DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
September 12-13, 1994

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The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-ninth meeting at 9:00 a.m. on September 12, 1994, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on September 12 from 9 a.m. until 5 p.m. and September 13 from 8:30 a.m. until 3:30 p.m. In accordance with Section 552 b(c)(4), Title 5, U.S.C. and Section 10(d) of Public Law 92-463, the meeting was closed to the public on September 12 from 5-5:30 p.m. to review, discuss, and evaluate proprietary information. The following were present for all or part of the meeting:

Committee Members:

Alexander M. Capron, University of Southern California
Gary A. Chase, Georgetown University Medical Center
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, The Foundation of Human Genetics
Robert P. Erickson, University of Arizona
David Ginsburg, University of Michigan
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington
Robertson Parkman, Children's Hospital of Los Angeles
Gail S. Ross, Cornell University Medical Center
Bratin K. Saha, Emory University
R. Jude Samulski, University of North Carolina
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, Virginia Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).
Ad Hoc Consultant

Harold Ginsberg, National Institutes of Health/Columbia University

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health staff:

Bobbi Bennett, OD
Michael Blaese, NCI
Diane Bronzert, NCI
Sarah Carr, OD
Jan Casadei, NCI
Judith Castellucci-Muhlhauser, NIA
Daryl Chamblee, OD
John Chiorini, NHLBI
Chin-Shyan Chu, NHLBI
Marinee Chuah, NHLBI
Corrado Cirielli, NIA
Stephen Epstein, NHLBI
Toren Finkel, NHLBI
Judy Fradkin, NIDDK
Jay Greenblatt, NCI
Barry Goldspiel, CC
Christine Ireland, OD
Sachiko Kajigaya, NHLBI
Masako Kawase, NHLBI
Robert Kotin, NHLBI
Becky Ann Lawson, OD
Catherine McKeon, NIDDK
Suzanne Medgyesi-Mitschang, OD
Hiroaki Mizukami, NHLBI
David Nelson, NCHGR
Jay Ramsey, NCHGR
Melissa Rosenfeld, NCHGR
Brian Safer, NHLBI
Gwen Shafer, NCI
Dorothy Soew, NCRR
Debra J. Wilson, OD
Thomas Shih, OD
Thierry Vanden, NHLBI
Harold Varmus, OD
Stephen Wiener, NHLBI

Others:

Paul Aebersold, Food and Drug Administration
Sandra Afiome, Johns Hopkins University
Abass Alavi, University of Pennsylvania
Jane Alavi, University of Pennsylvania
Stephen Albelda, University of Pennsylvania
Tom Alonzo, GenVec, Inc.
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Carlos Arteaga, Vanderbilt University
Estuardo Aguilar, Baylor College of Medicine
Robert Beall, Cystic Fibrosis Foundation
Bari Bialos, New York Hospital
Bridget Binko, Cell Genesys
John Bishop, Food and Drug Administration
Gary Boch, Life Technologies, Inc.
Ernst Boehnlein, Progenesys
Arindam Bose, Pfizer Central Research
Andrew Braun, Harvard University
Gracia Buffleben, Breast Cancer Action
Parris Burd, Food and Drug Administration
James Bylund, Quality Biotech
Ira Carmen, University of Illinois
Barry Carter, Targeted Genetics Corporation
Jan Chappell, Genetic Therapy, Inc.
Saswati Chatterjee, City of Hope National Medical Center
Yawen Chiang, Genetic Therapy, Inc.
Carol Conrad, Johns Hopkins University
Ronald Crystal, New York Hospital
Kenneth Culver, Iowa Methodist University
Karen Darcy, Magenta Corporation
Wanda deVlaminck, Avigen
Blythe Devlin, Duke University
Lori Doyle, University of Pennsylvania
Anne Driscoll, Fox, Bennett, & Turner
Stephen Eck, University of Pennsylvania
Jim Embree, Systemix
Suzanne Epstein, Food and Drug Administration
Terry Flotte, Johns Hopkins University
Jeffrey Fox, Private Journalist
Joyce Frey, Food and Drug Administration
Ingo Georgoff, Quality Biotech
Eva Giesan, French Cystic Fibrosis Association
Christine Ginsberg, Georgetown University

Igor Gonda, Genentech, Inc.
Christine Gorman, Time Magazine
Angus Grant, Food and Drug Administration
Tina Grasso, GenVec, Inc.
Nicolaas Groot, The Netherlands Health Department
Mariann Grossman, University of Pennsylvania

Bill Guggino, Johns Hopkins University
Jeff Gustavson, Act Up, Goldengate
Paul Hallenbeck, Genetic Therapy, Inc.
Jacqueline Hampton, Weinberg Consulting Group
Elie Hanania, MD Anderson Cancer Center
Rebecca Harmon, University of Pennsylvania
Jeffrey Holt, Vanderbilt University
Joseph Hughes, Quality Biotech
Edie Irvine, Genetic Therapy, Inc.
Jeffrey Isner, St. Elizabeth's Medical Center
John Jaugstetter, Genentech, Inc.
Alice Johnson, Virginia Breast Cancer Foundation
Susan Jones, Virus Research Institute
Larry Kaiser, University of Pennsylvania
Katherine Kaufmann, GenVec, Inc.
Connie Kirby, Canji, Inc.
Steve Kradjian, Vical, Inc.
Toshi Kotani, Genetic Therapy, Inc.
Imre Kovesdi, GenVec, Inc.
Alex Kuta, Food and Drug Administration
Eugene LaBrec, E.H. LaBrec & Associates
Jane Lebkowski, Applied Immune Sciences, Inc.
David Levitt, University of Maryland
Charles Link, Iowa Methodist University
H. Kim Lyerly, Duke University
Gail Maderis, Genzyme Corporation
Tamie Malaska, Targeted Genetics Corporation
Dan Maneval, Canji, Inc.
Phillip Maples, Baxter Healthcare Corporation
Tony Marcel, TMC Development
Stephen Marcus, Genetic Therapy, Inc.
Michael McCaughan, FDC Reports
Gerard McGarrity, Genetic Therapy, Inc.
R. Scott McIvor, University of Minnesota
Bruce Merchant, Viagene, Inc.
Andra Miller, Food and Drug Administration
Ron Morales, Harvard University
Richard Moscicki, Genzyme Corporation
Philip Noguchi, Food and Drug Administration
Sheryl Osborne, Viagene, Inc.
John Park, University of California, San Francisco
Virginia Parks, Project Inform
Nicholas Pelliccione, Schering-Plough Research Institute
Anne Petruska, The Pink Sheet
Ramila Philip, Applied Immune Sciences, Inc.
John Powderly, Georgetown University
Raj Puri, Food and Drug Administration
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
Cindy Richards, Burroughs Wellcome Company

David Robertson, Vanderbilt University
Mark Roffman, GenVec, Inc.
Carolyn Roitsch, Transgene
Joseph Rokovich, Somatix Therapy Corporation
Beryl Rosenstein, Johns Hopkins University
Bruce Schackman, The CTI Group
Richard Schifreen, Life Technologies, Inc.
Richard Scotland, Genzyme Corporation
Steve Shak, Genentech, Inc.
Terry Sharrer, National Museum of American History
Tomiko Shimada, Ambience Awareness International, Inc.
Juliet Singh, Baxter Healthcare Corporation
William Small, BioConferences International, Inc.
Steve Sternberg, Private Journalist
Margi Stuart, Breast Cancer Action
Franck Sturtz, Progenitor, Inc.
Nevin Summers, Ingenex, Inc.
James Symas, St. Elizabeth's Medical Center
James Taylor, Taylor Associates
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Joseph Treat, University of Pennsylvania
Brent Treiger, Applied Immune Sciences, Inc.
Cynthia Utley, GenVec, Inc.
Dominick Vacante, Magenta Corporation
Deborah Vaz, Virus Research Institute
Alan Venook, University of California, San Francisco
Samuel Wadsworth, Genzyme Corporation
Kenneth Walsh, St. Elizabeth's Medical Center
Robert Warren, University of California, San Francisco
Ted Waugh, Foundation on Economic Trends
Rick Weiss, The Washington Post
Judi Weissinger, Applied Immune Sciences, Inc.
Kathleen Whitaker, Quality Biotech
Lisa White, The Blue Sheet
Chet Whitley, University of Minnesota
Sharon Williams, Life Technologies, Inc.
Carolyn Wilson, Food and Drug Administration
James Wilson, University of Pennsylvania
Kamehameha Wong, City of Hope National Medical Center

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting and proposed actions were published in the *Federal Register* on August 23, 1994 (59 FR 43426) as required by the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the proposal, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted

written statements prior to the meeting, followed by the public at large.

Dr. Walters welcomed two new scientific members to the RAC: (1) Robert P. Erickson, M.D., Professor, Department of Pediatrics, Molecular and Cellular Biology, University of Arizona, Tucson, Arizona; and (2) R. Jude Samulski, Ph.D., Director, Gene Therapy Center, University of North Carolina, Chapel Hill, North Carolina.

Overview

Dr. Walters mentioned that the Office of Recombinant DNA Activities (ORDA) has moved to a new office space located at 6006 Executive Boulevard, Suite 323, MSC 7052, Bethesda, Maryland 20892-7052. The telephone and FAX numbers are unchanged, Phone (301) 496-9838/FAX (301) 496-9839. Dr. Harold Varmus, Director of NIH, is to address the RAC regarding RAC review criteria. It was noted that 8 protocols will be reviewed at this meeting, 2 on cystic fibrosis, 4 on cancer, 1 on mild Hunter syndrome, and another on peripheral artery disease. One protocol by Dr. Jack L. Gluckman has been withdrawn. There will be a discussion on the proposed actions to the *NIH Guidelines* regarding NIH and Food and Drug Administration (FDA) consolidated review of human gene transfer protocols.

Dr. Walters stated that the RAC has approved 57 gene therapy protocols (47 approved by the NIH Director) and 24 gene marking protocols (22 approved by the NIH Director).

Dr. Walters noted an article published by Dr. James Wilson and his colleagues on their study of familial hypercholesterolemia (Protocol #9110-012) in *Nature Genetics* (Vol. 6, pp. 335-341, 1994), with an accompanying commentary by David Weatherall in the same issue of the journal (pp. 325-326). There is correspondence in the same journal (Vol. 7, pp. 349-350) by Michael S. Brown, Joseph L. Goldstein, Richard J. Havel and David Steinberg questioning the interpretation of the results from the first patient study. These authors also proposed a set of criteria for evaluating success of gene therapy data on familial hypercholesteremia.

Dr. Walters announced that the FDA will hold a public meeting to discuss gene vector production issues immediately following the first day of the RAC meeting. Notice of this meeting was published in the *Federal Register* on August 30, 1994 (59 FR 44739).

In his review of approved protocols, Mr. Capron noted a discrepancy in the number of gene marking protocols that was approved by the RAC and that by the NIH Director. The total should be 24 for both approvals.

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

Dr. Walters stated that 10 minor modifications were approved to the following human gene transfer protocols since the June 9-10, 1994, RAC meeting:

DATE	PROTOCOL#	INVESTIGATORS
6/23/94	9206-018	Malcolm Brenner
7/05/94	9312-059	Edward Oldfield, Zvi Ram

7/11/94	9306-043	Hilliard Seigler
7/11/94	9306-048	Jeffrey Galpin, Dennis Casciato
7/11/94	9312-062	Richard Haubrich
7/11/94	9403-068	Joseph Rosenblatt
7/13/94	9303-037	Kenneth Culver, John VanGilder
8/31/94	9303-041	Robert Wilmott, Jeffrey Whitsett, Bruce Trapnell
8/31/94	9212-035	James Wilson
8/31/94	9303-038	Helen Heslop, Malcolm Brenner, Cliona Rooney

III. CHAIR REPORT ON ACCELERATED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that the RAC and ORDA have approved the first human gene transfer protocol under the newly adopted *Accelerated Review* process. On August 3, 1994, NIH/ORDA approved the protocol submitted by Dr. Jonathan Simons, Johns Hopkins Oncology Center, Baltimore, Maryland, entitled: "*Phase I/II Study of Autologous Human GM-CSF Gene Transduced Prostate Cancer Vaccines in Patients with Metastatic Prostate Carcinoma (Protocol #9408-082)*. Dr. Simons' protocol was eligible for Accelerated Review under the category entitled: "Lethally Irradiated Tumor Cells/No Replication Competent Virus--RAC-approved vector constructs with minor modifications/additional tumor cells."

IV. MINUTES OF THE JUNE 9-10, 1994, MEETING

The RAC approved a motion made by Dr. Smith and seconded by Dr. Dronamraju to accept the June 9-10, 1994, RAC minutes by a vote of 17 in favor, 0 opposed, and 1 abstention.

V. DATA MANAGEMENT UPDATE/DR. SMITH

Dr. Smith, Chair of the Working Group on Data Management, noted the following items of correspondence that were included in the meeting materials. Dr. Michael Knowles (Protocol #9303-042) reported in his cystic fibrosis study that adenovirus vector (Ad5-CB-CFTR) could be cultured on 293 cells from nostril swabs up to 1 to 2 days after dosing; and by a more sensitive polymerase chain reaction (PCR) assay, the vector sequences were detected up to 5 days. There are two adverse events reported in association with the brain tumor protocols. In Dr. Oldfield's study to treat leptomeningeal carcinomatosis by the *Herpes simplex* thymidine kinase gene/ganciclovir (GCV) strategy (Protocol #9312-059), one patient developed symptoms of acute meningitis after injection of the vector producer cells into the meninges through an Ommaya reservoir. In Dr. Culver's study to treat glioblastoma multiforme by a similar method (Protocol #9303-037), serious adverse reactions of neck pain, fever, and hypertension (238/128) were experienced after injection of the vector producer cells through the Ommaya reservoir.

Ms. Meyers asked if a health care worker could be infected with the adenovirus vector during those 5 days when the virus is detectable. Dr. Smith said that in the cystic fibrosis trials, the patients are kept in isolation and are required to wear masks during that period.

Dr. Straus inquired about the frequency of adverse effects when patients receive cells through the Ommaya reservoir. Dr. Smith said only 1 patient has been treated in Dr. Oldfield's protocol, and Dr.

Culver reported that he treated 4 patients with only 1 patient experiencing adverse effects. Dr. Smith added that 15 patients have been treated by Dr.Oldfield. Dr. Straus asked if the adverse effects had been ever observed in preclinical studies with animals. Dr. Culver said that he did not have first hand information; but in other studies, no similar effects have been observed in rodents and rhesus monkeys. The injected vector producer cells are of mouse origin. Dr. Straus expressed his concern about the acute adverse reactions in these types of gene therapy protocols and stated that the cause of the adverse effects should be determined. Dr. Chase asked if patients are told about the adverse effects and if there is a threshold of adverse events that will trigger RAC review of the safety issues. Mr. Capron asked if the injection rate or the injection method could contribute to the adverse effects. Dr. Straus suspected that there is some kind of immediate hypersensitivity reaction. Mr. Capron remarked that a revised Informed Consent document should reflect this new knowledge about risks. Ms. Meyers asked if there is any benefit to the treated patients. Dr. Culver said that the first patient is in satisfactory condition although there is no sign of tumor size change by magnetic resonance imaging (MRI). The second patient's tumor is close to the ventricle, and Dr. Culver suspects that cell leakage into the meningeal space may have contributed to the adverse effects. Tests showed no evidence of infection. Dr. Parkman reminded RAC members that these are Phase I toxicity studies not designed to evaluate efficacy, and it is not appropriate to ask investigators to demonstrate efficacy.

Dr. Smith said that the adverse effects will be closely followed up in the upcoming data management report at the December RAC meeting. Dr.Dronamraju asked if a revised Informed Consent document addressing the adverse effects should be submitted for RAC review. Mr. Capron said that the local Institutional Review Board (IRB) should be informed and necessary changes in the Informed Consent document can be made and reviewed by RAC members if necessary. Dr. Straus emphasized that if adverse events can be avoided in the future by a minor change of the procedure or premedication, no revision of the Informed Consent document is needed.

Dr. Walters welcomed Ms. Daryl A. (Sandy) Chamblee, Acting Deputy Director for Science Policy and Technology Transfer at NIH and Dr. Harold Varmus, the NIH Director, to the meeting.

VI. DISCUSSION REGARDING CRITERIA FOR RAC REVIEW AND APPROVAL OF HUMAN GENE TRANSFER PROTOCOLS/DR. VARMUS

Dr. Walters welcomed Dr. Varmus, the NIH Director, to the RAC noting that Dr.Varmus has a longstanding interest in gene therapy and was one of the original members of Human Gene Therapy Subcommittee of the RAC. Dr. Varmus made a proposal to form an *ad hoc* committee to review RAC activities, particularly the criteria by which RAC approves human gene therapy protocols. The reason for making this suggestion was threefold. First, during a meeting of the National Task Force on AIDS Drug Development chaired by Dr. Philip R. Lee, Assistant Secretary for Health, Department of Health and Human Services, which was held on July 18, 1994, at Arlington, Virginia, many concerns were raised regarding the slowness of the review process that affects investigators initiating gene therapy protocols to study aquired immune deficiency syndrome (AIDS). The current multiple procedures to review these protocols by both the RAC and the FDA were seen as unnecessarily slow. A one-stop shopping mechanism for consolidated review of human gene transfer protocols was proposed by Dr.Wivel, Director of ORDA at the NIH and Dr. Philip Noguchi, Director of the Division of Cellular and Gene Therapies of FDA. The proposal was endorsed by the AIDS Task Force. Second, many investigators have complained about undue delays in review of gene therapy protocols; for example, the review criteria are unclear as to the permissibility to use certain vectors. The third reason derived from Dr.Varmus' review of a disputed protocol which was approved by a split vote at the June 1994 RAC meeting (Protocol #9406-073 by

David Curiel). Dr. Varmus was concerned about lack of consensus in review criteria for approval of this protocol, i.e., the minimum criterion of no harm to the subjects or the additional requirement that useful scientific information to be obtained from the experiment in order to justify the study. He was disturbed by the lack of adequate information to make this decision. Relevant information of a human study of the same gene by other researchers was not made available to the investigators in this case, and there were significant questions regarding the preclinical experiments in mice.

Dr. Varmus proposed the formation of an *ad hoc* group that could include current or past RAC members, and those who previously served on the Human Gene Therapy Subcommittee. At least two major areas would be reviewed. The first area concerns the domain of RAC activities and the composition of its membership (scientific and public). The second task is to define the criteria by which gene therapy protocols are evaluated, e.g., safety, scientific credibility, scientific utility or therapeutic promise. Another issue is coordination of an application to the RAC with an application to NIH for extramural funding that is reviewed by an initial scientific merit review group. Should RAC require an application to first pass a merit review to assure scientific credibility of the proposal before its own review? The issue will become important as consolidated review by NIH and FDA develops. A determination has to be made as to which proposal would require public review by the RAC. Dr. Varmus said that he is committed to the idea of retaining RAC purview of gene transfer protocols, especially when the methodology is novel. Finally, Dr. Varmus wanted an outside committee to provide some guidance for the NIH Director about how to respond to RAC recommendation for protocol approval, for example, in the event of a split vote.

Dr. Parkman said that consistency of criteria in evaluating protocols is crucial. The *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects (Points to Consider)* is used as a yardstick to evaluate a protocol, but the medical, scientific and ethical criteria required for approval are not clearly defined.

Mr. Capron advanced an idea that review of novel protocols by the RAC should set standards and expectations that could be used in the future by the FDA in its closed review of similar protocols not going to be deliberated by the RAC in a public forum. *Points to Consider* requires the investigators to address certain issues but does not provide guidance to judge the adequacy of responses from investigators to these questions. He said it is an appropriate time for an *ad hoc* group to articulate these review criteria based on the accumulated experience the RAC acquired while reviewing individual protocols.

Dr. Varmus agreed that Mr. Capron identified an important problem. He said he is unsure whether a committee is able to put together a series of guideposts for protocol review. Even with a seemingly routine cytokine therapy for cancer, an investigator may propose a slightly different vector that warrants RAC deliberation. Without examining the submitted application, the appropriateness of a single review by FDA may not always be a straightforward decision. This is the reason that in the proposed NIH/FDA consolidated review procedure, a protocol will be evaluated by ORDA/RAC/FDA in order to determine its suitability to bypass the RAC process.

Dr. Motulsky was strongly in favor of redefining the function of the RAC in dealing with scientific issues, ethical issues, and its relationship to other committees such as NIH Study Sections which review proposals for their scientific merit. He noticed the scarcity of scientifically competent peer reviewers within the RAC to deal with a wide range of applications, and often in need of referring to *ad hoc* outside consultants. He proposed the establishment of RAC to deal with issues unique to gene therapy. RAC was initially established to address special recombinant DNA aspects of gene

therapy as a safety committee. Dr.Motulsky favored a return to this basic function. Currently, there is a division between the RAC functioning as a scientific committee and as a broader committee dealing with informed consent issues. This mixed function has caused some uneasiness among investigators.

Dr. Miller asked what prompted the AIDS investigators to demand revamping the whole system of protocol review since there are only a few modest HIV protocols. In his opinion, RAC has conducted a good job in bringing out all of the salient points of reviewed protocols. He agreed with Dr. Motulsky that the RAC occasionally gets bogged down by the informed consent issues. It was pointed out that proposals previously approved by the IRBs still contain serious problems that demand RAC attention. FDA provides more of a technical decision point in the final review process, while RAC reviews provide protocol evaluation within a larger context. Dr. Miller said when a good protocol is submitted, it goes through the RAC process very quickly.

Dr. Varmus was concerned about review criteria by which the decisions are made, and the timing of RAC review vis-a-vis NIH peer review and IRB approval.

Dr. Smith commented that management of data reporting will be expanded in the future since all protocols, even those that bypass RAC review, will have to report their progress to the RAC. He asked if there are other functions that are not performed by FDA that need to be addressed by the RAC.

Dr. Straus said that from his experience of chairing both anIRB and the Institutional Biosafety Committee (IBC) of NIH, he realized that each committee cannot succeed by confining itself strictly to ethical or safety issues alone. The RAC should deal with both aspects of protocols. There is a place for a public debate about the nature of new approaches, and this aspect of public safety debate is not readily available from FDA. Dr. Straus said that the RAC is already moving in the direction of redefining itself, and he does not object to the idea that the RAC would confine itself to safety issues.

