DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
December 1-2, 1994

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixtieth meeting at 9:00 a.m. on December 1, 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 December 1 from 9 a.m. until 5 p.m. and December 2 from 8:30 a.m. until 3:50 p.m. The following were present for all or part of the meeting:

Committee Members:

Constance E. Brinckerhoff, Dartmouth Medical School
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
Robert P. Erickson, University of Arizona
Joseph C. Glorioso, University of Pittsburgh
Robert Haselkorn, University of Chicago
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).

Liaison Representative:

Barbara Levin, National Institute of Standards and Technology, Department of Commerce

National Institutes of Health staff:

Bobbi Bennett, OD
Diane Bronzert, NCI
Kenneth Cowan, NCI
Barry Goldspiel, CC
Donna Huggins, NIDDK
Christine Ireland, OD
Becky Ann Lawson, OD
Harry Malech, NIAID
Catherine McKeon, NIDDK
Hiroaki Mizukami, NHLBI
David Nelson, NCHGR
Tadatsugu Sato, NHLBI
Thomas Shih, OD
Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
Estuardo Aguilar-Cordova, Baylor College of Medicine
Bob Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Dale Ando, Chiron Corporation
Elizabeth Ashforth, Genetic Therapy, Inc.
Greg Baigent, Chiron Corporation
Peter Ballard, The Blue Sheet
James Barrett, Genetic Therapy, Inc.
Edward Beecham, Human Gene Therapy Research Institute
Mitch Berger, University of Washington
Bridget Binko, Cell Genesys
John Bishop, Food and Drug Administration
Ernst Boehnlein, Progenesys
Arindam Bose, Pfizer, Inc.
Rosemary Browar, Genetic Therapy, Inc.
Jeff Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
Ira Carmen, University of Illinois
Mike Casey, Genetic Therapy, Inc.
Joe Catino, Schering-Plough Corporation
Yawen Chiang, Genetic Therapy, Inc.
Kim Changmin, Korea Cancer Center Hospital
Jan Chappell, Genetic Therapy, Inc.
Shuhsia Chen, Baylor College of Medicine
Theresa Chen, Genetic Therapy, Inc.
Tnjae Chung, MD Anderson Cancer Center
Dorothy Clarke, Fox, Bennett and Turner

Gary Clayman, MD Anderson Cancer Center
Kenneth Culver, Human Gene Therapy Research Institute
John Cutt, Schering-Plough Corporation
Karen Darcy, Magenta Corporation
Eileen Deist, Genetic Therapy, Inc.
Boythe Devlin, Duke University
Henry Dorkin, New England Medical Center
Earl Dye, Food and Drug Administration
Tom Eggerman, Food and Drug Administration
Bryan Finkle, Canji, Inc.
Reinhard Fleer, Rhone-Poulenc Rorer
Diane Fleming, Mid-Atlantic Biological Safety Association
Remi Gloecker, Transgene, Inc.
Jack Gluckman, University of Cincinnati
Raymond Goldberg, Public
Alan Goldhammer, Biotechnology Industry Organization
J. Clay Goodman, Baylor College of Medicine
Mario Gorziglia, Genetic Therapy, Inc.
Richard Gregory, Canji, Inc.
Robert Grossman, Baylor College of Medicine
Paul Hallenbeck, Genetic Therapy, Inc.
Susan Hamano, Cambridge Healthtech Institute
Yutaka Hattori, National Cancer Center in Tokyo
Evan Hersh, Arizona Cancer Center
Douglas Hickman, T. Rowe Price Associates, Inc.
Marc Horowitz, Texas Childrens Hospital
Yukihito Ishizaka, Cleveland Clinic
Jolynda Jones, Genetic Therapy, Inc.
Nancy Kan, Pharmacia, Inc.
Susumu Kanazawa, Kaken Pharmaceutical Company
Kathy Kaufmann, GenVec
Gary Kikuchi, Genetic Therapy, Inc.
Rachel King, Genetic Therapy, Inc.
Connie Kirby, Canji, Inc.
Rebecca Kolberg, Journal of NCI Research
Hitoshi Kotani, Genetic Therapy, Inc.
Robert Kozak, Miles, Inc.
Karen Kozarsky, University of Pennsylvania
Steve Kradjian, Vical, Inc.
Alan Lapey, Massachusetts General Hospital
Ming-Fan Law, Canji, Inc.
Fred Ledley, Gene Medicine, Inc.
Gloria Lee, Rhone-Poulenc Rorer
Charles Link, Human Gene Therapy Research Institute
Zhifeng Long, Genetic Therapy, Inc.
Russette Lyons, Genetic Therapy, Inc.
Dan Maneval, Canji, Inc.
Tony Marcel, TMC Development
Stephen Marcus, Genetic Therapy, Inc.
Alan McClelland, Genetic Therapy, Inc.

C. Bruce McCullough, Schering-Plough Research Institute
Gerard McGarrity, Genetic Therapy, Inc.
Amy McKee, FDC Reports
Janice McTeague, Genzyme Corporation
David Meeker, Genzyme Corporation
Amy Melnick, American Society for Microbiology
Setsu Mikumo, Japan Broadcasting Corporation
John Miller, Cambridge Healthtech Institute
Karen Millison, Genetic Therapy, Inc.
Masaaki Mizuno, Nagoya University
Robert Moen, Geneic Sciences, Inc.
Donald Moorman, Human Gene Therapy Research Institute
Richard Moscicki, Genzyme Corporation
Annemarie Moseley, Ingenex, Inc.
Aniceto Navarro, Smithsonian Institution
Susan Nemeth, Schering-Plough Research Institute
John Nemunaitis, Texas Oncology
Philip Noguchi, Food and Drug Administration
Rachel Nowak, Science Magazine
Carol Ohmstede, Burroughs Wellcome
Hideho Okada, Nagoya University
Sheryl Osbourne, Viagene, Inc.
Jeffrey Ostrove, Microbiological Associates, Inc.
Ed Otto, Genetic Therapy, Inc.
Keiya Ozawa, Jichi Medical School
Guy Page, Promega Corporation
John Park, University of California, San Francisco
Virginia Parks, Act-up Goldengate
Amy Patterson, Food and Drug Administration
Nick Pelliccione, Schering-Plough Corporation
Michael Pensiero, Genetic Therapy, Inc.
Stephen Pijar, University of Maryland
Charles Prussak, University of California, San Diego
Raj Puri, Food and Drug Administration
Abdur Razzaque, Food and Drug Administration
Gene Resnick, Schering-Plough Corporation
Rex Rhein, Biotechnology Newswatch
Dwaine Rieves, Food and Drug Administration
Joseph Rokovich, Somatix Therapy Corporation
Gene Rosenthal, Public
Jack Roth, MD Anderson Cancer Center
Michael Roy, Agracetus, Inc.
Patricia Ryan, Genetic Therapy, Inc.
Alain Schreiber, Vical, Inc.
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambiance Awareness International, Inc.
H. David Shine, Baylor College of Medicine
Helen Shu, Systemix Corporation
Judith St. George, Genzyme Corporation
Peter Stambrook, University of Cincinnati

Margi Stuart, Breast Cancer Action
Franck Sturtz, Progenitor, Inc.
Nevin Summers, Ingenex, Inc.
Yoshiyuki Takahara, Ajinomoto Company
Fumimaro Takaku, International Medical Center of Japan
Wayne Talton, Burroughs Wellcome
Thomas Tarlow, Chiron Corporation
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Yuri Tsuzuki, Japan Broadcasting Corporation
Dominick Vacante, Magenta Corporation
Alan Venook, University of California, San Francisco
Samuel Wadsworth, Genzyme Corporation
Trish Waitschies, MD Anderson Cancer Center
Kathryn Whartenby, Food and Drug Administration
Lisa White, The Blue Sheet
Sharon Williams, Life Technologies, Inc.
Savio Woo, Baylor College of Medicine
Diane Zezza, Schering-Plough Corporation
Shuyuan Zhang, Genetic Therapy, Inc.
Wei Zhang, MD Anderson Cancer Center

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that the notice of the meeting and proposed actions were published in the Federal Register on November 8, 1994 (59 FR 55796) as required by the National Institutes of Health (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 reviewers, other RAC members, and ad hoc experts, followed by responses from the principal investigators (PIs). The Chair will recognize other NIH and Federal employees, and the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters welcomed Joseph C. Glorioso, Ph.D., as a new scientific member of the RAC. Dr.Glorioso is Professor and Chair of the Department of Molecular Genetics and Biochemistry at the University of Pittsburgh, Pittsburgh, Pennsylvania.

Dr. Walters stated that 4 major items of business would be discussed during this meeting: (1) the review o 6 new human gene therapy protocols, (2) review and discussion of the December 1994 Data Management Report, (3) a presentation on inutero somatic cell and gene therapies by Dr. Amy Patterson of the Food and Drug Administration (FDA), and (4) discussion of the proposed amendments to Appendix B of the NIH Guidelines, "Classification of Etiologic Agents and Oncogenic Viruses on the Basis of Hazard."

Dr. Walters made the following announcements: (1) A public FDA meeting on somatic cell and gene therapy production issues will be held at the end of the first day of the RAC meeting. (2) Dr. DavidCuriel's human gene transfer protocol entitled: Phase I Trial of a Polynucleotide Vaccine to Human Carcinoembryonic Antigen in Patients with Metastatic Colorectal Cancer (Protocol #9406-073) was recommended for approval with contingencies by the RAC at its June 1994 meeting; however, Dr. Varmus deferred approval of Dr. Curiel's protocol based on insufficient preclinical data. (3) The ad hoc RAC review committee proposed by Dr. Varmus at the September 1994 RAC meeting is currently being formed and a tentative agenda approved. The first meeting of this ad hoc committee will be held in late

January or early February. Several meetings of this ad hoc committee are expected, at least one of which will coincide with a RAC meeting.

Dr. Walters noted that on September 28, 1994, a congressional hearing was held on the subject, "Human Gene Therapy-Status, Prospects for the Future, and Government Policy Implications," before the Committee on Science, Space, and Technology, chaired by Representative George Brown. Drs. Wivel, Philip Noguchi (FDA), and Walters testified before the committee. At this hearing, Ms. AshantiDeSilva, an adenosine deaminase deficiency (ADA) patient entered on Dr. Michael Blaese's Protocol #9007-002, Mr. DeSilva (her father), and Dr. Kenneth Culver were present at the hearing and testified. Dr. Walters informed the RAC that an article entitled: "Gene Techniques and the Shape of Future Generations," was printed on the front page of the November 22, 1994, New York Times in response to a study by Ralph Brinster of the University of Pennsylvania published in Proceedings of the National Academy of Sciences, U.S.A. in November 1994. Dr. Brinster's study involved the introduction of genetic modifications into the murine spermatogonia that develop into mature sperm cells. He stated that the National Academy of Sciences Institute of Medicine is considering funding for a report designed to analyze the implications of human germ line genetic intervention. This report would be considered a companion report to a study that was recently published by a National Academy of Sciences committee (Chaired by Dr. Motulsky) entitled: "Assessing Genetic Risks."

Dr. Walters noted that a total of 93 human gene transfer protocols (68 gene therapy and 25 gene marking) have been reviewed and recommended for approval by the RAC to date (See Attachment II-Human Gene Therapy Protocols). Forty-three are targeted at cancer, 5 directed towards human immunodeficiency virus (HIV) infection, 18 for inherited genetic disorders, and 2 other therapeutic interventions. TheNIH Director has approved 81 of the 93 RAC-approved studies following review and submission of data submitted in response to the RAC's stipulation requirements. Dr. Motulsky inquired about the reason for the discrepancy in the number of protocols approved by the RAC and that approved by theNIH Director. Dr. Wivel explained that most protocols are approved by the RAC, contingent on the review of data submitted in response to RAC's stipulations. The time required for investigators to adequately respond to these stipulations varies. The RAC is advisory to the NIH Director. The NIH Director retains the final authority to approve/disapprove any human gene transfer protocol.

II-A. CHAIR REPORT ON NIH-APPROVED ACCELERATED REVIEW PROTOCOLS/DR. WALTERS

Dr. Walters explained that the following 3 Accelerated Review protocols were approved since the September 12-13, 1994, RAC meeting: (1) Adenovirus Mediated Gene Transfer for Cystic Fibrosis: Safety of Single Administration in the Lung (lobar instillation) (Protocol #9409-091). This study will be conducted by Dr. Henry Dorkin, New England Medical Center, Tufts University, Boston, Massachusetts; and Dr. Allen Lapey, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. Drs. Straus and Ginsburg, and Mr. Capron reviewed and approved the lobar instillation arm of the protocol through the accelerated review process. However, the reviewers decided that the aerosol administration arm of the study required full RAC review. The reviewers' recommendation was based on the fact that aerosol administration is an unprecedented route of vector delivery that may raise significant safety issues. (2) High Dose Chemotherapy and Autologous Bone Marrow plus Peripheral Blood Stem Cell Transplantation for Patients with Lymphoma or Metastatic Breast Cancer: Use of Marker Genes to Investigate the Biology of Hematopoietic Reconstitution in Adults (Protocol #9411-092). This study will be conducted by Dr. Dan Douer, Kenneth Norris Jr. Comprehensive Cancer Center and Hospital, University of Southern California, Los Angeles, California. Drs. Haselkorn, Saha, and Secundy approved this protocol. Dr. Haselkorn commented that the investigators are serious and capable, and he noted that such an approval may not have been granted to less qualified investigators. (3) A Phase I Study of Vaccination with Autologous, Irradiated Melanoma Cells Engineered to Secrete Human Granulocyte-Macrophage

Colony Stimulating Factor (Protocol #9411-093) will be conducted by Dr. Glen Dranoff, Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts. Drs.Secundy, Brinckerhoff, and Smith approved this protocol which closely parallels Dr. Jonathan Simons' RAC-approved (Protocol #9303-040). Dr. Walters remarked that transition to Accelerated Review has been smooth and has led to a significant reduction in the number of protocols reviewed by the full RAC. This allows the RAC to focus on broader issues.

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

Dr. Walters stated that 10 minor modifications were approved to RAC-approved human gene transfer protocols since the September 12-13, 1994, RAC meeting (see Attachment III for a complete listing of minor modifications).

III. SEPTEMBER 12-13, 1994, RAC MINUTES

The RAC approved a motion made by Dr. DeLeon and seconded by Dr. Chase to accept the September 12-13, 1994, RAC minutes (with the incorporation of minor editorial changes) by a vote of 18 in favor, 0 opposed, and no abstentions.

IV. UPDATE ON NIH/FDA CONSOLIDATED REVIEW OF HUMAN GENE TRANSFER PROTOCOLS/DRS. WIVEL AND NOGUCHI

Dr. Walters stated that on November 22, 1994, Dr. AlanGoldhammer, Director of Technical Affairs of the Biotechnology Industry Organization, Washington, D.C., submitted comments on the proposedNIH/FDA consolidated review process. Dr. Walters called on Drs. Wivel and Noguchi to present an update on this process.

Dr. Wivel noted that the RAC recommended approval of proposed amendments to theNIH Guidelines at its September 12-13, 1994, meeting. These amendments accommodated theNIH/FDA consolidated review process through the inclusion of minor editorial changes. Although the NIH Office of General Counsel accepted the RAC's recommendation, FDA legal counsel has not yet agreed to the language of these proposed actions based on concern regarding the release of proprietary information. TheNIH and FDA are continuing discussion on these actions to resolve these issues.

Dr. Noguchi stated that the FDA endorses theRAC's Data Management activities and discussion of pertinent global issues, e.g., in utero gene therapy. The NIH/FDA consolidated review process will not preclude public disclosure of information that is currently submitted to the RAC. He stressed the importance of the RAC semiannual data reporting process since the FDA does not have an equivalent process for regular reporting. On October 27, 1994, the FDA published proposed amendments to 21CFR Part 312, Investigational New Drug (IND) regulations, in the Federal Register (59 FR 54046). These proposed amendments would require semiannual data reporting (currently annual) to the FDA and would emphasize adverse event reporting. There is an absolute necessity for "real-time" reporting of adverse events. The FDA will continue to propose amendments to the IND regulations in order to accommodate public disclosure of adverse event reporting.

Other Comments

Dr. Motulsky noted the following statement in Dr. Goldhammer's letter, "Since all gene therapy protocols will now be submitted initially to FDA, the provision should exist for sponsors not receiving NIH funding to

elect for or against NIH-RAC review." Dr. Motulsky inquired whether the NIH/FDA consolidated review process would change the current status of RAC review. Dr. Wivel responded that any industry sponsor that collaborates with an investigator whose institution receives any funding from the NIH for recombinant DNA research is required to comply with the NIH Guidelines. Therefore, any such collaborative efforts will still require NIH oversight. Under the consolidated review, however, there will be protocols that will require submission to NIH Office of Recombinant DNA Activities (ORDA) but will be exempt from full RAC review. Such protocols will be reviewed by the FDA while the documentation is maintained by both the NIH and FDA.

Dr. Straus noted that any company that voluntarily submits a protocol for RAC review should accept responsibility for data management and other follow-up activities.

Dr. Miller inquired about the human studies involving vaccinia virus vectors that were not reviewed by the RAC. Dr. Wivel explained that these vaccinia studies were initiated prior to the revision of the footnote in Section V-U of the NIH Guidelines. The recently revised definition restricts exempt vaccines to vector-encoded "microbial" immunogens. This revised definition does not exempt the "cancer vaccine" protocols.

Ms. Meyers asked if a company could conduct human "enhancement" gene transfer experiments and not be required to comply with the NIH Guidelines, e.g., experiments that introduce a "fat" gene for the treatment of obese people or introduce a growth hormone gene to "treat" short stature. Dr. Wivel responded that NIH would not require review of such studies; however, FDA review is mandatory. Ms. Meyers remarked that the FDA does not review ethical issues. Dr. Noguchi responded that the FDA requires Institutional Review Board (IRB) review of such protocols. The FDA reviews Informed Consent documents on an ad hoc basis. The FDA retains the option to bring any protocol for public discussion by an FDA advisory committee. The FDA can require RAC review for such proposals under the NIH/FDA consolidated review process.

Ms. Meyers inquired whether the FDA could bring such an enhancement gene therapy protocol to the RAC without explicit permission from the sponsoring company. Dr. Noguchi answered that the FDA can obtain advice from any review body that is deemed to be appropriate. Ms. Meyers asked if such a review would require a closed RAC session. Dr. Noguchi responded that the FDA's intention would be to have open discussion within current FDA regulations. There is no ready mechanism to demand that a company commit to an open meeting. Ms. Meyers expressed concern about the likelihood that investigators who are not collaborating with an NIH-funded institution could conduct germ line gene therapy or enhancement gene therapy without public awareness or discussion. Dr. Noguchi remarked that there is the legal possibility that such experiments could be conducted without public disclosure.

Dr. Walters noted that a couple of years ago there was a protocol submitted by a company to the FDA and discussed at a public FDA advisory committee meeting. Dr. Noguchi explained that the protocol described by Dr. Walters was the first HIV gene therapy protocol submitted by Viagene, Inc., San Diego, California. That study did not involve any NIH funding. Dr. Noguchi noted that in the future, FDA will bring such protocols to the RAC for public review as a part of the NIH/FDA consolidated review process. Dr. Walters said that up to now, the public has been informed of every gene therapy study that has been conducted in the United States by the FDA or the NIH.

Mr. Capron asked whether the FDA would reject Dr. Goldhammer's request for optional RAC review. Dr. Noguchi responded that Dr. Goldhammer's letter was not addressed to the FDA. The FDA does not have legal authority to accept or reject that particular concept under its current regulations. Dr. Noguchi noted that the pertinent issue is uncertainty regarding the definition of a trade secret or confidential information. I

is unclear what information can be disclosed to the public by the FDA. Public review of the overall concept and the ethical questions poses less of a problem for FDA. If a safety concern is related to vectors, it is unclear under the current regulations whether FDA can make that information public without the company's permission. Mr. Capron asked if the information can be shared with FDA's own advisory committees. Dr. Noguchi responded that confidential information can be discussed in closed session. Mr. Capron asked if RAC members will be co-appointed by both the NIH and the FDA and whether the RAC will become advisory to both agencies. Under such a scenario, would the RAC have access to FDA information? Information reviewed in a closed RAC session could be summarized for public disclosure after the session.

Mr. Capron said it is legal for a company to proceed with an experiment without RACs approval if reviewed on a voluntary basis. However, if an adverse event occurred when a sponsoring company was informed by the RAC that the research was inadequate and would raise unreasonable risks, the company would not have a defensible action.

Dr. Straus asked if an investigator who develops a drug or a biologic in his own laboratory is permitted to use that agent in any therapeutic, diagnostic or prophylactic setting without FDA approval? Dr. Noguchi responded that any such agent (with exception of diagnostics) is subject to IND regulation based on the October 14, 1993, Federal Register announcement. Any intrastate or interstate drug or biologic is subject to IND regulation. Dr. Straus asked whether regulation is intended for investigational use and commercial licensure? Dr. Noguchi responded that the regulation covers all uses. The FDA exercises discretion over regulation, e.g., bone marrow purging with monoclonal antibodies is not regulated. The more cellular manipulation is involved, the greater the likelihood that the experimental reagents will be subject to FDA regulation. Any reagent developed in an investigator's laboratory that has the potential to produce extremely potent effects may require other government oversight in addition to local IRB approval (if intended for human use). Dr. Straus inquired whether an investigator would be permitted to use a retrovirus vector encoding the interleukin (IL)-2 gene for the treatment of diseases other than those for which the vector was originally approved? Dr. Noguchi answered that the FDA allows latitude for other uses. Dr. Straus asked if FDA regulation would permit use of an approved gene therapy product for novel uses, i.e., enhancement or even germ line modification? Dr. Noguchi responded that although such a scenario is legally possible, there are no gene therapy products approved for commercial purposes. All gene therapy experiments are currently in the Phase I clinical trial stage. Dr. Straus was concerned that such a scenario could occur if there is no other mechanism for oversight.

Dr. Parkman agreed with Mr. Capron's suggestion that the RAC should be advisory to the FDA in order to facilitate the flow of information. In response to Ms. Meyers concern about confidential proprietary information, Dr. Parkman said the NIH Guidelines allow the RAC to review confidential information in closed session. A summary of the deliberations can be made available to the public that does not include any proprietary information.

