respond to them; these documents are included in the RAC meeting materials. Dr. Walters added that comments regarding the Informed Consent document can be raised for protocols reviewed by ORDA staf

Ms. Meyers asked if the FDA reviews the Informed Consent document. Dr. Noguchi responded that FDA regulations require that the local IRB approve the Informed Consent documents. For gen therapy protocols, the Informed Consent documents are reviewed as part of the process. Responding to a question by Dr. Zallen about the specific requirements of gene transfer researc stated in the Appendix M-III-B-2 of the *NIH Guidelin*, Dr. Noguchi stated that FDA would make efforts to include those requirements in future revisions of the FDA guidelines. Responding to a question by Ms. Rothenberg on including women in protocols, Dr. Noguchi stated that FDA makes special efforts to ensure that women are not excluded from clinical trials unless there are absolute medical reasons.

Ms. Meyers noted that there was an HIV protocol from Viagene (IND #5107 by M. Conant solely reviewed by FDA, and that it did not enroll any woman among the 40 patients accrued in the trial. Ms. Sheryl Osborne (Viagene) stated that the trial was completed several years ago; at tha time, there were no females in the patient population. In the current trials, there is 20% enrollment of females. Ms. Rothenberg stated that it would not be acceptable to omit women from AIDS trials at the present time. Ms. Knorr noted that the Informed Consent document of the Conant Protoco not reviewed by the RAC.

XIII. DISCUSSION OF PROPOSAL TO TREAT CANAVAN'S LEUKODYSTROPHY / MEYERS

Dr. Walters noted that Ms. Meyers in her letter dated October 16, 1995, raised a concern about a gene therapy experiment contemplated by a Yale University investigator to treat children with Canavan's leukodystrophy . A newspaper artic*New York Times*, Connecticut Edition, October 29, 1995) reported on a local physician who is attempting to develop a gene-therapy approach to Canavan's leukodystrophy . The Connecticut physician is affiliated with the disease. The scien Dr. Mathew During, apparently does not intend to seek permission from the RAC for this protocol. In a letter dated October 25, 1995, Dr. Wivel responded to Ms. Meyers' concern. Dr. Wivel state Dr. During is a citizen of New Zealand, and he is planning to return to New Zealand on January 1, 1996, as the Director of a gene therapy research unit. Dr. During is actively involved with others in the development of a set of guidelines for use in the oversight of human gene therapy research in New Zealand. There is a clear indication that the safety practices to be employed abroad will be consistent with the *NIH Guidelin*

Dr. Lysaught inquired if the Yale IRB and IBC are aware of the protocol. Dr. Wivel explaine during a recent meeting of the New England Regional Genetics Society, he had a conversation with Dr. Margretta R. Seashore, Professor of Genetics at Yale University, about Dr. During's exper The Yale IRB and IBC are well aware of the situation but have not yet formally reviewed th protocol; therefore, the proposal was not ready to be submitted for RAC review.

Dr. Smith inquired if there is a review procedure to deal with this kind of emergency protocol. Dr. Wivel explained that the RAC established a single patient expedited review procedure earlier t deal with unusual protocols; with the advent of consolidated NIH /FDA review, there is no provisio for expedited review.

Dr. Ross was disturbed to notice a solicitation for donations in the *New York Times* article stating: "Donations to the Yale University Canavan Project may be sent to Dr. Mathew During, Canav Project, Post Office Box 208039, New Haven, Connecticut 06520-8039."

Ms. Meyers was concerned about the present case as a precedent that will allow investigators to evade the current rules that provide oversight for human gene transfer experiments in the United States; some investigators might take their vectors to countries where there is no adequate oversigh to perform the gene transfer experiments on human subjects. The *NIH Guidelin* are not a legal regulation and do not have the force of a law; however, she was concerned that the investigators might attempt to evade RAC oversight.