Dr. Parkman said that 80% of RAC reviewed protocols have yet to pass theNIH initial peer review. RAC review usually is the first scientific review outside the protocol's sponsoring institution. He noticed that PIs very often are the only experts in the field of gene therapy in their local institutions, and they have to exclude themselves from reviewing their own protocols. As a matter of fact, RAC is the first place that all facets of a protocol are closely scrutinized, even though the protocol has been administratively approved by the local committees.

Dr. Dronamraju said that scientific and ethic issues are interconnected, and he would favor RAC to be concerned with both type of issues.

Ms. Meyers was concerned about deleting the *Points to Consider* from the *NIH Guidelines*, particularly since the RAC has just revised the informed consent portions of the document. RAC review of human gene transfer experiments is to assure public accountability and to protect patients' rights. She has been very concerned about the Informed Consent documents.NIH has been sued by families of patients who died in recent FIAU or fialuridine hepatitis drug trials for inadequate disclosure in the Informed Consent documents. Ms. Meyers said that the Informed Consent document is an important part of the protocol, and the RAC as a public body is responsible for assuring patient protection. More than 50% of Informed Consent documents submitted to the RAC are inadequate. The AIDS community now realizes that it was a mistake to accelerate approval of the class of drugs known as reverse transcriptase (RT) inhibitors without waiting for the efficacy

data, and the same mistake should not be repeated in gene therapy. Only one disaster will damage the entire new field of human gene therapy.
Dr. Varmus emphasized that redefining the role of the RAC is not totally driven by the need for fast track approval of human immunodeficiency virus (HIV) protocols. The current dual review system involving both RAC and FDA is cumbersome. Considering the rapid increase of gene therapy protocols, it is time to make this process more efficient in order to maximize the use of resources of NIH and FDA. He reiterated the need of RAC review since new kind of protocols are being proposed to treat diverse diseases.
Dr. Erickson noted that gene therapy was designed to treat genetic diseases caused by single gene defects, and now it has expanded into the areas of AIDS and cancer. False hope for children of genetic diseases is detrimental and the concern for terminally ill patients with AIDS or cancer is different. He agreed that it is time to redefine the review criteria.

Dr. Chase agreed that streamlining the review process is a good idea that will permit RAC to deliberate general issues involved in gene therapy. He pointed out three general areas for discussion: (1) quality and value of information to be obtained from gene therapy experiments, (2) indemnifying the subjects for research related costs, and (3) coordination and information exchange among multiple centers studying the same disease by the same approach.

Mr. Capron did not share the viewpoints expressed by Dr. Miller and Ms. Meyers that the system needs no change because of increasing demands of AIDS activists and AIDS invetigators, but he agreed with the opinion that most Informed Consent documents are inadequate. The recent revision of the *Points to Consider* concerning the Informed Consent states more specifically what is to be included in the document. Mr. Capron expressed his disappointment with the IRB system in that it has not met its responsibility to amend all the deficiencies found in the Informed Consent documents. The issue of compensation for research-injuries is a problem area for most Informed Consent documents, and there is no government policy to guide this compensation issue. Mr. Capron stated that the RAC should deliberate gene therapy issues of public concern such as genetic enhancement and germ line alteration. The time has come for the RAC to divest itself from reviewing all protocols, not just because of the AIDS community. The RAC needs to articulate the standards for FDA to approve several types of protocols. This standard would allow time to deal with the real issues involving intended changes of inheritable human characteristics, the concern that led to creation of the RAC in the first place.

Dr. Noguchi stated the FDA's viewpoint about the role of the RAC. FDA derives its regulatory oversight over gene therapy by promulgation of regulations based on the Food, Drug and Cosmetic Act (revised) (FD&C Act) and Section 351 of the Public Health Service Act (PHS Act). Historically, FDA is mandated by law to do things that are codified in the Code of Federal Regulations (CFR). The RAC by contrast is not a creation of law and is most appropriate to deal with issues not readily addressed at FDA. Dr. Noguchi said that publicly funded gene therapy studies should be reviewed publicly. One imminent issue is gene enhancement therapy. Human fibroblasts can be gene-modified to produce human grown hormone. Should this kind of gene therapy be tried in children of short stature? There is no easy mechanism to have timely debate of these issues at FDA. The public advisory committees of FDA are geared towards approval of Phase II/III clinical trials and final approval of drugs. There is no equivalent committee functioning like the RAC to deliberate issues of Phase I studies or issues such as prenatal gene therapy and enhancement therapy. The RAC is a societal body to consider the question of whether the society is ready to permit these types of gene therapy.

Ms. Meyers inquired if FDA has authority to turn down an experiment with questionable ethics such

as transferring human growth hormone gene in a short child. Dr. Noguchi said that FDA has little designated authority to do that, and no ready mechanism to deliberate this issue publicly in a timely fashion. Furthermore, FDA does not have bioethicists available to review this kind of ethics question. Ms. Meyers asked if FDA reviews the Informed Consent document. Dr. Noguchi said that FDA does have a regulation that requires investigators to obtain approval from their IRB, but that RAC can more easily recommend useful guidelines for evaluation of informed consent. Dr. Ross said that knowledge of the functions of other government agencies is helpful in defining the RAC role.

Dr. Tony Marcel (TMC Development, Paris, France) commented that the RAC has international impact. Through the open RAC meetings and distribution of its minutes, international audiences are informed about the present concerns relative to gene therapy.

Mr. Jeff Gustavson from ACT-UP-Goldengate, San Francisco, California, said that the AIDS community favors the RAC review in a public forum so that the community's concern can be channeled into the review process.

Dr. Varmus said that an important task of the *ad hoc* group to review RAC activities is to define the criteria by which a protocol can be reviewed by the one-stop shopping mechanism without public review in a RAC meeting. This issue is important since too many applications are expected in the future for the RAC to consider in its public meeting. Ms. Meyers said that most of these repetitive kinds of studies are very similar, except consistency of the quality of Informed Consent documents. Dr. Varmus said that efforts should be made to identify elements, either scientific or related to informed consent, that require close scrutiny in the *Accelerated Review* of gene transfer protocols.

Dr. Noguchi commented that even in repetitive type of studies the collection of data regarding adverse effects will be an important RAC activity.

Dr. Parkman said that recent RAC efforts to define the categories of experiments for *Accelerated Review* are in keeping with what Dr. Varmus has just suggested. Mr. Capron asked whether the proposed NIH/FDA consolidated review will be put in place before the *ad hoc* committee review is completed. Dr. Varmus said that he comes to the RAC to solicit feedback ideas about this proposal. The implementation of the consolidated review will have to be coordinated with FDA. The *ad hoc* review is a long-term process; and it can proceed in a series of phases, dealing with the immediate streamlining problem first and then other more difficult issues. Dr.Doi said different *ad hoc* groups can be formed to deal with different problems. Dr.Varmus emphasized that he is not coming to the RAC with a concrete proposal but rather to initiate a process to respond to the changing field of gene therapy. Dr. Zallen said that no matter what the final outcome of these changes, the tradition of public openness and public involvement of the RAC should be continued. Dr. Varmus indicated that he is committed to this tradition. Dr. Walters thanked Dr.Varmus for his comments to the RAC.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I STUDY OF AN ADENO-ASSOCIATED VIRUS-CFTR GENE VECTOR IN ADULT CF PATIENTS WITH MILD LUNG DISEASE/DR. FLOTTE*

Review--Dr. Samulski

Dr. Walters called on Dr. Samulski to present his primary review of the protocol submitted by Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland. Cystic fibrosis (CF)

is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR gene product is required for regulation of epithelial chloride transport in multiple organs including the lung airways. CF lung disease develops gradually over many years as abnormally viscous secretions lead to airway obstruction, infection, inflammation, and fibrosis. It ultimately may lead to respiratory failure, which is the cause of death in more than 90% of CF patients. Several protocols have been approved to employ adenovirus vectors or liposome-based vectors to transduce the CFTR gene to replace the missing gene function in CF patients. These investigators have developed an alternative vector system based on adeno-associated virus (AAV). AAV vectors can have long-term persistence in the host cells, and AAV-CFTR vectors have been shown to confer stable correction of the physiological defects when administered to CF bronchial epithelial cells *in vitro*. Long-term vector expression up to 6 months has been observed in a New Zealand white rabbit model. An additional advantage of the AAV vector is the absence of wild-type viral coding sequence in the vector construct that eliminates the possibility of vector-induced inflammatory reactions. The current protocol is a Phase I study of AAV-CFTR vector administered to the nose and bronchial epithelium of adult CF patients with mild lung disease. This protocol will be a dose escalation study to evaluate vector expression and safety. Vector doses ranging up to 1010 particles will be administered to the nasal epithelium and through a fiberoptic bronchoscope to a single lung lobe.

Dr. Samulski asked 6 specific questions. (1) Study cohorts. What is the rationale for the dose escalation schedule for the nose and lung since the number of target cells are different? Will patients treated with AAV be excluded from treatment with other vectors, specifically adenovirus vectors? The investigators answered that the plan is to escalate the nasal dose in advance of the pulmonary dose escalation. The issue of exclusion has been discussed at the CF Foundation/FDA Williamsburg Conference, and the consensus was that no patients participating in any of the gene therapy studies would be excluded from future studies. (2) General design of the vector. Dr. Samulski explained that AAV is a defective, nonpathogenic human virus. This virus has a strict requirement for a helper virus in order to go through alytic infection. Without a helper, the virus persists in host cells that makes it an attractive vehicle for gene transfer. Most of the viral coding sequences (96%) have been deleted with only the 145 base pair inverted terminal repeats (ITR) remaining at both ends of the vector. The ITR serves as the promoter to initiate gene transcription. Dr. Samulski asked if the ITR at the opposite end of the CFTR gene initiates transcription of an antisense RNA. The investigators wrote in a response that several lines of evidence indicate that this phenomenon, if it occurs, does not block CFTR expression. (3) Overall production process. Why is the wild-type adenovirus used as the helper to produce this vector? Since the investigator is using 293 cells for production of AAV, it is not necessary to use the wild-type adenovirus helper. An Ela-deleted adenovirus will be sufficient since the 293 cells already have E1a sequences. The investigators noted that in the present procedure, the adenovirus will be inactivated by heat treatment. Dr. Samulski commented that the E1a- deleted adenovirus helper offers another level of biosafety since the vector will not be mobilized in the presence of a wild-type AAV and a defective adenovirus. (4) Primate studies. The investigator detected positive vector sequences in the liver of a monkey after a dose of 1011 AAV particles. How widespread is the presence of vector sequences in the liver and what types of liver cells express the AAV sequences? How does the vector spread to liver since it is administered to the lung? (5) *In vivo* rescue of AAV-CFTR recombinant. The investigator presented data in monkeys showing that after administration of the vector to the lung, the monkey was challenged with adenovirus and wild-type AAV in the nose. Mobilization of the vector was localized to the nose only. Since the protocol proposes vector delivery to the nasal passages, does the investigator have any results pertaining to the spread of vector after delivery to the nose and challenge with adenovirus and AAV? Dr. Samulski said that the probability for all three viruses to infect the same host cells in order to effect a productive infection is very small. (6)

Efficiency and site-preference of AAV-CFTR vector integration in a CF bronchial epithelial cell line. What is the nature of vector integration? Does the integrated vector express its gene? What is the nature of the integration site? The investigator addressed these questions in writing. Overall, Dr. Samulski was pleased to see that the new vector system had progressed to the stage of a clinical trial in human subjects. It is a biologically safer type of vector since it is derived from a defective nonpathogenic virus.

Review--Dr. Straus

Dr. Straus commented on the outstanding review by Dr.Samulski. AAV is a potentially useful vector, and the investigator has performed excellent preclinical studies and presented a well written proposal. Dr. Straus pointed out his major concern regarding the persistence of theAAV vector. AAV is a very hardy virus that persists in the environment. This property does offer an easy means to inactivate other contaminating viruses by a heating procedure in the production process. Studies performed in the late 1960's indicated that children with adenovirus respiratory infections shed AAV for a long period of time in their stool. Dr. Straus stressed that persistent shedding of virus is the very issue needed to be discussed further. The investigator indicated that recipients ofAAV-CFTR are still shedding the virus several days after administration. The decontamination procedure is not articulated. Dr. Straus said that these procedures should be discussed in terms of their effectiveness and practicality. Overall, Dr. Straus expressed his satisfaction of the well written protocol.

Review--Dr. Dronamraju

Dr. Dronamraju was satisfied with the written response by the investigator regarding the question of transduction rate and vector expression. The concern about virus shedding was not mentioned in the Informed Consent document, and it is unclear why patients should be advised not to talk to the reporters about their participation in the study. Dr.Dronamraju asked the investigator to elaborate on the toxicity study in primates.

Other Comments

Dr. Miller indicated that he would abstain from voting on this protocol since he is associated with a company involved in a part of this study.

Dr. Parkman said this protocol employs a new vector system to treat CF. Since the RAC has approved several protocols using adenovirus vectors, it would be informative to the RAC to have investigators of those adenovirus protocols to offer a comparative assessment as to the pros and cons of these vector systems. He asked if animal studies are available in which these two vector systems have been compared in parallel. Dr. Doi asked the investigator to elaborate on the question of immunological responses to the administration of the AAV vector. Dr. Parkman noted immunological responses will be particularly pertinent to repeated vector administration. Mr. Capron asked how a subject would be treated if he or she withdrew from the study while still shedding the virus.

Investigator Response--Dr. Flotte

Dr. Flotte presented animal data to address the question of vector shedding. After administration of 1011 AAV-CFTR particles to the right lower lung of a monkey, there were no detectable vector sequences from day 3 to day 21. The vector assay involved aPCR using a lysate of 293 cells co-cultured with nasal or bronchial fluid from the infected monkey in the presence of both adenovirus helper and wild-type AAV. The assay has a sensitivity of detecting 10 infectious units of

AAV-CFTR present in these samples. The dose of AAV-CFTR used in this experiment is 10 times higher than the highest dose proposed for the human study.

Responding to the question of decontamination, Dr. Flotte said that in the unlikely event if a patient is discharged while still shedding the vector, the patient will be instructed to decontaminate his/her nasal secretions and sputum with a 1% bleach solution. According to a study performed at Targeted Genetics, Inc. (Seattle, Washington), such a bleach solution will totally inactivate any wild-type AAV. Dr. Flotte welcomed suggestions from RAC members as to how to address this problem keeping in mind patient's overall physical and psychological well-being.

Dr. Flotte said that a patient's used nasal tissues and sputum will be disposed in a bucket of bleach solution. Dr. Straus noted that this procedure will not prevent virus spreading by other routes such as aerosol created by coughing or hand to nose contact involving other individuals. Dr. Parkman expressed his concern about discharging patients who are actively secreting a vector. All RAC approved protocols require patient isolation until absence of virus shedding.
Dr. Smith asked if there was virus shedding at sites other than the nose in the monkey experiment. Dr. Flotte said that samples have been collected from urine and stool but have not been tested for the presence of vector.

Dr. Miller commented that there is little danger in spreading an AAV vector carrying the CFTR gene. Ninety percent of the human population already is infected with AAV as well as adenoviruses. AAV is nonpathogenic, and the vector is completely replication defective. There is little consequence of transducing a normal CFTR gene to other individuals since the gene is normally expressed. Dr. Parkman was concerned about the household contacts when the patients return to their home. The household members do not sign the Informed Consent document to have gene transfer from the research subjects. Dr. Miller said that this is the same argument advanced during the approval of other adenovirus CF protocols. Dr. Samulski said that there are published reports regarding cellular effects of CFTR being expressed in cells that do not normally express this protein. It is common sense to play it safe for the first trial of a new vector system. Dr. Chase remarked even if the AAV-CFTR is harmless, knowingly spreading the laboratory-produced virus to other individuals is a cause for concern to the general public.

Dr. Flotte said it is unreasonable to isolate patients indefinitely if they continue to shed virus. It has to be balanced with the problem of patient recruitment and patient's right to leave hospital if the patient withdraws from the trial. The contingency position is to give pertinent information to patients about how to decontaminate the virus. Considering there are no known adverse effects of spreading the vector, putting great burden on that particular virus-shedding patient would seem to be inappropriate.

Ms. Meyers asked if it would be acceptable to amend the protocol to state that patients will be isolated until there is no shedding. Dr. Flotte agreed to this change, but Dr. French Anderson of University of Southern California raised the issue of prolonged shedding, i.e., for more than 6 months. Dr. Parkman said that there is a theoretical possibility that a patient would have to be discharged before shedding stops. Would household members be required to sign the Informed Consent document for this unlikely event? Dr. Samulski suggested that the experience of virus shedding from the trials of adenovirus vectors is a useful reference. In the case of AAV, the likelihood of generating productive infection is very small since it needs simultaneous infection with 3 viruses.

Dr. Smith asked for clarification of two points: (1) the frequency of subject testing for virus shedding,

and (2) the likelihood that subjects will return not only to home but will go back to school or work. He noted that it is not possible to obtain Informed Consent document from all the potential contacts. Dr. Flotte said that at time zero, right after vector administration, nasal fluids, bronchial fluids, urine and stool samples will be collected; and at day 3 and day 10 all samples except the bronchial fluid will be assayed for the presence of vector. At day 30 and day 60 patients will return for repeat bronchoscopy, and samples will be taken for vector assay.

Dr. Straus did not believe one could fairly impose prolonged hospitalization. If patients test positive for vector shedding after 7 or 10 days, they will be sent home but no additional patients should be recruited until the biology of the vector is better understood. He would not favor keeping a patient indefinitely, nor could he approve a protocol that allows many people to go home shedding virus.

Dr. Dronamraju questioned the adequacy of the monkey model in terms of social behavior. Dr. Parkman said the monkey model is adequate to address the duration of vector secretion. Patients will be isolated initially for 10 to 14 days to observe vector shedding either from the initial vector inoculum or due to vector replication. Differences in the behavioral pattern of monkeys and humans are not significant in this case. But if there is a long-term persistent secretion, then behavioral pattern will be a significant factor in the spread of the virus. Regarding Dr.Dronamraju's question on the number of patients, Dr.Flotte said that 16 patients are required to test dose escalation with 2 patients in each dose group.

Regarding the question of virus shedding, Dr. D. Ginsburg suggested a study of 1 patient. If there is no long-term virus shedding in the first patient, RAC members may feel more comfortable in allowing treatment of additional patients. Dr. Flotte said that each cohort is separated by an interval of 1 month, and it is agreeable to report back to the RAC between each cohort. Dr. Straus said if long-term vector shedding is observed in the first cohort, no other cohort should be started. However, the patients should be allowed to go home.

Mr. Capron said that the Informed Consent document should be revised to incorporate a statement that informs subjects that in the event they choose to withdraw from the study after the vector administration, they will be strongly discouraged from leaving the hospital until lack of virus shedding has been demonstrated. Although the patients still have the legal right to leave the hospital, they would be encouraged not to do so under such circumstances. Dr.Flotte agreed to modify the Informed Consent document to reflect this concern. Ms. Meyers said that the revised Informed Consent document should be reviewed by RAC primary reviewers.

Regarding the question of transgene expression, Dr. Flotte made a slide presentation of a monkey study. *In situ* PCR was used to detect the AAV-CFTR sequences in bronchial epithelial cells after vector administration. At 3 dose levels up to 1011 particles, 13 to 51% of epithelial cells were found to contain the vector DNA for up to a period of 21 to 90 days. The result was very similar to a more thorough study in rabbits on the question of transgene expression.

In regard to the question why the vector was detected in liver after administration to the lung of a monkey, Dr. Flotte said that the vector was not detected in other parts of the gastrointestinal system. He speculated that in this particular instance, the vector might have spread to liver through the blood circulation. Since hepatocytes normally express the CFTR gene, no adverse effects will be expected.

Responding to a question on the comparative merit of AAV versus adenovirus vectors, Dr. Flotte said that the main impetus for pursuing CF gene therapy with the AAV vector was its ability for

persistence in infected cells and for long-term expression. AAV-CFTR can function as an episome and integrates at lower frequency than wild-type AAV but is still capable of long-term persistence. Since the AAV vector does not contain any viral coding sequences, it avoids the problem of inflammatory effects caused by expression of viral proteins of the adenovirus vectors.

Committee Motion

Dr. Straus made a motion to approve the protocol with several contingencies. Dr. Smith asked for a clarification about the contingency of virus shedding. Dr. Straus said that the investigator is permitted to proceed with the study cohort by cohort. If the subjects are still shedding virus 7 to 10 days after vector administration, the subjects will be permitted to go home with instructions about the decontamination procedure. In addition, the family members should be asked for consent to be screened for the presence of vector. The recruitment for other cohort studies will be discontinued until the RAC makes further recommendations to proceed with the study.

A friendly amendment, as suggested by Mr. Capron, was added to the motion for approval. This amendment requests a revision of the Informed Consent document with regard to patient withdrawal. Dr. D. Ginsburg seconded the motion.

With regard to the statement in the Informed Consent about communication with the newspaper or television reporters, Dr. Flotte said that the statement is to alert the patients about the possibility of publicity.

The RAC approved a motion made by Dr. Straus and seconded by Dr. D. Ginsburg to accept the protocol submitted by Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland, by a vote of 16 in favor, 0 opposed, and 1 abstention. The RAC approval is contingent on review and approval of the following by the RAC primary reviewers: (1) Submit a revised protocol that explains that each cohort will be evaluated for virus shedding. If virus shedding is detected at 10 days post-vector administration, vector administration to subsequent cohorts is prohibited. Any subject in whom virus shedding has been detected 10 days post-vector administration will be released from the hospital; however, family members and close contacts will be informed of the possibility that they may be screened for the virus. The RAC recommended that if a subject is released from the hospital while actively shedding virus, family members and close contacts should be evaluated for the presence of the vector. (2) Submit a revised Informed Consent document incorporating a statement that informs subjects that in the event that they choose to withdraw from the study after the vector construct has been administered, they will be strongly discouraged from leaving the hospital until the lack of virus shedding has been demonstrated. The revised Informed Consent document must be reviewed and approved by the RAC primary reviewers.

(Dr. Miller abstained from voting due to his association with Targeted Genetics, Inc., a sponsoring company of the protocol.)