Dr. Chase agreed with Dr. Goldhammer's comments that the RAC should focus on major societal issues and not individual review of routine protocols and that novel uses of approved gene therapy reagents should be addressed. He said that the RAC should preserve the option to review confidential information in closed session, but the general recommendations should become public.

Dr. Zallen disagreed with Dr. Goldhammer's suggestion that the requirement for IRB approval prior to RAC submission should be eliminated. The RAC should not be in the position of providing a pre-review for IRBs. Dr. Zallen agreed with Dr. Goldhammer's suggestion that the FDA should incorporate the RAC's recommendations on Informed Consent into the FDA's IND regulations. Dr. Zallen noted a similar petition to the Office for Protection from Research Risks (OPRR) of NIH. Dr. Noguchi said that a joint meeting

between the IRBs, FDA, and OPRR is planned to discuss Informed Consent issues. Dr. Noguchi agreed to present Dr. Zallen's suggestions at this meeting.

Dr. Glorioso questioned if PIs could use vectors that have been approved for a specific disease to treat other types of diseases. Dr. Noguchi said if a gene therapy vector is approved for a specific indication, it would require a label to state that it has not been shown to be effective in some other types of diseases; however, if a physician chose to use that product for other indications and an adverse event resulted, the physician could be sued. Dr. Parkman stated that local IRBs are required to review protocols involving the application of FDA-approved drugs or biologics for nonapproved uses.

Dr. Walters said that an NIH and FDA staff meeting (including legal counsel) is scheduled to address implementation of the consolidated review process.

Dr. Wivel stated that a response will be sent to Dr. Goldhammer on behalf of the RAC. Dr. Wivel stated that the outcome of the NIH/FDA meeting will be transmitted to the members of the RAC.

Dr. Walters emphasized that the RAC's major concern about the consolidated review process is that the review process will be as open to the public as possible. Public accountability is extremely important. Ms. Meyers added that the RAC exists to provide a public forum to debate the use of this technology and its implication for the future of humanity, not to regulate gene therapy products. Mr. Capron stated that although he is assured by the FDA's commitment that the consolidated review process will remain as open as possible, there is concern about such a process within the limits of FDA regulations. Mr. Capron said IRB-approved Informed Consent documents submitted to the RAC are quite inadequate; however, it is unreasonable for Dr. Goldhammer to request RAC to be the first committee to review these documents. IRBs are legally required to review Informed Consent documents. The requirement for prior IRB approval serves as a filtering mechanism for Informed Consent documents before RAC review. Dr. Walters noted that this requirement avoids the review of documents that are not in final form.

Dr. Haselkorn asked whether the possibility exists under the NIH/FDA consolidated review process that the FDA would approve a protocol that the RAC did not approve. Dr. Noguchi responded that the RAC serves in an advisory capacity, and that the circumstances would be rare that the FDA would not accept the recommendations of an advisory committee.

Dr. Noguchi said that the FDA does not require IRB approval before starting its deliberation; however, IRB approval is required before a clinical trial may be initiated. The RAC should consider elimination of this requirement. Dr. Straus strongly disagreed with Dr. Noguchi's suggestion since FDA does not review Informed Consent documents as rigorously as the RAC and IRBs are charged to review these documents by law.

Dr. Fumimaro Takaku

Dr. Walters recognized Dr. Fumimaro Takaku, President of the International Medical Center of Japan in Tokyo, Japan. He is chair of the committee responsible for human gene therapy oversight. Dr. Takaku stated that this committee plans to hold open meetings similar to the RAC in the near future.

V-A. DECEMBER 1-2, 1994, DATA MANAGEMENT REPORT/DR. SMITH

Dr. Walters stated that the RAC Data Management Report system monitors the results of RAC and NIH approved human gene transfer protocols, particularly adverse events and patient accrual. This semiannual reporting system was first initiated by the late Dr. Brigid Leventhal. Dr. Walters thanked

members of the working group and Ms. Debra Wilson of theORDA for their expanding efforts to collect, categorize, and review this substantial quantity of information. Dr. Walters called on Dr. Smith, Chair of the Working Group on Data Management, to present the December 1994 Data Management Report.

Dr. Smith stated that due to the increasing volume of information that is being submitted by investigators, the protocols have been subdivided into categories. Each category was reviewed by one or more members of the working group. Dr. Smith thanked Dr. Ross for providing a comprehensive written critique of all studies prior to the meeting. This timely critique allowed Ms. Wilson to contact investigators for follow-up information.

Working group assignments were as follows: (1) All human gene transfer protocols -- Drs. Smith, Ross, and Noguchi; (2) Gene Therapy/Cancer/Non-Vaccines -- Drs. Smith and Samulski; (3) Gene Therapy/Cancer/Vaccines -- Drs. Erickson and Samulski; (4) Gene Therapy/HIV -- Dr. Straus; (5) Gene Therapy/Inherited Genetic Disorders -- Dr. Motulsky; and (6) Gene Marking -- Dr. Parkman.

Dr. Smith noted the Data Management Report which summarizes the individual reports submitted by investigators (see Attachment IV-Data Management Report).

Dr. Smith stated a total of 329 subjects have been treated on the 91 RAC-approved protocols. Fourteen protocols are currently closed and 2 protocols are currently placed on hold either by FDA or by the investigators. A total of 36 academic institutions are participating in the 91 RAC-approved studies. Several issues should be considered by the RAC: (1) Protocols involving the G1TkSvNa and G1Tk1SvNa retrovirus vectors have been associated with numerous toxicities. (2) A total of 103 deaths have been reported; however, only 7 autopsies were reported by investigators. A 5% autopsy rate is extremely low. Important information related to gene transfer such as transmission to sites other than target tissues has not been obtained from autopsy specimens. (3) Several investigators have failed to enroll any patients on their NIH-approved studies or have failed to enroll any patients for more than one year. (4) There appears to be some confusion regarding the approvals that are required prior to initiating these studies, e.g., all 3 of theViagene sponsored studies were initiated prior to the NIH-Director's approval. To be in compliance with the NIH Guidelines, any investigator who receives NIH funding or collaborates with an investigator at an institution that has receives NIH funding must receive NIH approval to initiate human gene transfer protocols. (5) Biological efficacy is difficult to determine for some studies. Although the results observed in Dr. Blaese's protocol (#9007-002) on severe combined immunodeficiency (SCID) due to ADA deficiency are promising, the efficacy data is complicated by the fact that all of the subjects are concurrently on polyethylene glycol (PEG)-ADA enzyme replacement therapy.

Ms. Meyers was concerned over the poor autopsy rate. The RAC has made great efforts to ensure that investigators include the autopsy request statement in the Informed Consent documents; frequently such requests have been overlooked by investigators. Potential scientific information is lost forever due to the lack of proper autopsy. This negligent situation must be corrected.

Dr. Parkman commented that it is less of a concern to insist on a statement in the Informed Consent document to request autopsy than to require it. After a protracted illness of most patients, the family members may choose not to have autopsy performed on their deceased relatives. Dr. Parkman noted that most patients who died in gene transfer studies are those who died from advanced cancers in the gene marking protocols; persistence of genetically marked cells are not expected in these patients. Ms. Meyers' argument has much greater significance if patients are involved in gene therapy protocols where persistence of the transferred gene is expected.

Dr. Zallen expressed concern about the lack of pertinent information derived from the autopsy results, e.g. presence of replication competent viruses or gene transfer to sites other than the target tissue. Dr. Secundy asked if the autopsy rate for gene therapy protocols is different from the rate for gene marking studies. Clear communication to investigators is critical about the need for and type of information to be obtained from autopsy.

Dr. Straus noted that autopsy is particularly problematic in the clinical setting. He was concerned with the lack of cooperation by investigators in reporting complete data regarding safety and efficacy, perhaps due to competitive pressure. Dr. Walters inquired if any serious adverse event was not reported. Dr. Straus responded that no such instances have been documented. Dr.Motulsky noted the importance of communication between the investigator and the pathologist who perform the autopsy. The investigator should inform the pathologist about the type of scientific information sought, the tissue to preserve, and appropriate procedures for tissue preservation. Dr.Motulsky said that this 5% autopsy rate observed for gene transfer studies is below the national average of between 5 and 10%. Dr.Wivel said that the national average could be as high as 15 to 20%. To correct the problem of inconsistent reporting, Dr. Ross suggested that the questionnaire sent to investigators should be expanded.

In response to Ms. Meyers's concern about the low autopsy rate, Dr. French Anderson (University of Southern California) stated that for Dr. Steven Rosenberg's protocol #8810-001, extensive and extremely careful autopsies were conducted on all the deceased patients which resulted in greater research costs. In Dr. Rosenberg's study, there was no evidence that thetransgene had spread to any unintended site or evidence of any replication competent retrovirus (RCR). In the absence of adverse effects, costly autopsies for all patients in gene transfer studies are not justified. Autopsy alone is unlikely to uncover any serious adverse effects. Ms. Meyers noted that Dr. Rosenberg failed to report the autopsy information described by Dr. Anderson.

Mr. Capron stated that in the early stages of gene therapy development, negative results may be as valuable as positive information in documenting the lack of untoward effects. It is the very reason to demand autopsy. NIH and sponsoring companies should provide the funding necessary to gather such data in order to assure the safety of the nascent scientific technology. In response to Dr. Anderson's statement regarding the cost of autopsy, Dr. Straus said that an added cost of between $10,000 and $20,000 for autopsy is inconsequential compared to the total cost of developing a new gene therapy protocol.

Dr. Walters remarked that he was not aware that autopsies had been conducted on the first gene transfer protocol #8810-001. Dr. Anderson responded that the results were presented in a simple statement in a paper published in the New England Journal of Medicine. Dr. Miller commented it is preferable that the autopsy data be published in a more extensive format. Dr. Anderson said that he cannot defend his colleagues in terms of publication of their data. Dr. Miller said the data cannot be considered as part of the literature unless it is peer-reviewed and published. Dr. Anderson said that he would urge the RAC to encourage Drs. Rosenberg and Blaese to publish their results.

Ms. Wilson noted that the importance of inclusion of autopsy information had been reiterated in the cover letter that was sent out to investigators. Dr.Motulsky said that a brief narrative report from the investigators rather than just filling out the report may be more informative.

Report on Gene Marking--Dr. Parkman

Dr. Parkman stated that he was encouraged by the results of the gene marking studies. There have been 4 substantial publications in major medical journals from these marking studies. The RAC should

consider whether the data that have been generated should be used to reevaluate ongoing studies and/or make recommendations regarding the study design of ongoing and future studies. For example, the data clearly demonstrate that there is no evidence of tumor-specific migration of tumor infiltrating lymphocytes (TIL). The RAC should consider: (1) whether they should review any future TIL studies; and (2) whether the currently open protocols should be revised to focus on clearly defined scientific objectives. Dr. Parkman stated that one of the most important questions that has been answered by the bone marrow transplantation/gene marking studies is the aggregate data about human stem cell development. This data is an excellent example of the unexpected (yet valuable) information that can be obtained from these studies. Although these experiments were designed to determine the origin of disease relapse, this unexpected knowledge about stem cell development is extremely important. Many marking protocols appear on the surface to be "repetitive" studies; however, the unexpected results about stem cells from these "repetitive" studies have contributed important aggregate data.

Dr. Parkman identified two problematic areas involving gene marking studies that need improvement in the future: (1) Several protocols have not been initiated or are not progressing at the expected rate due to problems with vector availability. (2) Future Data Reporting Forms should be modified to request more detailed information about gene marking, i.e., polymerase chain reaction (PCR) data should be requested in a schematic (time-course) format.

Dr. Erickson commented that different categories of gene transfer experiments require different types of information to be obtained. He suggested that separate questionnaires should be used for each category. Dr. Ross noted that the data obtained through this semiannual reporting mechanism is useful to the RAC, the public, and investigators. Dr. Parkman stated that the data generated from these studies should eventually be published in peer-reviewed journals, and in the interim, the RAC data reporting process would serve as a mechanism to inform the public about the current state of the art. Dr. Smith stated that Dr. Parkman has identified problems that need to be addressed in the future review of gene marking protocols and their modifications. He noted the problem previously identified by Dr. Haselkorn regarding failure to accrue patients on an approved study for more than 1 year.

Report on Gene Therapy/Cancer/Non-Vaccines--Dr. Samulski

Dr. Samulski stated that 21 gene therapy/cancer/non-vaccine protocols have been approved by the RAC to date. The majority of these studies employ retrovirus vectors. Adenovirus vectors are used for 3 protocols in this category and 5 involve direct injection of "naked" DNA into target cells in vivo. Four strategies have been used to date: (1) incorporation of cytokine genes to stimulate an antitumor response, (2) antisense strategies designed to turn off oncogenes, e.g., myc, (3) tumor suppressor gene strategies, i.e., p53 to protect cells from converting to a tumorigenic state, and (4) drug enhancement therapy, i.e., introduction of the multi-drug resistance gene and Herpes simplex virus (HSV) thymidine kinase (TK)/ganciclovir (GCV) strategy. The primary endpoints of these Phase I studies are to address questions of safety and gene expression at the target sites. Sensitive techniques such as PCR are used to evaluate gene expression. Efficacy is not a primary endpoint for these Phase I studies. Dr. Samulski noted that significant toxicities have been reported for the HSV-TK studies. These toxicities will be addressed later in the meeting.

Report on Gene Therapy/Cancer/Vaccines--Dr. Erickson
Dr. Erickson stated that 17 of the 21 RAC-approved protocols on gene therapy/cancer/vaccine (lethally irradiated tumor cells) have been approved by the NIH Director. Only 11 of these studies are currently active. A total of 70 subjects have been entered onto these 17 NIH-approved studies, with 38 deaths (due to progressive disease). Dr. Erickson recommended that future Data Report forms should request information about humoral and cellular immunity. He stated that with one possible exception (Dr.

Brenner's Protocol #9206-018), there has been no evidence of biological efficacy in any of the studies. No significant toxicities have been observed.

Report on Gene Therapy/Inherited Genetic Disorders--Dr. Motulsky

Dr. Motulsky noted that approximately 20% of the RAC-approved studies involve inherited genetic disorders. The data derived from these studies are limited due to the fact that monogenic diseases are extremely rare. The information derived from Dr.Blaese's SCID-ADA protocol (#9007-002) is encouraging; however, gene transfer into stem cells has not been demonstrated, and all subjects are concurrently receiving PEG-ADA enzyme replacement therapy. Dr. Motulsky stated that 5 subjects were entered on Dr. Wilson's familial hypercholesterolemia study (#9105-005). Although the data from Dr. Wilson's study were published in the peer-reviewed journal, Nature Genetics, these data have been challenged by experts in the field who contend that the observed decrease in serum cholesterol may not be due to gene transfer. Such an effect was not considered in the published data. Dr.Motulsky was concerned about the invasive procedure involved in this gene therapy protocol such as removing part of the liver and reinfusing the gene-modified liver cells to the patients. Dr. Motulsky recommended that ad hoc experts should be included in the review of future protocols involving novel strategies in which the RAC may have limited expertise. Dr. Motulsky explained that the 8 cystic fibrosis (CF) studies have yielded valuable information regarding biochemical and biological evidence of corrections. Although toxicity was observed in one patient, the vector dosage was modified, and the subject's symptoms were transient. No subjects have been entered on the RAC-approved studies for Gaucher disease, Fanconi anemia, alpha-1 antitrypsin deficiency, rheumatoid arthritis, or peripheral artery diseases. Dr.Motulsky noted that there are many groups working on the same disease such as CF; therefore, a coordinated effor between investigators would facilitate progress. Dr. Walters noted that there is some evidence of gene transfer in bone marrow cells (between 2 and 5% colony forming units) tested at 1 year after transduction of 2 infants in the SCID-ADA protocol. Dr. Noguchi commented that if there is a limited population of patients, more than one of these studies must be synergistic not antagonistic. He was pleased to note that such coordination was encouraged by the Cystic Fibrosis Foundation at its annual meetings. Dr. DeLeon asked if there is any protocol that has progressed beyond the Phase I stage. Dr. Noguchi responded that there are no Phase II studies approved to date.

Ms. Meyers asked if there is any evidence of long-term gene expression. Dr. Parkman responded that long-term expression is not an objective for these CF studies. Persistence of marked normal hematopoietic progenitor cells has been demonstrated for up to 18 months in Dr. Brenner's acute myelogenous leukemia patient gene marking study. There is opportunity to learn from these marking studies whether long-term expression occurs in adults compared to children. Ms. Meyers was encouraged by the results and asked why the SCID-ADA protocol has not been modified to enter additional patients or to eliminate concurrent PEG-ADA enzyme replacement therapy. Dr. Parkman said once the long-term persistence in stem cells is established, it will be medically appropriate to consider reducing the PEG-ADA dosage.

Dr. Saha asked if gene therapy efficacy can be assessed in these SCID-ADA patients. Dr. Parkman responded that the observed clinical benefits are probably attributed to the synthetic PEG-ADA enzyme replacement. In order to definitively establish whether this clinical benefit is due to gene therapy, it is necessary to reduce or discontinue the PEG-ADA therapy. Such experimentation poses an ethical dilemma for physicians since they are obligated to do no harm to patients. One possible endpoint to assess the contributing effect of gene transfer would be to determine whether there is an increase in the number of the circulating T lymphocytes bearing the transgene since these cells are of donor origin. Dr. Walters commented that the results need to be documented in a scientific publication; there has been anecdotal account of this treatment in Larry Thompson's book, Correcting The Code (Simon & Schuster,

New York, 1994). Dr. Chase expressed his uneasiness about the inconclusive nature of the experimental design as well as the anecdotal account of the study. He stressed the need to have the general public more accurately informed about the outcome of gene therapy studies, and the Data Management Report has served this purpose very efficiently. Dr. Saha noted that a similar study involving Gaucher disease may cost an additional $1,000 per day for concurrent enzyme replacement therapy.

Dr. DeLeon asked whether efficacy is relevant for a Phase I study. Dr. Noguchi responded that efficacy is not the primary endpoint. Dr. Noguchi noted that the parent of one subject demanded concurrent enzyme treatment. Ms. Wilson noted that Dr. Blaese's Data Reporting form stated that if the inserted gene persists for 10-12 additional months, PEG-ADA therapy will be cautiously withdrawn, one patient at a time.

Dr. Anderson suggested that the RAC should request that investigators publish their data in the scientific literature. Dr. Ross recommended that investigators should report data in a timely manner. She noted that several closed protocols have not submitted data.

Report on Gene Therapy/HIV--Dr. Straus

Dr. Straus stated that a total of 40 patients have been entered on 5 gene therapy/HIV studies. The major problem with these studies is that the investigators have failed to provide timely data reporting. Minimal data have been provided regarding biological efficacy.

The RAC approved Dr. Wong-Staal's protocol (#9309-057) in September 1993, but it is still waiting for final NIH and FDA approvals. Mr. Capron inquired if there is any particular reason for the long delay. Ms. Wilson noted that the protocol has been approved by NIH after the deadline of the data reporting. Dr. Wong-Staal's protocol was recommended for approval contingent on the submission of additional preclinical data involving primary human CD34(+) cells. These data were only recently submitted for approval. Dr. Noguchi could not comment on the question of FDA approval.

Dr. Samulski suggested that the information contained in the Data Management Report should be published in a scientific journal to benefit others who are interested in gene transfer studies.

V-B. UPDATE ON ADVERSE EVENT REPORTS--PROTOCOLS INVOLVING PA317/G1Tk1SvNa VECTOR PRODUCER CELLS/DRS. BERGER AND MARCUS

Dr. Walters called on Dr. Steven Marcus, Director of Regulatory Affairs of Genetic Therapy, Inc. (GTI), Gaithersburg, Maryland, to provide an update of adverse events regarding the use of vector producer cells (VPC), PA317/G1Tk1SvNa, in the treatment of brain tumors. These VPC have been used in Protocols #9206-019, 9303-037, 9306-050, and 9312-059. Before discussing the adverse events, Dr. Marcus provided an update of patient accrual on these studies. The initial clinical trial was conducted at NIH on 15 patients with malignant brain tumors (13 of them had recurrent glioblastoma (Dr. Oldfield's Protocol #9206-019)). Subjects received intratumoral stereotactic administration of VPC. Based on the encouraging results from this first trial, additional clinical trials were initiated with a new generation of VPC which produced higher retrovirus vector titers. One subject was treated on Drs. Oldfield and Ram's leptomeningeal carcinomatosis protocol #9312-059 which resulted in a very serious adverse event. Nine subjects have been treated on Dr. Culver's protocol #9303-037 involving monthly VPC injections through an Ommaya catheter into the tumor cavity of brain tumor patients, followed by systemic GCV administration.

Dr. Marcus described the adverse events that have been observed on these studies to date. Two patients developed hemorrhage after stereotactic injection of VPC; both have recovered. Two patients had serious

hypertension, fever, and headache, which were believed to be related to intraventricular injection of VPC. Four patients developed seizures while on study, a common event occurring in patients with recurrent glioblastoma. Two patients developed serious infections associated with catheter insertion; most patients were on high dose steroid medication and were susceptible to infection. Autopsy results from one of these patients revealed multiple abscesses throughout the body. The cause of death was staphylococcal bacteremia caused by infection from the Hickman catheter. Autopsy of another patient revealed the cause of death by a pulmonary embolus. There were additional 2 deaths in the ongoing study. A patient developed pancytopenia; studies of the antiplatelet antibodies in the patient's serum ruled out VPC reaction as a cause of death. One patient had ischemic infarct in the opposite cerebral hemisphere believed to be unrelated to the injection. There have been a total of 11 deaths in the 15 patients entered on Protocol #9206-019; and 2 deaths for the 9 subjects entered on Protocol #9309-037. Dr. Marcus concluded that it is always possible that the causes of death are related to VPC; however, data indicates that this relationship is improbable. Mr. Capron remarked that the word "possible" means that the association cannot be ruled out.

Dr. Marcus stated that the average survival rate for patients entered on Protocol #9206-019 was longer than the 3 months; this survival rate is greater than expected. Four patients have survived for periods of 21, 13, 11, and 7 months respectively.