Dr. Noguchi stated that this protocol could be a case in which the RAC might ask the FDA Commissioner for a review since there are issues of transport of a regulated product across state and international boundries . Public awareness of cases like the present one can help FDA initiate review.

Dr. Wivel noted several precedents for international collaborative research in gene therapy. Th reagents for the SCID -ADA trial in Japan came from the United States; however, these are reage approved by the RAC and the FDA.

Dr. Anderson noted that under the *NIH Guideline* any reagents to be transported to a foreign country from an institution, i.e., Yale University, which receives NIH funding for recombinant DN research must have approval from the Yale IBC. Dr. Samulski noted that scientists frequently sha their reagents with investigators around the world. He asked if the scientists will then be liable for the experiments the recipients performed with their reagents. Dr. Anderson stated that the Material Transfer Agreement (MTA) stipulates that the materials should be used under the appropriat regulations; the institution is the responsible scientific official for the MTA . Dr. Samulski expres his concern about liability because one of Dr. During's vectors proposed for his experiments cam from his laboratory.

Dr. Zallen suggested sending a letter to the NIH / OPRR to issue an advisory to IRBs to investigators that appropriate regulations should be observed when they collaborate with foreign

investigators in their human gene transfer experiments. Dr. Wivel said it is a concern if the reagen exchange involves clinical gene transfer trials.

Responding to Dr. Samulski's concern of sharing reagents, Ms. Knorr noted that if the receiv institution is under the purview of the *NIH Guidelin*, the IBC of that institution is responsible for reviewing research conducted with his vector. The *NIH Guidelin* provide guidance for research performed abroad under Section I-C-1-b, specifically: (1) if research is supported by NIH funds; (it involves reagents developed with NIH funds and if the institution sponsors or participate in thos projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials; and (3) if the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH -approved IBC or equivalent review body and accepted in writing by an appropriate nationa governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the *NIH Guidelin*

Ms. Rothenberg noted that if Yale University does not continue to collaborate with Dr. During in New Zealand, then his research is outside the purview of the *NIH Guidelin*. Dr. Wivel agreed. Ms Meyers noted that it will be of concern if the patients treated in New Zealand return to Yale University for follow-up or if Dr. During maintains some faculty appointments with Yale University.

Ms. Rothenberg noted an hypothetical scenario where an investigator has been denied approval of his/her human experiment by an IRB . The investigator then accepts a position in a foreign institut and terminates all the collaboration with the former employer. Ms. Rothenberg asked if then the experiment is then outside the purview of the *NIH Guidelin*. Dr. Wivel responded affirmatively. Meyers said that this scenario is precisely what concerns her. Dr. Chase said that the protection of the American public from becoming a victim of an unsafe treatment abroad is the free flow of information to educate the American public about this danger.

Dr. Noguchi shared Dr. Samulski's concern that if the reagents are used under an unsupervise situation, they could be contaminated by impurities that could be harmful to the patients. Dr. Hirschhorn noted an element of hubris in assuming that only the United States has high standard for therapeutics. She noted drugs that are available in Europe and Canada 10 or 15 years before FDA approves them for this country.

Dr. Samulski stated that the investigators are bound by the rule to share reagents if their researc papers are published in scientific journals. There is little recourse to guarantee against any misuse of their reagents. Dr. McIvor stated that the *NIH Guidelin* are clear regarding its purview of experiments performed abroad. A proper MTA should be executed to include the reagent itself ar the vector sequence for the protection of investigator's liability for misuse.

Ms. Knorr noted that an investigator would be prudent to ask if the receiving institution has any funding for recombinant DNA research, or in case of no NIH funding, if the institution and the hos country have a written commitment to comply with the *NIH Guidelin*. Dr. Wivel said that it i

uncertain that it is legally enforceable to ensure that another country will comply with all elements of the *NIH Guidelin* Ms. Rothenberg responded that there would be many legal challenges to the enforcement.