Summary

Dr. Terence R. Flotte of the Johns Hopkins Children's Center, Baltimore, Maryland, may conduct gene transfer experiments on 16 subjects (18 years of age) with mild CF. A vector derived from AAV will be used to transduce a human CFTR gene. The vector construct is termed tgAAVCF. The vector will be administered by direct application to the nasal epithelium and by bronchoscopic delivery to the right lower lobe of the lung. This is a dose escalation study in 8 cohorts of patients. Each individual will receive a single nasal dose of 1 x 106 to 1 x 109 and a single lung dose of 1 x 107 to

1 x 1010 vector particles. The primary goal of the study is to assess safety of vector administration. As a secondary objective, brushed respiratory and nasal epithelial cells will be evaluated for gene transfer, gene expression, and physiologic correction of the CF defect. Pulmonary function testing and lung imaging studies will be used to assess clinical impact.

VIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: GENE THERAPY FOR THE TREATMENT OF METASTATIC BREAST CANCER BY IN VIVO INFECTION WITH BREAST-TARGETED RETROVIRAL VECTORS EXPRESSING ANTISENSE C-FOS OR ANTISENSE C-MYC RNA/DRS. HOLT AND ARTEAGA*

Review--Dr. Miller

Dr. Walters solicited help from RAC members to review the information collected for data management to be presented at the coming December RAC meeting. He then called on Dr. Miller to present his primary review of the protocol submitted by Drs. Jeffrey Holt and Carlos B. Arteaga of the Vanderbilt University, Nashville, Tennessee. Dr. Miller stated that the investigators propose to use retroviral vectors that express antisense *fos* or *myc* oncogene sequences to treat malignant breast cancer cells in the meninges, peritoneum, or pleura. The vectors would be directly injected into these areas. The antisense sequences are expressed by using a tissue-specific mouse mammary tumor virus (MMTV) promoter to direct expression to malignant breast cancer cells. The investigators have shown that cultured MCF-7 human breast cancer cells have reduced tumorigenicity in animals after vector transduction, and that not all cells need to be modified in order to see this antitumor effect. The investigators stated that experiments in animals to demonstrate efficacy of this technique on established tumors are in progress. Dr. Miller stated that these data will be important for evaluating this protocol. Dr. Miller said that the oncogene sequences used to construct the antisense vectors are relatively short and have no potential for oncogenicity if the oncogene sequences are inadvertently expressed in the forward direction. The use of the MMTV promoter is to express the antisense sequences specifically in breast cells, and the retroviral vectors target specifically to the actively dividing cancer cells in the injected body spaces. Dr. Miller raised several specific questions: (1) Vector design and production. The vector sequence submitted on the computer disk does not match the description in the text or in the diagrams. The overall structure of the vector is fine but it is based on the old N2 retroviral vector. There was concern that the old N2 vector, when produced in the PA317 cells, has a high probability of generating replication competent retrovirus (RCR) due to recombination with other viral sequences in those cells. Dr. Miller asked why the newer LXSN vector was not used. The investigators responded in writing that they are concerned that the MMTV promoter may not be appropriately expressed in this new vector. Dr. Miller stated that the investigators should provide quantitative data regarding the lack of RCR production or the criteria to be used for testing clinical grade vector preparations. Dr. Miller asked what culture medium will be used to produce the clinical grade vector preparations. The standard culture media contain bovine serum which is not acceptable for human use. The investigators indicated in writing that human serum will be used or alternatively the vector will be produced in serum-free media. (2) The IBC has recommended a Biosafety Level (BL) 2+ physical containment for the present experiments. Dr. Miller recommended a BL1 containment level based on lack of oncogenic sequences in the vectors. (3) The IRB initially classified this study as high risk. The investigators have explained that the IRB means the protocol deals with high risk cancer patients, rather than the vector or the experiment being of high risk. (4) Have the investigators used the antisense vectors to transduce primary breast cancer cells? The investigators provided data to show that antisense RNAs were made, and they could inhibit *fos* and *myc* oncogene expression and could reduce cell growth. Dr. Miller was satisfied with these *in vitro* data. (5) Will body fluids of the

vector injection sites directly inactivate the retroviral vectors? Human complement in blood can directly inactivate retroviruses. The investigators have provided data to show that at 37o C pleural effusions have little effect on virus infectivity, a twofold decrease after 24 hours. Overall, Dr. Miller was satisfied with the written response provided by the investigators. He asked to see the correct vector sequence and quantitative RCR data.

Review--Dr. Haselkorn (presented by Dr. Miller)

Dr. Haselkorn stated in his written review that since this new protocol is substantially different from others already approved by the RAC, it requires a thorough review. The protocol involves the administration of retroviral constructs expressing antisense RNAs of oncogenes to treat metastatic breast cancer. The vector has a MMTV promoter that requires estrogen for expression, so the transgenes should be transcribed only in cells such as breast cells that contain the estrogen receptor. Dr. Haselkorn raised several specific questions: (1) Are the vectors targeted to breast cancer cells from the prospective recipients? (2) Are the antisense RNAs expressed in those cells? (3) If expressed, do they prevent expression of the *fos* and *myc* gene products? and (4) Is growth of the tumor cells affected? Dr. Miller said that these questions have been affirmatively answered by the investigators in their written response. The investigators noticed a "bystander effect" in that tumor growth was affected more than the number of cells transduced. Dr. Miller mentioned additional data provided by the investigators to demonstrate antitumor effect on established tumors in the nude mouse experiments. The tumor was established in the peritoneal space. The untreated animals developed a tumor size of 700 mg, and in the anti-*myc* mice, the tumor was reduced to 400 mg. In mice treated with anti-*fos*, the tumor was reduced to 100 mg. There are preliminary experiments in 6 mice, but the data is encouraging.

Dr. Walters mentioned a fax letter from Dr. Haselkorn stating that he was impressed with the recent data provided by Dr. Holt and, therefore, he would withdraw his initial objections to this protocol.

Review--Mr. Capron

Mr. Capron, in his written review, raised several questions including incomplete preclinical studies, spreading of vector to non-targeted cells, and effective targeting of vectors in human cancer. Most of these questions were addressed in the reviews by Drs. Miller and Haselkorn. Since this study would involve a direct vector application to patients, Mr. Capron was concerned about any potential risk to others through vector spreading. The investigators responded in writing that the vector is replication incompetent and will be injected into a closed body cavity that should not present any risk to others. He would like to have clinicians on the RAC comment if treated body fluid could be released in any way. Mr. Capron pointed out several weaknesses of the Informed Consent document: (1) the format is difficult to follow because of the use of different font sizes, (2) the form lacks any statement on long-term follow-up, (3) the warning about not paying for injuries is in a small note, (4) there is an inadequate statement about withdrawing patient consent, and (5) the #6 item on alternative treatment is awkward. Mr. Capron said the investigators have addressed most of his concerns. Mr. Capron had provided specific wording for the Informed Consent document to address his concerns. The investigators have submitted a revised Informed Consent document.

Other Comments

Dr. Dronamraju asked how many patients will be treated. Dr. Holt said they will enroll 10 patients. Ms. Meyers stated that there are many shortcomings in the Informed Consent document: other chemotherapeutic drugs for breast cancer are not mentioned under alternative treatments; there is

no mention of contraception, autopsy, or long-term follow-up. Mr. Capron said that these points have been corrected in the revised Informed Consent document.

Dr. Saha asked questions regarding the rationale for targeting c-*fos* and c-*myc* among other oncogenes, the ratio of antisense expression to the c-*fos* and c-*myc* mRNA, whether the antisense expression is constant from experiment to experiment, and inhibition of oncogene translation. Dr. Parkman asked the investigators to clarify how transduction efficiency will be quantitated in pleural effusions or cerebral spinal fluid (CSF). He asked about the time points for sampling the body fluids.

Investigator Response--Drs. Holt and Arteaga

Dr. Holt said that marker rescue assays are used to detect RCR in their vector preparations. More stringent assays will be used to conform with the requirement of FDA including feline PG-4 S+L- assay, extended S+L- assay, and co-cultivation of test cells with *Mus dunni* cells with detection by the PG-4 S+L- assay. Dr. Miller said the criteria should be less than one RCR per 100 ml of the patient dose. Dr. Holt said that the RCR assay has been performed for 10 ml of supernatant, and it will be scaled up to one patient dose.
Dr. D. Ginsburg said that since the LXSN vector constructs are being developed for antisense expression, it would be preferable to wait and use the better vectors for the human study. Dr. Miller said that if RCR is not detected by the stringent tests, there is no reason not to use the present vectors. Dr. Holt explained that the reason not to change the vector is that MMTV promoter may not function as well in the LXSN vector. He agreed with Dr. D. Ginsburg that it is a reasonable point to try the new vector in the future.

Dr. Miller raised another question about the open reading frame of the *gag* gene in the N2 vector. Expression of *gag* proteins could complicate the interpretation of the results of *myc* and *fos* antisense expression due to potential immunogenicity. Dr. Holt explained that this complication is avoided by using the same vector with *gag* expression in the control studies.

Dr. Miller said that in the new data, a diagram is presented to show the vector construct. A *neo*R gene is driven by the long terminal repeat (LTR) of the vector. The MMTV promoter drives the expression of the anti-*fos* in the opposite direction and the RNA is terminated with a polyadenylation signal from the globin gene. Dr. Holt said that a complete vector sequence is provided.

Regarding the experiment of vector stability in pleural fluid, Dr. Holt said one explanation for the vector stability is that the level of complement, which inactivates the virus, may be lower in pleural fluid than in blood. As to the nude mouse experiment on established tumors, Dr. Holt said antitumor effects have been observed in a preliminary experiment with 6 mice, but additional studies with 20 nude mice are ongoing.

Regarding the statement of alterative therapy in the Informed Consent document, Dr. Holt said that there is no alternative therapy for breast cancer metastasis in pleural or peritoneal effusions. Dr. D. Ginsburg commented that sclerosing treatment is quite effective for the symptom of pleural effusion by closing the pleural space, although it is not directed toward breast cancer itself. This treatment, however, would affect the vector access to the tumor cells, and Dr. D. Ginsburg asked if it should be considered as one of the exclusion criteria.

Dr. Arteaga said that the sclerosing treatment will be useful for patients with serious symptoms of pleural effusion. The majority of patients to be enrolled in the study are not expected to have such severe symptoms. Patients will spend 4 days in the Clinical Research Center for the initial 3

infusions of retroviral vector. Blood will be drawn each day and tested for the presence of retroviral vector. Fluid sampling will be performed the day after infusion to obtain cell samples to determine the percentage of cells taking up the vector constructs and to assess the transgene expression. After the 4 day period, patients can receive other types of therapy.

Responding to Ms. Meyers' question on an alternate therapy statement for breast cancer in the Informed Consent, Dr. Holt said there are other systemic therapies for metastatic breast cancer, but the present protocol is directed to treat local disease. Dr. Parkman said that a statement indicating that there are no other investigational regional therapies would clarify this issue. Dr. D. Ginsburg said that pleurocentesis for malignant pleural effusion is a standard regional therapy. Dr. Arteaga said that these are therapies that patients can receive after completion of 4 days of vector infusion. Dr. Holt accepted Ms. Meyers' suggestion that there should be statements indicating that there are other systemic therapies that are available.

Dr. Parkman asked if all the scientific experiments will be performed in the first 4 days of the study. Dr. Arteaga said that the primary endpoint is to determine vector integration and expression in mammary cancer cells, and it will be done over 4 days. The second endpoint is the appearance of a retrovirus in the blood stream. The third endpoint is clinical toxicity, i.e., local peritonitis or pleuritis, and systemic symptoms such as fever and blood tests. Dr. Parkman commented that the local inflammatory response will be more critical in the study with meningeal infusion.

Dr. D. Ginsburg said that since this treatment is local and will not have any potential benefit to patients, patients may not want to enroll in this study. He asked if subsequent therapies such as intrathecal chemotherapy will complicate the interpretation of data regarding retroviral infusions. He questioned if it is acceptable to ask patients with carcinomatosis meningitis to undergo 4 days of experiment before starting intrathecal chemotherapy. Dr. Smith remarked that the vector dose for meningeal study is much lower than that used in animal studies. Dr. Holt said that meningeal patients would account for less than 5 % of eligible patients, and he agreed to delete this arm of the study.

Regarding the pleural effusion patients, Dr. Arteaga said that the protocol will not enroll patients with serious pleural effusions that require other immediate therapies. Dr. D. Ginsburg remarked that in this aspect the present protocol is different from other studies aiming at patients who have failed other standard therapies. This local therapy, which is similar to pleurocentesis, will not affect the outcome of the systemic metastasis of breast cancer. Dr Arteaga said that the local site will allow assessment of vector treatment over other local chemotherapies. Dr. Miller agreed that it is a reasonable approach. Dr. Smith said that this protocol will yield data addressing some questions about the use of antisense oncogenes in human cancer.

Responding to the question on contraception in the Informed Consent document, Dr. Holt said that the vast majority of patients will be post-menopausal. Ms. Meyers noted not all patients will be post-menopausal, and Dr. Holt agreed to add this statement to the Informed Consent document.

Responding to Dr. Saha's question about choosing c-*myc* and c-*fos* as target oncogenes, Dr. Holt said about 15 to 20% of breast cancers have c-*myc* amplification. c-*Myc* and c-*fos* are oncogenes encoding transcriptional factors contributing to cancer cell growth. Dr. Holt said that the preclinical studies showed data on the level of antisense expression. The ratio of antisense sequences to the endogenous cellular oncogene mRNAs is important since antisense decreases the stability of oncogene mRNA and reduces its cellular levels.

Dr. Parkman said that the preliminary tumor model data was limited to 6 mice, and that the study on another 20 mice is still ongoing. The data on the additional animals is needed for approval of the protocol. Mr. Capron suggested that Dr. Parkman review that data. Dr. Holt agreed to the suggestion.

Committee Motion

Dr. Miller made a motion to approve the protocol with a provision to provideRCR data, vector sequence on disks, and to revise the Informed Consent document. Dr. Parkman added a friendly amendment to request data on the additional 20 mice. Dr.Motulsky seconded the motion. Dr. Walters said that the stipulations should include deleting the meningeal arm of the study.

Dr. Parkman reminded the RAC that in the future consolidated review with FDA, the stipulations will be approved by FDA and will not come back to the RAC.

The RAC approved a motion made by Dr. Miller and seconded by Dr. Motulsky to accept the protocol submitted by Drs. Jeffrey Holt and Carlos B.Arteaga of Vanderbilt University, Nashville, Tennessee, by a vote of 14 in favor, 0 opposed, and 2 abstentions. RAC approval is contingent on the review and approval by the primary RAC reviewers of the following: (1) data demonstrating the absence of helper virus in a single patient dose, i.e., 100 ml; (2) the complete vector sequence submitted on three 3 1/2 inch diskettes in ASCII format; (3) a revised Informed Consent document incorporating the changes suggested by the RAC members; (4) deletion of thecarcinomatous meningitis arm of the study; and (5) data from ongoingmurine preclinical studies.

Mr. Capron noted that there are no clinicians among the three primary reviewers of this protocol, and that several of the clinical questions brought to the discussion have been missed by the primary reviewers. He requested at least one clinical reviewer for every protocol. Dr.Wivel commented that because of the shortage of RAC members with a clinical background, occasionally there are not enough clinical reviewers to be assigned to all protocols.

Summary

Drs. Jeffrey Holt and Carlos B. Arteaga of the Vanderbilt University, Nashville, Tennessee, may conduct gene transfer experiments on 10 female patients (over 18 years of age) withmetastatic breast cancer. Patient effusions from pleura or peritoneum will be drained and the fluid will be replaced with a supernatant containing retroviral vectors. The retroviral vectors, XM6:antimyc and XM6:antifos, are constructed with the N2murine retroviral vector to expressantisense sequences of c-*myc* and c-*fos* under the control of a mouse mammary tumor virus promoter. The vectors are designed for expression in breast cells. The primary endpoints are: (1) to assess uptake and expression of vector sequences in breast cancer cells of pleural and peritoneal fluids and to determine if the expression is specific to breast cancer cells; (2) to determine ifviremia occurs following vector infusion; (3) to assess the local toxicity of vector infusion; and (4) to assess any reduction of malignant cells in pleural or peritoneal fluids.

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: EVALUATION OF REPEAT ADMINISTRATION OF A REPLICATION DEFICIENT, RECOMBINANT ADENOVIRUS CONTAINING THE NORMAL CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR cDNA TO THE AIRWAYS OF INDIVIDUALS WITH CYSTIC FIBROSIS/DR. CRYSTAL*

Review--Dr. D. Ginsburg

Dr. Walters welcomed Dr. Harold Ginsberg, an *ad hoc* consultant on adenovirus vectors, to the meeting.

Dr. Walters then called on Dr. D. Ginsburg to present his primary review of the protocol submitted by Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York. Dr. D. Ginsburg said that this is the first protocol that he has reviewed for the RAC. This study is to treat CF with an adenovirus expressing the CFTR gene. The main differences from Dr. Crystal's previous study involve a change of the adenovirus vector with a cytomegalovirus (CMV) promoter and a test of escalating repeat doses of vector administration. Dr. D. Ginsburg said that these investigators are highly qualified with considerable expertise in the use of adenovirus vectors to treat CF patients. There are 3 distinct parts of the protocol involving a total of 26 patients. In Part A, escalating doses of adenovirus vector will be administered to 3 sites in large airways of the same lung. Two patients will be treated at each dosage level, beginning with 2 x 106 plaque forming units (pfu) per site and increasing to 2 x 109 pfu per site, for a total of 14 patients. In Part B, an additional 12 patients will be studied in 3 groups with the dose and schedule of repeat administration determined from the results of the first part of the study. In Part C, the same patients as in Part A will receive a repeat dose at a 10-fold higher level on days 90 and 180. In all patients, safety and CFTR expression will be monitored. The aims of this study are to examine the effect of more localized vector administration and to determine the responses to repeated treatment and increasing vector dosage.

Dr. Crystal provided a preprint of a manuscript in press for publication describing results of his previous study. Dr. D. Ginsburg was pleased to see the published work. He raised several specific questions regarding the present protocol, and most of them were satisfactorily answered by the investigator. (1) Since the critical target cells within the lung itself are unknown, will a 5-10% overall transduction efficiency of epithelial cells necessarily translate into the same transduction rate in the critical target cells of the lung? Dr. D. Ginsburg was not totally convinced that this issue was sufficiently addressed in the published results of the previous study. (2) Have the levels of expression with the new vector (AdGVCFTR.10) been compared to results with the previous work? What is the data demonstrating the superiority of the CMV promoter in the new vector? (3) Can the investigators provide data to validate quantitative PCR assays of the transduction rate in the lung? This is the measurement used for a primary biologic parameter of efficacy, and there are technical difficulties in performing this assay. (4) The investigator claimed that the vector doses given in the cotton rat experiments were 100-fold greater than the highest dose to be administered to humans in this study. Dr. D. Ginsburg was not comfortable with the calculation of the relative dose. The calculation was based on the body weight difference but this is not a systemic therapy but topical application to the pulmonary epithelium. The basis of this calculation may not be valid for estimating toxicity in humans. (5) The investigator has reported an adverse reaction in one patient in his previous trial. This adverse reaction was not adequately described in the published paper. Even a minor respiratory infection is potentially much more serious in a CF patient. Any inflammatory reaction might have more serious sequelae in patients with compromised lung function than in normal individuals. (6) Since there are large number of eligible patients with this common disease, why not exclude all minors from the study? The investigator responded in writing that there is no added risk to the minors.

In summary, Dr. D. Ginsburg said this is a relatively small change from the previously approved protocol. Dr. D. Ginsburg was satisfied with the responses to most of his questions. He pointed out two outstanding issues that need further responses from the investigator, i.e., the issue of relative dosage between the rat and humans, and the issue of greater risk to a patient with compromised

lung function.

Dr. Walters noted Dr. Crystal's paper has been published in *Nature Genetics*, Volume 8, pp. 42-51, 1994.

Review--Dr. DeLeon

Dr. DeLeon found the proposal to be well presented and most of her comments were mentioned in Dr. D. Ginsburg's review. She still had some concern about the statistics. With a population of 26 patients, 2 in each cohort, and 14 of them being used repeatedly in both Part A and Part C of the study, Dr. DeLeon said that better statistical methods could be applied. She would like the investigators to elaborate on this point. She had questions about the use of quantitativePCR. If it is not going to be used, the protocol should be revised to reflect this change. Most of the issues raised regarding the Informed Consent document have been answered by the investigator. Dr.DeLeon would favor approval of the protocol.

Review--Dr. Zallen

Dr. Zallen commented on two major areas, the experimental design of the study and the informed consent process. For the experimental design, she questioned if the Part C, which uses the same subjects who are in Part A but at a scaled up dose, is needed in this study. The investigator responded that it is necessary to use rare patient resources to obtain most scientific information. The upgraded exposure is to see if increasing vector dose will overcome immunity in these previously exposed subjects. Dr. Zallen asked Dr. Crystal to explain why increasing the vector dose could overcome immunity. She was concerned about the number of bronchial biopsies to be performed on these patients who already have damaged lungs. One biopsy will be performed after each vector administration. Dr. Zallen calculated that patients in Part A would have 1 biopsy; in Part C, a total of 4 biopsies; and in Part B, as many as 6 biopsies. Dr. Zallen asked the investigator to comment on the safety aspect of bronchial biopsy in CF patients. As to the consent process, the Informed Consent document is very long and elaborate. She said it is well written and covers most points. The risk/benefit ratio is reasonable. Dr. Zallen had an initial concern about the acceptability of this lengthy document by the patients. She was satisfied with the response from the investigator that he has successfully used a strategy for addressing this problem in a step-wise fashion. Most of the changes suggested for the Informed Consent document were made in the revision except the item on compensation for research-related injuries. Dr. Zallen said the statement is not clear enough to inform the patients that no such compensation is available. The wording should be for "medical treatment" not for "compensation" that will not be provided by the institution. The individuals who do sign up need to know that if there are injuries that medical costs will be their responsibility.