Dr. Haselkorn asked if any of these "extended survival" patients are off the study at present. Dr. Marcus said that these patients received a single injection of VPC. Dr. Secundy asked if the quality of life has improved for these patients. Dr. Marcus said their quality of life is quite good considering that these patients have received prior craniotomy and radiation therapy. Dr. Chase commented that this study is uncontrolled; without some formal analysis of these results, any conclusion of efficacy (even for a Phase I study) is unfounded. Dr. Marcus stated that in the new protocol the study design has been improved to assess the survival data, taking into account the factors mentioned by Dr. Chase. Dr. Straus asked if any patient is disease-free. Dr. Marcus said that one patient in Dr.Oldfield's protocol remains free of the disease.

Dr. Mitchell Berger (Protocol #9303-037) stated that the survival rate of these 4 patients is encouraging. Dr. Smith cautioned that referral-based studies automatically eliminate the sickest patients; similar bias has been observed in leukemia studies. Dr. Smith agreed with Dr. Chase's comments that these results do not provide statistically significant data. Dr. Saha asked about the stage of tumors these patients have. Dr. Berger responded that most patients have Grade 4 tumors. Ms. Meyers inquired if there is any magnetic resonance imaging (MRI) indication that the tumors have disappeared in these 4 surviving patients. Dr. Berger answered that a residual abnormality is normal for the long-term survivors and stressed that the stable condition of the subject should be observed as a positive outcome.

Dr. Berger addressed the adverse effects observed in Protocol #9303-037. There are 2 possible causes for these adverse effects: (1) the rate of VPC infusion, and (2) contiguity of the resection cavity with the ventricular system. Rapid injection of VPC may cause leakage into the third ventricle that could irritate the hypothalamus causing a hypertensive crisis and fever. The protocols has been modified to include an isotope tracer to monitor for leakage and to administer the VPC at a slower rate.

Commenting on the seizure that occurred in one patient, Dr. Berger said if the routine anticonvulsant had been given to that patient after brain tumor surgery, this episode of seizure might have been avoided. He said that other complications are unlikely to be related to gene therapy, i.e., pulmonary embolism, infarct in the contralateral brain, and infection of the Ommaya reservoir area.

Ms. Meyers asked if autopsy results indicated the presence of vector sequences in the surrounding tissue

Dr. Marcus responded that 2 autopsies have been conducted; however, the preserved tissue has not yet been analyzed, and the brains are being fixed in formalin but not yet examined.

Dr. Smith asked the investigators to comment on the report of finding vector sequences in the lung during a hemorrhagic episode. Dr. Ed Otto (GTI) presented PCR data of blood samples from all 15 patients on protocol #9206-019. PCR can differentially detect the vector and potential recombinant RCR generated from the vector. The sensitivity of this assay is at a level of detecting 10 vector copies per 500,000 blood cells with an assay competence of 99.99%. There is no evidence that any blood samples contained vector or RCR sequences. A false positive result was obtained using a single neomycin resistance (neoR) gene marker; however, when a second HSV-TK gene marker was included, none of the samples tested positive for both markers.

Dr. Smith asked whether there was any evidence in the preclinical animal studies that GCV alone evoked a reduction in tumor size, an effect described in Dr. Grossman and Woo's protocol. Drs. Berger and Miller (GTI) said they do not have definitive data in regard to this question.

Dr. Brinckerhoff asked if an immune response has been observed to the murine VPC. Dr. Yawen Chiang (GTI) explained that immune responses to VPC were documented in several patients who have received multiple VPC treatments. Some patients had VPC antibodies after a single treatment; however, these patients apparently did not have any adverse hypersensitivity reactions. No immune responses, either humoral or cellular, were observed in the rat model. Rats were treated with multiple intradermal VPC injections, and no obvious hypersensitivity reactions were observed. Dr. Parkman commented that the rat is not a good model for human hypersensitivity reactions; the guinea pig is the appropriate animal model and should be challenged with subcutaneous injections, not intradermal injections.
Dr. Straus asked whether any retroviral sequences had been identified in the brain tissue obtained at autopsy. Dr. Otto said that one autopsy analysis was incomplete due to failure to extract DNA from formalin fixed tissue. A second cryopreserved specimen revealed no retrovirus sequences or RCR. Investigators have been informed that all specimens should be cryopreserved not fixed in formalin. Additional autopsy information is unavailable because the majority of the families would not consent to autopsy.

Dr. Noguchi commented that FDA is concerned about the adverse effects, particularly infections relating to either insertion of the Ommaya reservoir or that have been associated with the GTI G1Tk1Sv.Na vector. This protocol is a multicenter study, and the FDA is cautious about treating more patients, especially children.

Dr. Miller was concerned about the inference of efficacy by GTI for this Phase I study since there is no control group and the comparison to the historical data may be biased due to selective patient enrollment. Dr. Berger said that a formal Phase III study is required to answer the question of efficacy. Dr. Marcus commented that it is very difficult to conduct randomized control trials for fatal diseases. Dr. Berger noted a precedent in which the FDA approved a drug based on an expanded Phase II study since the drug was found that a definite prolongation of survival over a matched historical control. Dr. Chase was concerned about a scenario in which a drug could become a standard therapy although it had never undergone thorough evaluation of efficacy.

V-C. FUTURE DIRECTIONS OF DATA MANAGEMENT REPORT--DRS. NOGUCHI AND SMITH

Dr. Walters called on Drs. Smith and Noguchi to provide an update on Data Management of human gene transfer protocols. Dr. Noguchi explained that public RAC discussion of the semiannual Data Management Reports serves a critical function. Public discussion of this information, particularly adverse

event reporting, is imperative to the FDA. The information accumulated by Ms. Wilson and the working group has been proposed as a pilot project for the FDA's $26 million Submission Management and Review Tracking project. Dr. Noguchi thanked Ms. Wilson for her significant contributions to this ongoing effort. Dr. Noguchi recommended that the RAC forward a letter of support for this project to the FDA Commissioner.

Committee Motion

A motion was made by Dr. Smith and seconded by Dr. Haselkorn to send a letter (as recommended by Dr. Noguchi) to the FDA Commissioner. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

Dr. Parkman recommended that each member of the working group should submit their recommended changes to the Data Reporting Forms to ORDA that would capture information specific for their assigned category.

Mr. Capron stated that the RAC should discuss implementation of Dr. Motulsky's recommendation regarding the inclusion of ad hoc experts for the review of novel protocols in which the RAC may have limited expertise. Mr. Capron said the key issue was whether the RAC has the foreknowledge to determine the necessity for such expertise. The RAC was unaware of the necessity for such review when Dr. Wilson's familial hypercholesterolemia study (#9110-012) was reviewed. Dr. Motulsky said that new target diseases or novel applications of gene delivery may be triggers for ad hoc review. Dr. Parkman said that ad hoc experts would benefit protocol review in the case where an assigned reviewer does not possess adequate expertise for the proposed study.

Dr. Secundy expressed concern about the low autopsy rate for all gene transfer studies to date. There must be increased communication between the RAC and investigators about the necessity of autopsy. Dr. Saha said the investigators are the ones best able to make the scientific judgment about the need for specific autopsies. Dr. Smith commented that the real issue is the reporting of autopsy results, not whether the autopsy was conducted. Dr. Anderson's account of detailed autopsies conducted on Dr. Rosenberg's protocol #8801-001, have never been reported to the RAC or published in the literature. Dr. Secundy suggested that 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) should be amended to include more specific autopsy reporting requirements. Mr. Capron said that the RAC should specify required post mortem data for individual protocols. Dr. Parkman agreed with Mr. Capron that biologically relevant data can be obtained by requesting specific autopsy information for a particular protocol. Dr. Smith suggested that each member of the working group should provide a list of required information to be transmitted to the investigators for the next data reporting period. Dr. Zallen suggested that the page limitations described in the Points to Consider should be modified to allow investigators to provide detailed responses to questions. Dr. Walters said that the RAC could include specific autopsy recommendations for a particular protocol, and that these recommendations could be included in their NIH approval letter.

VI. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: ADENOVIRUS MEDIATED GENE TRANSFER FOR CYSTIC FIBROSIS: SAFETY OF SINGLE ADMINISTRATION IN THE LUNG/DRS. DORKIN AND LAPEY

Review--Drs. Straus and Ginsburg (presented by Dr. Straus)

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

Dorkin of the New England Medical Center, and Dr. Allen Lapey of Massachusetts General Hospital, Boston, Massachusetts. In Dr. Ginsburg's absence, Dr. Straus presented his primary review in combination with Dr. Ginsburg's written comments. Dr. Straus explained that this adenovirus aerosol administration proposal was originally submitted in combination with the lobar instillation arm as a request for Accelerated Review. Although the primary reviewers approved the lobar instillation arm of the study, the reviewers recommended that aerosol administration of the adenovirus vector should be reviewed by the full RAC.

The adenovirus vector, Ad2/CFTR-2, has been previously approved by the RAC for direct installation (Protocol #9212-036). The proposed Phase I study is designed to determine safety of lobar vector followed by subsequent aerosol administration 2 months after lobar treatment (same vector dose). A total of 16 subjects will be entered over a 10 month period and divided into 8 cohorts. In this dose-escalation study, subjects will receive between 8 x 106 and 2.5 x 1010 infectious units (IU). Subjects will be evaluated for clinical responses by x-ray and epithelial brushings. Gene expression will be assessed by PCR. Subjects will be monitored for viral shedding for 3 days following aerosol administration; outpatient follow-up will continue up to 10 years following treatment. The investigators possess extensive experience in the clinical management of CF. Major scientific and logistic support is provided by Genzyme Corporation, Cambridge, Massachusetts. The investigators have provided adequate written responses to many of the technical issues raised in the primary written review.

This protocol is the first study involving aerosol administration that has been submitted for RAC review. The protocol provides a very vague description of the aerosol administration process. The RAC should consider the following issues: (1) the mechanics of aerosol administration, (2) performance standards of the aerosol devices, (3) possible penetration of the adenovirus vector into the small airways of the lung, (4) the possibility of horizontal transmission and subsequent environmental impact, (5) individuals who will be permitted to enter the facility during vector administration, (6) necessary protective clothing, (7) nebulizing masks, (8) airflow and filtration devices, (9) proposed procedures for environmental decontamination and air sampling, and (10) the potential for vector dissemination outside the containment facility. The investigators have provided written responses regarding recommended protective equipment and techniques and have noted that experiments are currently being conducted to determine the possibility of environmental transmission.

Dr. Straus described the canopy that the investigators have proposed to place over subjects as a filter. The investigators have not provided any data demonstrating the effectiveness of such a canopy, i.e., fluorescein labelled vector. Dr. Straus suggested the adoption of some of the techniques developed by Dr Mark Sawyer of the University of California at San Diego who detected environmental spread ofvaricella zoster virus and respiratory syncytial virus. In their written responses, the investigators indicated that health care workers who are present during aerosol administration will be monitored for vector contamination; however, Informed Consent will not be requested.

"Sentinel" mouse studies were conducted to address the potential risks of aerosol administration. "Sentinel" mice were placed in the cage with vector-treated mice. Low level adenovirus sequences were detected in the "sentinel mice" by PCR. The investigators assume that these adenovirus sequences were acquired by direct contact. Dr. Straus suggested that this experiment should be revised so that these two groups do not have any physical contact but share the same air space. Such studies would have to be conducted before approval could be recommended.

Review--Mr. Capron
Mr. Capron stated that the original Informed Consent documents were verbose and difficult to follow. The revised Informed Consent documents are improved over the original version. Since the proposed study

involves 2 separate institutions, the language in these 2 documents differs significantly, particularly with regard to the provision of medical care in the event of research-related injury. The New England Medical Center Informed Consent document indicates the provision of medical care for injury "directly caused by Genzyme material." Such a statement falsely suggests that no other compensation would be available, e.g., in the case of negligence. The Massachusetts General Hospital Informed Consent document indicates that medical care will be provided at no cost but does not address other forms of compensation. Mr. Capron noted that the investigator's name was omitted as the contact person in case of injury from the Massachusetts General Hospital document. With the exception of these specific issues, Mr. Capron stated that the revised Informed Consent documents are acceptable.

Other Comments

Dr. Zallen noted that the investigators have not provided proper responses to several specific questions asked in the recently revised Points to Consider (Appendix M-I-D). Considering the risk/benefit ratio of CF gene therapy, Dr. Zallen questioned whether the ratio is appropriate to treat CF individuals with very mild symptoms as proposed in this study.

Dr. Zallen expressed concern that the investigators stated in their written responses that a single day of patient isolation should be sufficient based on data indicating that adenovirus cannot be cultured 24 hours following nasal administration (Protocol #9212-036). Dr. Parkman commented that the proposed change should be considered in determining the isolation period. The first several subjects entered on the study should be monitored for virus shedding before being released. Dr. Parkman asked the investigators to comment on the proposed period of time that will be allowed for decontamination of the facility prior to release of the treated subject. Dr. Straus noted that similar precautions have been recommended for other RAC-approved protocols. Data must be obtained regarding persistence of the vector in the environment. Dr. Samulski recommended that an infectious particle assay would provide a more accurate assessment of environmental persistence rather than PCR analysis.

Investigator Response--Drs. Dorkin and Lapey

Dr. Dorkin described the containment equipment proposed to protect health care workers. The proposed system involves 3 levels of containment. The first containment level is provided by the nebulizer unit. The vector will be delivered by intermittent nebulization activated by inspiration. Subjects will wear nose clips and protective clothing and exhale through a High Efficiency Particulate Air (HEPA) filter with an 99.999% efficiency. The system will approximate a closed system as long as the patient is breathing through the mouthpiece. Nebulization is limited to 2 seconds during inspiration. The second level of containment is provided by the "Demistifier 2000" canopy. Nebulization of the vector will occur within this canopy in which air is drawn upward from the bottom of the canopy and out through the filter at a rate of between 4 and 6 air exchanges per minute. The third level of containment is provided by the negative pressure room which exceeds 12 air exchanges per hour. Health care providers will be required to wear similar protective clothing such as the HEPA-filter masks. Dr. Dorkin said that the recommended techniques developed by Dr. Sawyer will be used to sample both the inside and outside air. The subject will undergo nebulization of the vector for 30 minutes. Following the nebulization procedure, the subject will be required to remain within the canopy for approximately 1 hour to minimize the possibility of exhaling virus particles into the environment. Following release from the canopy, the subject will be required to remain in the negative pressure facility for 24 hours.

Dr. Dorkin stated that the subjects will be monitored for viral shedding by culture conducted 24-hours post-vector administration. Culture results will be available at 3 days; if the culture results are negative, the subject will be discharged. If the culture results are positive, the subject will be required to remain in

isolation until viral shedding is no longer detected.

Dr. Parkman stated that the stipulations for approval of Dr.Flotte's (#9409-083) adeno-associated virus-CFTR protocol should be applied to this study. The following stipulations should apply: (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 the subject may be secreting 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. Dr.Dorkin agreed to revise the protocol according to Dr. Parkman's suggestions.

Dr. Dorkin stated that Dr.Zallen's request for an autopsy is in the revised Informed Consent document. Dr. Dorkin stated that complications unrelated to gene therapy, e.g., small bowel obstruction (a common complication of CF patients) will be covered by third-party insurance. Medical care will be provided for other complications (such as respiratory infection) for 3 months following treatment.Genzyme will not indemnify complications that occur after that 3 month period. Mr. Capron stated that such a policy is acceptable provided that these provisions have been clearly disclosed to subjects who are considering participation in the study. Dr. Dorkin agreed to clarify this issue in the Informed Consent document.

Dr. Dorkin explained that decisions regarding patient accrual will be discussed weekly at the CF center and will involve all the members of the center who are involved in the care of patients. Accrual will not be based on a single physician's recommendations. Dr. Zallen stated that it is preferable that a neutral person obtains Informed Consent. Dr. Dorkin asked if it would be acceptable to have the patient's primary care physician or another physician at the center, other than the patient's principal consultant, to obtain the informed consent. Dr. Zallen said that Dr. Dorkin's suggestion is an acceptable arrangement. Dr. Lapey noted that the Massachusetts General HospitalIRB requires that a patient advocate obtain Informed Consent.

Dr. Parkman suggested that health care workers should be required to provide Informed Consent since they will be acting as "sentinel" individuals with regard to monitoring for viral spread. Dr. David Meeker (Genzyme) explained that health care workers will be tested by virus culture.

Mr. Capron asked whether the period of virus shedding is related to dosage. Dr. Meeker said that there was no evidence of virus shedding in Dr. Welsh's nasal administration study using vector doses up to 1 x 1010 IU. Dr. Richard Moscicki (Genzyme) stated that the cohorts for dose escalation will be staggered between the bronchoscopic and aerosol administration arms of the study. If evidence of virus shedding is observed following lobar instillation, no patients will receive aerosol administration.

Dr. Miller asked if fluorescein studies were conducted to determine the extent of virus spread. Dr.Dorkin said that similar studies have been conducted involving aerosol administration of ribavirin. Dr. Straus cautioned that major concerns have been raised by pulmonary physicians as a result of theribavirin study because ribavirin was detected on the subject's face and in the environment. Dr.Dorkin responded that HEPA filtration will provide more effective containment. HEPA filtration was not employed in the ribavirin study. Dr. Miller emphasized that additional studies must be conducted to determine the extent of virus transmission. Dr. Parkman agreed that a mock experiment involving aerosol administration offluorescein labelled particles would be the preferred experimental design. Dr. Dorkin agreed to conduct the proposed fluorescein experiments and provide this data to the RAC.

Dr. Glorioso noted that the extensive precautions proposed by the investigators is impressive, and

provides a greater level of containment than the Biosafety Level (BL)-2 that is required for vector production.

Committee Motion 1

A motion was made by Dr. Straus and seconded by Dr. Erickson to defer approval of the protocol until the investigators return to the full RAC with additional safety data regarding virus transmission following aerosol administration.

Dr. Judith St. George (Genzyme) requested permission to present additional safety data derived from a "sentinel" mouse experiment. The original experiment was flawed because the animals were allowed to come in contact with each other. The experiment was redesigned such that animals shared the same air but were physically separated by a porous barrier. Two mice received 1 x 1010 IU of the adenovirus vector in the nose and were placed opposite (separated by the porous barrier) 3 "sentinel" mice. On Day 3, the animals were sacrificed and assayed for vector sequences by PCR. Vector sequences were detected in the lung and nose of the treated animals, whereas there was no evidence of virus sequences in the "sentinel" animals at any site. There is no evidence of virus spread through the air. Dr.Samulski asked about the sensitivity of the PCR assay. Dr. St. George responded that lung tissue spiking experiments demonstrated a level of sensitivity between 1 in 1 x 103 and 1 x 104 particles. Dr. Parkman noted that the level of sensitivity is very low and does not adequately address virus spread through aerosol administration.

Dr. Haselkorn noted that the proposed adenovirus vector is replication-defective and that the proposed safety precautions exceed the requirements for previous adenovirus vector/CF studies. Dr.Dorkin requested that the requirement for additional safety studies be included as a contingency for approval.

Dr. Walters reminded the committee that the current motion involves deferral of the protocol until the investigators return to the full RAC with additional safety data. Dr. Miller suggested approval of the protocol contingent on review and approval of the additional data. Dr. Straus accepted Dr. Miller's recommendation to approve the protocol with stipulations.

In formulating the stipulations, Dr. Straus noted the necessity for data demonstrating safety to health care workers both inside and outside of the nebulization chamber. Dr. Meeker emphasized that the multiple levels of containment represent "state of the art" technology to prevent virus spreading. Dr. Straus noted that this protocol is a precedent setting study for aerosol adenovirus vector administration, and he would prefer to review the data before approval. Dr. Parkman stated that aerosol administration is obviously crucial to the long-term potential applicability of gene therapy for CF, and the burden of proof is on this precedent-setting case.

Mr. Capron asked for clarification of the proposed experiments that would be required. Dr. Straus stated that a mock experiment would be required involving either fluorescein marking or a sensitive PCR assay to detect vector DNA and to monitor virus spread inside and outside of the treatment room over a specifie time course. Dr. Miller expressed concern that coughing during the nebulization procedure could introduce potential hazard.

Dr. Motulsky proposed an amendment to Dr. Straus's motion to defer the protocol. A motion was made by Dr. Motulsky and seconded by Mr. Capron to approve the protocol contingent on submission of safety data and subsequent review by a subcommittee via a telephone conference call. The amendment passed by a vote of 18 in favor, 0 opposed, and no abstentions. Ms. Meyers remarked that the entire RAC should review this data before recommending approval. The RAC should not rush approval of this study.

Dr. Parkman remarked that the subcommittee should have the option to approve/disapprove the study based on subsequent data or request that the data be presented to the entire RAC if deemed necessary. Drs. Erickson and Parkman requested inclusion on the subcommittee and expressed their reservations regarding the sensitivity of the PCR assay.

Dr. Zallen asked the investigators to explain their decision to treat subjects with mild disease. Dr. Dorkin responded that data derived from mild to moderate disease states is very important since the likelihood of exacerbations, which could complicate interpretation, is less for this population. Dr. Moscicki added that the mild disease patients are the eventual target population for this therapy; therefore, the safety study should include this group of subjects.

Dr. Straus asked the investigators to submit a specific written description of the proposed safety experiment for approval by the subcommittee prior to initiation of the experiment. Dr. Walters agreed that prior approval of the experimental design will avoid any misunderstanding between the RAC and the investigators.

Committee Motion 2

A substitute motion was made by Dr. Straus and seconded by Dr. Erickson to accept the protocol submitted by Drs. Dorkin and Lapey contingent on the review and approval of the following by a subcommittee of the RAC: Data derived from "mock" experiments demonstrating that health care workers (both inside and outside of the treatment room) will be protected from inadvertent exposure to the adenovirus vector during nebulization. These mock experiments will not be conducted by the investigators until the experimental design has been mutually agreed upon between the investigators and the members of the subcommittee via a telephone conference call (Drs. Ginsburg, Erickson, Straus, and Parkman). The substitute motion passed by a vote of 17 in favor, 0 opposed, and no abstentions. The RAC will be informed of the accepted experimental design.

