DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING

June 8-9, 1995

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-second meeting at 9:00 a.m. on June 8, 1995, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided; Dr. Doris Zallen (Acting Chair) presided in Dr. Walters absence. In accordance with Public Law 92-463, the meeting was open to the public on June 8 from 9 a.m. until 5 p.m. and June 9 from 8:30 a.m. until 4:00 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
Krishna R. Dronamraju, Foundation for Genetic Research
Robert P. Erickson, University of Arizona
Joseph C. Glorioso, University of Pittsburgh
Michael M.C. Lai, University of Southern California
M. Therese Lysaught, Bioethics Associates
Kathleen M. McGraw, State University of New York at Stony Brook
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
Karen Rothenberg, University of Maryland School of Law
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).

Non-Voting Representatives:

Barbara Levin, U.S. Department of Commerce
Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Scott Abrams, NCI
Bobbi Bennett, OD
R. Michael Blaese, NCHGR
Diane Bronzert, NCI
Jan Casadei, NCI
Shan Chu, NHLBI
Robert Cowherd, NCI
Judith Dipietro, NCI
Judy Fradkin, NIDDK
Eric Francoeur, OD
Judith Greenberg, NIGMS
Jay Greenblatt, NCI
Taizo Hoshino, NHLBI
Patrick Hwu, NCI
Christine Ireland, OD
Judith Kantor, NCI
John Lam, NCI
Becky Lawson, OD
Catherine McKeon, NIDDK
Hiroaki Mizukami, NHLBI
Shinichi Muramatsu, NHLBI
David Nelson, NCHGR
Gopal Potti, CC
Mark Reeves, NCI
Thomas Shih, OD
Sonia Skarlatos, NHLBI
Jonathan Treisman, NCI
Debra Wilson, OD
Jim Yang, NCI

Others:

Paul Aebersold, Food and Drug Administration
Ronald Alvarez, University of Alabama
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Dale Ando, Chiron Corporation
Bart Barlogie, University of Arkansas

James Barrett, Genetic Therapy, Inc.
Mary Helen Binger, Gene Medicine, Inc.
Bridget Binko, Cell Genesys
Lars Bolund, University of Alabama
Katyna Borroto-Esoda, Glaxo, Inc.
Arindam Bose, Pfizer Central Research
Andrew Braun, Harvard University
Debra Brinckerhoff, Public
Stephanie Broome, Agracetus, Inc.
Rosemary Browar, Genetic Therapy, Inc.

Jeff Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
Michael Casey, Genetic Therapy, Inc.
Joy Cavagnaro, Food and Drug Administration
Jan Chappell, Genetic Therapy, Inc.
Saswati Chatterjee, City of Hope National Medical Center
Yawen Chiang, Genetic Therapy, Inc.
Robert Conry, University of Alabama
Kenneth Culver, Public
David Curiel, University of Alabama
Eileen Deist, Genetic Therapy, Inc.
Robert Desnick, Mt. Sinai School of Medicine
Nathalie Dubois-Stingfellow, Chiron Corporation
John Efthimiou, Glaxo, Inc.
Tom Eggerman, Food and Drug Administration
David Ennist, Genetic Therapy, Inc.
Alexandra Filipovich, University of Minnesota
Bernard Fox, Providence Portland Medical Center
Shawn Gallagher, Magenta Corporation
John Gardner, Systemix, Inc.
Martine George, RPR GenCell
James Hawkins, Public
Nunoi Hiroyuki, Tokyo University
Shigekazu Ichinose, Kasho Company, Ltd.
Helen Jenkins, MegaBios Corporation
Jolynda Jones, Genetic Therapy, Inc.
Susan Jones, Virus Research Institute
Christopher Juttner, The Hanson Centre for Cancer Research
Fran Kahane, Onyx Pharmaceuticals
Katherine Kaufmann, GenVec
Gary Kikuchi, Genetic Therapy, Inc.
Connie Kirby, Canji, Inc.
Stuart Kopperman, Systems Research and Applications Corporation
Toshi Kotani, Genetic Therapy, Inc.
Karen Kozarsky, University of Pennsylvania
Steven Kradjian, Vical, Inc.
Michael Langan, National Organization for Rare Disorders
Allen Lapey, Massachusetts General Hospital
William Larchian, Duke University