Review--Dr. H. Ginsberg

Dr. H. Ginsberg commented on the safety issue of using the replication deficient recombinant adenovirus. The comment will apply to most of the protocols using adenovirus vectors. The adenoviruses deleted in the E1 region are not truly replication deficient, they are only crippled. The published literature shows that if a high multiplicity of infections (MOI) (over 80) are used to infect cells, the viruses will replicate in cell culture as do the wild-type viruses. When one is using a high vector dosage such as 109 pfu, as is necessary for this type of gene therapy, it raises a very important point. It is very difficult to determine theMOI in the human situation since the number of target cells is unknown. Some cells may get an MOI of over 250, 500, or even 1,000. Dr. H. Ginsberg referred to toxicity associated with glycerol. The Ad.CFTR vectors are frequently stored in 10%

glycerol. Such concentration of glycerol will kill a cotton rat. It has to be diluted to 1 to 2% to avoid the induced inflammation. Dr. H. Ginsberg noted that adenovirus vectors with an additional E3 deletion, such as the one proposed in this study, are markedly more pathogenic than the wild-type virus. E3 deletion increases the problem of inflammatory response. Dr. H. Ginsberg said that examining the vector-induced cytokines in serum is inadequate. In the cotton rat experiments, tumor necrosis factor (TNF)-, interleukin (IL)-1, and IL-6 appear in the lung very early after infection, and only IL-6 has been detected in the serum. TNF- is very critical in causing the inflammatory reaction. Regarding the animal experiments used to assess toxicity, the animal's lungs were examined 30 days after infection. Dr. H. Ginsberg said most early inflammatory responses occur in the first week and disappear after 10 days in cotton rats. This point is particularly pertinent in repeated inoculations. Bronchial alveolar lavage should be performed early after inoculation to determine if the vector induces any inflammation. Besides cytokines, cellular immunity plays a very critical part in the inflammatory response particularly with the E3-deleted adenovirus vectors. One of the gene products of E3 reduces the expression of Class I major histocompatibility (MHC) antigen on the cell surface, and thus reduces cellular immune response. The E3 deletion increases pathogenicity in cotton rats.

Other Comments

Dr. Parkman said that repetitive vector administration is a logical step toward clinical fruition in the CF study. Dr. Crystal's original submission did include both single and multiple vector administration, although the latter was deleted. There were animal experiments for multiple vector administration, and they showed that the second dose of vector produced a more virulent inflammatory response. Since it is proposed to give 6 repeat doses to humans, the minimum amount of animal data should include at least 6 administrations. Repeat administration increases the cellular type of immune response.

Ms. Meyers asked Dr. H. Ginsberg to clarify his assessment about the safety of the adenovirus vector. Dr. H. Ginsberg said that the vector does express the CFTR gene in animals and in humans. He was concerned about the safety problem because E3 deletion of the vector increases its pathogenicity, and this effect does not require virus replication even though the vector is a crippled virus.

Dr. Ross stated that it would be more understandable to patients if the procedures and time schedules of the clinical protocol were summarized in a flow chart in the Informed Consent document. It would be particularly useful for a complicated protocol like this one. Ms. Meyers commented that this protocol has one of the best Informed Consent documents that she has reviewed.

Dr. Marcel asked if patients' seropositivity or seronegativity to adenoviruses should be listed in the exclusion/inclusion criteria. Dr. Parkman remembered this question has been asked when the RAC reviewed Dr. Crystal's previous protocol, but it was deleted from the protocol. Dr. H. Ginsberg said that unless the antibody levels were extremely high, it would be unlikely that there would be a direct effect on adenovirus replication.

Mr. Capron asked if Dr. Crystal would comment on the relative merit of the adenovirus vectors versus AAV vectors.

Investigator Response--Dr. Crystal

Responding to the question about a flow chart, Dr. Crystal said that such a chart had been prepared and included in the appendix of the submission material.

Regarding the question of immune status, Dr. Crystal clarified thatseropositivity is listed as an inclusion criterion. According to a literature report, people who are seropositive have less adverse effects from live adenovirus infection of the respiratory tract. He did not know whether it was critical.

In terms of adenovirus versus AAV, Dr. Crystal pointed out that adenovirus has no potential for malignancy and has been widely used as a vaccine. AAV does, at least in a limited way, integrate into the host cell chromosomes and has potential for insertional mutagenesis and potential for malignancy. Adenovirus vectors are very effective for transducing genes into target cells. Another advantage is that adenovirus vectors only cause transient expression, but treatment would have to be repeated for long-term effects.
Regarding the patient number in Part A of the study, 7 dosage escalations with 2 in each group will be carried out. It is necessary to have some consistency before moving to the next higher dosage level or to Part C of repeat dosage study. 12 patients will be needed in Part B of the study with 4 patients in 3 groups. The study will start with 1 patient, and then a second at the same dose. If no efficacy is seen, it will move to the next higher level using a half log dose increase. There will be a 3 week interval between each cohort. For repeat administration, there will be a 2 week interval.

Responding to the question of increased adverse reactions in repeat vector administration, Dr. Crystal said that there are two parallel animal studies, both for a duration of 6 months. There is no increased inflammation with multiple doses at 6 months.

Regarding the question of comparative animal and human dosage, Dr. Crystal stated that lung surface is proportional to the height of the individual. Yet it is difficult to compare directly to animals. In animal studies, a small volume of vector is either dripped directly into the trachea or expelled under light pressure. It is different from administration to humans. The surface tension of the lung is such that the applied liquid will soon spread out, so there is no accumulation of the volume over a small area. Dr. Crystal contended that it was not likely to have a few cells getting an extremely high multiplicity of infection. The dosage chosen for the present study is based on the original RAC approved protocol and subsequent discussion with FDA officials.

Dr. D. Ginsburg asked how widely the liquid applied through a bronchoscope will spread. Dr. Crystal said when 100 µl of methylene blue dye marker is applied to the lung, it spreads to a cylinder area of 2 inch in diameter and 4 cm in length. From this data, the MOI is estimated to be 250.

Responding to the question of vector replication at high multiplicity of infection, Dr. Crystal said the literature report described infection of transformed cells such as HeLa cells. For normal airway epithelial cells or cells from CF patients, no replication was detected with the present vector, up to a MOI of 1,000. It is true in his study in cotton rat airways.

As to the expression level with the new vector using a CMV promoter, Dr. Crystal said that there is 10-fold difference in expression of the CFTR gene as compared with the old vector.

Regarding the toxicity to the lung of the CF patients, Dr. Crystal said he is starting at a low dose of 106 pfu and in a smaller volume. The toxicity of the particular patient in the other study occurred with 20 ml of vector at high dose. The volume has been reduced to 100 µl per site for a total of 3 distinct sites. The toxicity can be due to spread of liquid to the alveolar sac, and the smaller volume of vecto

inocula will avoid this complication.

Quantitative PCR will be deleted from the protocol. Regarding the question of subjects being immunized by repeat vector administration, Dr. Crystal said that in animal experiments as well as in the current human study, no neutralizing antibodies have been detected after vector administration.

As to the concern about bronchial biopsy, Dr. Crystal said there will only be bronchial brushing to obtain lung cells rather than biopsy to remove lung tissue. There is no added toxicity by using this procedure.

Regarding the consent process, the patients are allowed 2 weeks to decide about the study.

In terms of glycerol, it is diluted to 3.3%; and no toxicity has been observed at this level. As to the bronchial alveolar lavage procedure in the protocol, it has not been applied to any patient in the CF protocol. Putting the saline solution to the lung will cause some inflammation. The procedure is included in the protocol as a potential means of obtaining samples for analyzing cytokines. Dr. Parkman inquired about the toxicity studies on animals and requested additional data.

Mr. Capron asked if the patient population of the present protocol was the same as the previous CF study. Dr. Crystal said that the new protocol will recruit patients in the New York metropolitan area. As to Mr. Capron's question about the present study aiming to cure CF, Dr. Crystal said that CF is a genetic disease and cannot be cured by the present approach. Repeat vector administration is essential to produce long-term relief of the lung symptoms.

Dr. Walters asked for a clarification of the question of vector replication at high dosage. Dr. H. Ginsberg said that at 1010 pfu, he has observed replication of E1-deleted adenovirus in the cotton rat experiments. It is not a prolonged replication. Dr. Crystal emphasized that he has never seen replication of his vector in several monkey and cotton rat experiments. Mr. Capron asked if different results were due to different vectors. Dr. H. Ginsberg said he is discussing E1a and E1b deleted mutant adenovirus and not the vector Dr. Crystal constructed. Dr. Crystal said the clinical grade adenovirus vector preparations have to pass a test of less than one replication-competent virus per patient dose. With this clinical grade vector, no replication has been observed in animal experiments. Mr. Capron inquired if this result is published. Dr. Crystal said it is included in the RAC submission. Ms. Meyers expressed her discomfort about the contradictory experimental results, especially when the vector is to be used for humans. Dr. Crystal said that in his study with 9 human subjects, no replication-competent virus has been detected. This result was not obtained with the present modified vector with CMV promoter. Dr. H. Ginsberg asked how the viruses are assayed. Dr Crystal said that secretions from patients are tested for adenovirus on 293 cells. Dr. H. Ginsberg said that proper sampling should be bronchial alveolar lavage. Ms. Meyers asked if there were any adverse effects on the 9 patients studied. Dr. Crystal said only the one that has been reported previously; after dose and inoculum volume reduction, no additional adverse effects have been observed.

Dr. Samulski asked to compare the different adenovirus vectors used in all approved CF protocols, some are E3 plus, some are E3 deleted, and some are temperature-sensitive mutants besides the common E1 deletion. Dr. Crystal said that E3 expression required the presence of E1; and in all the E1 deficient vectors, it was not relevant if the E3 was present or absent unless it was under the control of a constitutive promoter. Regarding the temperature-sensitive mutants, they are leaky; and they work in mice but not in humans. The other strategy is to delete E4 and E2b, but these vectors are still under development.

Dr. Samulski asked about the stopping rule for the present study if an adverse effect is observed. Dr Crystal said that if there was no safety issue, the study would continue. No virus shedding was observed when the vector was applied to the lung.

Dr. Miller said that a published work reported vector replication observed in human epithelial cells reconstituted in nude mice. Dr. Crystal said that no vector replication has been seen in human studies. Dr. Miller asked questions about recombination with adenovirus sequences present in host cells. Dr. H. Ginsberg said it does not happen since 293 cell sequences are integrated. Dr. Crystal stated that the criteria for clinical grade preparations are to assure that there is less than one replication-competent virus per patient dose. The clinical grade vector is prepared from a plaque-purified virus and treated with DNase to eliminate any contaminating adenoviral sequences. Dr. Miller said that the efforts to assure vector quality appeared adequate. Dr. H. Ginsberg said that his preparations used in animal experiments have not been as thoroughly prepared as the clinical grade materials.

Mr. Capron asked if the patients were being treated withDNase. Dr. Crystal said that 70 to 80% have proceeded through this kind of treatment. The patients will continue to receiveDNase while on the study since it does not interfere with the present trial.

Dr. H. Ginsberg asked if the cotton rat experiments have been performed with the original vector as well as the new CMV vector. Dr. Crystal answered that both of them have been tested.

Dr. Smith asked if day 7 and day 30 are proper time points to look for inflammatory reaction with the E3 deleted vector. Dr. Crystal said the experiments have been conducted at both time points, and there is no difference. He will provide the data to the RAC.

Committee Motion

Dr. DeLeon made a motion to approve the protocol on the contingency that Dr. Crystal would remove the quantitative PCR assay from the protocol. Dr. Crystal agreed to this stipulation. Dr. Parkman added a friendly amendment to ask the investigator to provide the toxicology data from the cotton rat experiments. Dr. Crystal said this is a completed study.

Ms. Meyers said that she will abstain from voting because of the conflicting statements from the *ad hoc* expert and the investigator. Dr. Walters noted that Dr. Ross abstained due to her employment by Cornell University.

The RAC approved a motion made by Dr. DeLeon and seconded by Dr. Parkman to accept the protocol submitted by Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, by a vote of 12 in favor, 1 opposed, and 2 abstentions. Approval of the protocol is contingent on review and approval of the following by the primary RAC reviewers: (1) removal of the quantitative PCR assay from the study, and (2) toxicology data derived from cotton rat experiments (6 doses of adenovirus vector) obtained at 1 week and 1 month post vector administration.

Summary

Dr. Ronald G. Crystal of New York Hospital-Cornell Medical Center, New York, New York, may conduct gene transfer experiments on 26 patients (15 years of age) with CF. A replication deficient recombinant adenovirus vector will be used to transduce the human CFTR gene to the epithelium of

large bronchi. The vector to be used, AdGVCFTR.10, is an E1-E3- adenovirus-5 based vector with an expression cassette in the E1 region that includes the CMV promoter. The study will initially define the safety and kinetics of expression of the normal CFTR cDNA in the airway epithelium following single dose administration of ascending doses to the airways in different individuals. Once the dose schedules are defined, it will evaluate repeat administration on these individuals. Differences from Protocol #9212-034 are: (1) administration of vector to more localized areas of airways, (2) more careful definition of pharmacodynamics of CFTR expression, (3) evaluation of CFTR expression following repeat administration, and (4) use of a more active promoter/enhancer in the expression cassette.

X. AMENDMENTS TO SECTIONS I, III, IV, V AND APPENDIX M OF THE *NIH GUIDELINES REGARDING NIH AND FDA CONSOLIDATED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DRS. WIVEL AND NOGUCHI*

Dr. Walters mentioned that several written comments were submitted in response to the proposal of NIH/FDA consolidated review. Included in the meeting materials are a letter dated September 7, 1994 from Ms. Wendy L. McGoodwin, Acting Executive Director of Council for Responsible Genetics, Cambridge, Massachusetts, and a letter dated September 12, 1994, from Mr. Jeremy Rifkin, President, and Mr. Theodore Waugh, Staff Attorney of the Foundation on Economic Trends, Washington, D.C..

Dr. Wivel (Executive Secretary) said in response to a question by Dr. Parkman that the NIH/FDA consolidated review and the *ad hoc* committee to review RAC activities are two different proposals. The streamlined review will be implemented while the *ad hoc* review will be planned for the future. Dr. Wivel explained the background and the revised review process. On July 18-19, 1994, the National Task Force on AIDS Drug Development held an open meeting for the purpose of identifying barriers to AIDS drug development that included a proposal to streamline the dual review process for human gene transfer experiments. One of the problems the AIDS investigators identified was that the RAC and FDA require different formats for their submission of applications for review. To streamline this process, one-stop "shopping" mechanism was proposed. Dr. Varmus, the NIH Director, and Dr. David Kessler, the FDA Commissioner, expressed their support for streamlining the review process as did Dr. Philip Lee, Chair of the AIDS Task Force and DHHS Assistant Secretary for Health. As a result of the Task Force's deliberations, recommendations were adopted in order to eliminate any unnecessary overlap between FDA and NIH review of human gene transfer proposals. Both Drs. Varmus and Kessler noted that their respective agencies would cooperate fully to effect the changes necessary to implement these recommendations. The recommendations of the Task Force were:

"The NIH and FDA recommend that the RAC become advisory to both the NIH Director and the FDA Commissioner with regard to the review of human gene transfer protocols. In the interest of maximizing the resources of both agencies and in simplifying the method and period of review of research protocols involving human gene transfer, it is planned that the FDA and the NIH institute a new consolidated review process that incorporates the following principal elements:

"(1) All gene transfer protocols shall be submitted directly to the FDA. Submission will be in the format required by the FDA and the same format will be used by the RAC when public review is deemed necessary.

"(2) Upon receipt, FDA review will proceed. The NIH Office of Recombinant DNA Activities (ORDA) staff will simultaneously evaluate the protocol for possible RAC review.

"(3) Factors which may contribute to the need for RAC review include: (i) novel approaches, (ii) new diseases, (iii) unique applications of gene transfer, and (iv) other issues that require further public review.

"(4) Whenever possible, principal investigators will be notified within 15 working days following receipt of the submission whether RAC review will be required. (RAC reviewed applications will be forwarded to reviewers 8 weeks prior to the next quarterly RAC meeting.)

"(5) Semi-annual data reporting procedures will remain the responsibility of NIH/ORDA. Semi-annual data reports will be reviewed by the RAC in a public forum."

Dr. Wivel explained that the RAC very often approves a protocol provisionally with a list of contingencies to be fulfilled by the investigators before final approval by the NIH Director. Under the new system, the contingencies will be followed up by FDA and the RAC will have no further input. Minor modifications of approved protocols will also be handled by FDA without input from RAC members. Dr. Wivel emphasized that data collection on approved protocols will be continued by ORDA and with reports to the RAC at six-month intervals to maintain public accountability of gene transfer experiments. Dr. Wivel proposed amendments to Sections I, III, IV, V and Appendix M of the *NIH Guidelines*, to reflect this consolidated review process.

Dr. Miller asked a procedural question about the plan to streamline review that has been endorsed by respective agencies, and whether it requires the RAC to vote on this plan. He questioned whether the consolidated review will effectively shorten the review process. Dr. Wivel said that it is clear from the AIDS Task Force meeting that both the NIH Director and the FDA Commissioner are committed to the consolidated process. The RAC is specifically asked to amend the pertinent sections of the *NIH Guidelines* to facilitate the streamlined process. In the new review system, applications will be processed as soon as they come in; and they will be sent out to the reviewers immediately. All the protocols that require RAC review will be collected by a batch method and will be presented at the next quarterly RAC meeting. Dr. Secundy asked why the whole review process has to be changed to accommodate the demand of a single AIDS group. Dr. Wivel said that, as indicated by Dr. Varmus, the current dual review system needs streamlining not simply to meet the demand of AIDS protocols, but to respond to the expected increase in gene therapy proposals. Responding to a question by Ms. Meyers, Dr. Wivel said that the RAC was created by the NIH Director after the Asilomar conference to review recombinant DNA research, not as a result of any statutory action. Dr. Motulsky expressed his sympathy with the concept of streamlining the process. Dr. Zallen was concerned about the deletion of the *Points to Consider* from the *NIH Guidelines* that will deprive the RAC of its ability to utilize its recent revision of the sections dealing with informed consent issues. Mr. Capron shared the concern raised by Dr. Zallen and asked whether there is formal commitment by FDA in this regard. Dr. Noguchi from FDA said that the *Points to Consider* will be adopted as part of the Investigational New Drug Application (IND). There are 11 sections in this IND submission and the *Points to Consider* will be included in Section 11, Relevant Information. He proposed that a working group be formed to solicit public, academic, and corporate input to facilitate the long-term consolidation. Mr. Capron expressed his inclination to abstain from voting on the proposed guideline changes, indicating that the FDA document to adopt the *Points to Consider* has not gone through publication and public comment process. Several outstanding questions regarding review criteria, and the new structure of the review system are still evolving. He suggested that the word "to" should be changed to "under" for the proposed guideline amendments in "Section III-A-1. Major Actions ***to*** the *NIH Guidelines*. Dr. Ross expressed her concern about the triage process in the new review system. Dr. Wivel reassured her that the triage process will involve RAC

members. Dr. Noguchi said that the RAC should make decisions that will have major impact on the field of gene therapy such as establishing criteria for prenatal gene therapy rather than attempting to review all the submitted protocols. Dr. Miller said that he was uncomfortable with the current proposal since the RAC already had adopted an *Accelerated Review* procedure to address the overload problem. Procedurally, the new system will limit the RAC's ability to amend its own guidelines. Dr. Erickson made the observation as someone who had served on the RAC before, that the present proposal might not greatly simplify the review process. Dr.Doi indicated his inclination for deferral until the FDA finalizes its guidelines for IND submission. Dr. Noguchi said that consolidated NIH/FDA review system is a radical idea that requires joint effort from both agencies and the public in order to finalize its plan. Dr. Anderson agreed on the principle of the simplified review system but expressed his concern that the public will lose track of all gene therapy protocols including minor modifications if all submissions are routed through the confidential FDA process. He stressed that the data management function should remain in the public domain within theORDA. Dr. Noguchi said that data monitoring will be enhanced with its pilot project to create a comprehensive computer data base for itsIND process, and that these data will be made available to the public. Dr. Anderson said that FDA data base is confidential and not all information can be made public. The final agreement has to assure that the RAC is able to make the information available even though the submission is to FDA. Dr. Noguchi said that public access is a crucial point of the proposal.

Dr. Walters called on Dr. Noguchi to present his prepared remarks. Dr. Noguchi acknowledged that the joint agreement for theNIH/FDA consolidated review was drafted in a very short order during the AIDS Task Force meeting. Dr. Noguchi said that from the FDA's viewpoint, the public nature of the oversight process has allowed the field of human gene therapy to progress very rapidly in the past. The public dissemination of the information regarding the adverse effects of Dr. Crystal's protocol on the use of an adenovirus vector to study cystic fibrosis resulted in a timely readjustment of the dosing schedule for several other similar clinical trials. FDA has a congressionally mandated regulatory authority over certain areas, but it cannot act in areas where there is no legal authority. The RAC is not a creation of law and, therefore, can complement the restrictions imposed on the FDA. FDA gets its authorization when there is a disaster and the RAC has prevented disasters. Dr. Noguchi said that the joint review is a real opportunity for both parties.

Dr. Secundy favored deferral for the present and suggested a small group of RAC members to work with FDA for the final plan. Ms. Meyers expressed her concern about the fact that FDA needs to keep trade secrets confidential. Confidentiality will impede public accountability of gene therapy studies. She questioned whether FDA has enough resources to perform the additional responsibility relative to human gene therapy, and whether FDA has appropriate staff to evaluate the ethical issues and the Informed Consent documents. She mentioned and Mr. Capron recalled a lawsuit against the RAC in 1989 for failing to duly announce a RAC meeting in the *Federal Register.* Dr. Walters said that a lawsuit was brought by the Foundation on Economic Trends and was settled out of court at the time of RAC approval of the first gene marking protocol. Ms. Meyers asked if the proposed arrangements will be a problem for the confidential FDA process. She considered that public oversight is still needed at the present stage of development of human gene therapy since the long-term possibly untoward effects are not clearly understood. Ms. Meyers indicated she could not vote for the proposal at present.

Dr. Miller asked what portions of theIND submission will be made available to the RAC. Dr. Noguchi responded they will include clinical protocol, *Points to Consider*, Informed Consent document, and enhanced data reporting. All communication with the investigators will be handled by FDA. Dr. Miller was unsure that FDA, without the RAC input, will be able to adequately follow-up

the stipulations attached to RAC approval of each protocol. He was concerned about the closed discussions inherent in the FDA review. Dr. Miller reiterated his view that the current system functions well. FDA and the RAC already have fruitful interactions in the development of criteria for evaluating retroviral vectors.