Summary

Dr. Henry Dorkin of the New England Medical Center, Tuft University, Boston, Massachusetts, and Dr. Allen Lapey of Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, may conduct gene transfer experiments on 16 subjects (18 years of age) with CF. The replication-deficient adenovirus vector AD2/CFTR-2 will be used to deliver the human cystic fibrosis transmembrane conductance regulator (CFTR) gene to the lung of CF patients by aerosol administration. AD2/CFTR-2 is an E1/partial E4 deleted type 2 adenovirus. The adenovirus construct includes the phosphoglycerate kinase promotor which drives CFTR expression. AD2/CFTR-2 is identical to the vector used in Dr. Michael Welsh's protocol #9312-067 and Drs. Dorkin and Lapey's protocol #9409-091. This aerosol administration protocol will not be initiated until an initial safe dose has been determined in the lobar instillation arm of the study (Protocol #9409-091). Subjects will receive between 8 x 106 and 2.5 x 1010 IU of the adenovirus vector. The objective of the study is to evaluate the safety of a single aerosol dose of AD2/CFTR-2. Subjects will be monitored for evidence of virus shedding and transgene expression.

VII. DISCUSSION OF ETHICAL CONSIDERATIONS RELATIVE TO IN UTERO SOMATIC CELL AND GENE THERAPIES/DRS. PATTERSON (FDA), ZALLEN, MOTULSKY, AND MR. CAPRON

In a letter dated November 3, Dr. Noguchi submitted a letter regarding the ethical considerations of in utero somatic cell and gene therapies. Dr. Noguchi's letter states:

"The extension of gene therapy to the treatment of fetal disease inutero is imminent. In view of the recent realignment between the RAC and the FDA in the consolidated review process for new gene therapy protocols, the many ethical considerations raised by in utero gene therapy would optimally be addressed in a public forum. The expertise and the resources of the RAC will be invaluable in laying the groundwork for the consideration of future protocols which will include in utero somatic cell and gene therapies."

Dr. Walters called on Dr. Motulsky to initiate the discussion. Dr. Motulsky said the issue of in utero gene therapy is an outgrowth of fetal stem cell transplantation proposals. Fetal stem cell transplantation is currently under consideration because the fetus is immunologically tolerant. In utero gene therapy should be considered only in cases of imminent life-threatening disease from birth through the first few years of life, e.g, Tay-Sachs and alpha-thalassemia. One of the major difficulties that will be encountered is gene delivery to the fetus. It probably will be impossible to target the gene in a fetus younger than 4 months. In order to be effective, the gene would have to be delivered between 4 months and birth.

Dr. Motulsky stated that both a fetal advocate and a maternal advocate would be required. The most appropriate choice for a fetal advocate would be a geneticist with expertise in the target disease. The most problematic issues will be: (1) the most appropriate time for gene delivery, and (2) the most appropriate candidates to receive this therapy. Should this therapy be made available only to those mothers who refuse abortion so that the fetus will be provided the best available treatment options? Should the therapy only be made available as an alternative option to mothers who have elected to have an abortion? There are convincing arguments for and against both options.

Dr. Glorioso noted that animal experiments have shown that retrovirus gene delivery results in the correction of stem cell defects in fetal lung cells which results in long-termtransgene expression after birth. In situations where immune rejection oftransduced cells is a problem for gene therapy, inutero gene transfer could prove beneficial in cases were immune rejection is problematic.

Mr. Capron stated that fetal gene therapy is a very pertinent issue for the RAC to address and suggested that experts in the field should be invited to educate the RAC on relevant issues. Mr. Capron disagreed with Dr. Motulsky regarding the necessity for a fetal advocate. Given the current legal and moral system, the pregnant woman should be provided with state-of-the-art information so that she and her mate (if involved in the decision making process) can make an informed decision. Mr. Capron said that it is inappropriate to have an individual who possesses authority to argue the case of the fetus as opposed to the mother's choice. There are people who will disagree with this point of view, which is the reason that further public discussion is necessary. Dr. Walters stated that this discussion will be continued following Dr. Amy Patterson's in utero gene therapy presentation.

VIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: INTRATUMORAL INJECTION OF HERPES SIMPLEX THYMIDINE KINASE VECTOR PRODUCER CELLS (PA317/G1Tk1SvNa.7) AND INTRAVENOUS GANCICLOVIR FOR THE TREATMENT OF LOCALLY RECURRENT OR PERSISTENT HEAD AND NECK CANCER/DR. GLUCKMAN

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Jack Gluckman of the University of Cincinnati Medical Center, Cincinnati, Ohio. Dr. Parkman stated that this protocol is similar to other GTI sponsored protocols involving the injection of murine VPC secreting the HSV-TK vector followed by the intravenous administration of GCV. Preclinical studies have demonstrated that intratumoral injection of HSV-TK VPC (intracranial tumors) followed by GCV administration resulted

in the tumor regression whereas no effect was observed on control tumors injected withVPC expressing LacZ.

Dr. Parkman explained that this Phase I/II proposal involves the administration ofHSV-TK VPC for persistent or recurrent head and neck tumors. Fourteen days followingVPC injection, subjects will receive 5 mg/kg GCV twice daily for 7 days. Subjects will be reevaluated on Day 30. Subjects who have evidence of antitumor response or stable disease will be eligible for 2 additional cycles of VPC/GCV therapy. Subjects must be greater than 18 years of age, have recurrent or persistent disease not amenable to curative therapy, not have received alternative therapy within 30 days prior to the initiation of this study or evidence of disease progression, and their tumors must be accessible to direct injection.

Dr. Parkman raised several specific questions regarding the present protocol, some of which have been addressed by the investigators in their written response. The major principle used for cytokine evaluation in gene therapy protocols is that the preclinical data should correlate directly with the proposed tumor target. Different responses will be observed based on the tumor type. Minimal data have been provided to demonstrate an antitumor effect in the proposed target cell. Approximately 60% of the data involves hepatocellular carcinoma cells, about 30 to 35% involves squamous cell carcinoma, and 5% involves lung cancer and brain tumor cells.

Dr. Parkman noted that the nude mice experiments are invalid. These mice received either a combination of human hepatocellular carcinoma cells and PA317 alone or PA317 VPC (HSV-TK vector). Following GCV administration, the amount of tumor regression observed withVPC was equivalent to the regression observed with non-modified PA317 cells. If the effect observed with the modified and unmodified cells is the same, why should patients be subjected to any potential risk relating to the recombinantmurine retrovirus? In their written response, the investigators state that PA317 cells contain anHSV-TK expression plasmid which could elicit a "bystander" effect. Dr. Parkman said that the data do not demonstrate any significant benefit over the controlVPC PA317 alone. The investigators have recently provided additional data indicating a differential effect; however, the difference is not statistically significant. The investigators have performed an additional experiment with the NIH3T3TK- cell line (HSV-TK negative), but the results of this experiment are inconclusive.

The investigators have provided data involving human squamous cell carcinoma, a tumor type that is more relevant to the protocol. One animal was injected with a combination of 2/3 non-transduced cells and 1/3 transduced cells in one flank and with non-transduced cells on the contralateral side. After GCV treatment, tumors on both sides regressed. The small difference observed in tumor regression between the two flanks can be attributed to variability in the number of tumor cells that were injected. The data suggests that GCV alone may act as a therapeutic agent forsquamous cell carcinoma.

Dr. Parkman concluded from these data thatGCV results in tumor regression by a mechanism that is not clearly understood. It remains to be determined whetherVPC co-injection is necessary to elicit tumor regression. Dr. Parkman said that he would not be comfortable approving this protocol since the preclinical data are inconclusive.

Review--Dr. Ross

Dr. Ross expressed concern that the proposed study employs the sameVPC that have been administered to subjects in other protocols that have reported many adverse effects. These adverse effects have been attributed tointratumoral injection, biopsies, or rapid rate of VPC infusion. However, the causes for these events have not yet been determined. To what extent the adverse effects will be limited to patients with brain tumors is unknown. She asked if it is advisable to use theVPC to treat the head and

neck tumors. She suggested that tumors should be independently evaluated by more than one individual by computer tomography (CT) or MRI. Independent blinded tumor measurement would ensure reliability of the data.

Dr. Ross made several comments in regard to the Informed Consent document: (1) Pronouns should be used consistently throughout the Introduction To The Procedures section, (2) The term "response" should be replaced with "reduction in tumor size" throughout the Purpose Of This Study section, (3) Tumor evaluation should be explained thoroughly in the Screening section, (4) The discriptor "teaspoon" should be added to "5 ml" and additional biopsy information should be added to the Treatment section, (5) The request for autopsy information should be expanded to include specific details about information that will be obtained in the Follow-Up section, (6) Phone call follow-up is not adequate, and should be included in the Amount Of Time Required For This Study section, (7) Other possible risks, i.e., renal impairment, retinal problem, and headache should be included in the Risks Of Cytokine Therapy section, (8) The cost of participation should be thoroughly explained in the Cost section, and (9) Name and phone numbers of other contacts should be added in the Questions section.

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

Dr. Parkman presented Dr. Motulsky's written comments. Dr. Motulsky states that the investigators are using a biologically plausible gene therapy approach for cancer therapy yet they do not define the exact nature of the head and neck tumors to be studied. The exact type and extent of the tumors to be treated should be thoroughly defined. Dr. Parkman stated that the other issues raised in Dr. Motulsky's written review have been previously addressed.

Other Comments

Dr. Brinckerhoff asked the investigators to comment on the possible immune reactions that could occur in response to these murine cells. VPC injection into head and neck tumors is very different than administering these cells in brain tumors which are insulated by the blood brain barrier. The proposed study could induce serious humoral and/or cellular immune responses and anaphylaxis. Dr. Parkman remarked that even for the brain tumor protocols, inflammatory response in the closed brain space could be serious; however, sometimes the inflammatory responses against the tumors are beneficial to the therapy. The special concern for the head and neck is that a local edema can sometimes be a serious problem. Dr. Parkman suggested that a skin test should be performed to determine the possibility of a delayed hypersensitivity reaction in patients. Dr. Brinckerhoff agreed that such a skin test would provide a beneficial screen for hypersensitivity.

Dr. Miller questioned the persistence of VPC in head and neck tumors. Will VPC persist long enough in situ to secrete enough vector to transduce the tumor cells? The injected VPC might be immediately lysed by human complement. He explained that complement lysis was less of a concern for the brain tumor studies since there is a low complement level in cerebral spinal fluid. Many studies suggest that VPC are lysed immediately in peripheral tissue. Dr. Miller commented that the rat is not a valid animal model for evaluating complement activity since rat complement does not lyse murine VPC. An appropriate model for complement activity would be a larger animal, i.e., a canine or primate model. The investigators should study the persistence of VPC in human tumors.

Ms. Meyers asked for clarification regarding the antitumor effects observed with GCV alone. She inquired whether the antitumor responses observed in other studies could be attributable to GCV alone. Drs. Parkman and Smith noted that these GCV effects have not been observed in the HSV-TK/VPC brain tumor studies. These effects have been only observed in the preclinical human squamous cell/nude mice

studies.

Dr. Miller asked the investigators to clarify the difference between this modified vector and the previously approved vectors.

Investigator Response--Dr. Gluckman
Dr. Gluckman stated he would address the clinical and Informed Consent issues, and that Drs. Peter Stambrook (University of Cincinnati) and Chiang would respond to vector related issues.

Dr. Gluckman explained that the majority of head and neck tumors involve squamous cell carcinoma that arises from the mucosa of the upper digestive tract, i.e., cancer of the mouth, tongue, pharynx, larynx, and the cervical esophagus. When these tumors are diagnosed early, current therapies may be effective; if tumors are presented at later stages, treatment is extremely difficult and requires extensive surgery in combination with radiation therapy. There is about 50% recurrence rate, and recurrent tumors usually occur locally, causing tremendous problems in the head and neck areas. These patients have no other alternative therapy. Since recurrence usually occurs locally, the tumors are easily accessible for evaluation and treatment, which may offer an advantage to the proposed gene therapy approach. MRI, CT scans, and ultrasonography can be effectively utilized to guide VPC injection and monitor antitumor response. The toxicity associated with the HSV-TK/VPC brain tumor protocols is related to cerebrospinal fluid circulation and will not be a problem for head and neck tumors. Since the majority of these patients have tracheostomy and gastrostomy, any potential complications due to local edema can be safely corrected. He noted that the Informed Consent document has been revised in response to the primary reviewers' recommendations and approved by the IRB.

Dr. Chiang explained that this modified vector incorporates a 3' non-coding HSV-TK deletion which circumvents any potential for RNA splicing and results in higher vector titers. In response to Dr. Parkman's question regarding the necessity for VPC, Dr. Chiang stated that both VPC and GCV are essential for the full therapeutic effect. Dr. Chiang described an experiment that was conducted to determine the relationship between HSV-TK enzyme activity and the "bystander" effect. The 9L glioblastoma cell line was added to: (1) VPC expressing variable levels of TK enzyme activity, (2) PA317 (some endogenous TK activity), and (3) NIH3T3TK(-) (no endogenous activity). Data demonstrate that a threshold level of TK activity is required for the GCV "bystander" effect and that little difference is observed beyond this threshold level. Dr. Parkman commented that the experiment described by Dr. Chiang involved a glioblastoma cell line, not a human squamous cell carcinoma line.

Dr. Chiang presented data demonstrating PA317 (endogenous TK) sensitivity to GCV killing. In contrast, NIH3T3TK- and untransduced 9L cells were insensitive to GCV killing. She noted that similar in vitro studies have been conducted using a squamous cell carcinoma cell line; however, the in vivo experiments involved only the hepatocellular carcinoma model.

In response to Dr. Brinckerhoff's concerns regarding immune reactions, Dr. Chiang stated that VPC immune responses were observed in the rat model; however, no serious adverse effects were identified upon pathological examination.

In regard to Dr. Miller's question about VPC persistence, Dr. Chiang said that no primate data is available. Dr. Anderson stated that he has conducted experiments in which VPC were injected into porcine livers and no complement lysis was observed for 5 days. Porcine complement lyses murine VPC in vitro, which indicates that the complement levels within the liver are low. VPC are lysed within 30 minutes when injected directly into the blood; however, VPC will remain viable up to 5 days in the lymphatic system.

Dr. Stambrook presented data demonstrating the "bystander" effect in asquamous cell carcinoma cell nude mouse model. The first experiment involved mixing tk+ and tk- squamous carcinoma cells at a 1:3 ratio and injecting them into the left flank of the mouse; an equal number oftk- cells (5 x 106) were injected into the right flank. Following a period to allow for tumor growth, treatment withGCV was carried out, and both the right and left flanks showed tumor regression. Upon histological examination of the regressing tumor, there was some evidence of lymphocyte infiltration. The significance of this observation remains to be determined. In the second experiment,squamous cell carcinoma cells were injected in both right and left flanks of the mouse, followed by injection of PA317 cells; following treatment withGCV, there was tumor regression in both flanks. A third experiment was conducted as a control; whenHSV tk- 3T3 cells were used instead of PA317 cells, there was no evidence of tumor regression following GCV treatment. This result was consistent with the suggestion of Dr. Parkman that PA317 cells may contain HSV-tk.

Dr. Parkman noted that the investigators may have discovered a new mechanism by whichTK and GCV may be able to destroy distant tumors, but it seemed unlikely that the killing effect was the same as the "bystander" effect observed by other investigators. For example, it was unlikely that there would be any gap junction established between human and murine cells. Furthermore, there was no data to suggest the persistence of the VPC for a sufficient period of time in vivo to allow for gene transduction to occur. Additional experiments to explain the "bystander" effect would be necessary.

Dr. Brinckerhoff agreed with Dr. Parkman. She was concerned about the lack of immunological data in humans. The data from the rat model does not adequately address the question of immune reaction. If an anti-VPC reaction does occur, these cells could be lysed even more quickly by complement.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Erickson to defer the protocol. Although the proposal is a logical extension of the HSV-TK/GCV strategy previously used in the brain tumor protocols, too many questions remain regarding safety and potential efficacy. The "bystander" effect must be more clearly understood since the mechanism appears to be different from the effect observed for brain tumors. There is no rationale for exposing subjects to possible risks of the retroviral vector since PA317 (no vector) are equally effective as VPC in combination with GCV therapy. The investigators must demonstrate: (1) VPC are clinically superior to PA317, and (2) persistence of VPC for an appropriate period of time in a large animal model or equivalent in vitro experiments involving appropriate lysing antibodies to murine cells.

Dr. Gerard McGarrity (GTI) made several comments: (1) The blood brain barrier inglioblastoma patients is not absolutely intact; therefore, similar immune reactions would be expected for both studies. (2) Rat serum does contain anti-murine antibodies that inactivate VPC; therefore, the rat data are valid. (3) The proposed head and neck protocol is an ideal system to obtain data regarding VPC persistence since tumor and tissue samples will be readily accessible. (4) Regarding the "bystander" effect, the 9L rat glioblastoma data was shown because this system is where most data is available; whereas, the hepatocellular carcinoma is the worst case scenario.

Dr. Parkman noted that the biology of one tumor type does not predict response in another tumor type; therefore, preclinical data derived from an appropriate tumor model is critical. The rat model is not an adequate model to determine VPC persistence; larger animals, i.e., primates are more appropriate. The ongoing porcine experiments will be acceptable if the serum can be shown to kill the murine fibroblasts and the VPC are persistent for greater than 30 minutes. Dr.Haselkorn agreed with Dr. Parkman's comments about the necessity to conduct the proper experiments. Dr. Miller stated that data should be

provided demonstrating that transduction of the TK gene is responsible for the therapeutic effect of GCV. In addition, Dr. Parkman stated the data should be provided to explain the mechanism of the "bystander" effect on tumors at other sites. Dr. Erickson said that the data must demonstrate that VPC are clinically superior to PA317.

A motion was made by Dr. Parkman and seconded by Dr. Erickson to defer the protocol submitted by Dr. Gluckman by a vote of 16 in favor, 0 opposed, and no abstentions. The protocol was deferred until the investigators return to the full RAC with additional preclinical data in a squamous cell carcinoma/large animal model demonstrating the persistence of PA317/G1Tk1SvNa.7 VPC and that administration of PA317/G1Tk1SvNa.7 VPC is clinically superior to PA317 cells alone. The RAC strongly recommended that the mechanism of the distant "bystander" effect should be established.

IX. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: PHASE I TRIAL OF INTERLEUKIN-2 PLASMID DNA/DMRIE/DOPE LIPID COMPLEX AS AN IMMUNOTHERAPEUTIC AGENT IN SOLID MALIGNANT TUMORS OR LYMPHOMAS BY DIRECT GENE TRANSFER/DRS. HERSH, AKPORIAYE, HARRIS, STOPECK, UNGER, AND WARNEKE

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Evan Hersh, Emmanuel Akporiaye, David Harris, Alison Stopeck, Evan Unger, and James Warneke, of the Arizona Cancer Center, Tucson, Arizona. Dr. Miller explained that the proposed study involves direct intratumoral injection of a liposome/plasmid DNA vector encoding human IL-2. The investigators hypothesize that local IL-2 production will stimulate an antitumor response. A similar strategy has been employed for other RAC-approved protocols. Although there is little evidence that this strategy works in humans, there is sufficient preclinical data to support the protocol. The vector is liposome based; therefore, concerns about virus spread and transmission are negligible.

One potential concern is the possibility that the plasmid could enter and integrate into normal immune cells. If such integration were to occur, constitutive IL-2 production (a growth stimulatory cytokine) could promote uncontrolled cell proliferation. The argument that the proposed DNA administered does not integrate is probably incorrect; stable transfectants can be isolated in cell culture by using this DNA transfer technique. The rate of integration may be low; therefore, an argument can be made that transformation of immune cells to IL-2 independent growth is unlikely, but presumably there will still be some risk. The possibility that gene transfer might result in uncontrolled cell growth was not a safety concern for the human leukocyte antigen-B7 human gene transfer experiments. The supporting preclinical data is derived from short-termmurine experiments. Long-term primate experiments would have been preferable to demonstrate safety. Dr. Miller said that the plasmid DNA poses little risk to others, but there could be some harm to patients in term of stimulation of T-cell growth in those patients. The safety issues need to be addressed in animals prior to use in humans.

Review--Dr. Saha

Dr. Saha said that this protocol is very similar to other RAC-approved protocols involving IL-2. One major difference is that the present study will utilize administration of IL-2cDNA in liposomes without the benefit of a viral vector. One advantage of the proposed gene transfer method over viral vectors is that there is less likelihood of insertional mutagenesis. Liposome delivery is a less expensive means of gene transfer than viral delivery. There are no direct comparative studies of these two gene transfer methods. He said that most of his questions have been adequately answered in the investigators' written responses. He

strongly suggested that potential subjects should be encouraged to seek a second opinion regarding their options for further treatment before being enrolled in this protocol. He did not agree with the investigators' written response that a second opinion is unnecessary because subjects will have failed all other available therapeutic options. Dr. Saha recommended approval of the study based on the life expectancy of the patient population and the precedent that has been set by approval of other IL-2 human gene transfer protocols.

Other Comments

Dr. Zallen commented that the investigators have provided adequate responses to Appendix M-I-D Informed Consent of the Points to Consider. She inquired about the limited amount of time in which subjects are required to make an informed decision about their participation in the study, i.e., 24 hours to 2 weeks.

Dr. Parkman noted that the proposed study involves unspecified solid tumors and lymphomas. However, there is significant data in the literature that suggests that melanoma and renal cell carcinoma are more responsive to IL-2. Will the inclusion of numerous tumor types complicate the study design and a subsequent interpretation of data? Is there any evidence that IL-2 is effective in treating lymphoma? There are alternative therapies available for lymphoma, i.e., autologous bone marrow transplantation.

Ms. Meyers thought that the Informed Consent document was very poorly written and should be revised. A request for autopsy should be included, and the patient responsibility for medical cost of untoward effects should be clearly disclosed. Mr. Capron agreed that the statements regarding medical costs are difficult to follow and should be clarified. Ms. Meyers stated that it is unacceptable to exclude patients from participation in the study who are unable to pay for such costs. Mr. Capron remarked that Phase I studies are not intended to benefit patients.

Dr. Miller asked the investigators to clarify inconsistencies between the actual IL-2 sequence and the sequence that has been published in the literature.