Jane Lebkowski, Applied Immune Sciences
Gloria Lee, GenCell
Geeta Lingam, Glaxo, Inc.
Charles Link, Human Gene Therapy Research Institute
Albert Lobuglio, University of Alabama
H. Kim Lyerly, University of Alabama
Russette Lyons, Genetic Therapy, Inc.
Christopher Maack, Onyx Pharmaceuticals
Libbie Mansell, Burroughs-Wellcome Company
Phillip Maples, Baxter Healthcare Corporation
Wayne Marasco, Dana-Farber Cancer Institute
Tony Marcel, TMC Development
Stephen Marcus, Genetic Therapy, Inc.
Elliot Marshall, Science Magazine
Stephen McCormack, Georgetown University
Gerard McGarrity, Genetic Therapy, Inc.
R. Scott McIvor, University of Minnesota
Amy McKee, FDC Reports
William McVicar, Sandoz Pharmaceuticals Corporation
David Meeker, Genzyme Corporation
Andra Miller, Food and Drug Administration
Fred Miller, Food and Drug Administration
Karen Millison, Genetic Therapy, Inc.
Atsushi Miyanohara, University of California, San Diego
Susan Mize, Mize Information Enterprise
Robert Moen, Public
David Moorman, Iowa Methodist Medical Center
Ron Morales, Harvard University
Richard Moscicki, Genzyme Corporation
NIkhil Munshi, University of Arkansas
Susan Nemeth, Schering-Plough Research Institute
Andrea Neuman, Technology Catalysts
Jeffrey Ostrove, Microbiological Associates, Inc.
Amy Patterson, Food and Drug Administration
Jerri Perkins, Perkins & Perkins
Ramila Philip, Applied Immune Sciences
Tony Phillips, Glaxo, Inc.
M. Lynn Pritchard, Glaxo, Inc.
Raj Puri, Food and Drug Administration
Harvey Rabin, Pro-Neuron, Inc.
Urban Ramstedt, Virus Research Institute
Rex Rhein, Biotechnology Newswatch
Cary Robertson, Duke University
Joseph Rokovich, Somatix Therapy Corporation
Richard Schifreen, Life Technologies, Inc.
Becky Seufert, Genetic Therapy, Inc.
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International, Inc.
Juliet Singh, Baxter Healthcare Corporation
Alan Smith, Genzyme Corporation

Franck Sturtz, Progenitor, Inc.
Yoshikazu Sugimoto, Japanese Foundation for Cancer Research
Nevin Summers, Novation
Thomas Tarlow, Chiron Corporation
Bruce Trapnell, Genetic Therapy, Inc.
Guido Tricot, University of Arkansas
Yoshitaka Uchijo, Kyodo News Service
Walter Urba, Providence Portland Medical Center
Dominick Vacante, Magenta Corporation
Karen Valentino, IDUN Pharmaceuticals
Debra Vaz, Virus Research Institute
Samuel Wadsworth, Genzyme Corporation
Janet Walker, Genzyme Corporation
Judy Ways, Glaxo, Inc.
Judi Weissinger, Applied Immune Sciences, Inc.
David Wheeler, The Chronicle of Higher Education
Kathleen Whitaker, Quality Biotech
Lisa White, The Blue Sheet
Kam Wong, City of Hope National Medical Center
Thasia Woodworth, Systemix, Inc.
Shuyyan Zhang, Genetic Therapy, Inc.
Robert Zimmerman, Chiron Corporation