Responding to a question by Ms. Meyers, Dr. Noguchi said that FDA has adequate resources to deal with gene therapy protocols. His Division of Cellular and Gene Therapies has 14 Ph.Ds, 10 M.Ds (or M.D.-Ph.D.) research scientists, and an additional 6 to 8 Ph.D. review scientists. There are 4 to 5 physicians with subspecialty certification from the Clinical Trial Designs Group, and 4 Ph.D. scientists from the Development Group. There is a total of about 70 full-time equivalent personnel available to review the human gene therapy protocols. Dr. Noguchi admitted that FDA does not have ethicists on its staff, and the contribution of ethicists and public members on the RAC will complement FDA shortcomings in dealing with issues related to Informed Consent documents.

Dr. Parkman said that one point that is unique to the RAC is its ability to evolve its review criteria and process as each protocol is reviewed. He was concerned about the rigidity of the review process that will be codified in FDA regulation. The present categories of *Accelerated Review* can serve as a dividing line to have those protocols reviewed by FDA. The RAC should be notified at its meeting of the accelerated protocols reviewed by FDA and be able to question its appropriateness. Dr. Parkman indicated that he would be more comfortable in approving the consolidated proposal if the detailed plan of implementation had been presented.

Mr. Capron commented that the factors which may contribute to the need for RAC review are described in a very elastic language. It is important to have FDA commitment that all accelerated protocols and its follow-up on data reporting and minor modifications will be reported back to the RAC, and the information will be made publicly available. Dr. Wivel clarified a question by Dr. Anderson that data reporting will include all protocols that are submitted to FDA and not limited to those proposals reviewed by the RAC. Mr. Capron was concerned that if *Points to Consider* is deleted from the *NIH Guidelines*, the RAC would lose its ability to require certain information from the investigators and to effect the continuing evolution of this document. He asked if there is any provision for investigators to demand public RAC review of their protocols to minimize their risk of any future untoward adverse effects. Mr. Capron asked is there any protocol that has been approved by FDA while still pending RAC review. Dr. Noguchi replied that FDA waits for final RAC/ NIH approval before making its own approval. Dr. Anderson noted a single exception of the expedited review of a single patient protocol submitted by Drs. Robert Sobol and Ivor Royston in 1993. Mr. Capron expressed his support of the concept of consolidated review.

Responding to a question by Ms. Meyers about public access to the information, Ms. Wilson (ORDA) explained that for those IND applications submitted to FDA but not reviewed by the RAC, the public portions of the INDs including *Points to Consider* and Informed Consent document will be kept at the ORDA and will be made publicly accessible. The protocols will be tracked by using the FDA's IND numbers. Dr. Noguchi said this public accessibility is the aspect that FDA by law cannot do by itself. He proposed an FDA/ORDA/RAC working group to address problems of long-term consolidation.

Dr. Walters said that the discussion on the proposal for NIH/FDA consolidated review will resume tomorrow morning, and the RAC should achieve cloture for a final vote.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PILOT STUDY OF AUTOLOGOUS HUMAN INTERLEUKIN-2 GENE MODIFIED TUMOR CELLS IN PATIENTS WITH REFRACTORY OR*

RECURRENT METASTATIC BREAST CANCER/DR. LYERLY

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina. Dr. Walters remarked that the discussion will be divided in two parts: an open session and a closed session to discuss the proprietary information regarding the construction of the vector and its sequence. This is the second time in the review of gene therapy that the RAC has to hold an executive session. The other occasion was when confidential patient information was discussed during a single patient expedited review.

Dr. Smith said that this protocol is another cancer vaccination. This protocol proposes to utilize an AAV provirus based plasmid DNA complexed with a cationic liposomal vehicle to transduce autologous breast cancer cells with the gene for human IL-2. Thetransduced cells will be administered subcutaneously to patients in an accelerating dose schedule for 4 doses (0.1, 0.5, 1.0, 5.0 x 108 cell every 4 weeks for 4 months). The endpoints to be assessed include: (1) toxicity, (2) *in vitro* immunological reactivity to the breast cancer cells, (3) duration of clinical response, and (4) patient survival. Patient selection will include those with metastatic disease who have failed all conventional therapy. The plan would require 20 patients.

There are two major differences from previously approved protocols: (1) it involves breast cancer; and (2) it uses a plasmid DNA derived from the AAV to deliver the IL-2 gene in a liposome complex. In terms of the disease, it has long been held that breast cancer, unlike melanoma and renal cell carcinoma, is not particularly immunologically responsive to vaccination therapy. The investigator has provided encouraging preclinical data in the mouse model to demonstrate that this approach might be useful in preventing tumor establishment and treating established tumors.

This protocol was initially submitted to the 1994 June RAC meeting but it was subsequently withdrawn prior to consideration since there was a need for an executive session of the RAC to consider proprietary information. There was insufficient time to announce such a session in *Federal Register*. Most of the questions raised in that initial review have been adequately answered by the investigator. Dr. Smith had two remaining questions. (1) Is the radiation dose sufficient to kill the transduced autologous cell line while still permitting adequate expression of the transduced IL-2 gene? (2) The second question concerned the origin of IL-2 produced in the primary tumor cell population. The investigator transduced a primary cell culture established from breast cancer, which is a mixture of tumor cells and lymphocytes. Although most of the T-lymphocytes have been eliminated from the culture, the IL-2 produced could potentially originate from the remaining T-lymphocytes, either by lymphokine production stimulated by the transduction procedure or by expression of the IL-2 transgene itself. Dr. Smith asked if the investigator had data to show that IL-2 is produced by the transduced tumor cells. Dr. Smith said this information is needed for eventual scientific interpretation of the results of the trial but is not crucial for RAC approval of the protocol. The preclinical data is adequate to justify the present technology. Dr. Smith said the investigator has supplied most other data, and barring further discussion with respect to the specific vector, this is an approvable protocol.

Review--Dr. Doi

Dr. Doi said that he had a few questions, but that most of them had been satisfactorily answered by the investigator in his written response. He favored approval of the protocol. The questions Dr.Doi

asked were as follows: (1) What is the reason for the transient nature of high expression (i.e., 1-3 days)? (2) Is the level of IL-2 production sufficient for obtaining the desired immune response? (3) Is there any control study with unmodified tumor cells? Is the toxicity expected to be only from IL-2 or from other "cellular" effects? (4) Is there any plan to inject the DNA/liposome complexes directly into tumors? and (5) Has the plasmid DNA vector construct been totally sequenced?

Review--Ms. Meyers

Ms. Meyers said her comments were all answered satisfactorily, and that the Informed Consent document was acceptable.

Other Comments

Dr. Parkman asked if there was a minimal level of IL-2 production for administration to patients. There is a hundredfold difference in IL-2 production between mouse cells and human tumor cells. Will this difference impact on clinical outcome? Dr. Smith said that IL-2 production in human cells is roughly at the same level in one of the animal experiments. Dr. Miller said the information in the submitted data regarding the IL-2 levels is unclear. Dr. Doi asked the investigator to explain the statement in his response that the level of IL-2 required to show clinical benefit is unknown at present.

Investigator Response--Dr. Lyerly

Responding to a question by Dr. Smith regarding the origin of IL-2 production, Dr. Lyerly said that after transducing the primary cell culture, tumor cells were purified and T cells were isolated by phenotypic markers. No measurable IL-2 production was observed in T cells. The other experiment used an irrelevant plasmid as a control for transfection, and no IL-2 production was obtained. Thus, it was not due to nonspecific activation of the residual T cells. The other types of studies in which the IL-2 gene delivery and expression into tumor cells is to look for intracellular IL-2 expression. Such studies are ongoing.

Dr. Lyerly explained that the unit for expressing IL-2 production is defined as pg/ml/106 cells/24 hours. Dr. Miller said that this unit is not interpretable since one cannot have exactly 106 cells in one ml. Dr. Lyerly said it refers to one ml supernatant from a tissue culture dish of approximately 106 cells. The IL-2 level is corrected for actual number of cells in each dish. Dr. Miller suggested leaving out the "ml" in the definition to avoid confusion.

Responding to the question of IL-2 levels required for clinical response, Dr. Lyerly said that in animal experiments, 1,000 to 2,000 pg IL-2/106 cells/24 hours, demonstrated protection against tumor metastasis. Initially, the level of IL-2 production in primary tumor cells was 200 to 800 pg/106 cells/24 hours. After improving the techniques, IL-2 levels comparable to the animal studies, i.e., 1,000 to 2,000 pg were achieved. Dr. Lyerly noted the problem of IL-2 production in this kind of therapy, and that was the reason for choosing the present vector. In these primary breast cancer cells, the production of IL-2 was undetectable using the retroviral vectors.

In response to Dr. Doi's question about the optimal level of IL-2, Dr. Lyerly said that there has been no reported data suggesting that there is an optimal level for T cell immune response. The consensus is that the more the better, and the reasonable starting level would provide the protection against tumors in the mouse model.

Dr. Miller asked about the explanation for the extremely high level of IL-2 production shown in one of the experiments. Dr. Lyerly said the high level, i.e., 200,000 pg/106 cells/24 hours, was obtained from a human breast cancer cell line, MCF-7, which can be grown as a monolayer in a tissue culture dish. That level has not been achieved with primary tumor cells. Dr. Parkman asked what IL-2 level was used when animal experiments demonstrate efficacy. Dr. Lyerly said it is about 1,000 units, and it is a level achieved with primary tumor cells.

Committee Motion

Dr. Smith made a motion to approve the protocol pending review of the vector in the closed session. Dr. Doi seconded the motion.

OPEN SESSION: The RAC approved a motion made by Dr. Smith and seconded by Dr. Doi to accept the protocol submitted by Dr. H. KimLyerly of Duke University Medical Center, Durham, North Carolina, by a vote of 13 in favor, 0 opposed, and 1 abstention. Approval of the protocol is contingent on approval of proprietary information presented during the closed session.

Dr. Samulski abstained from voting since the protocol was submitted before he joined the RAC.

EXECUTIVE SESSION/CLOSED: The RAC approved a motion made by Dr. Miller and seconded by Dr. Erickson to approve the proprietary information presented during the closed session by a vote of 14 in favor, 0 opposed, and no abstentions.

Summary

Dr. H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiment on 20 subjects (18 years of age) with refractory or recurrent metastatic breast cancer. The autologous primary breast cancer cells will be transfected with a plasmid DNA vector in a liposome complex to produce human interleukin-2 (IL-2). The plasmid DNA vector termed pMP6-IL2, encoding the human IL-2 gene is derived from theAAV. After transfection, the tumor cells will be lethally irradiated and administered subcutaneously to the patients in an escalating dose schedule. The primary objective is to evaluate the safety of treating patients with thetransduced cells. The secondary objectives are to determine the effects oncytotoxic T lymphocytes and to evaluate clinical response and duration of responses to the treatment.

XII. CHAIR REMARKS/DR. WALTERS

Dr. Walters solicited input from the RAC regarding Dr. Varmus' suggestion about an *ad hoc* committee to review RAC activities. Since this item was not announced in the *Federal Register*, no formal vote could be taken but suggestions were to be sent to Dr.Wivel or Dr. Walters in order to be transmitted to Dr. Varmus.

Dr. Walters stated that the RAC recommended approval of Dr.Lyerly's protocol following review of the proprietary information about the structure and sequence of the vector in the executive session of the RAC.

Dr. Anderson found it ironic that the RAC as a public body had to hold a closed session to review a portion of Dr. Lyerly's protocol. He suggested that Section IV-E-5,*Protection of Proprietary Information,* should be deleted from the *NIH Guidelines*. Companies should provide public access to all protocol information to allow a level playing field. If every company starts to request executive

sessions, it would be contrary to the mission of the RAC to provide an open forum for discussion of human gene therapy protocols. The reason for the closed session cited by Dr.Lyerly's sponsoring company was to protect patent information. Dr. Anderson said that once a patent application is submitted, company's rights are protected. There was sufficient time for the company to file for patent protection before the RAC meeting. Dr. Anderson said that the closed session should be discontinued for the RAC meetings except for exceptional circumstances (e.g., patient confidentiality). Dr. Miller remarked that Dr.Lyerly's company has yet to file for patent application, but he conceded that there was nothing discussed in yesterday's closed session that could not be reviewed in a public meeting. Dr. Walters remarked that this was the first occasion in recent history of RAC meetings that proprietary information was reviewed in a closed session since the last instances in the early era of recombinant DNA research of 1980 and 1981. The other occasion of a closed session was to protect patient confidentiality in the discussion of a single patient expedited review in 1993.

XIII. CONTINUATION OF THE DISCUSSION REGARDING PROPOSED AMENDMENTS TO SECTIONS I, III, IV, V AND APPENDIX M OF THE *NIH GUIDELINES REGARDING NIH AND FDA CONSOLIDATED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DRS. WIVEL AND NOGUCHI*

Dr. Walters noted that there was no one from the audience who had submitted written comments on the consolidated review and wanted to make a comment. He introduced a revised proposal submitted by Dr. Noguchi following the previous discussion on the NIH/FDA consolidated review system. The FDA proposal reads as follows:

Appendix M, *Points to Consider*, will not be deleted from the *NIH Guidelines*. The *NIH Guidelines* will be modified to require Appendix M, *Points to Consider*, to be submitted directly to FDA before the IND. FDA will update their guidance documents in a similar manner. When necessary, the RAC will continue to be responsible for modifying Appendix M, *Points to Consider*.

FDA/ORDA/RAC will decide on the necessity for full RAC review. The submitted Appendix M, *Points to Consider*, will be publicly available for all human gene transfer submissions even if RAC review is not required.

RAC/FDA will broaden their scope of review in gene transfer to jointly and prospectively address global issues on a regular basis, e.g., ethical considerations in the implementation of a gene therapy patient registry, access for "orphan" genetic disease patients to therapies, criteria for prenatal gene therapy, and transgenic technology for xenotransplantation.

FDA/ORDA/RAC will establish a working group to enhance data monitoring efforts that will be maintained by ORDA.

An FDA/ORDA/RAC working group will be established to consider long-term consolidation. The working group will have input from public, academic and corporate sources.

Dr. Walters called on Dr. Noguchi to present the FDA proposal. Dr. Noguchi used a slide to illustrate the different logistical backgrounds in the creation of the RAC and FDA initiatives. FDA initiatives are very much in response to hazards that have been known. In 1902, the Biologics Control Act was enacted following an episode of contamination of diphtheria antisera with tetanus, resulting in 11 deaths in St. Louis. For most biologics, the efficacy has never been in doubt; the regulation is mainly to ensure safety. In 1981, it was demonstrated that a recombinant protein could

be produced in bacteria, and very shortly a recombinant growth hormone was produced. On the other hand, the RAC was created following a moratorium on recombinant DNA research at the Asilomar Conference and approved its first gene transfer protocol in 1988. The FDA responded in 1991 by issuing its own *Points to Consider* for human gene transfer studies; and in 1992, created the Division of Cellular and Gene Therapies within the Center for Biologics Evaluation and Research of FDA. A recent notice on Application of Current Statutory Authorities to Human Somatic Cell Therapy was published in the *Federal Register* on October 14, 1993, and on Regulation of Somatic-Cell Therapy by the FDA was published in the *New England Journal of Medicine*, Volume 329, pp. 1169-1173, October 14, 1993.
Dr. Noguchi noted that in the last couple of years,NIH and FDA had started productive interactions, and the RAC has provided a public forum to discuss gene therapy issues. Under the Biologics Control Act and the Food, Drug, and Cosmetic Act is a large body of proprietary information that FDA has to protect. Dr. Noguchi said that in gene therapy area, this should not be an issue since the crucial development is the biology of gene transfer which can be discussed in public rather than the proprietary information of vector preparation. In response to concerns raised by Dr. Miller and Ms. Meyers, Dr. Noguchi said that in the revised FDA proposal, the Appendix M,*Points to Consider*, will be retained in the *NIH Guidelines* and will be allowed to continuously evolve as new ethical and societal issues are raised. The investigators will submit responses to the *Points to Consider* simultaneously to both FDA and ORDA in order to determine its need for RAC review.

Dr. Noguchi used a slide to illustrate adverse reactions encountered in clinical trials of biologics. Dr. Jonas Salk personally immunized over 100,000 individuals with his polio vaccine without any adverse events. It was not until the company started to produce this vaccine en masse for the polio campaign that large-scale contamination by simian virus 40 (SV40) occurred. The amount of formalin used in the large scale production process was not sufficient to inactivate SV40, and hundreds of thousands of individuals were exposed to this virus. Fortunately, nosequelae have been directly linked to that exposure. Yellow fever vaccine was contaminated by retroviruses. Many fatalities were associated with the vaccine for respiratory syncytial virus. Most recently, an incidence of outbreak of replication competent retroviruses has been experienced in the production of retroviral vectors, and there were instances of adverse events in gene therapy trials with adenovirus vector and in the treatment of brain tumors with retrovirus producer cells.

Addressing concern that RAC will cede its oversight role to FDA, Dr. Noguchi suggested that RAC continues to provide public review of the emerging issues. Dr. Noguchi mentioned a concern about the prohibitive cost of biosafety testing of retroviral vectors raised by the public testimony in the retroviral production meeting held by FDA following the RAC meeting. The high cost of vector testing will hinder access of "orphan" disease patients to gene therapy. Dr. Noguchi suggested that the RAC can influence public policy about this concern. Other areas the RAC can address through public discussion are a gene therapy patient registry, criteria for prenatal gene therapy, gene therapy for enhancement, and transgenic technology for xenotransplantation, in which transgenic baboons and pigs will be used as organ donors for human transplantation.

Dr. Noguchi addressed concern about the logistics and merits of the consolidated review system. In order to maintain the public nature of gene therapy protocols, the FDA will adopt the current Appendix M, *Points to Consider*, and the investigators will be required to submit this document to FDA/ORDA before submission of an IND. Dr. Noguchi said that once an IND is submitted, FDA reviewers are assigned; and it will be given a response within 30 days under a statutory mandate. FDA/ORDA/RAC will decide on necessity for full RAC review. The RAC review will proceed in the pre-IND period. Whether reviewed by the RAC or not, the*Points to Consider* submitted by the investigators will continue to be publicly available. FDA has resources to enhance data monitoring

efforts, and these data will be made available to the public through the ORDA. FDA/ORDA/RAC will establish a working group to implement a long-term consolidation with input from public, academic, and corporate sources.

Dr. Parkman expressed the feeling of the RAC about the consolidated review as being ambivalent. The political reality is that both Drs. Kessler and Varmus have committed to the idea of a one-stop "shopping" mechanism. He was delighted that Dr. Noguchi responded to the RAC concern by adopting the *Points to Consider* and assured the role of the RAC in the continued evolution of the document by keeping it as Appendix M of the *NIH Guidelines*. Dr. Parkman said that the list of all protocols, including those deemed not to require RAC review, should be reported to the RAC at its next quarterly meeting, and that the RAC should retain its ability to recall any of those protocols for full RAC review, if necessary. Dr. Parkman expressed the desire to wait until the next meeting to vote on this issue when the detailed procedures of the review process are worked out. Mr. Capron said the revised FDA proposal is an evolution in the right direction, but he still favored deferral of any formal action at present. Ms. Meyers said Dr. Noguchi has responded to her two major concerns, i.e., public access to the information submitted by investigators in response to the *Points to Consider*, and the RAC's role in its continuous evolution. Dr. Chase said that it is fruitless to resist the change of the review process, and he lauded the administration's efforts to refocus the RAC's role to deal with the global issues of gene therapy in a public forum.

Mr. Capron recalled that the creation of the RAC effectively made congressional legislation to regulate a nascent scientific field unnecessary. The RAC was created in response to the issue of potential dangers of recombinant DNA research expressed in the Asilomar Conference of 1975, and to the recommendation by the President's Commission report, *Splicing Life*, regarding human gene therapy. At those times, Congress held several hearings and was considering legislation to regulate these areas of concern. To ease these concerns, the RAC was formed consisting of scientific and public members; later a Human Gene Therapy Subcommittee provided public oversight in these areas. The industry demonstrated its voluntary compliance to the *NIH Guidelines*. The present reform of the review process in response to the National AIDS Task Force is a continuation of the RAC's own *Accelerated Review* reform. Mr. Capron was comfortable with the arrangement that all submissions would be routed through FDA. But he still expressed concern about the current state of the art regarding patient outcome in the gene therapy trials,e.g, safety and other patient follow-up data. Given the relative paucity of data, lesser scrutiny by RAC may not be justified. Ms. Meyers asked if efficacy criteria should be required for so many cytokine studies. Dr. Parkman said no efficacy has to be demonstrated in these Phase I studies since the patient's cancer is very often too advanced to respond to gene therapy; efficacy is not the primary endpoint of these trials.

Committee Motion

Dr. Walters asked if there was a motion for approving the concept of consolidated review. Dr. Miller said he would be ready to propose such a motion. He said that the revised FDA proposal has addressed his main concern that the RAC will maintain control over its *Points to Consider*, and it is not important which agency receives submissions. At present, the RAC-approved categories of *Accelerated Review* protocols can be adopted as a guideline for proposals that will not require RAC review. Dr. Parkman reminded that the categories served only as guidelines. For unusual experiments, even those falling within categories such as the administration of retinoblastoma cells secreting interleukin-2 into a child's eye, would not be exempted from RAC review. Dr. Miller said that he would vote for the proposed guideline changes within the general concept of NIH/FDA consolidated review. Specifically, he would sanction the revised FDA proposal submitted by Dr. Noguchi. Dr. Zallen seconded the motion.

Discussion

Dr. Walters considered that it was important to keep the *Points to Consider* within the *NIH Guidelines.* The document has evolved in the last 10 years under the purview of the RAC. Ms. Meyers expressed her remaining concern about lack of ethicists in FDA staff in dealing with ethic issues even for those "me too" experiments in regard to the Informed Consent documents. Dr. Noguchi assured Ms. Meyers if there is any question concerning safety and subjects' rights, the RAC will be consulted. These issues are of paramount importance to FDA's review. Dr. Noguchi mentioned as an example, the ethical dilemma in their approval of gene therapy for newborn infants in the adenosine deaminase (ADA) deficiency protocol. The RAC provided guidelines for the FDA process.