Investigator Response--Drs. Scheiber and Hersh

Dr. Alan Scheiber (Vical, Inc.) responded to Dr. Miller's concern about the safety of the IL-2 plasmid DNA. Dr. Scheiber said that in vitro transfection produces stable transfectants in fibroblasts at a frequency between 1 and 5 x 10-5. Lymphocytes are more difficult to transfect by this liposome/DNA technique. Short-term murine safety studies of intravenous injections have been conducted. The primate study is still ongoing (Day 65 at the present time), and no hyperproliferation of T-cells has been observed. Dr. Miller said that T-cell transfection by IL-2 cDNA should be evident by Day 65.

Dr. Scheiber clarified Dr. Saha's concern about the sensitivity of the PCR assay (60 copies of the plasmid in 1 mg sample of total DNA). With this assay, plasmid DNA sequences can be detected at 1 week and 1 month following intravenous injection of high vector doses. Intravenous injection is intended to represent a worst case scenario, i.e., leakage to the blood circulation. Most of the plasmid DNA injected into tumors is rapidly degraded in situ. Once the supercoiled circular DNA is nicked by the enzyme, expression of IL-2 cDNA is greatly reduced.

Dr. Hersh said that patients will be offered multiple treatment options including no further therapy (supportive care at home), referral to other institutions, and gene therapy. The patients are referred to the Arizona Cancer Center by community medical oncologists; therefore, a second opinion has already been provided. Dr. Hersh agreed to revise the Informed Consent document according to Dr. Zallen's

suggestions. Dr. Hersh stated that subjects referred to the Arizona Cancer Center have already made the decision to participate in this study; therefore, the 24 hour minimum waiting period protects subjects from making a hasty decision about participation. The 2 week maximum period is included to minimize any substantial changes in the patient's disease status and maintain consistency for data evaluation. Patients are informed that this protocol is a Phase I study designed to determine safety and the maximally tolerated dose and the biological activity of the construct. Potential subjects are informed of the experimental nature of the study and that no therapeutic benefit is expected. The history of most Phase I therapeutic studies suggests that approximately 20% would show some evidence of therapeutic effect. Dr.Hersh agreed to delete this Phase I trial information from the Informed Consent document if the RAC has concerns about this statement. Dr. Zallen requested that the statement be deleted from the Informed Consent document.

Dr. Hersh explained the following reasons for not limiting the types of solid tumors to melanoma and renal cell carcinoma. Animal model studies have shown that IL-2 may be effective against other solid tumors, e.g., lymphoma and colon cancer. He stated his preference for maintaining the broader approach. A variety of tumor types will provide an opportunity to obtain a broader range of information that will be useful for the design of future Phase II trials. Theantitumor mechanism of IL-2 is different for immunotherapy than systemic IL-2. Systemic delivery induces lymphokine activated killer cells. IL-2 expression has been achieved with a variety of tumor cells in vitro including hepatocellular carcinoma, melanoma, renal cell carcinoma, breast cancer, and ovarian cancer.

Dr. Hersh agreed to clarify the Informed Consent document regarding the patient's responsibility for medical costs. When subjects are referred by their oncologists, the basic medical tests have been already conducted. Once the subject enters the protocol, essentially all costs for physician visits, administration of the agent, follow-up CT scans and other services are covered. The patient's third party insurance is expected to cover the costs of conventional follow-up.

Dr. Hersh agreed to clarify the statement in the Informed Consent document regarding the cost of treating any adverse effects. He said that two minor adverse events were observed in the previous trial (Protocol #9403-072). One patient required overnight hospitalization for treatment of severe pain in the injected nodule. All of the expenses for this hospitalization were covered by the sponsor. Efforts will be made to admit patients who do not have any health insurance. In such cases, the physicians charges will be waived, an effort will be made to obtain free medication from pharmaceutical companies, and the hospital will be petitioned to waive the cost of hospitalization. Since 90% of the anticipated costs will be covered by the sponsor, cost should not be a decisive factor to subjects considering participation.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Haselkorn to approve the protocol contingent on incorporation of suggested changes to the Informed Consent document and clarification of minor inconsistencies in the IL-2 DNA sequence.

Dr. Parkman said the investigators have made a reasonable argument for inclusion of a broad range of solid tumors.

The RAC approved a motion made by Dr. Miller and seconded by Dr. Haselkorn to accept the protocol submitted by Drs. Hersh, Akporiaye, Harris, Stopeck, Unger, and Warneke, by a vote of 13 in favor, 0 opposed, and 1 abstention. Approval of the protocol is contingent on review and approval of the following by Drs. Miller, Saha, and Zallen: (1) verification of the vector sequence, and (2) a revised Informed Consent document incorporating the changes suggested by Dr. Zallen.

Dr. Erickson abstained from voting due to his affiliation with the same institution. Mr. Capron commented that the investigators have efficiently responded to the questions raised by the RAC, and that the protocol was reviewed within the time limit of the agenda. He reminded other investigators to follow the same rule to limit their oral responses to the RAC's questions. The presentation of data not submitted 2 weeks prior to the meeting is prohibited. The investigators for the previously reviewed protocol overlooked these criteria.

Summary

Dr. Evan Hersh, Emmanuel Akporiaye, David Harris, Alison Stopeck, Evan Unger, and James Warneke, of the Arizona Cancer Center, Tucson, Arizona, may conduct gene transfer experiments on 25 subjects (18 years of age) with advanced solid malignant tumors or lymphoma. Subjects will receive intratumoral injection of the plasmid DNA/lipid complex, VCL-1102, which encodes the IL-2 gene in an attempt to induce an antitumor response. The objectives of this study are to determine: (1) safety and toxicity associated with escalating doses of VCL-1102; (2) IL-2 expression in tumor cells; (3) biological activity and pharmacokinetics; and (4) whether expression of IL-2 stimulates tumor regression.

X. ADDITION OF APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: CLINICAL PROTOCOL FOR MODIFICATION OF TUMOR SUPPRESSOR GENE EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) WITH AN ADENOVIRUS VECTOR EXPRESSING WILD-TYPE P53/DR. CLAYMAN

Review--Dr. Brinckerhoff

Dr. Walters called on Dr. Brinckerhoff to present her primary review of the protocol submitted by Dr. Gary Clayman of MD Anderson Cancer Center, Houston, Texas. Dr. Brinckerhoff stated that head and neck squamous cell carcinoma (HNSCC) is among the most frequent cancer, accounting for nearly 45,000 new cancers per year in this country and throughout the world. Mortality remains at nearly 55% and has not changed since contemporary radiation therapy was implemented over 30 years ago. This disease may have profound effects on speech, swallowing, and the appearance of affected individuals. Patients who have failed local and regional therapy usually have a median survival of 6 months. Subjects with recurrent head and neck cancer exhibit readily accessible tumors that can be measured, treated, and biopsied without significant discomfort to the patient.

The replication defective adenovirus vector will be Ad5CMV-p53, introduced into the patient's tumor cells to determine whether a normal copy of the p53 tumor suppressor gene can slow or inhibit tumor cell growth. The objectives of this study are to: (1) determine the maximum tolerated dose of the adenovirus vector, (2) determine the qualitative and quantitative toxicity and reversibility of toxicity of this treatment, and (3) document the antitumor activity. Subjects will be divided into 2 groups: (1) those who have advanced inoperable HNSCC cancer, and (2) those who are surgically resectable but not surgically curable. Subjects will receive an initial vector dose of 1 x 106 plaque forming units (pfu). Three subjects will be entered at each dose level with 6 patients entered at the maximum tolerated dose. Treatment will be repeated 3 times per week for 2 weeks. Treatment will continue on a monthly basis in the absence of tumor progression or adverse reactions.

Dr. Brinckerhoff stated that the rationale and experimental design for the proposed study are reasonable. The small font size used for the Points to Consider is unreadable; investigators should succinctly summarize their responses with a readable font. She noted the following concerns regarding the preclinical data: (1) Data has not been provided demonstrating that all tumor types have receptors for the adenovirus vector. (2) The investigators indicate that transduction of the p53 gene induces apoptosis of

tumor cells; however, the data are indirect and only suggestive of this mechanism. (3) Data has not been submitted demonstrating PCR sensitivity capable of detecting 1 x 109 replication competent virus particles. Additional data must be provided regarding tumor specificity, apoptosis, and PCR sensitivity before recommending approval of the protocol.

Review--Dr. Haselkorn
Dr. Haselkorn agreed with the comments presented by Dr.Brinckerhoff. The most significant concern regarding this study is the ability of the adenovirus vector to infect the target tumor cells.

Review--Dr. Secundy

Dr. Secundy said that the Informed Consent document should provide a clearer description of the number and types of procedures that will be conducted, the anticipated time frame for these procedures, the possible risks and benefits of the study, and the subject's responsibility for costs. Will the ability to pay exclude subjects from participating in the study? She recommended clarification of the last sentence in the non-technical abstract.

Other Comments

Dr. Zallen said that the Informed Consent document language relating to costs associated with medical care is much improved as compared to other protocols previously submitted by MD Anderson Cancer Center investigators. She suggested that the description of the waiting period between surgery and study eligibility should be clarified. A 6 week delay is inappropriate.

Dr. Walters noted that the revised Informed Consent document has clarified the medical cost statement, i.e., the experimental treatment and related costs will be provided free to the patients.

Dr. Parkman asked the investigators to provide additional information regarding in vivo transduction of Ad5CMV-p53 in the microscopic residual disease flap model. What is the mechanism of the "bystander" effect by which nontransduced cells are killed?

Dr. Samulski said that introduction of the wild-type p53 gene into tumor cells that contain a p53 mutation is a reasonable therapeutic strategy. Multiple p53 mutations have been identified, some of which result in very stable p53 proteins. Have the p53 mutations been characterized to determine their effect on tumor cell proliferation following wild-type p53 transduction? What are the cellular effects of high level wild-type p53 protein expression? Dr. Walters noted that the wild-type p53 gene functions as a checkpoint control mechanism, causing the cell to rest between cell cycles.

Dr. Saha said that some individuals are predisposed to tumor development because of a hereditary mutation in one allele of the p53 gene. Following a somatic mutation of the second allele, tumors develop. Is preferential mutation of thetransduced wild-type p53 gene a possibility in predisposed individuals? Dr. Samulski said that such mutations are random events, and it is unlikely thetransduced gene will result in preferential mutations. Dr. Walters remarked that the patients with LiFraumeni syndrome have germ line p53 mutations rendering them susceptible to tumor development.

Dr. Smith explained the importance of determining the effect of thetransduced wild-type p53 gene on normal cells since the vector will transduce normal and tumor cells. Proper autopsy of these subjects would provide an opportunity to obtain information regarding the persistence and distribution of adenovirus sequences in advanced cancer patients that is not readily available from subjects in CF studies due to a longer life expectancy.

Dr. Frank Sturtz (Progenitor, Inc.) raised several concerns regarding the use of the wild-type p53 gene to treat cancer patients. The p53 gene was first recognized as anoncogene. Mutation of theCFTR gene render the gene nonfunctional. Mutation of the wild-type p53 gene at several "hot spots" can produce mutant p53 proteins that are veryoncogenic. The proposed strategy has the potential for risk. Ifoncogenic mutations in the p53 gene occur during vector preparation, both subjects and health care workers could be at possible risk.

Dr. Miller inquired about the frequency of transforming p53 mutations in the adenovirus vector preparations. Dr. Sturtz responded that he was not knowledgeable about the frequency of such mutations. However, introducing a mutant p53 gene can transform anastrocytoma to a higher grade malignant tumor. Dr. Miller said that adenovirus vector stocks should be screened for such mutations. Dr.Saha said that 50% of human cancers have p53 mutations at several "hot spots," and that he is not aware of any assays available to detect such mutations in adenovirus vector stocks. Dr.Glorioso noted that the frequency of stable human transformation with this vector is extremely low since two separate low frequency events are required for transformation to occur, i.e.,oncogenic mutation of the p53 gene and integration of adenovirus sequences. Dr. Samulski agreed that adenovirus sequences do not normally integrate into host cell chromosomes. However, high level expression could interfere with the cell cycle and move the cell along a hyperplastic pathway. Between 4 and 5 p53 mutation "hot spots" have been identified; therefore, aPCR assay could be developed to detect such mutations in the virus stocks.

Dr. Haselkorn said that the RAC should focus its discussion on the two significant issues: (1) development of an assay to identify the potentiallyoncogenic viruses, and (2) duration of p53 expression in both normal and tumor cells. The period of p53 expression will determine the degree of risk that a p53 mutant poses to normal cells. Dr.Glorioso said thatHSV has a mutation frequency of 1 in 1 x 106 per cell cycle; therefore, a 1 x 1010pfu virus stock would have an enormous number of mutations. Dr.Samulski added that p53 mutations will accumulate since this gene is nonfunctional. An experiment should be conducted involving the transduction of normal cells with a mutant p53 construct to determine the effect of high level expression.

Dr. Walters asked the reviewers to clarify what are the principal differences between the present protocol for squamous cell carcinoma of head and neck and Dr. Roth's non-small cell lung cancer protocol that was reviewed by the RAC at the June 1994 meeting (Protocol #9406-079). Dr. Smith stated that these same safety issues were raised during the review of Dr. Roth's protocol.Bronchoscopic delivery of Ad2CMV-p53 to the lung (Dr. Roth's protocol) could present a higher degree of risk than this direct injection head and neck tumor study. Dr. Roth's protocol includes a greater possibility of horizontal transmission due to aerosol distribution of the vector. Dr.Saha expressed serious concern about the possibility of oncogenic p53 mutations in Dr. Roth's previously reviewed protocol. Dr. Walters noted that Dr. Roth's protocol was approved by the RAC contingent on the review and approval of additional safety data. The NIH Director has not yet approved Dr. Roth's study.

Dr. DeLeon asked the investigators to address the issue of p53overexpression in normal cells. Dr. Saha suggested Dr. Arnold Levine of Princeton University should be invited as an ad hoc consultant to address p53 issues. Dr. Ross asked if there is any concern about the present protocol that is not pertinent to Dr. Roth's study. Dr. Smith noted that Dr. Roth's contingencies were: (1) intra-pleural administration of the adenovirus vector will be eliminated from the protocol; therefore, a revised protocol and Informed Consent document are required; (2) the protocol will be revised to include patient sputum titration assays on 293 cells (for both wild-type and mutant vector) to be conducted until virus is no longer detectable (patients wil be isolated for a period of 1 week). The RAC did not address the safety issues raised during this discussion as part of Dr. Roth's contingencies for approval. Dr.Secundy noted the necessity for adequate

autopsy data for this protocol. Dr.Samulski said that most of the safety concerns could have been avoided if the adenovirus vector had been constructed differently, i.e., include a promoter that preferentially expresses p53 in tumor cells and not in normal cells.

Investigator Response--Dr. Clayman

Dr. Clayman stated that 20 tumor cell lines have been assayed in his laboratory; all of these cell lines can be equally transduced by the adenovirus vector. The "bystander" effect, i.e., cell killing without direct transduction, has been characterized by electron microscopy, DNA fragmentation, and nuclear fluorescence studies. All of these observations are consistent with the apoptosis mechanism. With regard to transduction efficiency, an experiment was conducted in which a 1.5 cm solid tumor nodule was injected with 100 microliters of 1 x 1010 pfu of vector. 25% of the tumor cells in that nodule expressed the transgene.

In response to Dr. Zallen's concerns about the Informed Consent document, Dr.Clayman said that there will be no costs incurred to patients for surgery, post-operative follow-up, injections, or vectors. However, treatment and procedures not directly related to the protocol will not be covered by MD Anderson Cancer Center, e.g., standard therapy and long-term follow-up. Medical Care will be provided for residents of Texas regardless of their insurance coverage.

Dr. Clayman said that in vitro experiments were conducted using head and neck tumors and non-small cell lung cancer cells. The "bystander" killing effect of nontransduced cells was demonstrated for all experiments. Subjects will be characterized for p53 mutations; however, specific mutations will not be included as an eligibility criterion. In vitro experiments with cultured tumor cell lines suggest that this "bystander" effect is not p53 mutation-specific. The adenovirus vector is anepisomal vector that produces transient expression of p53; therefore,overexpression should not be a concern for subjects with limited life expectancy.

Dr. Clayman stated that tumor accessibility is a major advantage with regard to autopsy information. Autopsy analysis will include examination of the tumor and surrounding tissue. He agreed to perform PCR screening assays to identify p53 mutations in the adenovirus vector stocks.

Dr. Parkman inquired whether 2 copies of the wild-type p53 gene can affect normal cell growth. Dr. Clayman responded that no growth effect was observed on either normal human fibroblasts or oral keratinocytes transduced with the adenovirus vector; however, transient p53 expression was observed for 5 to 7 days. Dr. Roth added that no morphological orproliferative effects were observed in non-immortalized human epithelial cells. Dr. Glorioso asked about the multiplicities of infection (MOI) used for the preclinical experiments. Dr. Roth answered that these studies were conducted using anMOI of 100 pfu per cell. A titer of 1 x 109pfu was analyzed.

Dr. Clayman said that adenovirus is tropic to epithelial tumor cells but thecytomeglovirus (CMV) promoter is not specific for the cell type that is targeted. Dr.Samulski mentioned that there are promoters that are specific for prostate or liver cells.

Dr. Clayman presented data involving 17 cell lines which have either homozygous mutations of both p53 alleles or have two wild-type alleles transduced at an MOI of 100 to 1. Although the wild-type parental lines show a slight delay in cell death, killing occurred equally in all of the cell lines tested. There was no effect on the normal fibroblast cell lines. Dr. Clayman showed data demonstrating apoptosis and infect efficiency. In a dose response experiment, expression of the p53 protein was shown by an immunohistochemistry technique. There was some inflammatory response to virus infection at hig

multiplicity. Antitumor effect was demonstrated in murine experime

Dr. Wei Zhang (MD Anderson) presented data derived from consecutive HeLa cell infection experi No replication competent adenovirus was detected in the vector stocks. Similar results were obtained using a sensitive PCR assay specific for the E1a region of the wild-type adenovirus. Dr. Miller commen that PCR data is acceptable since the assay has been properly validated by spiking experiments. Th sequential amplification assay has not been validated. Dr. Samulski commented that radioactive labe uptake by newly replicating adenovirus is a delayed phenomenon, requiring up to 48 hours to observe uptake. The experiment demonstrating lack of replication at high MOIs is invalid because the data wer collected at 24 hours.

Dr. Zhang presented data demonstrating the lack of any growth effect on a normal human fibroblast cell line whereas, tumor cells were killed. p53 gene expression in the normal fibroblasts lasted between 5 and 7 days. He showed additional data demonstrating apoptosis. Addressing the issue of p53 mutations in the vector stocks, Dr. Zhang showed data derived from in vitro transformation assays on NIH3T3 cells. No transformed foci were observed indicating the lack of oncogenic effect of the adenovirus vector. Th likelihood of any oncogenic effect is slight because gene expression is transien

Dr. Roth said that mutant p53 alone cannot transform normal cells; transformation occurs in cooperation with other oncogenes . No evidence of NIH3T3 transformation has been observed at high MOI comp to the positive transformation observed with the Abelson retrovirus. Transgenic murine experime suggest that the dominant negative effect of mutant p53 is very weak.

Dr. Roth stated that the protein molecule which appears to mediate the "bystander" effect ranges between 30 to 100 kilodaltons . This protein causes tumor cell killing through a mechanism that is consistent wit apoptosis. This protein is inactivated by trypsin digestion, heating, acetonitrile , and 0.1% sodium d sulfate. De novo protein synthesis is required for the "bystander" effect to occur, as suggested by cycloheximide inhibition. Progress is ongoing in an attempt to isolate this protei

Dr. Parkman said that the biological significance of p53 mutations is the critical issue. He inquired whether the primary reviewers of this protocol are comfortable with the results of the transformation assays using replication competent adenovirus. Dr. Miller said that retrovirus transformation can be observed by focus formation in the NIH3T3 cells 2 weeks post-infection; similar assays cannot be performed with the adenoviruses since expression is transient. Dr. Samulski suggested that "hot spot p53 mutations should be monitored using a properly validated PCR assay. Dr. Roth stated that P sensitivity for detecting a particular p53 exon mutation or a single strand conformational polymorphis assay for point mutations is approximately 10%. Dr. Miller asked if animal models are available to test for p53 mutations. Dr. Roth responded that such experiments had been conducted in mice but cotton rat experiments were not conducted. Dr. Glorioso suggested that a mutant p53 adenovirus vector should b constructed to use as a transformation assay control. Dr. Roth said that other p53 mutant vectors have been shown not to cause cell transformation. p53 requires oncogenes to transform most normal cells, K- ras

Dr. Parkman said that a validated and sensitive transforming assay is preferable to a biochemical assay for the detection of p53 mutations. The important factor is the biological significance of these mutations. A transformation assay could be constructed using helper oncogenes to assay mutant p53 oncogen activity. Dr. Samulski agreed that cell transformation requires multiple events; a system to detect p5 oncogenic mutations has to be set up in cooperation with other oncogenes . Dr. Glorioso said s biological system to assess the risk is necessary, i.e., to test the vector stocks in a cooperation type of transformation assay. Dr. Miller commented that constructing an adenovirus with an oncogenic p53 is

risky experiment. Mr. Capron suggested an alternative strategy for providing a biological containment, i.e., tumor-specific promotor which could limit mutant p53 expression to target cells. Dr. Miller said that th albumin promoter elicits some degree of specificity for liver cells; however, development of a tumor specific promotor would be difficult. Dr. Samulski agreed with Mr. Capron that development of suc vector would be preferable. Dr. Roth responded that a cancer specific promoter is a complex issue and should not distract the present proposal.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Brinckerhoff to approve the proto contingent on the submission of a validated assay which can detect potentially oncogenic p53 mutation in the vector stock. Dr. Haselkorn expressed concern that the construction of an oncogenic adenovirus would be more hazardous than the theoretical risk of the proposed study.

Dr. Saha asked about the frequency of p53 mutations in the general population. Dr. Roth responded th hereditary p53 mutations are rare. Li- Fraumeni syndrome represents a rare predisposition for th development of certain cancers due to a hereditary mutation. Dr. Roth stated that none of the 100 patients who were screened had a p53 germ line mutation.