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

Dr. LeRoy Walters (Chair) called the meeting to order and stated that the notice of the meeting and the proposed actions were published in the *Federal Register* on May 22, 1995 (60 FR 27206), 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 indicated his intent to 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 the following new RAC members: (1) Michael Lai, M.D., Ph.D., Professor, Department of Microbiology and Neurology, University of Southern California, Los Angeles, California; (2) M. Therese Lysaught, Ph.D., Assistant Professor, Department of Religious Studies, University of Dayton, Dayton, Ohio; (3) Kathleen M. McGraw, Ph.D., Associate Professor, Department of Political Science, State University of New York at Stony Brook, Stony Brook, New York; and (4) Karen Rothenberg, J.D., Marjorie Cook Professor of Law, University of Maryland School of Law, Baltimore, Maryland.

Dr. Walters thanked Ms. Debra Wilson of the Office of Recombinant DNA Activities (ORDA) for her efforts in compiling the data for the semiannual data management review.

Dr. Walters noted several documents included in the meeting material and activities: (1) a March 21, 1995, letter from Ms. Wilson to the PIs of RAC-approved human gene transfer protocols describing the detection of low levels of a presumptive human retrovirus in Protocol #9209-027 (as requested by the RAC at its March 1995 meeting); (2) a March 21, 1995, letter from Dr. James Barrett, Genetic Therapy, Inc. (GTI), regarding the issuance of a broad *ex vivo* gene therapy patent by the U.S. Patent Office to the NIH with exclusive licensing to GTI (Cooperative Research and

Development Agreement); (3) a letter from Dr. Gerard J. McGarrity, GTI, entitled: *Human Gene Therapy Protocols: RAC Review* (*Science*, volume 268, page 1261, 1995); (4) the Institute of Medicine of the National Academy of Sciences is about to initiate a study of germ line genetic intervention; and (5) a study of issues concerning research related injuries will be initiated within the Office of the Director at NIH.

Ms. Meyers inquired if all investigators are asked to test their cancer patients for the presence of the new human retrovirus reported by Dr. Miller. She asked what the patent signifies as to the future of independent gene therapy research and whether academic scientists would have to pay a royalty to GTI if they wish to conduct *ex vivo* experiments.

Dr. Wivel explained that the investigative phase of the human gene therapy research will have no infringement on the patent, and licensing agreement would have to be negotiated for commercialized applications. Dr. Miller expressed his reservation about the validity of this patent and its negative impact on investigators developing *ex vivo* gene therapy vectors and procedures, particularly if they need a biotechnology industry sponsorship. Mr. Capron recited an early recombinant DNA patent by Drs. Stanley Cohen and Herbert Boyer that is very broad. It has not impeached on recombinant DNA research because it imposes a minimal fee for its use. Mr. Capron asked if the licensing procedure of the gene therapy patent has been publicly announced.
Dr. Barrett responded to questions raised by Ms. Meyers, Mr. Capron, and Dr. Miller. The patent is deemed valid since it is issued by the U.S. Patent Office. In terms of clinical academic research, it should not infringe on the patent. With respect to the involvement of commercial entities, investigators should contact GTI to obtain a license to the patent. Ms. Meyers reminded that such a licensing agreement should not discourage academic scientists from pursuing gene transfer research. Dr. Miller said in reality such a licensing procedure will inhibit commercial companies to develop vectors in collaboration with academic scientists. Mr. Capron said that it would not be in GTI's best interest to have such a scenario occur because the patent will not yield any revenues.

Responding to Ms. Meyers' question on the human virus, Dr. Wivel explained that the finding is still preliminary and no pathogenicity has been found to be associated with this virus; there is no compelling reason at present to require all the investigators to test their cancer patients for the presence of this virus. Dr. Parkman noted that it is inappropriate for the RAC to require the investigators to address this issue since the new virus is found in cells before undergoing transduction; therefore, it is not associated with gene transfer. Mr. Capron said that if gene transfe procedures should increase the pathogenicity or titer of this virus by any mechanism, the issue would raise a concern for the RAC. Dr. Miller said that his study on this virus is still ongoing, and he has not yet developed any molecular or antibody assay suitable for use in screening cancer patients. Dr. Samulski said that it is important to study if the virus that is observed in cancer patients is observed in normal individuals.