Dr. Zallen asked Dr. Noguchi two questions: (1) The investigators presumably are still required to produce as many documents as in the old review system. Will the new system be more efficient? (2) The RAC will continue to evolve its *Points to Consider*. Will FDA amend its document? Dr. Noguchi said that FDA's intent is to abide with the *Points of Consider* as much as possible and has no intention of unilaterally revising this document since it is a part of the *NIH Guidelines*. Responding to a question by Dr. Miller, Dr. Noguchi said that FDA will keep its own *Points to Consider* dealing with the vector manufacturing process. The joint review process will be developed with public, academic, and corporate inputs to make it as efficient as possible. Dr. Ross suggested re-evaluation of the new system after it is implemented.

Dr. Straus said that the RAC is eager to see this conceptual process move forward. He was not sure what is to be voted on since the administrative decision to implement this new system was already made, and Dr. Varmus has proposed the formation of an *ad hoc* committee to review RAC activities. Mr. Capron explained that the thrust of the motion is to endorse the revised FDA proposal presented by Dr. Noguchi to keep the *Points to Consider* under the *NIH Guidelines* and to disapprove the proposed deletion of this document from the *NIH Guidelines*, as announced in the *Federal Register* on August 23, 1994. Mr. Capron would vote for Dr. Miller's motion, but he agreed with Dr. Straus' assessment that the major action will come from Dr.Varmus' proposed *ad hoc* committee. Dr. Walters said the RAC is endorsing the concept and will pass the endorsement to the *ad hoc* committee.

Mr. Capron said that under the new system, only those essentially repetitive experiments will be exempt from RAC review. Most AIDS gene therapy protocols represent new approaches and will continue to have full RAC review. The proponents of the new review system in the National AIDS Task Force may not be terribly satisfied. All the submitted *Points to Consider* will be filed at ORDA, and the master list of all the approved protocols and data reporting will be maintained as a public record.

Dr. Parkman commented that Dr.Varmus had touched on two important issues: (1) the global philosophical question of what are the pertinent RAC activities; and (2) to refine the review criteria and establish consistency in applying these criteria to the review of each protocol. Dr.Wivel said that the elements that define RAC review have been deliberately left loose enough so that they provide guidance rather than restriction. The whole process is contingent on a case-by-case review with a flexibility inherent in this type of approach. Dr. Walters remarked that Dr.Varmus' proposal for *ad hoc* review originated from his recent review of a RAC recommendation regarding the Curiel protocol, and it is independent from the NIH/FDA consolidated review proposed at the AIDS Task Force meeting. Dr. Parkman agreed that the expected criteria for RAC approval such as preclinical

data are not clearly defined in the *Points to Consider*, and what Dr. Varmus has suggested is to define these criteria more closely in dealing with different kind of diseases.

Dr. Walters called for a vote on Dr. Miller's motion. Dr. Wivel stressed that the motion will nullify the proposed deletion of the Appendix M from the *NIH Guidelines*. Mr. Capron said that retention of Appendix M is stated in Dr. Noguchi's proposal, and the motion is to approve this proposal. Dr. Noguchi remarked that the last element of his proposal is to form a working group to propose long-term consolidation, and this element possibly can be combined with the *ad hoc* committee proposed by Dr. Varmus. Mr. Capron pointed out that Dr. Varmus' proposal is an "external review" of RAC activities, and it is a prerogative for a NIH Director to perform this kind of review. The vote to endorse the FDA proposal is to endorse the concept and the direction of the NIH/FDA consolidated review. Dr. Chase said that the minutes will reflect deliberation of the intent of the motion that Appendix M will not be deleted from the *NIH Guidelines*, and the motion endorses the process proposed by the FDA. Dr. Wivel said there is no need for another *Federal Register* announcement in order to have a vote on this proposal. The results of the RAC action, when approved by the NIH Director, will be published in the *Federal Register*.

Committee Vote

The RAC approved a motion made by Dr. Miller and seconded by Dr. Zallen to accept the following: (1) the FDA proposal submitted by Dr. Noguchi; (2) adopt the *Categories for Accelerated Review* that were approved by the RAC at its March 3-4, 1994, meeting, as guidelines for proposals that will not require RAC review (until such criteria have been established by an *ad hoc* review committee proposed by Dr. Varmus); (3) FDA and the RAC will establish a subcommittee to examine the consolidated review process for human gene transfer protocols; and (4) accept the proposed amendments to the *NIH Guidelines* to reflect this revised consolidated review process (including acceptance of Appendix M and incorporation of necessary editorial changes).

The motion was approved by a vote of 15 in favor, 0 opposed, and 1 abstention. Acceptance of the proposed amendments to the *NIH Guidelines* is contingent on review and approval of these amendments by NIH and FDA legal counsel, the NIH Director, and the FDA Commissioner.

Ms. Meyers thanked Dr. Noguchi for his efforts in crafting the FDA proposal. Dr. Merchant (Viagene, Inc., San Diego, California) commented from his vantage point as a former NIH and FDA employee that the RAC vote is very pertinent for the rapidly evolving field of human gene therapy. By not allowing the RAC to evolve into a Study Section that involves itself constantly with "nuts and bolts," the RAC will be able to really concentrate on the novel applications of gene therapy. It was Dr. Merchant's opinion that the more time that the RAC spends in a truly deliberative and advisory capacity and the less time with simple review issues, the more effective the RAC can be in helping the American public.

XIV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: RETROVIRAL-MEDIATED TRANSFER OF THE IDURONATE-2-SULFATASE GENE INTO LYMPHOCYTES FOR TREATMENT OF MILD HUNTER SYNDROME (MUCOPOLYSACCHARIDOSIS TYPE II)/DR. WHITLEY*

Review--Dr. Erickson

Dr. Walters called on Dr. Erickson to present his primary review of the protocol submitted by Dr. Chester B. Whitley, University of Minnesota, Minneapolis, Minnesota. The overall purpose of this

study is to evaluate the possibility of treating Hunter syndrome (mucopolysaccharidosis type II) , a severe heritable disease, by a form of gene therapy using aLXSN-class vector, L2SN. This vector is a retrovirus genetically-modified to carry the normal gene for human iduronate-2-sulfatase (IDS), which is lacking in patients with Hunter syndrome. For treatment, lymphocytes will be removed from the patient, grown in the laboratory, and exposed to the L2SN vector. The treated lymphocytes will then be returned to the subject by intravenous injection. It is hoped that the treated lymphocytes will survive in the blood stream for several days or longer and will be able to partially replenish the IDS enzyme which is missing. It is hoped that some symptoms of Hunter syndrome will be slowed, prevented, or reversed by this treatment. The specific objectives of this study are: (1) to determine the amount of IDS enzyme that can be produced in the body after injection oftransduced lymphocytes; (2) to determine how long the modified lymphocytes can survive in the blood stream; (3) to determine if the gene modified lymphocytes will reduce the abnormal amounts of glycosaminoglycan storage material in urine; (4) to determine if the gene-modified lymphocytes will decrease the size of patient's enlarged liver and spleen, and if treatment will improve heart and respiratory functions; and (5) to determine if there are any other effects of this new form of treatment i.e., other improvement and side-effects.

Dr. Erickson said that children with Hunter syndrome are usually born looking normal. With increasing years, they start to look abnormal, and the disease was given the unfortunate name of gargoylism years ago. As the storage material accumulates in the internal organs, it affects lung and heart functions. Children usually die by the age of 10; but in the mild variant of the disease, these children will survive much longer. This is a lysosomal storage disease similar to Gaucher disease, and it is a reasonable target for gene therapy.

The investigator provided preclinical data which shows successful expression of the IDS enzyme in the transduced cells, and the enzyme produced by thetransduced cells can partially correct cells lacking IDS. Dr. Erickson's major concern was the patient selection. Some patients with the mild form of the disease have lived to the fifth decade and have reproduced. The "mild" has been used to designate a form of Hunter syndrome with normal and sometimes subnormal intelligence. Thus, it is frequently a matter of degree, and the choice of patients who would optimally benefit from this therapy is quite critical. Mere identification of the particular mutations, as proposed by the investigators, is probably not adequate to identify the appropriate cases. Although it may be rare, there has been moderate heterogeneity even within a single family.

Dr. Erickson's second critique was about the efficacy of this gene therapy. In preclinical studies, transduced cells were selected for high IDS expression by G418, and the actual proposed protocol, such a selection will not be used. IDS expression on average is about 50% of the normal level, and it is not certain how much efficacy would be achieved with such a level of IDS expression. Dr. Erickson said that bone marrow transplantation (BMT), particularly with the cord blood, will be a better alternative. The cross correction in reducing theglycosaminoglycan storage with the gene transfer is only about 60%. The usual classic correction would need to have a level of 20% reduction. But this protocol is a Phase I study, efficacy is not a major endpoint.

In conclusion, Dr. Erickson said that this vector has been previously approved by the RAC, the approach of gene modification of peripheral blood lymphocytes and treating patients with cell infusion are all well established procedures, and there can be some hope of efficacy. Dr. Erickson would recommend approval. But he would like to limit the patients population to adults that can give informed consent. There are enough of those patients for a trials of 4 or 5 patients.

Review--Dr. Saha

Dr. Saha agreed that Hunter syndrome is an excellent target disease for gene therapy. Most of the affected patients will be males. He asked several questions. (1) Patient selection. The patients with mild Hunter syndrome have a life expectancy of 30 to 40 years. Dr.Saha asked if the more severe Hunter syndrome patients are more appropriate target for this initial Phase I study. The mild patients can be treated after the efficacy and safety questions have been resolved. (2) Number of patients in the study. The study will involve 2 children and 2 adults. The numbers are too small, and he suggested to target the adults in the first trial to have 4 adults or at least 3 adults. (3) Transduction efficiency of lymphocytes. The transduction efficiency oflymphoblastoid cell lines in the preclinical studies is very good. The transduction rate of the peripheral blood lymphocytes without G418 selection as proposed in the protocol is quite low. The investigator responded in writing that the peripheral blood lymphocytes cannot be cultured for longer than 3 to 4 weeks to allow G418 selection. G418 inhibits lymphocyte growth. Dr. Saha said that based on the present state of art of transduction, he has some reservation about efficacy of gene transfer to the patients. (4) Vector rearrangement in the transduced cells in patients. The investigator needs to elaborate on it. (5) Data of RCR testings. Dr. Saha questioned if the testing data was from 5 ml of supernatant not the 100 ml patient dose required by the RAC and FDA.

Review--Dr. Ross

Dr. Ross agreed with other reviewers that the present protocol will be better to study 4 adults rather than to include children. She has questions about appropriate dosage of the gene-modified cells for adults and children. The present protocol calls for cell infusion to patients every month for a total of 12 months. Dr. Ross said it is very difficult to keep young children for such a prolonged study. The children, after consenting to enter the study, may regret later that they had to spend such a prolonged period of time to the study. For these reasons, Dr. Ross would recommend limiting the study to adults.

Other Comments

Dr. Doi asked about the validity of the idea oftransducing lymphocytes to correct forlysosomal storage diseases. Dr. Erickson said that the whole basis of the idea is that thelysosomal enzymes released from the transduced lymphocytes will be taken up by other cells. The alternative BMT treatment does not help the symptoms of the central nervous system in classic Hunter patients since the IDS enzyme released from lymphocytes cannot cross the blood brain barrier. The overall approach is similar to the treatment ofGaucher disease. But Dr. Erickson was not impressed by the cross correction data presented by the investigators.

Dr. Parkman said uptake of cross correcting enzymes varies from disease to disease. Most of the classic studies have been done for Hurler syndrome. Dr. Parkman asked the investigators to elaborate on data of a study conducted at the University of Minnesota in 1993 about survival after BMT for patients with Hunter syndrome. How many of these patients have a genotype similar to the present proposed study? What was the clinical response in this study? Most of the questions have been related to its effect on the symptoms of the central nervous system. It has some effects on organomegaly or airway obstruction.

Dr. Parkman agreed with the suggestion of limiting the present study to adults. Unlike the study of adenosine deaminase deficiency, there are adult patients available for the study of Hunter syndrome. The RAC could consider the question about whether children aged 13 to 18 are permissible. Dr. Walters remarked that children are included in the familial hypercholesterolemia

study. Mr. Capron commented that other diseases are fatal to patients in earlier ages, but in the mild Hunter syndrome, patients over 18 years old are good candidates for treatment. Dr. Erickson said that the present study will enroll patients with adequate mental capacity, and those patients will be able to make an informed consent. It is a strong argument that the study should be limited to adolescents. Ms. Meyers said that this is a very painful disease and should not exclude children under 18 years of age. She said that her own 8 year old son was able to make an informed consent to participate in a clinical study.

Ms. Meyers commented that the Informed Consent document should include barrier contraception for men as well as for women. Dr. Chase said that sincehumter syndrome is an X-linked recessive trait with very low fitness, it is largely confined to males. Ms. Meyers questioned the compensation for research related injuries. She asked the investigator to address the ethical aspect of limiting the treatment to 1 year period in case efficacy is demonstrated in the course of the study.

Dr. D. Ginsburg said that alternative treatments for Hunter syndrome are making progress. If in the near future BMT shows promise, the children who have enrolled in the present gene transfer study might be excluded from other kinds of treatments. This is another reason to hold off the proposal to treat children. Ms. Meyers said most orphan diseases have very few other research projects going on. Dr. D. Ginsburg noted that the use ofBMT is a very active area of research.

Dr. DeLeon said she would include children as suggested by Ms. Meyers since Hunter syndrome is a progressive disease. It is easier to assess the outcome of the treatment in children whose disease is in the early stage. Dr. Erickson reminded that this protocol is a Phase I study, and efficacy is not the major question. Dr. Saha stated he is not asking the investigators to give up children, just that the initial effort would be better performed on adults. Dr. Ross agreed on doing the initial study on adults.

Dr. Samulski said that the investigators indicated that they only had resources for 1 year experiment and that it was not good to include children in this short study. Dr. Parkman said that from a scientific point of view, it is better to perform a study on adults in order to obtain data on how long the transgene would persist in individuals and how often patients have to be treated. Children are not reliable subjects to commit themselves to complete the study. Dr.Samulski supported the idea that this study potentially can yield data on how long and how frequent gene transfer has to be performed to sustain gene expression.

On the point raised by Ms. Meyers on limiting the study to one year, Dr. Straus said it is a sensible decision to inform patients that depending upon outcome, there may or may not be further investigation. This point can be more clearly stated in the Informed Consent document to avoid false hope from the patients.

Dr. Chase said that patient's perception of benefit may be different from scientific consideration that no benefit is intended for the present study. Dr. Ross said that she has yet to see the revised Informed Consent document that informs the patients when they will learn about the outcome of the study.
Dr. Saha would leave the decision if the study will continue after 1 year period to the discretion of the investigators.

Investigator Response--Drs. Whitley and McIvor

Dr. Walters called on the investigators to address the issues raised by RAC members. He noted that

Dr. McIvor has in the past served on the RAC and its Human Gene Therapy Subcommittee.

Dr. Whitley said that they have extensive experience in treating the Hurler syndrome, the Type I mucopolysaccharidosis, with transplantation of normal bone marrow into children. The details of the study have been published. There is not only good somatic response, but some preservation of neurologic function. In contrast, BMT has seen some success in treating Hunter syndrome, the Type II mucopolysaccharidosis, in terms of organ shrinking and airway improvement, but little effect on symptoms of the central nervous system. This is the reason to choose this disorder for the present study.

Dr. Whitley said that patient selection is one of the most difficult issues. Regarding the genotype, there are few common gene mutations in this disease except a few hot spots of gene mutations, and the genetic mutations do not always predict the phenotypes. So relying solely upon genotype analysis is not a good way of selecting patients. There is some evidence indicating that patients with mild Hunter syndrome frequently have alternative splicing of mRNA of the IDS gene. A majority (99%) of the mRNA produces ineffective IDS enzyme, and only about 1% is making functional enzyme.

The primary patient selection criteria would be some assessment of the clinical severity. Even mild Hunter syndrome patients very rarely live beyond the age of 40 years. Although the disease appears mild, frequently there are severe internal organ problems.

The investigators debated among themselves the question of whether to include children in the study. One of the reasons in deciding to include children was to assess the dose effect. The laboratory capacity is limited to the production of enough transduced cells to provide 20% of a normal circulating lymphocyte enzyme level in an adult patient. Similar doses of cells would have a better effect on a child since the body weight is much smaller. If children are treated early, there is better chance of preventing complications. Considering that this is a Phase I study, Dr. Whitley said he would agree to limit the present study to 2 adults for a 1 year period and to evaluate the data at the end of the study.

This initial study is limited due to the constraint posed by the limited resources available to the investigators at their institution.

Responding to Dr. Saha's question on vector rearrangement, Dr. Whitley said Southern blot analysis of vector structure in transduced lymphoblastoid cell line was included in the submitted materials. No vector rearrangement has been observed in this system. There is a technical problem in performing similar analysis in the transduced peripheral blood lymphocytes since these cells cannot be established as cell lines for this kind of analysis.

Regarding the Informed Consent document, revisions will be made according to the RAC suggestions, including clarification of the barrier contraception statement, and a clear statement on the 1 year limit to the study. No firm funding is yet available for the present study. All the testings for vector preparations are very expensive.

Dr. Whitley agreed to limit this study to 2 adult patients, although some of his minor patients will be disappointed by excluding them in the trial.

Dr. Straus inquired about patient follow-up particularly in terms of assessing how long the transgene will persist and what proportion of the circulating white blood cells will have the transgene. Dr.

Whitley agreed to do so.

Dr. Chase asked about the cost of performing this study. Dr. Whitley said that just vector production and testing would cost $1.6 million to treat 4 patients, and additional $200,000 to hire a personnel to transduce the cells. Dr. Chase commented that the prohibitive cost of gene therapy would be a problem for wide-scale application of this form of treatment.

Dr. Parkman said the cost would be less if a single bone marrow stem cell protocol is proposed. The investigators chose to perform the peripheral blood cell study first to avoid the anticipated tough questions of attempting a bone marrow study with children.

Dr. Walters remarked on the cost of gene therapy. As the gene transfer technology is improved, it is hoped that simplified and cost effective methods will emerge out of this effort.

Dr. Whitley said that patients will be informed as soon as the study information is obtained regarding the results of RCR testings.

Dr. McIvor addressed questions regarding RCR assays. RCR was assayed by a marker virus rescue assay. In his assay, upon infection by RCR, a virus containing the *neo*R marker gene will be rescued. Using the assay, no RCR has been detected in aliquots of 5 ml supernatants from the L2SN producer cells. No S+L- assay has been performed yet. In the future, every vector production lot and master cell bank will be assayed.

Dr. Parkman asked if the RAC criterion of less than 1 RCR per 100 ml patient dose will be applied. Dr. McIvor said it has been planned to screen larger volumes of the supernatants. But he noted the FDA requirement is to screen for 5% of the production lot, i.e., 500 ml for a 10 liter lot. Dr. Whitley indicated that this kind of screening effort is excessive and expensive.

Ms. Meyers asked Dr. Noguchi from FDA to comment on this issue. Dr. Noguchi said that stringent requirement for RCR testing is a result of the primate studies that show that RCR can cause malignant lymphoma. It is the purpose of FDA's public meetings to encourage investigators and sponsoring companies to discuss these RCR testing requirements. There is not enough data to suggest which levels of testing are adequate. Lacking reliable safety data to suggest which level of testings such as 5%, 1%, or 0.5% of a production lot is adequate, FDA's position as a responsible body is to take a conservative stand.

Dr. McIvor commented that the amplification assay for RCR required by FDA is very costly, around $10,000 per specimen. The marker virus rescue assay is used in most research laboratory, but not for vector production. Dr. McIvor said that he is planning to do the S+L- assays for replication competent amphotropic virus. Dr. Noguchi emphasized that there is no inherent reason not to use the marker rescue assay if it can detect RCR to some degree of certainty.

Mr. Capron commented that RCR is more of a concern for children who have a long life span with a mild disease. Dr. McIvor said the question is how far does one have to go in sensitivity level in order to ensure that the research subjects are being given a safe stock. Ms. Meyers said that for research that is sponsored by commercial companies, there is less problem. But the cost is prohibitive for protocol like this one which does not have a commercial sponsor. Mr. Capron said that if the research is promising, it should be funded at an adequate level rather than cut the safety standard, and run the risk of harming people.

Dr. McIvor said the standard has not been established. Dr. Noguchi agreed it needs to be established. Ms. Meyers said there should be special federal funds for this type of project. Dr. Parkman said that clinical research has risks. Is there is a moral imperative to reduce the risks to as close to zero, disregarding the costs of doing the tests? Dr. Parkman asked if the marker rescue assay is much cheaper than the amplification test, would the RAC accept an assay with an error rate of 50% with the understanding that it can save 90% of the money. Ms. Meyers answered that she would not. Dr. Parkman said that if this information is disclosed in the Informed Consent document to the patients, would it be acceptable?

Committee Motion

Dr. Erickson made a motion to approve the protocol to treat 2 adult patients with the only other stipulation being a follow-up after the 12 month period of study. Dr.Saha seconded the motion. Ms. Meyers added that follow-up should be life long.

The RAC approved a motion made by Dr. Erickson and seconded by Dr. Saha to accept the protocol submitted by Dr. Chester B. Whitley, University of Minnesota, Minneapolis, Minnesota, by a vote of 15 in favor, 1 opposed, and no abstentions. RAC approval is contingent on the following: (1) the protocol will be limited to 2 adult subjects, and (2) patients will be monitored for the presence and expression of the transduced gene for over 1 year following their participation in the study.

Summary

Dr. Chester B. Whitley, University of Minnesota, Minneapolis, Minnesota, may conduct gene transfer experiments on two adult subjects (over 18 years of age) with mild Hunter syndrome (Mucopolysaccharidosis Type II). The autologous peripheral blood lymphocytes will be transduced *ex vivo* with a retroviral vector, L2SN, encoding the human cDNA for iduronate-2-sulfatase (IDS). The transduced lymphocytes will be reinfused into the patients on a monthly basis. The study will determine the frequency of peripheral blood lymphocyte transduction and the half-life of the infused cells. Evaluation of patients will include measurement of blood levels of IDS enzyme, assessment of metabolic correction by urinary glycosaminoglycan levels, clinical response of the disease, and monitoring for potential toxicity. This Phase I study is to demonstrate the safety of the L2SN-mediated gene therapy and to provide a preliminary evaluation of clinical efficacy.