Dr. Glorioso asked Dr. Roth to compare the adenovirus p53 "bystander" effect to HSV-TK / "bystander" effect strategy. Dr. Roth said that the present "bystander" effect is much weaker than the effec observed with HSV-TK . Dr. Glorioso asked about the rationale for using p53 instead of the HSV-T

Dr. Ross stated that any stipulations required for Dr. Clayman's protocol should be applicable to Dr Roth's previous lung cancer protocol (#9406-079), so that the assays will be conducted for all the studies involving the same adeno-p53 construct. Dr. Miller commented that the investigators have provided the best in vitro cell culture data for the previous approval.

As a point of clarification, Dr. Chase said the present stipulation does not include construction of a more risky vector with a mutant p53 gene. He said in this case he would support Dr. Haselkorn's motio

Dr. Samulski proposed a friendly amendment to the motion for approval of the protocol. In addition to t PCR assays that will be used to show that mutant p53's are not accumulating in the vector stock, h suggested that there be additional assays to characterize the different types of p53 mutations. One could be able to determine if there is a correlation between mutants that are stabilized with protein and accumulate versus those mutants that produce a truncated or nonfunctional protein.

Dr. Parkman was concerned about the requirement for inclusion of a biochemical assay for p53 mutations. Mutations occur in all genes. Although such a mutation would be expected in some virus stocks, the real concern is posed by mutations that have a biological effect. A biological assay for such mutations would be preferable to a biochemical assay. Dr. Samulski agreed with Dr. Parkman' suggestion regarding the biological assay. Dr. Roth agreed to discard any virus stock in which a p53 mutation is detected by PCR assay. Dr. Glorioso suggested that co- transfection with the ras could be used to screen for oncogenic p53 mutants. Dr. Clayman said that phenotypically "norm keratinocytes infected by transforming human papilloma virus (HPV)-16 and 18 exhibit normal g when transduced with the wild-type p53 gene. This HPV assay could be developed into co-transfor assay for the detection of oncogenic p53 mutants. Dr. Haselkorn said such an assay should be acceptable test for the adenovirus stocks. A subgroup of the RAC should review the pertinent data.

Mr. Capron asked the investigators to use consistent terminology throughout the Informed Consent

document, and he suggested that the p53 gene be called "normal" instead of "wild-type."

Dr. Ross asked the investigators to specify post-mortem analyses that will be conducted at autopsy. Dr. Clayman agreed to provide a complete description of such a pla

Regarding Dr. Roth's protocol (#9406-079) previously approved by the RAC, Ms. Meyers asked ORDA include a note to the NIH Director conveying the RAC's concern about potential oncogenic muta the p53 gene. Dr. Wivel explained that the NIH Director is always informed about contingencies approval and reasons for split votes when considering protocol approval. Dr. Smith said that the NIH Director should be informed that the RAC retrospectively recommends amending its approval of Dr. Roth's protocol (#9406-079) contingent on each vector lot should be assayed for the presence of p53 mutants, using the same biological assay recommended for Dr. Clayman's study. Dr. DeLeon said t similar stipulation about autopsy analysis should be included for Dr. Roth's protocol. Mr. Capron said that the FDA should monitor the vector lots in regard to the safety concerns raised by the RAC. As a point of clarification, Dr. Haselkorn stated the assay for a mutant p53 in the adenovirus vector should be biological assay such as the proposed HPV system and not a PCR biochemical assay. Dr. Park confirmed that a biological screening assay will be required. Dr. Smith said the RAC is giving the investigators a choice to propose a biological test that is acceptable to a RAC subcommittee; if a vector lo is found to contain any mutant p53, it will be discarded.

The RAC approved a motion made by Dr. Haselkorn and seconded by Dr. Brinckerhoff to accept protocol submitted by Dr. Clayman by a vote of 16 in favor, 1 opposed, and 1 abstention. Approval of t protocol is contingent on the review and approval of the following by the primary RAC reviewers: (1) Development of a sensitive screening assay (preferably biologic -- e.g., the HPV assay), to detect th presence of p53 mutants in the adenovirus vector stocks. In the event that a mutant is identified, that stoc will be discarded. (2) The specific p53 mutation will be characterized for all subjects undergoing gene transfer. (3) Submission of a detailed description of the analyses that will be conducted on available post-mortem tissue.

Ms. Meyers expressed her concern about approving this protocol before vector safety has been demonstrated in this biological system.

Dr. Walters noted that a vote for approval of the protocol requires that the investigator satisfactorily meet all stipulation requirements prior to forwarding the proposal to the NIH Director for approva

Summary

Dr. Gary L. Clayman of The University of Texas - M.D. Anderson Cancer Center, Houston, Texas, ma conduct gene transfer experiments on 21 subjects (> 18 years of age). Subjects will receive intratumoral injections of the replication-defective type 5 adenovirus vector, Ad5CMV-p53, which contains the normal human p53 tumor suppressor gene. The E1 region of Ad5CMV-p53 has been replaced with a p53 expression cassette containing the human CMV promoter. Subjects will be divided into 2 cohorts: (1 those with surgically accessible tumors, and (2) those with non- resectable tumors. This dose-escalatio study involves multiple administrations of Ad5CMV-p53. The objectives of the study are to: (1) determine the maximum tolerated dose of Ad5CMV-p53, (2) determine qualitative and quantitative toxicity, and (3) document antitumor activit

XI. CHAIR REMARKS/DR. WALTERS

On behalf of the entire committee, Dr. Walters presented 4 outgoing RAC members with certificates of

appreciation for their dedicated service to the RAC. He thanked the outgoing members for their tireless contributions to the rapidly developing field of human gene therapy. The outgoing members are: (1) Alexander Capron, LL.B., Professor, University of Southern California, Los Angeles, California; (2) Roy Doi , Ph.D., Professor, University of California, Davis, California; (3) Constance Brinckerhoff , Ph. Professor, Dartmouth Medical School, Hanover, New Hampshire; and (4) Robert Haselkorn , Ph.D. Professor, University of Chicago, Chicago, Illinois.

XII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GEN TRANSFER PROTOCOL ENTITLED: GENE THERAPY OF PRIMARY AND METASTATIC MALIGNANT TUMORS OF THE LIVER USING ACN53 VIA HEPATIC ARTERY INFUSION: A PHASE I STUDY/DRS. VENOOK AND WARR

Review--Dr. Erickson

Dr. Walters called on Dr. Erickson to present his primary review of the protocol submitted by Drs. Alan Venook and Robert Warren of the University of California, San Francisco, California. Dr. Erickso explained that this protocol involves adenovirus vector delivery of the human p53 gene to subjects with hepatic metastasis from colorectal cancer or hepatocellular carcinoma. Patient eligibility will be limited subjects who have p53 defects. p53 is a tumor suppressor gene. Correction of the defective gene may decrease tumor growth. The rationale for this proposal is similar to the rationale for Dr. Clayman's protocol; therefore, many of the concerns are similar. The protocol is presented very well. Some of the concerns regarding the extent of virus infection will be ascertained either by laparoscopic exploration or needle biopsy rather than evaluation of post-mortem tissue.

Dr. Erickson stated that initial concerns described in his primary review (i.e., efficacy, infection rates in different tumor cell lines, and inhibition of tumor cell growth) have been adequately addressed by the investigators. The investigators note that optimal responses to p53 transduction have been observed with breast cancer and osteosarcoma cell lines. Hepatocellular carcinoma and colorectal cancer ce demonstrated a positive response. The data derived from these preclinical studies adequately supports the protocol.

Dr. Erickson noted that ACN53 growth suppression was observed in some nonmalignant tissue culture cells and tumor cell lines not containing the p53 mutation. This observation is different from the results reported by Dr. Clayman . The correlation between ACN53 antitumor responses and p53 mutations easily obtained from this protocol. Dr. Erickson stated that the transduction efficiency obtained with hepatic artery delivery is acceptable.

Dr. Erickson expressed concern about toxicity that was observed in experimental animal studies at high doses of ACN53. The elevations of liver enzymes in serum corresponded to severe liver toxicity with histological evidence of liver cell necrosis. Such toxicity could decrease the subject's ability to tolerate subsequent chemotherapy if delivered by hepatic artery infusion. Nausea, vomiting, anorexia, and alopecia should not be excluded as dose-limiting toxicity. Such symptoms are characteristic of hepatic damage. In their written response, the investigators state that these symptoms are mostly non-specific and can be easily treated or prevented by medications; if such symptoms are severe, dose-related toxicity will be considered. Subjects should be carefully monitored to ensure that any hepatic toxicity is resolved before chemotherapy is initiated. Most of the concerns regarding oncogenic p53 mutations raised durin Dr. Clayman's review are relevant to this proposa

Review--Dr. Do

Dr. Doi stated this Phase I study is designed to evaluate the safety, biological efficacy, and the dosag effect of ACN53. ACN53 is a type 5, subgroup C recombinant adenovirus vector. The E1a, E1b, and protein IX coding sequences have been replaced with the wild-type p53 expression cassette. This recombinant plasmid will be introduced into the liver through the hepatic artery which supplies greater than 80% of the blood flow to liver tumors. Hepatic delivery should optimize vector delivery to the tumor and minimize the exposure to normal tissues outside the liver. This study is a Phase I open-label, non-randomized, dose-escalation trial. A maximum of 27 patients will receive the adenovirus vector with the 3 initial patients receiving a dose of 1 x 108 IU . If no dose-limiting toxicity is observed after 7 days, patients will receive 1 x 109 IU ; if no toxicity is noted after 7 days, this increasing dose-treatment wil continue until the last 3 patients have received 1 x 1013 IU . In addition to clinical follow-up, studies wil include an analysis of p53 transduction efficiency and stability, distribution of ACN53 to non-liver tissues, and evidence of tissue damage.

Dr. Doi said that many of his initial concerns have been addressed in the investigators' written respons Potential liver toxicity still remains a significant concern. The investigators have stated that liver toxicity was observed in animals at much higher doses than those that are proposed for the human study, i.e., 2,000 fold greater than the initial clinical dose. He asked the investigators to further elaborate on this issue. Another concern is the potential adverse effects on normal tissue. He asked the investigators to expand on the formal toxicology studies in rats and primates. Other questions have been addressed including vector targeting, persistence of the virus, and potential problems with over-expression in normal tissues. He inquired whether there is any evidence of vector outside the treated tumor mass. Transduction of peripheral normal liver cells was observed in the rat hepatoma mode

Review--Ms. Meyers

Ms. Meyers said most of her questions about the Informed Consent document have been addressed. She requested a rationale for selecting a total of 27 subjects to participate in this protocol.

Ms. Meyers expressed concerns that the effects of the chemotherapy might make it difficult to determine whether hepatocellular necrosis results from the gene transfer or conventional therapy. In their writte response, the investigators note that chemotherapy toxicity is well characterized and can be differentiated from vector-related toxicity; Ms. Meyers noted that side effects, such as nausea, will be difficult to differentiate between these two treatments.

Ms. Meyers noted liver cell necrosis occurred in mice through tail vein injection of the vector. How can hepatic delivery be compared to tail vein injection? The RAC should review primate hepatic infusion safety data before approving the protocol for humans. No side effects can be ruled out as being unrelated to gene transfer, including nausea. The investigators should be required to present the results from the ongoing primate studies.

Other Comments

Responding to Ms. Meyers' concern about tail vein injection, Dr. Erickson noted that the investigators have performed a relevant rat experiment by hepatic artery delivery of the vector.

Dr. Parkman stated that he shared the same concern about toxicity with Ms. Meyers. The serum chemistry analysis of liver enzymes in the mouse experiments is not adequate. Since a mouse is not a permissive host for the adenovirus vector, Dr. Parkman preferred to have the study performed in a permissive host, i.e., the cotton rat. The proposed dose escalation schedule is too aggressive. The rat and primate toxicity studies are critical for assessing acute toxicity and chronic hepatitis. Responding to Ms. Meyers' question

about differentiation of chemotherapy and vector-related toxicities, Dr. Parkman explained that peak liver enzyme elevation occurs on Day 7. Chemotherapy will not be initiated until Day 28; therefore, the effect of such therapy can be easily differentiated from acute vector toxicity. Questions regarding chronic hepatitis have to be answered in the long-term animal studies. He could not anticipate the outcome of the effect of liver toxicity to the subsequent chemotherapy. Dr. Parkman emphasized more data is needed in order to address the issue of acute toxicity.

Dr. DeLeon noted that a primate model was not required for approval of Dr. Clayman's protocol. Parkman said Dr. Clayman's study involves local vector injection. This proposal involves hepatic arter infusion which has the potential for exposing a substantial number of normal cells to the vector. Dr. Smith expressed concern about potential toxicity. Drs. Grossman and Woo's preclinical primate data demonstrated brain toxicity at high doses (adenovirus vector).

Dr. Samulski commented that the proposed vector construct has E1 (including the coding sequence fo protein IX), E1a, and E1b deletions. Both E1a and E1b deletions are common to other adenovirus constructs. Deletion of the protein IX coding sequence renders this vector more heat sensitive and poses less risk of horizontal transmission. He asked the investigators to address the issue of unintended vector spread to other organs, e.g., the lung, as observed in the preclinical animal studies. Dr. Samulski suggested that attention should be paid to vector construction for the primate experiments, to ensure that the vector is completely permissive in that host. He added that a single alteration in the DNA-binding protein coding sequences renders the vector more permissive in primates.

Dr. Doi asked if the timeframe for direct comparison of acute toxicity is comparable for mice and huma Dr. Parkman asked if there is any evidence of vector spread to ovaries or testes in the murine experiments. Similar studies should be conducted on primates since this protocol is the first to administer high does of adenovirus vector via the hepatic artery. Although systemic vector administration was proposed for Dr. Nabel's protocol (#9306-045), the risk of unintended transduction is higher for thi adenovirus vector than Dr. Nabel's nonviral DNA/liposome comp

Investigator Response--Drs. Venook and Warr

Dr. Venook stated that he is a medical oncologist and that Dr. Warren is a surgical oncologist. Dr. Ve stated that the murine tissue distribution studies (tail vein injection), demonstrated transgene expre in the spleen and adrenal glands but not in the lung. Viral DNA was detected in the lung. Additional information will be forthcoming from the ongoing hepatic infusion primate studies. The gonads were not examined for vector distribution in the murine experiment but will be examined in the primate study. Dr Dan Maneval (Canji , Inc.) stated that the ovaries were negative for -gal marker express

Dr. Warren stated that the starting dose for the human study is 2,000-fold lower than the dose that caused murine liver toxicity. The mouse is a relevant model to examine the toxicity of the replication-deficien adenovirus vector because it is nonpermissive for virus replication but permissive for virus infection. Dr Samulski commented that adenovirus toxicity can be observed even in nonpermissive hosts. This s generation adenovirus vector has reduced potential for toxicity because it is a temperature-sensitive mutant. Dr. Maneval said that serum liver enzyme levels were not examined in the hepatic artery toxici study.

Dr. Venook said that 27 patients are proposed for study was based on a dose-escalation scheme with patients in each cohort. The number of dose-escalation cohorts will be designed according to the results of the primate toxicology study. If toxicity is encountered in the human studies, higher dose cohorts will no be initiated; 27 subjects is the projected number at the present time.

Dr. Glorioso asked if the toxicology studies involved preimmunized animals. The severity of an imm reaction may vary depending on the immune status of the patient. Dr. Miller noted there is no mention of immune status in the eligibility criteria and expressed concern about the potential for a stronger immune response in preimmunized patients. Since most patients are expected to have prior adenovirus exposu experiments involving preimmunized animals is pertinent. Dr. Samulski remarked that in the CF pro the exclusion criterion of antibody positive patients was deleted because most patients have prior virus exposure. Dr. Glorioso said that the possible consequence of immune reaction in the CF studies is th loss of some transduced cell in the lung; however, the entire liver could be rejected in this proposed stu Dr. Parkman stated the issue of the preexisting immune response is twofold: (1) the possibility of decreased transduction efficiency, and (2) the more serious concern of increased inflammatory response in the liver. Preimmunized cotton rat studies are critical for determining hepatocellular damage. Dr. expressed concern that a severe reaction might occur during the dose-escalation schedule. Dr. Parkman said important information can be obtained from the experiment comparing the toxicity in the immunized versus non-immunized cotton rats.

Dr. Warren presented syngeneic Buffalo rat tumor model data demonstrating preferential uptake an expression of the transgene following hepatic artery vector delivery by tumor cells as compared to th surrounding liver parenchyma. This differential uptake and expression can be attributed to the preferential blood supply from the hepatic artery to the tumors. Dr. Samulski commented that an alternativ explanation for this phenomenon could be that these tumor cells have acquired the property of preferentia virus infectivity during in vitro cell culturing, and similar preferential virus infectivity may not exist in the natural liver cancer cells.

Dr. Venook stated not all patients are expected to receive both hepatic pump placement and subseque chemotherapy. These patients will provide information about long-term side effect of ACN53 treatment alone. Patients undergoing pump placement will receive chemotherapy 28 days following ACN53 treatment. This timeframe will allow for evaluation of the safety and biological efficacy of ACN53 treatment. The normal toxicity pattern associated with chemotherapy is predictable and well characterized; therefore, any unusual occurrence due to gene transfer should be readily recognizable. Dr. Venook emphasized that the liver function can be sensitively monitored by assaying serum liver enzym rather than depending on the less specific symptoms such as nausea and vomiting. This Phase I study will provide further toxicity data from humans.

Dr. Samulski asked if animal toxicity studies will be performed on both immunized and non-immunize animals. Dr. Parkman said if the cotton rat experiment demonstrates no difference in toxicity, then there will be no need to conduct preimmunized primate studies; however, if differential toxicity is observed additional primate studies will be required. Dr. Finkle (Canji , Inc.) said that a formal toxicology study has been negotiated with the FDA. Dr. Samulski noted a published animal study by Dr. James Wilso and his colleagues (Philadelphia, Pennsylvania) indicating that there is no gene transduction if the adenovirus vector is administered to preimmunized animals; transduced cells are eliminated by immune responses. Dr. Miller said that the human situation may be different since the adenovirus exposure may have happened long ago. In addition, Dr. Warren stated that not all the virus administered through the hepatic artery will pass through the liver and enter into the systemic circulation.

Dr. Miller stated that virus stocks should be assayed for oncogenic p53 mutations. Dr. Richard Gregor (Canji , Inc.), stated that wild-type p53 is dominant over the mutant p53; the co-transformation assays h been performed with the ras oncogene in cells that do not contain wild-type p53. In an endogen situation such as in the Li- Fraumeni syndrome, the wild-type and mutant alleles are expressed at th same level. If the mutant p53 is expressed at a high level by virus transduction, the cellular effect may be

significant. Dr. Walters asked if it is a concern that the vector has an oncogenic p53. Dr. Venook sa less of a concern for patients with advanced cancer, but Dr. Warren agreed to perform the same type of biological assays required of other protocols, e.g., the HPV assay. Dr. Bryan Finkle (Canji , Inc.) that a formal toxicology study will be completed before initiating the human trial; such data will be presented to the FDA.

Dr. Parkman stated that the RAC is equally concerned about the safety data, and the RAC should be careful in considering approving a protocol when a large amount of toxicity data is not presented. Mr. Capron remarked that the task of monitoring the vector lots can be delegated to the FDA, but the initial deliberation of safety testing should be conducted by the RAC.

Committee Motion 1

Ms. Meyers made a motion to defer approval of the protocol until the investigators return to the RAC with complete toxicology tests in mice and primates, safety testing of p53 mutations, and a detailed description of the analyses to be conducted on available post-mortem tissue. Dr. Secundy seconded the motion. D Smith added a friendly amendment that the immunized cotton rat experiment should include assessment of toxicity and efficiency of transduction. Dr. Parkman emphasized that the toxicity data of immunized versus non-immunized permissive hosts, i.e., cotton rats, is required. In addition, the data pertaining to germ line integration in non-immunized rats is needed. Dr. Smith asked to review the additional data from the monkey experiment.

Dr. Erickson made a substitute motion to approve the protocol with stipulations to be consistent with other approved adeno-p53 protocols. There is some concern about the systemic administration of the vector but a similar route of administration has been approved for DNA/liposome complexes.

Dr. Miller stated that the risk in the present study is greater than other approved protocols: The therapeutic gene will be transduced by a virus vector at a high concentration, and there is a concern about th unpredictable immune response to the vector. There is a need to review the rat data before approving the study. In addition, hepatic artery administration is different from the local injection of the adenovirus vector into a tumor mass. Dr. Parkman said that he shared the same concern about safety: There are some toxicities from the mouse data, and some effects on normal cells of gene transduction, especially by the mutant p53. These effects are significant in the hepatic artery delivery of the vector. A detailed autopsy plan is appropriate for this study; information regarding peripheral persistence and integration after vector administration through the hepatic artery is valuable. Dr. Venook agreed that it is the goal of this study obtain information regarding vector distribution. Dr. Finkle stated that similar information will be obtaine from the primate study, and safety information has been included in the proposal. Dr. Miller emphasized that the critical preimmunized animal data is not availabl

Mr. Capron suggested several changes of the wording of the Informed Consent document, i.e., substituting the words "therapeutic agent" for "therapeutic drug." The autopsy plan should be specific in regard to questions of how to sample the tissue, under what conditions, and what to examine by what tests. Dr. Venook agreed to revise the Informed Consent documen
Dr. Walters noted that the immunity involves both the humoral and cellular immune responses. Dr Parkman explained that the immunized animal study is to address two issues: (1) how much less infection in the preimmunized animals, and (2) how much more toxicity in these animals. The stipulated immuniz cotton rat experiment will address these two issues.

Dr. Finkle said if the RAC approves the clinical part of the Phase I study, the investigators will perform animal studies on safety to meet all the RAC stipulations. Dr. Ross stated that there are too many

contingencies to approve this protocol; it should be deferred until more information is provided.