Dr. Walters asked if it would be appropriate for the RAC to follow up on the gene therapy patent issue. Dr. Miller said that the patent issue is not under RAC's purview. Dr. Ross said it would be of interest to see whether the patent has any impact on the development of the field. Dr. Miller reiterated his concern about the negative impact of this patent. Mr. Capron suggested that Ms. Rebecca Eisenberg at the University of Michigan would be a good person to address the patent issue.

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

Dr. Walters explained that a total of 5 minor modifications were approved since the March 1995 RAC meeting to the following protocols: (1)*A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene Modified Cytotoxic T Lymphocytes in HIV-infected Twins* (#9403-069), Robert Walker, M.D.; (2)*High Dose Chemotherapy and Autologous Bone Marrow plus Peripheral Blood Stem Cell Transplantation for Patients with Lymphoma orMetastatic Breast Cancer: Use of Marker Genes to Investigate the Biology ofHematopoietic Reconstitution in Adults* (#9411-092), Dan Douer, M.D.; (3)*A Repeat Dose Safety and Efficacy Study of HIV-IT (V) in HIV-1 Infected Subjects with Greater Than or Equal to 100 CD4+ T Cells and No AIDS Defining Symptoms* (#9503-105), Peter Frame, M.D., Mark Loveless, M.D., and WilliamPowderly, M.D.; (4)*Phase I Trial of Interleukin-2 DNA/DMRIE/DOPE Lipid Complex as an Immunotherapeutic Agent in Solid Malignant Tumors or Lymphomas by Direct Gene Transfer* (#9412-095), Evan Hersh, M.D.; and (5)*A Repeat Dose Safety and Efficacy Study of HIV-IT (V) in HIV-1 Infected Subjects with Greater Than or Equal to 100 CD4+ T Cells and No AIDS Defining Symptoms* (#9503-105), Peter Frame, M.D., Mark Loveless, M.D., WilliamPowderly, M.D, David Parenti, M.D., and Richard Haubrich, M.D.

II-B. STATUS REPORT - ACCELERATED RAC REVIEW AND NIH APPROVAL OF HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters noted that the following human gene transfer protocols were approved through the *Accelerated Review* process since the March 1994 RAC meeting: (1)*A Repeat Dose Safety and Efficacy Study of HIV-IT (V) in HIV-1 Infected Subjects with Greater Than or Equal to 100 CD4+ T Cells and No AIDS Defining Symptoms* (#9503-105), Peter Frame, M.D., Mark Loveless, M.D., and William Powderly, M.D (University of Cincinnati, Cincinnati, Ohio, Kelly Avenue Clinic, Portland, Oregon, and Washington University Medical Center, St. Louis, MO); and (2)*Autologous Marrow Transplantation for Chronic Myelogenous Leukemia Using Stem Cells Obtained After In Vivo Chemotherapy Cytokine Priming* (#9506-106), Catherine Verfaillie, M.D. (University of Minnesota, Minneapolis, Minnesota).

II-C. STATUS REPORT - HUMAN GENE TRANSFER PROTOCOLS CONTINGENT ON FULFILLMENT OF RAC STIPULATION REQUIREMENTS/DR. WALTERS

Dr. Walters stated that a total of 11 protocols have contingent approval pending fulfillment of RAC stipulation requirements. Three protocols reviewed at the March 1994 RAC meeting remain unresolved: (1) #9503-100 (Link/Moorman), (2) #9503-101 (Economou), and (3) #9503-102 (Gansbacher). Dr. Walters mentioned that the day after the March 6-7, 1995 RAC meeting, Dr. Russell Walker at Elexion Pharmaceuticals in New Haven, Connecticut, wrote a letter regarding Link/Moorman protocol about the lack of persistence of virus producer cells in primates. The PIs have not yet responded to his concern to date. Dr. Glorioso agreed that the letter raised a very relevant question about the proposal.