XV. CLARIFICATION ON NIH/FDA CONSOLIDATED REVIEW/DR. WALTERS.

Dr. Walters made a clarification regarding the motion concerning the NIH/FDA consolidated review voted for approval by the RAC. The motion included the necessary changes in the *NIH Guidelines* indicated in the proposed action to allow initial submission of the application to FDA. Dr. French Anderson inquired about when will the change taking place. Dr. Walters said that the guideline changes have to be approved by the NIH Director before they are effective. Dr.Wivel added that until that time, the old system will stay in place. Dr. Walters' clarification was accepted by Dr. Miller, who made the motion, and by the RAC.

XVI. REPORT FROM THE WORKING GROUP ON RETROVIRUS VECTORS/DR. WIVEL

On September 7, 1994, the Working Group on Retrovirus Vectors held a telephone conference call to discuss the letter dated December 2, 1993, submitted by the late Dr. HowardTemin regarding the adequacy of current methods to detectRCR. The Working Group members were Drs. Walters, Miller, Straus, Parkman and Brinckerhoff. Dr. Wivel summarized the discussion. Dr. Temin asked in

his letter, if new RCR are generated by recombination of viral RNA sequences, would such recombinant RCR be detectable by the assay systems that are currently in use? Unfortunately, Dr. Temin has died since he submitted his letter, and there was no opportunity to involve him in further discussion. There was background information in the meeting materials, and the subject has been informally discussed and recorded in the minutes of June 9-10, 1994, RAC meeting. The conclusion of the discussion is as follows: The assays which are currently in place and accepted by both the RAC and FDA for detection of RCR are adequate, irrespective of the mechanisms by which these recombinant RCR are generated. In view of this, the consensus of the Working Group members was that further discussions of this subject was not necessary.

Dr. Walters noted no further comment from participants of this telephone conference.

XVII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: ARTERIAL GENE TRANSFER FOR THERAPEUTIC ANGIOGENESIS IN PATIENTS WITH PERIPHERAL ARTERY DISEASE/DRS. ISNER AND WALSH*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Jeffrey M. Isner and Kenneth Walsh of St. Elizabeth's Medical Center, Tufts University, Boston, Massachusetts. Dr. Parkman said that this protocol is exciting because it deals with the very common disease of atherosclerosis. Instead of focusing on the heart, it is directed toward disease of the peripheral arteries. Patients with peripheral artery disease (PAD) have pain upon walking due to compromised blood flow to their muscles, particularly in their lower extremities. When the blood flow is severely compromised, they begin to have pain at rest and develop skin ulcers. The basis for the decreased blood supply is the presence of atherosclerotic plaques in their arteries. No drugs are now available that significantly reduce the symptomatology of patients who have muscle pain at rest. If the PAD becomes severe enough, patients may have portions of their extremities amputated. Such amputation, however, does not result in long-term clinical stabilization.

The investigators propose to test a new therapy in patients with PAD. This therapy is based upon the observation of Dr. Judah Folkman about 20 years ago that angiogenic growth factors are able to stimulate the production of new blood vessels. A series of angiogenic growth factors have been identified. The investigators propose to use a factor termed vascular endothelial growth factor (VEGF) which was initially isolated as a heparin binding factor secreted by bovine pituitary cells. It has been shown that VEGF stimulates angiogenesis *in vivo* in rats and rabbits. The investigators have performed a preclinical study in rabbits who have had ischemic injury induced by ligation of iliac arteries. The VEGF-cDNA is expressed in a plasmid DNA vector under the control of a CMV promoter. The plasmid DNA was introduced by an arterial catheter into the iliac artery. The cardiac balloon was expanded resulting in the transduction of a small percentage of the arterial cells. The treated animals had significantly increased collateral vessel growth. The use of the arterial catheters is based upon pre-clinical studies in which the investigators determined the ability of the VEGF plasmid DNA to bind to the hydrogel polymer coating of the angioplasty balloon.

The investigators now propose to study 12 patients with claudication at rest or non-healing ischemic ulcers. Such patients are not candidates for non-surgical or surgical re-vascularization. The patients will have the VEGF plasmid DNA introduced by arterial catheterization with the balloon catheter being expanded for one minute. The maximal calculated dose of the plasmid DNA will be 1.07 mg. The endpoint of the study will be a decrease in the amount of pain at rest and/or healing of the ulcers. Secondary endpoints will be based on arteriorography that will be done to patients before

and after gene transfer. Other physiologic measurements are being explored as surrogate endpoints.

Dr. Parkman raised several questions in his critique. Most of them have been satisfactorily answered by the investigators in their written response. The arteriograms were scored by more than one blinded observer, and consistent differences between control and treated animals were observed. The investigators provided copies of all arteriograms, and Dr. Parkman agreed with the investigators' assessment about improvement of blood flow. Dr. Parkman said that this protocol is very good, and he did not agree with all the criticisms made by Dr.Dzau in his written review. The investigators require the patients to keep a diary for pain medication but the baseline is not well defined. Dr. Parkman suggested the diary be kept for a month's period before treatment to define a baseline for pain relief. The diary will record a pain scale, and Dr. Parkman suggested a period of 1 month before and 1 or 2 months after gene transfer.

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

Dr. Walters called on Dr. Parkman to present a written review by Dr.Dzau in his absence. Dr. Dzau raised several issues, and the investigators have provided a detailed response to each question. Dr. Parkman said that most of the concerns have been addressed. Three major issues raised by Dr. Dzau were: (1) What is the imperative rationale for doing gene transfer rather than simple infusion of VEGF peptide? (2) The statement, "not satisfactory candidates fomonsurgical and surgical revascularization", in the Selection of Patients, must be clinically defined. (3) Is this protocol a Phase I study for safety or a therapeutic trial? If it is a efficacy study, a control group of patients should be included. On the matter of the control group in the study, Dr. Parkman said that this is a Phase I study using pain relief as an endpoint. The primary objective is to look for untoward effects. His suggestion of keeping a diary for pain and medication is to use each individual as his/her own control. In his opinion, it is better than a control group of patients in this study. On the question of exclusion criteria, the investigators have now listed the entities in the Exclusion Criteria of the protocol. Dr. Parkman commented that there are patients who are candidates for surgical intervention who are in fact potential candidates for gene transfer.

Review--Dr. Motulsky

Dr. Motulsky said this new approach using gene therapy is very exciting for PAD. This protocol will study the endpoints such as improvement of severe leg pain and healing of ulcers besides angiographic and physiologic measurements, and thus has some elements of a therapeutic protocol. Dr. Motulsky was concerned that mildly favorable effects on pain that patients notice in the study may be due to placebo effects. A control group of patients will be valuable although he recognized problems with this study design. Dr. Motulsky suggested that a small pilot study with about 2 to 4 patients will be useful if a control study is needed. Dr.Motulsky asked if there are extensive studies to examine for dissemination of the DNA from the artery into the blood stream and into other organs. Most of the people to be studied are old men witharteriosclerotic heart disease and many have diabetes. VEGF has a potential for contributing to diabetic retinopathy by stimulating new vessel growth. The Type I diabetic patients will be excluded but some Type II diabetics may be eligible since the risk posed by the protocol is low. The investigators provided satisfactory answers in writing that no plasmid DNA has been detected in brain, lung, heart, liver, and gonads in animal studies. Dr. Motulsky said that the statement regarding cost in the Informed Consent document should be clarified. Do the investigators imply that certain costs will be charged to the patients' insurance even though many additional tests related to the gene therapy are required? Dr.Motulsky had reviewed Dr. Dzau's comment, and Dr. Motulsky agreed with Dr. Parkman's opinion that the

investigators have addressed most of his concerns. In rejecting the idea of a control group in the study, the investigators responded in writing that the additional risk of catheter manipulation to the PAD patients is a factor for favoring the present study design.

Dr. Parkman added that the investigators answered the question about the use of the recombinant VEGF protein. Gene transfer is preferred over bolus injection of recombinantVEGF protein for two reasons: (1) the recombinant product is not available for clinical trial at present and will be expensive, and (2) the local continuous production of VEGF following gene transfer is favored over systemic peptide administration, since the latter will peak and decline quickly. Dr. Parkman noted that gene therapy may be cheaper in this case.

Review--Dr. Secundy

Dr. Secundy said that she had only a minor comment on the statement about autopsy in the Informed Consent document. It should always be stated that autopsy will be requested not required. The investigators have responded to this concern. Dr. Secundy was very pleased to see a clear Informed Consent document, and the statement on patient charges is what the RAC recommended. She favored approval of the protocol.

Other Comments

Dr. Erickson said it is important to point out that this is the first time that gene therapy would be applied to patients in advance of other types of therapy, including recombinant growth factors.

Dr. Straus would like the investigators to elaborate on the issue of retinopathy. He said that a theoretical risk still exists that VEGF DNA could circulate and produce VEGF to induce neovascularization particularly in Type I diabetic patients. He asked if this problem has been investigated in the animal models.

Dr. Miller said that the investigators stated that a modification has been made to the plasmid DNA vector after discussion with FDA officials. The modification involved change of the selectable marker and deletion of the SV40 replication origin. Does the new vector function as well as the old one? He asked the investigators to explain why there are many blank spaces in the submitted vector sequence.

Investigator Response--Drs. Isner and Walsh

Responding to Dr. Miller's question, Dr. Walsh said the SV40 origin of replication has been deleted according to FDA's suggestion to eliminate any chance of autonomous replication of vector DNA in animals. As to why there are N's in the DNA sequence, Dr. Walsh said that is due to sequences unreadable by the automatic sequencer. But the sequence is 98% in agreement with the predicted sequence, which accounts for the entire plasmid DNA. Dr. Miller commented that if there is no compelling reason to change the vector, it should not be changed since all other animal data were obtained from the original vector. Dr. Walsh said that the FDA routinely requests removal of the SV40 origin of replication and the -lactamase gene which confers ampicillin resistance in this type of vector. The concern relates to potentialampicillin contamination of the plasmid DNA preparations. Dr. Miller commented that it is a remote possibility.

Dr. Noguchi of FDA remarked that the FDA's position has been that the aforementioned sequences should be removed if they are not needed. He agreed in this case, that if removal affects the activity,

the vector should not be changed.

Dr. Samulski said that it was entirely possible that deleting the plasmid vector sequences could affect gene expression. He suggested that the human study should be performed with the original plasmid with which all the preclinical data were obtained.

Dr. Isner stated that a reasonable compromise would be to move into this initial clinical study with the original plasmid. In the future, if the study progresses into a product development phase, the more stringent safety issues will be readdressed. Dr. Miller agreed it is a reasonable compromise.

Dr. Samulski suggested that FDA issue some guidance to the investigators early on in the study proposal so that the investigators would not have to repeat all the experiments. Dr. Miller asked if FDA would provide consultation when the project is started. Dr. Noguchi said it is a good idea to start FDA negotiations before the project is started. He said in this case, the preclinical studies would be better performed with a plasmid without the SV40 replication origin.

Dr. Saha said this is an exciting protocol. He asked the investigators to compare their plasmid study with the recent paper published in *Science* by Gary Nabel and his co-worker on gene therapy for arterial restenosis.

Mr. Capron pointed out that the statement in Paragraph E about withdrawal from the study in the Informed Consent document, is repeated in Paragraph G regarding long-term follow-up. He asked about the reason for the repetitive statements. Paragraph F on angioplasty intervention was confusing.

Dr. Isner said that repetitive statement resulted from adding additional statements to the standard Informed Consent document of the hospital. He was willing to revise it. Regarding the angioplasty statement, Dr. Isner said it is a statement suggested by his IRB so that patients are not confused when they consent to a standard angioplasty procedure. He agreed to revise the statement to avoid ambiguity.

Responding to Dr. Straus's question on retinopathy, Dr. Isner said that retina was not examined in the rabbit experiment. In another animal experiment with 22 rabbits, no neovascularization was observed in many different organs after gene transfer or administration of recombinant VEGF. He appreciated Dr. Straus's concern, and funduscopic examination will be performed on the subjects before and after gene transfer. This risk in Type II diabetics is considered to be very low according to the advice received from ophthalmologic and endocrinologic consultants. It is unreasonable to exclude patients who have serious risk of limb loss.

Dr. Straus suggested a complete ophthalmologic examination before and after gene transfer. The issue of retina involvement should be addressed in the future animal studies. Dr. Motulsky said funduscopic photography of each patient will be useful. Dr. Isner agreed to the suggestions.

Dr. Parkman asked if observation has been carried beyond 30 days in animals for collateral neovascularization. Dr. Isner said his colleagues have observed the animals for 90 days, and there is no further vessel growth after 30 days.

Dr. Isner used a slide to show data on the time course of transgene expression. Expression peaks on day 14 and day 20, declines on day 21, and disappears by day 30.

Responding to Dr. Saha's question on comparison of the present study to that of Dr.Nabel's *Science* article, Dr. Isner said that the two approaches are very different applications of gene therapy for vascular disease. Dr. Nabel's study involved application of an adenovirus encoding the *Herpes simplex* thymidine kinase gene to artery endothelium after balloon angioplasty to prevent restenosis. It is a very different approach from the present study to stimulate new vessel growth.

Regarding the suggestion of pain scale, Dr. Isner said that there is a pain scale developed by the European Consensus Document, and it can be used as a valid endpoint to measure the effects of pain medication. He will incorporate that pain scale in the protocol. He will require the subjects to keep a diary about pain and medication, as suggested by Dr. Parkman.

Dr. Isner said that the severe narcotic dependent pain experienced by the PAD patients is unlikely to be relieved by a placebo effect. For those patients, pain relief is a reliable endpoint. Dr.Motulsky accepted this explanation.

Dr. Motulsky asked if the need for a control group in the study has been seriously considered. Dr. Isner replied affirmatively. The question was deliberated in the initial design of the protocol, and later in IRB and IBC reviews. It was deemed unconscionable to enroll patients who have 4 weeks of narcotic dependent pain, 4 weeks of ulcers, and marginal circulation to a sham transfection by balloon angioplasty. The risk of this procedure is higher in these patients, and the risk is not worth taking in a patient who stands to gain no benefit from that kind of intervention.

In response to the question of whether or not the protocol is a Phase I study, DrIsner said that safety is the principal objective of this initial study, but it does not seem wise to exclude the possibility to discover a potential benefit. Mr. Capron commented that from the small number of patients involved in this study, it is not possible to definitively address the question of efficacy. A control study group is needed in a study designed to show efficacy. But in this initial study, the decision not to include a control group of patients seems to be reasonable. Dr. Parkman commented that it is good science to have a control group, but the idea of using the patients as their own control is the only appropriate thing to do at this time.

Committee Motion

Dr. Parkman made a motion to approve the protocol with stipulations to requireophthalmological examination including funduscopic photography and a revised Informed Consent document to include a pain scale and medical diary. Dr. Miller added a friendly amendment to require the use of the original plasmid vector. Dr. Motulsky seconded the motion.

Ms. Meyers noted that there is no commercial sponsor involved in this study. Dr. Miller said that it is very inexpensive to produce a plasmid DNA vector and thus there is less need for a commercial sponsor. Dr. Motulsky was pleased that the protocol did not have the problems associated with retroviral vectors.

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Motulsky to accept the protocol submitted by Drs. Jeffrey M.Isner and Kenneth Walsh of St. Elizabeth's Medical Center at Tufts University by a vote of 15 in favor, 0 opposed, and no abstentions. RAC approval is contingent on review and approval of the following stipulations by the primary RAC reviewers: (1) revision of the appropriate sections of the protocol to clarify that the phVEGF165 vector will be the only vector used for the human study (the same vector used for the preclinical animal studies); (2) submission o a revised protocol and Informed Consent document that includes a statement that patients will

undergo complete ophthalmologic examination (including funduscopic photography) prior to, during, and following vector administration; and (3) changes in the Informed Consent document as suggested by the RAC members, e.g., quantification of pain scale, and requirement for completion of a medication diary 1 month prior to entry onto the study.

Summary

Drs. Jeffrey M. Isner and Kenneth Walsh of St. Elizabeth's Medical Center, Tufts University, Boston, Massachusetts, may conduct gene transfer experiments on 12 subjects (40 years of age) with PAD. A plasmid DNA vector, phVEGF165, encoding the human gene for VEGF will be used to express VEGF to induce collateral neovascularization. Percutaneous arterial gene transfer will be achieved using an angioplasty catheter with a hydrogel coated balloon to deliver the plasmid DNA vector to the artery. The objectives of the study are: (1) to determine the efficacy of arterial gene therapy to relieve rest pain and/or heal ischemic ulcers of the lower extremities in patients with PAD; (2) to document the safety of thephVEGF arterial gene therapy for therapeutic angiogenesis. The secondary objective is to determine the anatomic and physiologic extent of collateral artery development in patients receiving phVEGF arterial gene therapy.

XVIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: TREATMENT OF ADVANCED CNS MALIGNANCY WITH THE RECOMBINANT ADENOVIRUS H5.020RSVTK: A PHASE I TRIAL/DRS. ECK AND ALAVI*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania. Dr. Parkman said that this is a "recombinant" protocol in which two elements of previously approved protocols have been combined to produce this new protocol. The backbone of the adenovirus vector used by the investigators at the University of Pennsylvania totransduce the human CFTR gene for the treatment of CF has been employed in this protocol totransduce the *Herpes simplex* virus thymidine kinase (HSV-TK) gene to treat brain tumors. The approach is similar to Dr. Oldfield's study (Protocol #9206-019) to treat brain tumors using intratumoral transduction with the HSV-TK gene and intravenous GCV. The basis for this concept is that the introduction of the HSV-TK gene into brain tumor cells followed by the systemic administration of GCV will result in the local production of toxic metabolites of GCV that will cause the destruction of the transduced tumor cells as well as the destruction of the non-transduced tumor cells, due to a bystander effect. The mechanism of the bystander effect appears to be the transport of the GCV metabolites through intercellular channels, resulting in the destruction of tumor cells that have not beentransduced. The innovative part of this protocol is the use of adenovirus rather than a retrovirus based vector. Retroviruses require cellular replication for effective transduction while the adenoviruses may be able to transduce cells without cell division. Therefore, it is possible that a higher proportion of the brain tumor cells maybe transduced although it is possible that some normal neurons may be transduced. The risk is similar to neurosurgery where removal of a brain tumor may result in removal of some normal tissue. If both therapies are shown to be successful, it would be important to ascertain which one has the least side effects against normal tissues.

The investigators have a significant amount of preclinical data in animals showing that: (1) the injection of both rat and human tumors*in vivo* results in decreased tumor growth or in some cases destruction of all the tumors, and (2) the injection of the adenovirus vector does not result in demonstrable clinical toxicity.

The protocol has a lot of similarities to the Oldfield protocol. Two groups of patients will be studied, those who have resectable and non-resectable tumors. In both groups, the patients initially will have the adenovirus vector injected stereotactically and then will begin on systemic GCV treatment two days later. When patients have resectable tumors, the tumors will be resected 7 days later and local injections of the adenovirus vector will be given, and GCV continues for another 7 days. The removed tumor will be assayed for the presence of the adenovirus vector. The investigators will take advantage of the Positron Emission Tomography (PET) scanner available at their institution to measure the metabolic changes that occur in the transduced tumors. In an individual with multiple tumors, it will be possible to compare the metabolic effects of the injected tumor versus the non-injected control to determine if there is any distant bystander effect.

The major concern raised by Dr. Parkman was the potential inflammatory response in the closed space of the central nervous system when the vector is stereotactically injected into the brain of individuals who are already immunized to the adenovirus. This response is a serious concern when swelling occurs in the closed space after stereotactic injection, but is less of a concern when a tumor is resected since expandable space will be created. The investigators have not addressed the clinical sequelae of this complication.

Overall, most elements of the protocol have been previously approved by the RAC, i.e., general therapeutic approach, the adenovirus vector backbone, and the cDNA insert of HSV-TK gene. The vector was constructed by Dr. James Wilson's laboratory for his RAC approved CF protocol. The only remaining question is the potential inflammatory response to the adenovirus vector in the closed space of the central nervous system.

Review--Dr. Motulsky

Since many of the aspects of the protocol have been mentioned by Dr. Parkman, Dr. Motulsky did not see that any other novel issues needed to be raised. He said that this protocol is very well written and could be approved as is.

Review--Ms. Meyers

Mr. Meyers commented that this protocol was another gene therapy for brain tumors and questioned if there was any need to conduct another of this kind of study. The investigators responded that this approach uses a different vector. Ms. Meyers asked the investigators to elaborate on this issue. Regarding the Informed Consent document, Ms. Meyers pointed out the barrier contraception was not mentioned for males; and she suggested a statement to indicate that the patient may not benefit from the study, but knowledge may be gained that would benefit others. The investigators responded in writing that there is some therapeutic intent in this protocol. Ms. Meyers was concerned about adverse effects observed in other brain tumor studies. She was not comfortable with starting another brain tumor study unless it would provide unique and valuable clinical data that would not otherwise be forthcoming from similar experiments.

Other Comments

Dr. Parkman commented on Ms. Meyers' objection to another brain tumor study without waiting for the outcome of other similar studies. Dr. Parkman said the present approach is significantly different from other brain tumor protocols using a retroviral vector to deliver the HSV-TK gene. The target specificity of the adenovirus vector is different. Being able to transduce non-dividing cells in addition to dividing tumor cells is a significant change in the target of this therapy. Regardless of the outcome

of other retroviral studies, this approach is significantly different. Dr. Parkman and Mr. Capron both agreed with the point made by Ms. Meyers that no therapeutic benefit should be implied in the Informed Consent document for this Phase I trial.

Dr. Samulski stated that this vector is so different in its ability to transduce non-dividing cells, one would not accept any other protocols using the retroviral approach to treat brain tumors if this protocol shows promise. Dr. Miller added that the HSV-TK gene needs to get into a dividing cells in order to have the killing effect of GCV. (The GCV metabolites can only be incorporated into DNA of a dividing cell.)

Ms. Meyers asked about the chance of the adverse effects of the Oldfield protocol happening in the present study. Dr. Parkman said that the adverse effects of Oldfield protocol are related to the large number of injections of the vector producer cells, and it is independent from the nature of the vector. Dr. Wivel added that another variable is that some of the Oldfield trials involve the use of Ommaya reservoir.

Review--Dr. H. Ginsberg

Dr. H. Ginsberg said that this is an excellent protocol and new information will be obtained by using the present vector.