Mr. Capron asked how much time is needed to complete the safety study. Dr. Finkle said the ra experiment can be completed shortly, and the primate study will take several months. Dr. Wivel said th deadline for protocol submission for the March RAC meeting is January 9, 1995; if it is a deferral, the investigators have to meet the deadline. Ms. Meyers noted that the experiments will take time to complete therefore, there is no rush for approval. Dr. Finkle stated it is extremely important to know that th investigators are moving forward on the right track, and a positive opinion of the RAC is crucial. Drs. Miller and Parkman explained that deferral is not disapproval; the concept of the proposal is acceptable, but the safety data is inadequate. Mr. Capron asked if the immunized rat experiment will provide definitive information. Dr. Parkman responded affirmatively. Mr. Capron and Dr. Erickson asked if a subcommittee could review such data; therefore, the RAC could approve the protocol with contingencies; however, if there is toxicity in the rat experiment, the protocol should not go forward.

Dr. Secundy stated that she would not vote for approval of this protocol since the investigators did no provide the required materials outlined in the Points to Consider. A consistent standard should be held for all protocol submissions. Dr. Parkman said the proposal does include toxicity data; however, additional toxicity data is requested to assure its safety. Dr. Smith said that the requested information is important enough to require the full RAC review. Responding to Dr. Secundy's remark regarding submitte materials, Dr. Venook said all the Points to Consider questions have been addressed in the presen proposal. Dr. Parkman reiterated his conclusion that if the animal data are safe, the protocol could be approved by a subcommittee; if there is positive toxicity, it will be forwarded to the full RAC for review. He favored a conditional approval.

Dr. Walters called the motion. The motion is to defer approval of the protocol pending submission of additional data about toxicity and transduction efficiency in preimmunized cotton rats, data about ger line integration, p53 mutations, and a detailed autopsy plan. He noted that the stipulation does not require the monkey data.

The RAC disapproved a motion made by Ms. Meyers and seconded by Dr. Secundy to defer the proto submitted by Drs. Alan Venook and Robert Warren by a vote of 8 in favor, 9 opposed, and no abstenti The motion to defer the protocol failed.

Committee Motion 2

A second motion was made by Mr. Capron to approve the protocol with previously described contingencies. The motion to approve the protocol was seconded by Dr. Erickson.

Dr. Chase stated that he was concerned with the lack of critical information presented in this protocol. There are 3 categories of protocols: (1) the type with no problem, (2) the totally unacceptable type that should not have been brought to the RAC, and (3) the most time-consuming type like the present protocol He suspected some investigators have a strategy of submitting a protocol with just enough supporting data to obtain a contingent approval. He was not sympathetic to the strategy, but he recognized a legitimate need for it. Dr. Chase stated that he would vote for approval of the protocol. In the future, the technical points should be resolved by a subcommittee rather than by a time consuming discussion at the full RAC meeting.

Dr. Parkman said it is unfair to the investigators to fault them for bringing in the toxicity data; the investigators have been very responsive to the reviewers' questions. The prolonged discussion is due to complexity of the novel scientific issue: This protocol is the first systemic administration of an adenovirus

vector, and the deliberation will set a precedent for review of future protocols. The significant new issues should be resolved by the full RAC rather than by a subcommittee.
Dr. Secundy commented that if it is an important issue, the contingency of the present protocol should reviewed not just by a subcommittee. Dr. Miller indicated his interest in reviewing such data. Dr. Parkman clarified that the present contingency is very specific, and it should be resolved by a subcommittee. Dr. DeLeon appreciated the learning curve for the RAC in dealing with a new issue, concluding that th discussion was very worthwhile. Dr. Smith said the subcommittee for this protocol should include all RAC members who share concerns about the safety data.

Ms. Meyers expressed her concern regarding approval of this protocol with contingency; it will set a precedent for investigators to hastily submit a protocol for a provisional approval even with the knowledge of serious toxicity problems. Dr. Erickson said that such a statement is very unfair to the investigators; the responses from the investigators are very thoughtful. Dr. Miller said that the lack of toxicity data, particularly in regard to the preimmunized animals, is too serious to warrant an approval; he preferred t review the new data with an opportunity to discuss it at the full RAC meeting. Dr. Secundy agreed with Miller's statement. Dr. Walters suggested a large subcommittee for this protocol, and Dr. Zallen asked the subcommittee report their decision to the RAC.

The motion was made by Mr. Capron and seconded by Dr. Erickson to accept the protocol submitted by Drs. Alan Venook and Robert Warren, contingent on the review and approval of the following by th scientific members of the RAC: (1) Data derived from preclinical studies (toxicology and transduction efficiency) in both an immunized and non-immunized permissive host (i.e., cotton rats). (2) Data demonstrating that the adenovirus vector does not integrate into the germ line of a permissive host. (3) Development of a sensitive screening assay (preferably biologic, e.g., the HPV assay) to detect th presence of p53 mutations in the adenovirus vector stocks. In the event that a mutation is identified, that stock will be discarded. (4) Submission of a detailed description of the analyses that will be conducted on available post-mortem tissue. The motion to approve the protocol (with contingencies) passed by a vote of 10 in favor, 7 opposed, and no abstentions.

Summary

Drs. Alan P. Venook and Robert S. Warren of the University of California at San Francisco, Sa Francisco, California, may conduct gene transfer experiments on 27 subjects (18 and 75 years of age) with hepatocellular carcinoma or liver metastasis of colorectal cancer. The adenovirus vector, ACN53, transduce the human p53 tumor suppressor gene is derived from adenovirus type 5 by replacing the E region including E1a, E1b, and protein IX coding region with a p53 expression cassette driven by the CMV promoter. The vector is additionally deleted for 1.9 kb DNA sequences in the E3 region. ACN53 v be administered as a single bolus infusion via a hepatic artery catheter. This Phase I dose-escalation trial is designed primarily to assess the safety of ACN53 and secondarily its biological efficacy. Biological efficacy including efficiency and stability of gene transfer will be studied by analysis of tumor tissues obtained 7 days following gene transfer. Clinical evidence of anti-tumor efficacy will also be collected. The effect of ACN53 dosage on patient tolerance and toxicity will be evaluated in the dose escalation cohorts. As an important part of this objective, the pharmacokinetics of ACN53 will be studied.

XIII. ADDITION TO APPENDIX D OF THE NIH GUIDELINES REGARDING A HUMAN GEN TRANSFER PROTOCOL ENTITLED: PHASE I STUDY OF ADENOVIRAL VECTOR DELIVERY OF THE HSV-TK GENE AND THE INTRAVENOUS ADMINISTRATION OF GANCICLOVIR IN ADU WITH MALIGNANT TUMORS OF THE CENTRAL NERVOUS SYSTEM/DRS. GROSSMAN AND WOO

Review--Dr. Samulsk

Dr. Walters called on Dr. Samulski to present his primary review of the protocol submitted by Drs. Robe Grossman and Savio Woo of Baylor College of Medicine, Houston, Texas. This study is to test adenov vectors for the delivery of a cytotoxic gene to the tumors of the central nervous system (CNS). Th investigators have provided preclinical data demonstrating the use of this vector in the reduction of brain tumors in a rat model as well as important preclinical data demonstrating toxicity of the vector in non-human primates. The investigators stated that the ultimate goal is to develop more effective and less toxic adenovirus vectors. This Phase I trial is designed to study the safety and efficacy of gene therapy in patients with CNS tumors. The investigators will use a viral dosing regimen of 1 x 108 pfu followed b GCV treatment for 14 days. After monitoring the first cohort of 5 patients for one month, the dose will b escalated to 5 x 108, 1 x 109, and then 1.5 x 109 pfu . The primary objective is to monitor for an significant toxicity. The investigators will use MRI or CT scans in comparing survival times of vira transduced patients to the historical survival time. The primary objective, however, is to determine i treatment is associated with significant toxicity.

Dr. Samulski asked why the investigator did not use the more updated adenovirus vectors which hav been shown to have less toxicity. This new generation of vectors utilize temperature sensitive mutations of the adenovirus vectors and restore the often deleted E3 region. The investigators responded in writing that the rationale to use the vector ADV/RSV- tk is that all the preclinical data that demonstrated promis tumoricidal effects from this treatment used this vector construct. No data is available at the present tin that demonstrates efficacy when using the temperature-sensitive vectors. The investigators theorize that the excellent efficacy in rodent tumor models and the seriousness of the brain disease justify the intent to initiate the present limited clinical toxicity trial.

A major concern raised by Dr. Samulski was the toxicity observed at a high dosage in the baboon stud The toxicity study was suggested by FDA officials, and the investigators began the study in May 1994 to test the toxicity of the vector, ADV/RSV- tk , and GCV treatment in baboons. Three treatment groups established: (1) Moderate dose of ADV/RSV- tk with GCV , 6 week survival. A dose of 1.5 x 109 pf vector was injected into the brains of 1 male and 1 female baboon. Gross examination of the brains showed no abnormality. (2) High dose of ADV/RSV- tk with GCV , 3 week survival. 3 x 1010 pfu c vector was injected into the brains of 1 male and 1 female baboon. The male baboon died 5 days after virus injection. The female was sick and euthanized on Day 10. Both animals had 1.5 to 1.8 cm areas c liquefactive necrosis at the injection sites. (3) High dose vector, 3 x 1010 pfu , but no GCV . One was necropsied at 3 weeks after vector injection and another at 6 weeks. Both animals had 1.5 cm cys cavities. Significant toxicity difference in Groups 2 and 3 suggests a synergistic role of GCV in causing toxicity. Dr. Samulski said either the adenovirus vector is causing the cells to divide and sensitizing the to GCV , or alternatively, the vector is making the cell predisposed to GCV in causing this adverse e Although the mechanism of this vector/ GCV synergism is unclear, the phenomenon is alarmin

Dr. Samulski stated that this toxicity has to be taken into account in considering the protocol approval. investigators responded that the human trial will start at a lower dose and the maximum dose will not exceed the dose of 1.5 x 109 pfu where no toxicity was observed in the baboon experiment. Dr. Sam noted that baboons are not permissive hosts for adenovirus infection while humans are, and this maximum ceiling is artificial. Severe toxicity might occur in the permissive human hosts at a lower dose. Dr. Samulski asked the investigators to elaborate on this toxicity issu

Review--Dr. Smith

Dr. Smith agreed with Dr. Samulski's review that the toxicity is a major issue, particularly when the vec is to be administered to the brain rather than to other organs such as the lung or the liver. Toxicity in

baboons is very significant, and it raises even more concern in permissive humans as opposed to nonpermissive baboons as hosts for adenovirus infectio

Dr. Smith noted the preclinical data of experiments with nude mice and Fisher rats showed that GCV b itself has an antitumor effect in these brain tumor models, and the addition of "gene therapy" acts in a adjunctive fashion. In addition, adenovirus vector per se is directly cytopathic . The design of the preser human study has shortcomings and is unable to sort out whether any efficacy seen would be either due to GCV alone, to the vector without HSV-TK gene, or to the combination of the vector with

Since adenoviruses have been reported to recombine with or reactivate members of polyoma / papovavirus family such as SV40 and JC viruses, Dr. Smith asked the investigators to if there is an additional risk of reactivating the latent JC , SV40, or polyoma virus in the brain of patie who have latent infection. Since the present construct has potential toxicity, the health care workers, the investigators, as well as the patients should be monitored for virus shedding. Dr. Smith asked the investigators to explain the reason why a previously approved protocol (#9105-008) in which Dr. Woo is a co-investigator, has never been initiated.

Dr. Smith reiterated that the major concern of this protocol is the toxicity observed in the baboon experiment. Dr. Samulski credited the investigators for performing this baboon experiment at a high ve dosage in order to demonstrate the potential toxicity. Similar caution should be taken in regard to the protocol by Drs. Eck and Alavi (#9409-089) previously approved by the RAC that used a simila adenovirus vector to treat brain tumors. Dr. Glorioso stated the previous approval of Drs. Eck and Ala protocol was made without the benefit of the baboon toxicity data. In addition, the immune status of the patients might further aggravate the toxicity. Dr. Parkman said the question of immune status was discussed during the review of Drs. Eck and Alavi's protocol, and one of the stipulations of the approva was to submit data from a pre-immunized cotton rat experiment.

Review--Dr. Zalle

Dr. Zallen raised the same concern about the vector toxicity observed in the baboon experiment. She f endorsed the comments made by the previous reviewers. Dr. Zallen stated that the Informed Consen document is quite clear and addresses all the concerns that have been identified by the RAC in the Points to Consider. The few changes recommended by Dr. Zallen have been accepted by the investigators Once the toxicity issue is clarified, such information should be included in the Informed Consent document.

Other Comments

Ms. Meyers said the Informed Consent document is well written but the potential risks need to be stated more explicitly to include the adverse effects seen in the retroviral HSV-TK / GCV protocols and the t of adenovirus vectors in baboons. Dr. DeLeon said Dr. Harold Ginsberg (NIH) made a statement du previous RAC meeting that the adenovirus vectors with E3 deletion potentially has more toxicity; she asked the investigators to explain the reason for such deletion in the present vector construct.

Investigator Response--Drs. Grossman and Woo

Dr. Grossman identified himself as the Chief of Neurosurgery at Baylor College of Medicine. The toxicity issue is of the greatest concern to the investigators, and it is the reason to perform the baboon experiment There is no treatment that will not have toxicity at a certain dosage level. The purpose of the baboon experiment is to determine the dosage level that will be toxic. The adenovirus vector at a dosage of 3 x

1010 pfu is toxic in the baboon, and the clinical human trial will be conducted below this dosage level Liquefactive necrosis at the injection sites is not unexpected since all treatments of brain tumors are t destroy cells and to cause necrosis. An important factor to consider when comparing toxicity of baboon and human brains is that the baboon brain is very tiny and the necrotic reaction produces increased intracranial pressure, while the human brain is much larger and more tolerant to such necrotic lesions. Radiotherapy produces a necrotic lesion at the center of the tumor mass but does not kill the peripheral brain tumor cells. Hopefully, the gene therapy owing to its "bystander" effect will be able to kill the peripheral cells as well. The investigators theorize that the necrotic reaction will be controllable, and it will be tolerated by the patients. The starting dose in humans is 300 times smaller than the toxic dose in baboons. Furthermore, the investigators will be able to control the intracranial pressure and inflammatory reaction in the human study, and they are convinced that the proposed study is safe.

Dr. Samulski stated that his reservation about Dr. Grossman's rationale is that the baboon is not a vali model to assess the toxicity issue since it is nonpermissive to adenovirus infection. Dr. Woo thanked D Samulski for giving the investigators the credit for performing the baboon toxicity study that has not bee conducted in other brain tumor protocols. Responding to Dr. Samulski's concern of using the baboon, nonpermissive host, for the experiment, Dr. Woo said that an additional experiment using the permissiv cotton rat is ongoing. The rats were preimmunized with the wild-type adenovirus by intranasal infusion and the adenovirus vector was then injected into the brain at a dose of 1.5 x 109 pfu . Up to 7 day post-vector administration, the animals appear to be normal. Neuropathological analysis is planned to b conducted in a few days. Dr. Samulski stated that the experiment should be performed with a combina of the vector and GCV since there is a synergistic effect. Dr. Woo answered that such an experiment i planned; however, it has not been initiated. Dr. Samulski noted that the cotton rat experiment was initia in response to the reviewers' written comments.

Mr. Capron inquired that if a similar cotton rat experiment was requested as a stipulation to the approval o Drs. Eck and Alavi's protocol. He asked for clarification as to what would be considered a satisfactor outcome from this experiment since Dr. Parkman stated in the previous review that a negative result has to be obtained from this experiment while Dr. Noguchi from FDA commented that the cotton rat data is needed, but the results do not have to be negative. Dr. Samulski said the RAC now has the benefit o hindsight to be more sophisticated on this issue since the investigators have brought in the baboon toxicity data. Dr. David Shine, a co-investigator, confirmed that the cotton rat experiment has been performed with 5 immunized and 9 nonimmunized animals. Responding to Mr. Capron's question, Dr Samulski suggested using the following adenovirus CF studies as an example of a way to evaluate th present study: Once the toxic dosage is identified, the dosage should be adjusted in subsequent studies. Similar relevant toxicity information is not available for the present study. Dr. Glorioso stated that there published reports about destruction of brain tissue following adenovirus injection into the brain. Dr. Woo recited the contingencies required for approval of Drs. Eck and Alavi's protocol including negative resu to be obtained from intracerebral injections of preimmunized cotton rats as scored by either lethality dysfunction of the CNS. The results of the cotton rat experiment has satisfied this requirement in that no lethality nor CNS dysfunction has been observed in these animals although the pathological data has not yet been completed. The investigators will submit the data once the experiment is completed.

Dr. Smith noted that the new data from the baboon experiment has shown the synergism of the vector and GCV in causing the toxicity; similar experiments should be conducted in the cotton rats both in thi protocol and, retrospectively, in the previously approved study by Drs. Eck and Alavi

Dr. Miller was concerned about the adenovirus dosage that caused the brain damage in the published reports mentioned by Dr. Glorioso . Dr. Woo said they have performed an experiment with Fisher rats, a no toxicity was observed with the proposed vector dose. Dr. Parkman considered the question of what

would be acceptable as a beneficial approach to brain tumor therapy since there is no therapy for cancer that does not kill some normal cells. The major concern is the therapeutic ratio, a criterion to be used in evaluating the outcome of the cotton rat experiments. Dr. Samulski said the cotton rat is permissive t adenovirus infection and is an appropriate model. Similar experiments can now be performed in the monkey with an adenovirus genetically modified to be permissive in the monkey. Dr. Samulski said th investigators have provided a toxic ceiling dose in the animal model on which to base their human trial. Dr. Chase commented that the number of cotton rats in the experiment is appropriate if all the animals are without any toxic effect.

Dr. Walters asked for clarification if the injection to the brain will provoke less immune reaction. Dr. Glorioso said that in brain tumor patients, the blood brain barrier is frequently compromise

Dr. Woo explained that the design of the baboon experiment was a result of consultation with FDA officials to test for the vector dose that will produce toxicity in animals with and without GCV treatment. addition, distribution of vector sequences in these animals was examined; there was no virus spread except for a positive finding of the blood samples from the first 2 days of a high dosage group. Dr. Woo said the cotton rat experiment indicates no fatality in all test animals. Dr. Chase explained that a formal toxicity study for drugs would involve escalating dose series to determine the half maximum dose for toxicity. Dr. Miller said the investigators have performed the cotton rat experiment with a very high dose and have observed no toxicity. Dr. Samulski said the protocol could be approved contingent on providi the data from the cotton rat experiments. He was uncomfortable with using the baboon data alone as a basis for approval since the baboon is a nonpermissive hos

Committee Motion

Dr. Samulski made a motion to approve the protocol with contingencies. Dr. Smith seconded the motio Dr. Samulski said the cotton rat experiments should test the ADV/RSV- tk vector on nonimmunize immunized animals with and without GCV at an escalating dose regimen above the lethal dose for th baboon. Dr. Parkman said the data from the ongoing cotton rat experiment of nasal inoculation followed by intracerebral vector administration is acceptable. In addition, the data from a dose escalation study and without GCV is needed. Dr. DeLeon said the histological data regarding inflammatory respon should be included. Dr. Samulski said the preclinical study has demonstrated the efficacy of th adenoviral HSV-TK / GCV approach in treating brain tumors, and the present study is to define treatment window that is not associated with toxicity.

Dr. Samulski suggested that a plan for autopsy should be included in the Informed Consent document Ms. Meyers said a description of serious side effects observed in the retroviral HSV-TK / GCV stud should be included in the section of the Informed Consent document discussing side effects. Dr. Grossman accepted Ms. Meyers' suggestion.

Dr. Chase commented that the design of the present protocol is not to evaluate the efficacy of the treatment strategy since it would involve many factors that need to be evaluated. Dr. Grossman agreed completely with Dr. Chase's statement; this protocol is the first step to develop a new brain tumor treatment, and it is a Phase I toxicity study.

The RAC approved a motion by Dr. Samulski and seconded by Dr. Smith to accept the protocol submit by Drs. Robert Grossman and Savio Woo by a vote of 16 in favor, 0 opposed, and 1 abstention. Appro of the protocol is contingent on the review and approval of the following by the primary RAC reviewers: (1) Data (including histological analysis) derived from the ongoing preclinical cotton rat study in which animals undergo intranasal preimmunization with the adenovirus followed by direct injection of th

adenovirus vector into the brain (to determine the effect on the CNS). (2) Data derived from dose-escalation toxicology studies in non-immunized cotton rats (with and without the administration of GCV) up to a dose that is greater than the dose that was lethal for baboons. (3) The Informed Consen document will be revised to include a statement that informs potential participants that adverse events (toxicity) have been reported for a similar Phase I human gene therapy protocol involving a HSV-TK /retrovirus gene delivery syste

Summary

Drs. Robert G. Grossman and Savio L. C. Woo of Baylor College of Medicine, Houston, Texas, ma conduct gene transfer experiments on 20 subjects (18 years of age) with malignant tumors of the CNS. Patients with malignant brain tumors refractory to all potentially curative therapy will be treated with stereotactic intra- tumoral injections of a replication-defective adenovirus vector delivering the HS gene. The vector, ADV/RSV- tk , is constructed from adenovirus type 5 with E1 and E3 deletions; th expression of the HSV-TK gene is driven by a Rous sarcoma virus long terminal repeat. GCV w administered intravenously at 10 mg/kg/day for 14 days. Only one course of therapy will be administered. Five patients will be tested at an initial dose of 1 x 108 pfu , and they will be closely monitored for 1 mo for evidence of toxicity before other groups of patients are to be treated at higher doses of 5 x 108 and 1.5 x 109 pfu . Effectiveness of the treatment will be monitored by MRI and/or CT scans and by compar survival times to the historical survival times for patients with recurrent brain tumors. The primary objective of this initial study is to determine if the treatment is associated with any significant toxicity. The ultimate goal is to develop a more effective and a less toxic vector for brain tumor gene therapy.

XIV. PROPOSED AMENDMENTS TO APPENDIX B, CLASSIFICATION OF ETIOLOGIC AGENTS AND ONCOGENIC VIRUSES ON THE BASIS OF HAZARD OF THE NIH GUIDELINES/DR. FLE

In a letter dated June 24, 1993, Dr. Fleming requested that Appendix B should be revised to conform with the most recent taxonomy and agent risk groups as described in Biosafety in Microbiological and Biomedical Laboratories (BMBL), 3rd edition, May 1993, U. S. Department of Health and Huma Services. At its September 9-10, 1993, meeting, the RAC recommended that the revised Appendix B should not be adopted until letters of concurrence were submitted by the Centers for Disease Control and Prevention and the NIH Division of Safety. In a telephone conference on October 20, 1994, Dr. Flemin stated that Appendix B would be reviewed by experts from the Centers of Disease Control and Prevention and the American Society for Microbiology (ASM). The revised Appendix B was submitted for RA review.