Four protocols reviewed at the December 1994 RAC meeting remain unresolved: (1) #9412-094 (Dorkin/Lapey), (2) #9412-096 (Clayman), (3) #9412-097 (Venook/Warren), and (4) #9412-098 (Grossman/Woo). Two protocols remain unresolved from the September 1994 RAC meeting: (1) #9409-087 (Whitley), and (2) #9409-089 (Eck/Alavi). One protocol remains unresolved from the June 1994 RAC meeting: (1) #9406-079 (Roth).

Dr. Walters noted that the Dorkin/Lapey protocol (#9412-094) would be discussed later during this meeting to resolve the pending issues regarding aerosol administration of the adenovirus vector. Drs. Roth and Clayman's protocols (#9406-079 and 9412-096) required the development of a sensitive biological assay for the detection of p53 mutants in the adenovirus vector lots. Dr.

Samulski recommended that Dr. Arnold Levine, Princeton University, should be invited to address the RAC at its September 1995 meeting regarding fundamental safety issues regarding human application of p53 adenovirus vectors.

III. MINUTES OF THE MARCH 6-7, 1995, RAC MEETING/DRS. MOTULSKY, ROSS

The RAC approved a motion made by Dr. Motulsky and seconded by Dr. Ross to accept the March 6-7, 1995, RAC minutes (with the incorporation of Drs. Ross and Miller' minor editorial changes) by a vote of 22 in favor, 0 opposed, and no abstentions.

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

Dr. Wivel stated that the most recent meeting of theNIH *Ad Hoc* Review Committee was held on May 1, 1995. This Committee (chaired by Dr.Inder Verma) is charged with providing a comprehensive assessment of the past and current RAC activities in an effort to develop recommendations regarding the future role of the RAC in the review of human gene transfer experiments. The *Ad Hoc* Review Committee has identified the "quality of science" as a critical area that should be addressed by the RAC. A Cover Sheet is currently being developed that will introduce consistency and will facilitate the evaluation of protocols byORDA on the basis of "minimum" scientific standards. The*Ad Hoc* Review Committee will recommend that studies that do not meet the "minimum" scientific criteria delineated in the Cover Sheet will not be forwarded for RAC review. Dr. Parkman recommended that RAC comments or suggestions should be forwarded directly to Dr. Verma. Dr. Varmus suggested that while it is clear that the principal role o the RAC is in evaluating new applications of gene therapy, once an area had been approved the issue of raising the threshold is not within the charge of the RAC. Dr.Zallen said this issue will be further deliberated in the upcoming meetings of the*Ad Hoc* Review Committee.

Dr. Noguchi noted that the Food and Drug Administration (FDA) does not discriminate on the basis of the quality of a submission. The FDA accepts all applications and recommends changes as necessary. Dr. Chase stated that the majority of RAC members agree that the current standards for human gene transfer research may not be high enough to facilitate statistically significant data In the absence of such data, the public will not have access to valid scientific information regarding the problems and progress of this field to weigh against sometimes unsubstantiated media reports.

Mr. Capron said that the topic of how the RAC evaluates protocols is an important issue and he suggested formalizing a consensus statement of the RAC to be forwarded to the*Ad Hoc* Review Committee. Regarding the issue of quality of science, Dr. Noguchi noted that human transplantation has occurred in this country without federal oversight and without quality standards imposed by any government body. Ms. Meyers stated that it is important to delineate th proper role of the RAC in addressing both safety and quality issues of gene transfer protocols. Dr. Walters noted that there is a general agreement that every protocol must meet a certain minimal level of quality, and a debated point is whether that threshold should gradually be made higher as the field matures.

The RAC decided to continue its discussion regarding standards for RAC review of human gene therapy protocols later in the meeting (see Item XV).