Dr. H. Ginsberg reiterated a comment he made yesterday during the review of another adenovirus protocol about the importance of E3 deletion of the adenovirus vectors. He said the E3 region encoding 3 genes is important for protection of cells from the harmful effects of viral infection: (1) The gene for the 19 kd protein has the effect of reducing the cytotoxic T lymphocyte (CTL) response because it markedly decreases the Class I major histocompatibility antigen on the cells; (2) The gene for the 14.7 kd protein protects the host against cytokine induction; and (3) The gene for the 11.7 kd protein protects the cell against apoptosis. Deleting these genes is harmful to the host. The adenovirus vector used in the present study has partial E3 deletion, Dr. H. Ginsberg was uncertain which gene has been deleted. This is a critical point regarding the question of virus induced inflammation.

Dr. H. Ginsberg said that vector dose should be expressed by the number of pfu rather than by the number of virus particles since infectivity of the virus differs from each preparation.

Both adenovirus vectors used in this protocol and the previous CF protocols share the same viral backbone, and they have the same capacity to induce inflammation. It is not correct to assume that if the vector does not induce inflammation in monkeys, that it is safe for humans. Dr. Parkman added that the experiment was performed on naive monkeys not on immunized animals.

Dr. H. Ginsberg said that if a vector induces a CTL response, it may increase inflammation upon repeat administration.

Additional Comments

Dr. Parkman explained that stereotaxic injection of vector will be performed in all the patients; and in some with resectable tumors, the tumor will be removed, while GCV treatment continues. This variation is different from the other retroviral brain tumor protocols.

Dr. Samulski asked if the temperature-sensitive mutant developed in Dr. Wilson's laboratory will

decrease the chance of inflammation. Dr. H. Ginsberg said the mutant is leaky at body temperature, and it is inactivated only at 39o C.

Dr. Parkman asked Dr. H. Ginsberg to clarify if E3 expression requires the presence of E1. Dr. H. Ginsberg said E1 has an enhancer that affects E3 expression but it is not absolutely necessary for E3 expression.

Investigator Response--Dr. Eck

Dr. Eck conceded that Dr. Parkman's concern about inflammatory response in a closed space within the skull is a valid one. For those patients who are to receive only the stereotaxic injection and no resection, careful screening of the degree of brain edema will be performed to determine their eligibility for the study. The patients will not be admitted if they have substantial edema, which decreases the expandable brain space.

Dr. Parkman stated that a simple animal experiment will be able to address this toxicity question. Injecting the vector into the brains of 10 animals pre-immunized with the adenovirus would be helpful. Dr. Eck agreed that it is a technically feasible experiment; however, it will be difficult to predict brain swelling in human patients.

Dr. Parkman said that this animal experiment can be easily performed on cotton rats, and if there is response, it will raise some concern.

Dr. Eck said the question of inflammation will be examined from the brain tissues removed after vector administration, although it will be complicated by immunosuppression already existing in cancer patients. Dr. Parkman suggested *in vitro* systems of peripheral blood lymphocytes to observe for immune responses. Dr. Eck said it is included in the CTL assays they are planning to do.

Regarding the question of prior immune status of the patients, Dr. Eck said that previously immunized patients have less chance of spreading the vector, but they may have more severe adverse reactions.

Responding to Ms. Meyers concerns about duplication with other protocols, Dr. Eck said that it is a different vector and will have different toxicities. The knowledge about toxicity with this adenovirus vector will be useful. No additional surgical or lumbar puncture procedures are to be performed on these patients. All are standard procedures to treat brain tumors. So there is no undue stress on the family or the patients in proposing these procedures.

Regarding the benefit section of the Informed Consent document, Dr. Eck agreed to the suggestion by Ms. Meyers and Mr. Capron to not imply any potential benefit to patients, but to mention potential societal benefit.

Dr. James Wilson (University of Pennsylvania Medical Center) said that the most important scientific goal of these human studies of adenovirus vectors is to understand the interactions of host with this potentially therapeutic vehicle. An important aspect of these studies is to critically evaluate the immunological profiles of the recipients to adenoviruses before and after gene therapy. A series of serological tests for adenoviral antibodies and CTL assays will be used to assess the immunological responses. But how these *in vitro* assays correlate with clinical reactions is still unclear. Dr. Wilson agreed that enhanced inflammation in repeat administration is a concern, and he agreed to perform the experiments on pre-immunized animals suggested by Dr. Parkman.

Addressing the vector question raised by Dr. H. Ginsberg, Dr. Wilson said he has finished sequencing the whole vector DNA. The partial deletion in the E3 region involves the deletion of the gene coding for the 14.7 kd protein, but the gene for the 19kd protein is intact. The latter gene affects the level of class I major histocompatibility antigen expression. Dr. Wilson said that he has conducted experiments comparing side by side the E1-E3 deleted virus with the adenovirus deleted only at E1. No significant difference in pathogenicity to the lung and the liver has been observed. Dr. Miller asked what animal was used in this experiment. Dr. Wilson said it is a mouse experiment, and Dr. H. Ginsberg said it is a valid animal for this experiment. Dr. H. Ginsberg has conducted similar experiments in cotton rats but with wild-type virus and E3 deletion, he has observed some differences. Dr. Miller expressed concern about the interpretation of these somewhat conflicting results. He asked why any portions of the E3 region have to be deleted from the vector construct. Dr Wilson said it is easier to clone the E3 deleted DNA because some troublesome restriction enzyme sites are removed. Dr. Wilson said this vector has the same E1 deletion as the one for CF, but it retains 2.5 kb of the E3 region. In this sense, it is a safer vector.

Dr. Parkman said that for the sake of consistency in approving protocols, he asked for data on preimmune animals to assess the question of inflammatory response. Most human patients will be seropositive for adenoviruses, but the animal experiments were all performed with non-immune naive animals.

Dr. Wilson said that there are few correlates in animals that will be predictive in humans, and that is the main reason to perform this human study to assess toxicity. He suggested limiting the present study to those patients who have less risk for this complication.

Dr. Miller asked if the preimmune cotton rat experiments will be agreeable to Dr. Wilson. Dr. Wilson said different toxicities in different organs in different animal species will complicate the risk assessment. Dr. H. Ginsberg agreed that it is an important point. For example, gastrointestinal tract sensitivity to adenovirus in humans does not have a parallel in cotton rats.

Dr. Parkman said he would agree if the study is limited to those patients who do not have preexisting immunity similar to the animal experiments. But this patient population will be a very small percentage.

Dr. Eck said that there are practically no brain tumor patients who have never been infected by adenoviruses. He suggested proceeding first with the group of patients who will have brain resection. If there are serious untoward reactions, they could be taken for surgical debulking immediately. If there are no adverse effects in this group of patients, then the study would be performed with the group going for stereotaxic injection alone.

Dr. Noguchi said that FDA's toxicologists will be more supportive if the animal data is available. He would encourage the investigators to perform these toxicological studies. Dr. H. Ginsberg remarked that the term "toxic" may be not appropriate in this case since the inflammation is not caused by a toxic effect of virion proteins.

Dr. Straus said that the adverse reactions that occurred in other brain tumor protocols appear to be immediate hypersensitivity reactions, and similar reactions could happen in this case.

Dr. Chase said that considering the threshold of patient burden even for a Phase I trial, this protocol is very close to the margin and that he would approve it with a great deal of discomfort.

Responding to a question by Dr. H. Ginsberg about the unit of adenovirus, Dr. Eck said it is expressed as the pfu , plaque forming unit, throughout the protoco

Dr. Franck Sturtz of Progenitor, Inc., Athens, Ohio, commented that it is useful to have severa different trials to compare the results. The RAC should propose some index to monitor these studies. Dr. Eck said that this protocol is a toxicity study; but he agrees that in the future Phase II or III studies, the clinical trials should be so designed that data from different studies can be directly compared. Dr. Sturtz said that even for toxicity studies, indexes such as intracranial hypertensior headaches might be useful. Responding to a critique by Ms. Meyers, it is important to conduct different trials on the same disease in order to compare the outcome.

Committee Motion

Dr. Parkman made a motion to approve the protocol with two contingencies: (1) the protocol design should be revised so that the group of patients who receive stereotaxic injections followed b resection occur before the cohort of patients who receive the stereotaxic injections alone; and (2 negative results are to be obtained from intracerebral injections of pre-immunized cotton rats a scored by either lethality or dysfunction of the central nervous system. Dr. Motulsky seconded th motion.

Dr. Eck accepted a revised Informed Consent document suggested by Mr. Capron concerning wording of the Expected Benefit Section of the Informed Consent document. It says, "Since the purpose of this study is to determine the safety of new techniques, the investigators do not expect that I will benefit personally from participating, although knowledge may be gained that may benefit others."

Dr. Parkman said patients in group of 3 will start at the lowest dose, and then move up to a higher dose. Dr. Eck agreed to a 30 day period of follow-up before starting the next cohort.

Dr. Noguchi commented that the cotton rat data is needed but the results do not have to be negative. Dr. Parkman said that if all animals die, the protocol would have to be reconsidered.

Dr. Imre Kovesdi of GenVec , Inc., commented that it is simplistic thinking that leaving all t region intact will make a safer vector. Interaction of several genes in this region may be important. He was cautious not to make a definitive statement regarding which adenovirus vector is better, since most animal studies are not conclusive in regard to application to the human situations. Dr. H. Ginsberg said his detailed study has been performed in cotton rats by deleting one gene at a time in the E3 region.

The RAC approved a motion made by Dr. Parkman and seconded by Dr. Motulsky to approve th protocol submitted by Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvani Medical Center, Philadelphia, Pennsylvania, by a vote of 15 in favor, 0 opposed, and no abstentions. Approval of the protocol is contingent on the review and approval of the following by the RAC primary reviewers: (1) A revised protocol design in which the first low-dose cohort will receive stereotaxic injection of the adenovirus vector followed by surgical resection. The secon cohort will receive stereotaxic injection alone. Each dose of adenovirus vector will be administere in this manner. Each cohort will be monitored for a period of 30 days before entering the next cohort. If data indicate any serious untoward event, the PI will immediately notify the RAC and stop patient accrual onto the study. (2) Submit data from preclinical cotton rat experiments in which the

adenovirus vector is injected directly into the central nervous system of pre-immunized animals. These animals will be evaluated 1 week following vector administration for evidence of inflammation. (3) Submit a revised Informed Consent document incorporating the changes suggested by Mr. Capron.

Summary

Drs. Stephen L. Eck and Jane B. Alavi of the University of Pennsylvania Medical Center Philadelphia, Pennsylvania, may conduct gene transfer experiments on 18 subjects (>18 years of age) with malignant glioma . The adenovirus vector encoding the HSV-TK gene, H5.020RSVT be injected by a stereotactic guided technique into brain tumors. Afterwards, the patients will rece systemic GCV treatment. Patients eligible to undergo a palliative debulking procedure will rec the same treatment followed by resection on day 7, and a second dose of the vector intra-operatively. Brain tissues removed by resection will be analyzed for adenovirus infection, transgene expression, and signs of inflammation. The size and metabolic activity of tumors will b monitored by scanning with MRI and PET. The objective of the study is to evaluate the overall sa of this treatment and to gain insight into the parameters that may limit the general applicability of this approach.

XIX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GEN TRANSFER PROTOCOL ENTITLED* : *TREATMENT OF ADVANCED MESOTHELIOMA WIT THE RECOMBINANT ADENOVIRUS H5.020RSVTK: A PHASE I TRIAL/DR. ALBELD*

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania Dr. Straus said that this protocol is similar in many aspects to Dr. Eck's protocol for the treatment of brain tumors. This protocol is from the same institution, involves the same vector to transduce th same HSV-TK gene, and the same concept of using GCV to kill the HSV-TK transduced of the issues have been raised and answered during the review of the brain tumor protocol.

Dr. Straus said this proposal is for up to 12 patients with advanced mesothelioma . Mesotheliom a tumor in the lining of the pleural space. It spreads locally and causes obstruction and infection for which there are few satisfactory treatments. The patient survival from the time of diagnosis is a few years at most. Therefore, a novel approach to this disease is very much in order. The patients will have diagnosis performed by biopsy through a pleural scope into the pleural space. On the next day, if the biopsy is positive, the vector will be administered by a chest tube already in place into the pleural space. The vector will be given to 4 cohorts with 3 patients in each group. Each cohort will have a log-fold increment of doses ranging from 109 to 1012 pfu . It is hoped that mesothelioma normal pleural cells will be transduced and will express the TK gene. Five days after transduc patients will be treated with GCV intravenously at a dose of 5 mg twice a day for 14 days TK -expressing cells will become subject to GCV -mediated toxicity and death. It is hoped that will be extensive killing of transduced mesothelioma cells and that some collateral killing of tu cells will occur as well.

The preclinical data suggested that the present approach might work, and the protocol was sound. Dr. Straus raised several questions regarding timing of vector administration after biopsy diagnosis, issues in the Informed Consent document, issues about dealing with GCV toxicity, and severa points about preclinical studies. Most of these questions have been answered satisfactorily by the

investigators in writing.

One remaining question is about the potential management of GCV toxicity. Dr. Straus said tha GCV is a toxic drug that causes bone marrow suppression and has been fatal on rare occasions The revised protocol stated that if Grade 4 bone marrow toxicity persists for more than 5 days, the drug dose will be reduced to 75% of full dose. If toxicity persists for another 5 days, it will be reduced to 50%; and if it still persists, the treatment will be stopped. Dr. Straus said that he is uncomfortable with this prolonged exposure to drug toxicity. He said the toxicity management in the previous protocol is more agreeable. If toxicity is seen, the dose will be reduced to 75% without waiting for 5 days. If absolute granulocyte counts drops to less than 500/mm3, the GCV administration wil discontinue, and resume to a 50% level when the count comes back. He asked the investigators to explain the toxicity management schedule.

Review--Dr. Sah

Dr. Saha noted a discrepancy of the adenovirus vector nomenclature stated in the title of th protocol which is different from Dr. Eck's protocol. Dr. Straus clarified that it appears to be the same vector and is a typographical error in the present protocol title. Dr. Saha said the investigators ha performed excellent preclinical studies in both cell culture and in animal models. In the latter category, rats with the rat mesothelioma and severe combined immunodeficiency (SCID) mice the human mesothelioma were utilized. Dr. Saha was concerned about the rat data demonstra presence of vector DNA in the pleural cavity following intraperitoneal injection of the vector. In th human study, the vector will be injected into the pleural cavity rather than the peritoneal cavity. He was concerned about the spread of vector sequences from the peritoneum to liver and kidney in the rat experiments. The complete nucleotide sequence of the vector is not provided. Dr. Saha made general comment regarding the use of GCV as opposed to acyclovir (ACV) for killing the HSV- transduced cells. There is known toxicity for GCV . If other nucleoside analogues are available HSV-TK gene is going to become a routine strategy for cell killing, it is worth exploring othe alternative drugs for killing the HSV-TK transduced ce

Review--Dr. Zalle

Dr. Zallen said that the investigators have responded to each of the questions she raised in he original review. She anticipated seeing the data concerning the presence of vector sequences in gonadal tissues in the mouse experiments. The investigators have revised the inclusion/exclusio criteria. The exclusion criteria include patients who have had previous gene therapy, chemotherapy, or radiotherapy. Dr. Zallen said that there is no other gene therapy approved for mesothelioma protocol excludes all other treated patients, there will be few eligible candidates left. The investigators have amended the Informed Consent document. The statement about research costs is now satisfactory. The usage of "I" and "you" is not consistent in the Informed Consent document. Dr. Joseph Treat is given as the investigator in the Informed Consent and yet Dr. Albelda is listed the PI. It is not clear to the patients who is the physician in charge. Contraception is mentioned but the duration for its practice is not indicated. Dr. Zallen was concerned about the excessive ches X-ray to be given to the patients.

Review--Dr. H. Ginsberg

Dr. H. Ginsberg said that this protocol is very thoughtfully written for the treatment of mesothelioma Most of his concerns have been raised in the review of the last protocol. He questioned if the vector produced any inflammatory response in Fisher rat experiments. Some of the bowel obstruction and

fibrosis could have resulted from inflammation. Dr. H. Ginsberg suggested when tissues are obtained after vector administration to the patients, they should be carefully examined for signs of inflammation, and what multiplicity of infection of vectors will cause it.

Other Comments

Dr. Erickson said the present treatment is intended as the first treatment for mesothelioma patien and will exclude patients who have had prior treatments. He was concerned that the present treatment would preclude the patients from receiving other forms of treatment since the mesothelioma patients can have a long survival rate. Dr. Parkman asked the investigators t elaborate on the question if there is any pleuritis or pneumonitis following vector administratio Meyers said that the possible long-term effects and unknown side effects of gene therapy are not clearly disclosed to the patients in the Informed Consent document.

Investigator Response--Drs. Albelda and Tre

Dr. Albelda clarified that he is a pulmonary physician and is the PI of the project; and Dr. Treat, medical oncologist, will supervise the clinical trial.

Dr. Albelda presented data with a slide from a rat experiment demonstrating the absence of vecto sequences in organs two days following vector administration to the pleural cavity. Vector sequences were detected by a reverse transcriptase polymerase chain reaction (RT-PCR) assay No vector sequence was present in testes or ovaries. In other experiments, there was some uptake in tumors; and in the peritoneal model, some in liver and kidney probably due to mesothelial tissu that coats these organs. It should not be a problem for the lung. There was no toxicity found in extensive rat studies, and no vector sequences in gonads and all other tissues.

Dr. Albelda said that GCV is a better substrate for the HSV-TK enzyme than ACV. Since th is of a new therapy, he did not want to introduce another parameter to change the generally accepted use of GCV . He agreed it is worthwhile to explore other drugs if the strategy is proven t be useful for the treatment of cancer.

Dr. Albelda agreed to revise the Informed Consent document to delete the exclusion criteri regarding prior gene therapy and other items suggested by the RAC.

Dr. Albelda will revise the management schedule for GCV toxicity. He made a comment abou vector-induced inflammation. Inflammation in a closed space of the brain is undesirable; however, it is acceptable and is actually intended as a form of immunotherapy for pleural cancer. Perhaps inducing inflammation may induce a more therapeutic response in tumors. It should not cause any problem for the pleural space. Nevertheless, signs of inflammatory response is one of the major endpoints of the human studies with the adenovirus vectors. Pleural tissues obtained by biopsy following vector administration will be examined by immunohistochemistry for signs of inflammati The presence and expression of the transgene will be studied. An important aspect about th protocol is that a surgeon can easily access the pleural space to obtain biopsy samples for detailed studies to learn more about the scientific problems of host-vector interactions in the adenovirus vector system.

Dr. Wilson said that the compiled DNA sequences can account for all the components of DNA fragments, and they are in the process of completing actual sequencing of the entire vector DNA.

Dr. Treat accepted the GCV dose modifications as suggested by Dr. Straus. Addressing th question of prior therapies, Dr. Treat said that the major reason to exclude radiotherapy patients is the formation of adhesion or sclerosis of pleural cavity that can result from previous treatments. This mechanical problem will prevent successful placement of a chest tube. As to the chemotherapy patients, they may have worse performance status and overall condition. Gene therapy exclusion will be deleted.

Regarding the concern of at least 9 chest X-ray examinations for each patients, Dr. Lavi , co-investigator, commented that the total exposure of 10 chest X-rays would only be one 10th of the radiation exposure allowed by the FDA rule. He considered it is a safe level of radiation.

Dr. Albelda said that patients enrolled in this study will not be precluded from any other futur treatments including surgical procedures, chemotherapy or radiotherapy. He said he would agree to expand the statement in the Informed Consent document that the long-term effects of gene therapy are unknown. Mr. Capron said that the Informed Consent document would be preferable if the whole consent form were written with the investigators in the first person (I or we) and the subject as the second person (you) because of the complicated nature of the information. Dr. Albelda agreed t these suggestions.

Committee Motion

The RAC approved a motion made by Dr. Straus and seconded by Dr. Saha to accept the protoc submitted by Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelph Pennsylvania, by a vote of 14 in favor, 0 opposed, and 1 abstention. Approval of the protocol is contingent on review and approval of the following by the primary RAC reviewers: (1) a revised Informed Consent document incorporating the changes suggested by Mr. Capron, and (2) revision o the sections of the protocol concerning the management of GCV toxicit

Summary

Dr. Steven M. Albelda of the University of Pennsylvania Medical Center, Philadelphia Pennsylvania, may conduct gene transfer experiments on 12 subjects with advanced mesotheliom The adenovirus vector encoding the HSV-TK gene, H5.020RSVTK, will be administered by a che tube into the pleural cavity. Tumor biopsies will be harvested for analyses for evidence of gene transfer and expression and for immunological responses to vector administration. GCV will b administered by intravenous infusion for 14 days. The primary objective of this Phase I study is to evaluate the safety and feasibility of treating patients with malignant mesothelioma by direct deliv of the adenovirus vector into the pleural cavity.

XX. CLOSING REMARKS AND FUTURE MEETINGS OF THE RAC

Mr. Capron asked if the RAC will have a working group to establish guidelines for adenovirus vectors. There had been a great deal of discussion involving the *ad hoc* consultant and RAC members during the course of reviewing the protocols regarding the safety criteria of adenovirus vectors. He asked if a working group could be established to examine the adenovirus vectors. Dr. Straus noted that although a significant amount of animal data exists, little is known with regard to safety in humans. It would not be a productive effort at this time to try to work out a concrete set of guidelines for the adenovirus vectors. Dr. Walters and Mr. Capron agreed on this assessment.

Dr. Ross said that the issue about adenovirus vectors appears to be a procedural question on how

to resolve the different opinions of an expert in the field and the investigators. Ms. Meyers said that her position is that if there is a scientific argument and it is uncertain who is right, she prefers to err on the side of caution and wait until it is proved to be safe.

Dr. Parkman asked if the RAC should start the process to define the review criteria, or this task will be included in the *ad hoc* committee review of the RAC as suggested by Dr. Varmus earlier. Dr Wivel explained that from his understanding both the issues of review of RAC activities and revie criteria will be included in the proposed *ad hoc* external review. Dr. Walters said that, based on the RAC review experience, it is possible to provide some conclusive answers to the questions raised in the *Points to Consider*.
Dr. Walters announced that the next meeting of the RAC will be December 1-2, 1994, at NIH Building 31C, Conference Room 6.

XXI. ADJOURNMENT

Dr. Walters adjourned the meeting at 3:20 p.m. on September 13, 1994.

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.

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