Ms. Rothenberg asked if the Yale IRB has reviewed the protocol. Ms. Meyers said that Yale IRE not yet reviewed and approved the study. Ms. Rothenberg stated that she is uncomfortable in discussing the Yale's responsibility in this case without more data.

Dr. Zallen favored having OPRR advise the IRB and the investigators on proper rules in co human gene therapy trials. Dr. Smith said that in this case the investigator knows the regulatory requirements. The Yale IBC of which Dr. Smith is an *ad hoc* member has not yet received an application regarding this particular experiment. Ms. Rothenberg stated that if the patients are initially recruited at Yale and continue to be treated at Yale, then the university has a fiduciary duty to the patients.

Dr. Anderson stated that aside from the issues of institutional involvement, he is concerned if the investigator knowingly chooses to disregard the appropriate rules and goes ahead to perform the experiment. He was concerned that some investigators believe that they can circumvent the *NIH Guidelines* and no one will notice.

Dr. Erickson stated that there is a moral issue of giving the patients false hope. The patients have a life expectancy of 1½-2 years. It is unreasonable to assume that an entirely new gene therapy can be developed in such a short time-frame. Solicitation of contributions to the Canavan project shou be done in the context that there is little chance of these children benefitting from this project however, the contribution is to help other children with the same disease. Ms. Meyers noted that the father of one of these children is a medical doctor.

Ms. Knorr noted that under th*NIH Guidelin*the institution has the responsibility to establish the IBC and to ensure compliance at the institution. The institution is responsible for monitoring all the recombinant DNA experiments being performed under its jurisdiction.

Ms. Meyers said that the investigator needs to acquire permission from the IRB and IBC of Yal University, as well as a written letter from an appropriate oversight body in New Zealand. Dr. Wivel explained that the purview of the *NIH Guidelin*has to be based on the exact knowledge of the type of relationship between a United States institution and what goes on in New Zealand. The IRE and IBC of Yale University will be contacted to obtain further information.

Dr. Smith noted that the investigator has not yet made any definite proposal to perform the alleged experiment. He agreed that the parents should not be given false hope for treatment of their disease

Mr. Andrew Braun asked if it is legal to recruit patients in the United States for unapproved treatments abroad. Dr. Noguchi responded that the legal basis of this practice is ambiguous. The

studies that are performed in foreign countries, even if they are paid for by American sponsors, do not have to be formally under an Investigational New Drug (IND) applicatio

Dr. Wivel stated that Yale IBC and IRB will be queried to determine if this case is under the pu of the *NIH Guidelin*

Dr. Walters thanked Ms. Meyers for bringing this matter to the RAC's attentio

XIV. CLOSING REMARKS/DR. WALTERS

Dr. Walters stated that he has enjoyed serving as the RAC Chair for the past 3 years and that he appreciated the excellent work of RAC members and the ORDA staf

Dr. Samulski stated that it is prudent for the RAC to take the lead in considering the forthcomin proposal from Dr. Anderson regarding fetal gene transfer. This RAC meeting has set two precedents: approval of a protocol using an E1A viral oncogene for a gene therapy study an treating patients who do not have life threatening disease in the OTC deficiency protocol.

Dr. Walters noted that these are two generic issues regarding human gene therapy that will require further discussion in the future: (1) use of vectors carrying oncogenes for human gene therapy, a (2) target patient populations with diseases that are not life threatening.

XV. FUTURE MEETING DATES/DR. WALTERS

The next meeting of the RAC will be March 4-5, 1996 at NIH , Building 31C, Conference Room 1

[Note from the Executive Secretary: The March 4-5, 1996, meeting was canceled. The next meeting will be June 6-7, 1996]

XVI. ADJOURNMENT/DR. WALTERS

Dr. Walters adjourned the meeting at 12:30 p.m. on December 5, 1995.

	/ s /
	Nelson A. Wivel , M.

	Executive Secretary I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.
Date: 12-5-95	/ s / LeRoy B. Walters, Ph. Chair Recombinant DNA Advisory Committee National Institutes of Health