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

Dr. Wivel noted that the first meeting of theNIH Panel to Assess Investment in Gene Therapy Research was held on May 15-16, 1995. The co-chairs of this Committee are Drs.Arno Motulsky and Stuart Orkin. The Committee has been instructed to analyzeNIH funding of basic and clinical research (related to gene therapy) and to develop a set of recommendations about how best to use NIH resources to support gene therapy research. The Committee will have a series of speakers to assess the current status and to point out the future prospects of the field in both areas of fundamental research and clinical trials. Dr.Motulsky added that NIH has committed a large amount of resources to gene therapy research, and the Committee's charge is to recommend to the NIH Director on how to allocate its resources in various aspects of gene therapy research.

Ms. Meyers suggested that NIH should support gene therapy research on rare genetic diseases, because the private sector often sponsors research based on market consideration. Mr. Capron disagreed with Ms. Meyers' observation that the trend of gene therapy is primarily driven by the commercial interest; the disproportionate number of cancer protocols is more of a historical scientific development during the years of 1990 through 1993. Dr. Parkman said that theNIH initiative to set up national vector laboratories is a step in addressing Ms. Meyers' concern.

Dr. Samulski noted that as the field evolves, patient eligibility will be expanded to include subjects with minimal disease and/or longer life expectancies. Although it is necessary to consider such classes of patients in order to accurately assess the therapeutic potential of this technology, the RAC must increase its standards for approval as the potential for long-term gene expression and/or inadvertent germ line transmission increases. Dr.Samulski noted that a National Vector Laboratory is a preferable option for funding trials involving rare inherited genetic disorders that may not receive financial support from industry/commercial components.

Dr. Motulsky said the molecular principles of gene therapy have been developed by studying a very rare genetic disease. These principles have been applied to common diseases such as cancer with a tremendous impact on public health. Such a trend should be encouraged while at the same time pointing out the need ofNIH to support orphan diseases because of lack of commercial incentive in this area. Dr. Noguchi noted that insurance companies would have interest in rare diseases if the treatment has a potential of reducing the cost of patient care. The RAC's role is not to approve another cancer vaccine protocol sponsored by a company but rather to address a societal concern as raised by Ms. Meyers. Dr. Chase and Mr. Capron said that there are many forces shaping the development of gene therapy, and it is beyond the power of the RAC to direct this development.

Dr. Glorioso noted that the roadblock of gene transfer research is technology rather than commercial interest; the real problem is to have a gene transfer vector that will allow persistent and targeted gene expression via *in vivo* route of administration. Dr.Lysaught observed that there are three factors contributing to the imbalance of protocols in favor of common diseases: market forces, higher risk standard for childhood diseases, and diseases with no animal models. Ms. Meyers said the RAC has approved many protocols for children and for diseases with no proper animal models, and the main factor is the market force.

VI. REPORT FROM THE DATA MANAGEMENT SUBCOMMITTEE

Dr. Smith (Chair) noted two broad issues that should be addressed by the RAC: (1) theRAC's future role in Data Management, and (2) the necessity for public education regarding the current

status of the field of human gene therapy to explain "positive" results that are often inflated in media reports. Dr. Zallen emphasized the importance of the RAC's continued role in the Data Management process. Public accountability of gene therapy maintains public confidence and serves to validate/invalidate media claims. Dr. Zallen agreed to inform the NIH *Ad Hoc* Review Committee about the necessity for maintaining RAC's role in the Data Management process.

Gene Therapy Information Network (GTIN)/Dr. Noguchi:

Dr. Philip Noguchi, Director of Cellular and Gene Therapies, FDA, addressed the RAC regarding the status of the FDA-funded GTIN. Dr. Noguchi summarized the content of his memorandum dated June 8, 1995, regarding GTIN. GTIN was originally funded with the idea that it would serve as a prototype for the FDA for how to track patients with an initially small population involved in gene transfer trials. As with all government agencies today, each project and program is being scrutinized for relevance and meaning. Dr. Noguchi stated that he has received pressure from his supervisors for their impressions that FDA funding of gene therapy registry has been for NIH benefit rather than for facilitating FDA review. FDA is committed to the development of this project but its funding cannot be guaranteed beyond the end of this year. He has contacted both private and public foundations, and they have indicated an interest to support further funding of this project.