Dr. Wivel said that Appendix B is an essential guideline for the local Institutional Biosafety Committee (IBCs) to set the appropriate physical containment levels for etiologic agents. It is an important task tha Dr. Fleming has been undertaken to update this document. Appendix B is a classification based on human and animal pathogenicity of the organisms and the physical containment levels are assigne according to the known pathogenicities . Appendix B is intended to be used in conjunction with the BM book. The proposed Appendix B has been reviewed by a Committee to Revise Appendix B (CRAB) assembled by Dr. Fleming under the aegis of the ASM . CRAB members include the following: Ms. Mar Cipriano , Abbott Laboratories, Abbott, Illinois; Dr. Joseph H. Coggin , Jr., University of South Alaba Mobile, Alabama; Dr. Diane O. Fleming, Bowie, Maryland; Dr. Mary Gilchrist, University of Cincinnati, Cincinnati, Ohio; Ms. Janet Hindler , University of California, Los Angeles, California; Dr. Jonathan Y Richmond, Centers for Disease Control and Prevention, Atlanta, Georgia; Ms. Amy Melnick , America Society for Microbiology, Washington, D.C.; Dr. Moselio Schaechter , Tufts University, Bost Massachusetts; and Dr. Joseph E. McDade , Centers for Disease Control and Prevention, Atlanta Georgia.

Review--Dr. Straus

Dr. Straus agreed that the task to update the Appendix B is overdue. The intent of the updated Appendix B is to harmonize the classification between the existing Appendix B and the recent edition (3rd edition, 1993) of the BMBL book. Dr. Straus stated that there are one global and several minor issues. The glo issue involves the new concept of "risk group." It has meanings different from the old classification system. Different levels of physical containment can be assigned to an organism in a particular risk group depending on factors such as large volume, aerosol, or how it is handled. There are revised groupings of the listing for oncogenic viruses and other organisms. Dr. Straus noticed several inconsistencies in th listings, for example, classification of Chlamydia psittaci , Bacillus anthracis , adenoviruses, prions several retroviruses. He asked Dr. Fleming to explain the rationale behind the reclassification of organisms. He said it is premature for him to make any specific comment.

Review--Dr. Sah

Dr. Saha said a thorough review cannot be made since he received the revised Appendix B just a fe days ago. The concept of the new Appendix B is to classify organisms into "risk groups," and a particular "risk group" could be assigned to different physical containment levels. This document needs a taxonomist and other experts to comment on this new listing. Proposed Appendix B should be published in the Federal Register for public comments before the next RAC meeting.

Other Comments

Dr. Parkman asked if the underlying philosophy of the risk group and inconsistencies in the listings are the major problems. Dr. Straus said these two are part of his concerns, and the other concern is "risk group" versus " biosafety level." The factors necessary to assign an organism to different levels of phys containment are not clearly stated. The advantage of the existing classification is that it sets firm standards as to how a particular organism is handled. The risk group classification leaves too much room for interpretation. Dr. Saha agreed that this issue requires further discussio

Dr. Miller said the concept for risk group is reasonable since it assigns certain risk to an organism rather than a containment level that could vary depending on the circumstances in which the experiment is performed. Minor inconsistencies of the classification can be amended during the public comment period. Dr. Miller said that CRAB can serve as a standing committee to update Appendix B in the future. Dr. Straus said although there is some merit to the new risk group concept, there are experts who differ in their opinion regarding this classification concept. Dr. Miller said that RAC members can vote on approval or disapproval of this document.

As a point of clarification, Dr. Wivel said today's discussion is to identify the problems with this proposa The proposed Appendix B will be published in the Federal Register for public comment, and the RAC can make a decision at the March meeting. There were discussions among RAC members about whether to invite experts to comment on different parts of the proposed Appendix B. Dr. Miller stated that the proposa definitely has to be published for public comment. Dr. Saha agreed it should be published for commen and experts can be solicited to comment on specific areas, e.g., brucellosis. Dr. Saha stated that th concept of the risk group is reasonable; it conveys an assessment of the inherent risk of an organism that allows the local IBC to determine a physical containment level appropriate for a particular experiment. Dr. Wivel said a complication of this classification scheme can be illustrated by the example of handling th HIV. It is a Risk Group 2 organism that can be handled either at BL2, or at BL2 with BL3 practices, or at BL3 for large-scale production. The question is whether Appendix B should include this kind of

complicated directives.

Dr. Straus stated his major reservation about this document is its vagueness and ambiguity as illustrated with the HIV example, but he agreed that the proposal should be published for comment. He asked Dr. Fleming to provide a statement about the rationale for several proposed changes in the classification of organisms. Dr. Straus asked if the changes of Appendix B would require amendments to other appendices of the NIH Guidelines. Dr. Wivel explained that there will be minor changes to the Guidelines; however, no major changes will be required. Dr. Straus speculated that the IBC would welcome a more specific guidance from the NIH Guidelines. Dr. Miller said that changes involving othe parts of the NIH Guidelines are very simple, e.g., substituting the words "Class 2" with "Risk Group 2" i Section III-C-1-a that reads as the following: "Experiments involving the introduction of recombinant DNA into Class 2 agents shall be conducted at Biosafety Level (BL)-2 containment."

Dr. Walters asked if it is the RAC consensus to publish the proposed Appendix B in the Federal Register for comment in advance of the March RAC meeting. Mr. Capron favored the idea of publishing the proposal with accompanying explanation of the changes. Ms. Meyers stated that the amendment of Appendix B should be an ongoing process. Dr. Wivel explained that in the last few years, there have b several requests for reduction of containment level approved by the RAC. Dr. Parkman asked what percentage of organisms in the list would have containment levels other that indicated in the risk group number. Dr. Straus stated approximately around 10% of the listed organisms would need assigning to different containment levels according to circumstances of the experiments, e.g., simian immunodeficiency virus. Dr. Walters said if there is no objection, the proposal will be published in the Federal Register prior to the next RAC meeting. He called on Dr. Fleming to make her comment.

Dr. Fleming explained that the concept of risk group came from a recommendation by World Health Organization. The idea of putting together the revised Appendix B is to provide the IBCs with an updat guidance for handling etiologic agents. Appendix B is to be used in conjunction with the BMBL book. T book contains agent summary statements that recommend containment levels according to several factors, such as handling HIV in clinical specimens or in a production scale. She said that her group of Mid-Atlantic Biological Safety Association has experience on quantitative risk assessment. Dr. Straus suggested that the BMBL book should be included in the next mailing to the RAC members in order t facilitate the review of the proposal. Dr. Parkman suggested to have the proposal announced in the E-mai for wide public comment.

I.

XV. PRESENTATION OF ETHICAL CONSIDERATIONS RELATIVE TO IN UTERO SOMATI CELL AND GENE THERAPIES/DR. PATTERSON

Dr. Amy Patterson of FDA presented an overview of some of the ethical issues involving in utero cell and gene therapies with an overhead slide illustration. Her presentation covered 4 major areas: (1) In utero bone marrow transplantation, its rationale and the proof of concept; (2) Huma experience; (3) Experiments involving potential protocols; and (4) Ethical considerations inherent in prenatal cell and gene therapies, including informed consent process and patient enrollment and eligibility criteria. The meeting materials included an article by Drs. Morton J. Cowan and Mitchell Golbus entitled: In Utero Hematopoietic Stem Cell Transplants for Inherited Diseases, pub the Journal of Pediatric Hematology/Oncology, Volume 16, pages 35-42, 1994.

Overview--Dr. Patterson

The proof of the concept for in utero bone marrow transplantation relies on immuno -incompet

the fetal immune system early in gestation. Evidence for immuno -incompetence of the fetal immu system is as follows: (1) Aborted fetuses have decreased T-cell immunity in vitro; (2) Donor lymphoid cells reportedly engraft in fetuses transfused with unirradiated blood transfusions; (3) F liver or thymus transplants given to SCID children have shown significantly lower risks of graf versus host disease (GVHD) when fetal tissue is less than 10 to 12 weeks gestational age. Huma immunocompetence emerges at roughly 16 weeks of gestation. The rationale for doing in ute transplantation rests on the following provisions: (1) the genetic disease is diagnosed early enough in gestation; (2) the fetus would be relatively immuno -incompetent; and (3) engraftment could b achieved without the usual toxic preconditioning or the ablative regimen. In addition, there could be relatively low risk of damage to target organs.

At present, 14 attempts have been made at in utero transplantation in humans worldwide involvi genetically unmodified human stem cells. The sources of stem cells include parental, sibling, fetal liver and fetal thymus. 5 of those 14 attempts have shown evidence of engraftment. It is notable that 2 of those 5 engraftments were conducted at a gestational age of less than 16 weeks when the fetus was still immuno -incompetent. There are evidence of mixed chimerism in all instan

Mr. Capron asked at what gestational age the transplantation was performed in the 9 cases that failed to show engraftment. Dr. Patterson said they were conducted between 14 and 32 weeks; not all transplantations conducted before 16 weeks were successful. Dr. Parkman explained that most of these patients are SCID children, and there are many other variables contributing to the failure engraftment.

Of the 5 patients with evidence of engraftment, 1 infant had bare lymphocyte syndrome. This transplant was performed in France using fetal liver and thymus administered through the umbilical vein. The infant is now 16 months postnatal, and there is no evidence of GVHD , and 26% of it lymphocytes are of donor origin. An infant with - thalassemia was transplanted in France at 1 weeks gestational age using fetal liver and thymus. Another infant with SCID was transplanted i France with fetal liver and thymus at the gestational age of 28 weeks. This child is now 22 months old and remains out of the isolation chamber. Two families elected abortion at 20 to 24 weeks when testing failed to show engraftment. However, on examination of the aborted fetuses, it was found tha donor cells had indeed engrafted into fetal marrow. These 2 cases raise difficult medical and ethical issues when counseling parents for abortion.

The potential obstetrical complications of in utero bone marrow transplantation include both feta and maternal infection, fetal death, premature rupture of membranes, fetal trauma, and persistent fetal bradycardia . Reported outcome of 14 attempts includes 5 engraftments, 2 fetal deaths due t miscarriage 1 week after treatment, and septic abortion within 24 hours of treatment.

With the precedent of in utero bone marrow transplantation, one could envision the application o gene therapy in this setting. A potential Phase I protocol might propose to harvest whole marrow from the biological mother or father of a fetus with a documented inherited disease. The stem cells would be enriched from the marrow and transduced with a vector. The transduced stem cells be subsequently infused intravenously or by direct intraperitoneal injection into the fetus at an ag while it is still immuno -incompetent. The size of fetus at this stage would easily fit into the palm of hand.

With this background information, Dr. Patterson raised the major issues and questions that would be inherent in any prenatal gene therapy protocol:

Issue A: What is the optimal informed consent process for fetal somatic cell and gene therapy? In any clinical context, informed consent consists of a process and not simply Informed Consent document. The ethical problems of obtaining valid informed consent in fetal therapy have been developed in other contexts, such as surgical correction of fetal heart defects, hydrocephalus, and hydronephrosis . Due to the possibility that a mother may be likely to disregard her own well-bein for the sake of the fetus and the possibility that clinical investigators will communicate enthusiasm about new and unproven approaches to fetal therapy, special precautions for the informed consent process have been recommended. These recommendations include: (1) an impartial physician from the fetal medicine team to "speak for" the fetus; (2) the mother's own physician, available at least by phone to assist in the assessment and comprehension of maternal risks; (3) requisite involvement of a genetics counselor in the decision making process; and (4) documentation of the full informed consent process.

Issue B: This issue regards patient enrollment criteria. There are 4 questions in regard to this issue: (1) Should prenatal somatic cell and gene therapies (Phase I clinical trials) be restricted at this time to diseases in which it is a current standard practice to offer "therapeutic abortion?" (2) Should enrollment in prenatal somatic cell and gene therapies (Phase I clinical trials) be restricted to mothers who have been offered the option of "therapeutic abortion" and for religious purpose and/or personal motives decline this option? (3) Should enrollment be extended to include mothers who have already chosen "therapeutic abortion," and would this option potentially enhance the risk/benefit ratio of in utero therapy? (4) At what point are mothers informed about this protocol (e after already having made a decision about abortion)?

Issue C: This issue regards fetal enrollment criteria. There are 3 major questions related to this issue: (1) Should inclusion criteria be restricted to diseases with known early fatality? Of particular note is the instance of diseases with a known genetic defect diagnosed in utero but with wid phenotypic variability. As in the case with many of the inherited metabolic diseases, it is often difficult to made a diagnosis of fetuses with devastating diseases. (2) Will the studies be restricted to singleton pregnancies? In order to potentially safeguard the well-being of unaffected fetuses if multiple gestations are present, should the studies be restricted to cases where both fetuses are affected? (3) Should fetuses with other concomitant abnormalities detected by sonography karyotype , amniocentesis, or biochemical analyses be exclude

In terms of future directions, Dr. Paterson asked the RAC to consider the formation of a subcommittee to address the following issues: (1) expedited analysis of the ethical considerations inherent in prenatal cell and gene therapy clinical trials; (2) definition of an informed consent process in this clinical setting, and (3) definition of parameters for patient enrollment and eligibility criteria for both mother and fetus that would optimize the risk/benefit ratio.

Other Comments

Ms. Meyers and Mr. Capron commented on the thought-provoking presentation made by Dr. Patterson. Ms. Meyers was concerned that there will not be much financial incentive to develop the in utero gene therapy for rare diseases. Dr. Patterson said most of the ground work for in ute therapy including the vector and stem cell technology has been developed for the treatment of other genetic diseases such as Gaucher diseas

Dr. Parkman noted that many diseases such as SCID do not have in utero abnormality, and th no justification to perform in utero gene therapy when the same result can be achieved wit newborns. There are a limited number of diseases in which there is significant morbidity and

mortality prior to birth that will benefit from the in utero gene therapy. Dr. Motulsky noted seve examples, i.e., - thalassemia , and some fetal lung and CNS diseases. Dr. Parkman mentioned th fetal lamb studies by Dr. Esmail Zanjani of the University of Nevada School of Medicine that relevant to in utero gene therap

Dr. Straus stated that in utero therapy is a challenging issue. However, the RAC is not a prope forum to discuss fetal therapies that do not involve gene therapy. Since gene therapy still needs proof of efficacy in post-natal individuals, the issue of prenatal treatment is premature.

Dr. Samulski noted that the promising research of stem cell technology will have the best chance succeeding in prenatal therapy. Dr. Glorioso stated that the present discussion is related to th future of fetal research. The stem cell technology has potential applications to diseases of lung, liver, colon, and others. For example, a preventive gene therapy can be performed by transducing the p53 tumor suppressor gene to stem cells in utero in multiple copies since many more mutatio of this gene would have to occur in order for a tumor to develop.

Dr. Zallen stated the prospect of current investigation using stem cells in utero could lead dire attempts to genetically modifying stem cells and to conduct gene therapy in utero . The subject is considerable interest to the RAC and to FDA as well. Dr. Zallen supported Dr. Patterson' suggestion to form a subcommittee to examine these issues in details. The subcommittee could evaluate the issues with the public involvement and subsequently recommend appropriate amendments to the Points to Consider.

Dr. Zallen said there is no ethical impediment using medical technology to treat a fetus in ute under 2 conditions: (1) if the intervention directly benefits the fetus, and (2) if the medical outcome requires intervention in utero because of additional complexities of the disease if it is treated afte birth. The proposal would have to be judged by both the overall scientific merit and a favorable risk/benefit ratio. The informed consent process will guide the potential subjects to make their decision to participate in the study. Responding to the question of whether willingness of the mother to undergo abortion should be a factor of the enrollment criteria, Dr. Zallen said that women wh have decided to proceed with the pregnancy are the most deserving candidates since the decision to have the baby would allow medical evaluation of the treatment after birth. On the other hand, it could be argued that women who are willing to have an abortion will allow termination of pregnancy if serious problems of the intervention are detected before birth. In this case, the women should agree to an autopsy so that scientifically useful information could be obtained. Dr. Zallen stated th someone's willingness to consider abortion should not be the determining factor in eligibility for a protocol; all potential subjects should be presented with the protocol. Responding to the question of fetal enrollment criteria, Dr. Zallen said scientists and clinicians are most able to choose th appropriate diseases to be treated by in utero gene therapy. While fetal intervention should be fir given to extremely serious life threatening diseases, the degree of seriousness has to be factored into the degree of knowledge possessed and the likelihood that the intervention could produce a beneficial result. Responding to the question of the informed consent process, Dr. Zallen said tha timing of informing the potential subjects is the most critical factor since there is very little time lapse between genetic testing and the optimal time to perform the fetal intervention. The informed consent process should be planned with a long lead time. Both the pregnant woman and her husband should be invited to make their joint decision. In addition, there is a need to involve a neutral resource person such as the woman's obstetrician or an expert in fetal medicine to provide the information about the complicated aspects of the research protocol. Dr. Zallen emphasized that th person should have no conflict of interest. Dr. Zallen said the need for long-term follow-up i absolutely necessary in doing the in utero gene therapy. Since the treated subjects are expected

outlive the investigators, a mechanism has to be in place to keep track of the patients. In conclusion, Dr. Zallen stated that the RAC should form a subcommittee to address these issue

Mr. Capron stated that development of stem cell technology and gene transduction of spermatogonia point to a future for gene therapy. One additional legal and ethical consideration o utero gene therapy is the involvement of the body of the mother. Birth is recognized as a singl event in the achievement of a legal personhood. Fetal intervention cannot be imposed if there is a parental objection. Mr. Capron considered that the question of abortion as an entrance criterion is relevant in 3 ways: (1) If the disease is serious enough, the usual ethical and legal prohibitions against late term abortion are overcome by agreement between the doctor and the parents. (2) The relevance of abortion is to avoid harm to a born person. Abortion is a relevant criterion for Phase I or even Phase II trials. If there is any harm known, it should not be passed onto a born person. (3) Another way in which abortion is relevant is in providing a justification for this form of treatment. For autosomal recessive disease such as - thalassemia , it is possible to offer parents in vitro fertili and pretesting of the embryos with the implantation only of the unaffected one. For those peopl who absolutely object to the destruction of any embryonic life form, in utero gene therapy offers a alternative treatment.

Mr. Capron favored the idea of creating a subcommittee, and its purview should be extended to questions of in utero gene therapy that might lead to germ line alterations. Such questions arise f Dr. Ralph Brinster's work on sperm germ cells. The subcommittee is not only to speculate about t science itself but to ask the structure through which the issues can be analyzed from an ethical viewpoint. The deliberations would be carried out through a series of meetings with involvement of experts in the field. Mr. Capron said this moment is very important for him in observing the RAC reaching this point as he prepares to leave a decade of service to the RAC.

Dr. Chase commended Drs. Noguchi and Patterson on formulating the in utero gene therapy issu in such a way that the public can have some input. The research in this subject should not be confined to people who are already in the middle of a pregnancy, but it should be extended to individuals who are about to begin families. Dr. Chase suggested inviting individuals from the Ethical, Legal and Social Implications Program of the NIH National Center for Human Genom Research to the RAC for discussion since there is a potential for developing a research plan on this topic. There are medical, legal, and ethical issues regarding eligibility criteria for abortion. The issue of the right of the fetus versus the right of the mother deserves attention in a public discussion.

In agreement with Mr. Capron's statement that the present discussion leads to issues of germ line alteration, Dr. Parkman cited the work of Dr. Zanjani and his colleagues who have injected into th peritoneal cavities of immuno -incompetent fetal sheep a retroviral vector bearing the neoR g marker. The fetal sheep treated with the vector were born with the vector sequences in multiple organs. These vector sequences were then transmitted to the subsequent generation, apparently through the germ line. Similar unintended alteration of the germ line could occur by attempting to treat CF in the fetal lung. Dr. Parkman said the RAC should focus its attention on addressing the germ line issue in terms of the categories of how germ line alteration might occur, which are acceptable, and which are not acceptable.

Regarding the informed consent from the affected parents, Dr. Parkman said most of the families with serious genetic diseases have prior knowledge of their condition, and it would not be a traumatic decision to be made in a short order during pregnancy as suggested by Dr. Zallen

Ms. Meyers noted that fetal therapy appears to be more pertinent to some neurological diseases

since it prevents the irreversible brain damages in the early stage of the disease. Dr. Parkman pointed out that only a subset of neurological disorders will benefit from the fetal therapy. If the defect is primarily within the neurons, such as the Tay -Sachs disease, fetal therapy will not offer a additional advantage.

Mr. Capron said the RAC should start to discuss the issues of fetal or germ line therapy and should be prepared before it receives its first protocol. Dr. Chase agreed that such a preparation is needed for the RAC, and he suggested the subcommittee to include germ line issues in its purview. Dr. Parkman favored a focus on the more important germ line issues. Dr. Glorioso agreed that the fe gene therapy should be considered
within the context of germ line alteration. Dr. Parkman said the immediate question for the RAC is the unintended germ line insertion of vector sequences, and the RAC has to develop guidelines for the forthcoming protocols with this unintended germ line alteration. Dr. Zallen said that the prenat gene therapy is a very important area for the RAC. Ms. Meyers suggested a combined working group of prenatal and germ line gene therapy. Dr. Walters thanked Dr. Patterson for her excellent presentation.

SUMMARY

On December 2, Dr. Amy Patterson of FDA presented an overview of some of the ethical issues involving in utero cell and gene therapies. The RAC recommended the establishment of subcommittee to examine the ethical issues involving in utero gene therapy. Since the issue of ge line gene therapy and transmission is closely related to the issue of in utero therapy, th subcommittee should analyze both issues and their interrelationship to each other.

XVI FUTURE MEETINGS OF THE RAC

The next meeting of the RAC will be March 6-7, 1995, at the NIH , Building 31C, Conference Roo 6, Bethesda, Maryland.

XVII. ADJOURNMENT

Dr. Walters adjourned the meeting at 3:50 p.m. on December 2, 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