Mr. Capron asked Dr. Noguchi to clarify what is the complaint from FDA that it is being asked to do NIH's work. Dr. Noguchi explained that FDA is very interested in the idea of being able to track patients and have invested a great deal of resources into developing the prototype. Ms. Wilson said that the system will capture detailed information about clinical trials and will have the capacity to retrieve any type of information from it. She is serving as the NIH contact person for the development of this project. Mr. Capron said that the project looks like a NIH/FDA collaborative process rather than like the FDA's impression of funding the project for the NIH benefit. Dr. Noguchi said that the use of FDA funds is primarily to create a system that will make it easier and more facile for FDA reviewers to complete their work. He was not asking NIH for any funding of this project. A year after the mandate to streamline the review process, the RAC has not appeared to be as accommodating or as committed to sharing tasks in the consolidated review process.

Dr. Miller asked what the computerization of the database means. Dr. Noguchi responded that the system is designed to allow investigators to submit their data to FDA electronically through investigational new drug (IND) applications to all phases of clinical trials. A subset of this information will be made available to the public. The GTIN will be assessable by IBM compatible or Macintosh personal computers, and the hardware will reside on the FDA's VAX computer at the Center for Biologics Evaluation and Research. Subsequent GTIN phases will encompass world-wide-web access and on-line FDA submission and data entry. Dr. Walters inquired about the cost of this pilot project.

Dr. Noguchi responded that the start-up costs for this prototype average approximately $1,000 per patient. Mr. Stuart Kopperman, GTIN Project Manager, SRA Corporation, Rockville, Maryland, explained that this amount estimates the cost of development for the first year. As the system is defined and future requirements are understood, the cost will probably not remain at the current level. The current strategic vision estimates approximately the same level of funding for the next 3 years; subsequent years will largely encompass FDA requirements. Dr. Chase said the cost of $1,000 per patient is very high, and the task could be accomplished with much less money.

Dr. Noguchi said the problem is not money, and he can get support from outside sources. A

critical component to the success of the GTIN is whether the RAC will make a commitment to consolidate and streamline the gene therapy review process, i.e., the FDA prefers that the RAC take the necessary action to reduce the period of protocol review and cooperate with the FDA to provide complementary review between the two agencies. Dr. Noguchi said that his superiors answer to industry. Mr. Capron said that he thought the FDA answers to the American people. He inquired about the current impediments to the consolidated review process. Dr. Noguchi noted that the RAC has not been responsive to the proposed streamlining issues such as concurrent Institutional Review Board (IRB) and FDA approval. Dr. Noguchi said that by law and by statute, FDA is the final arbiter of clinical trials.

Dr. Chase said that the discussion is wandering through a larger issue beyond the question of preserving a data monitoring project. He said that data gathering of gene transfer protocols is vital and if it cannot be decided whether NIH or FDA is responsible for the task, it should be decided at a level above these two agencies. Ms. Meyers noted that the task of data management has grown much larger than the original intention of monitoring gene transfer patients on a long-term basis. Dr. Noguchi said the project is a prototype not only for gene therapy but as a model for other needs of the FDA. The project is being funded through the user fee program of FDA. Dr. Noguchi again emphasized that funding is not really the issue.

Overview/Dr. Smith:

Dr. Smith stated that a total of 106 protocols are encompassed in the June 1995 RAC Data Management Report, 105 RAC-reviewed protocols and 1 protocol not reviewed by the RAC (voluntarily submitted by Viagene, Inc., San Diego, California). A total of 597 subjects have undergone gene transfer to date at 37 clinical trial sites. 55 out of 85 protocols listed NIH as their major funding source. About 1/3 of the protocols have industry sponsors. The median time from Institutional Biosafety Committee (IBC)/IRB approval to RAC approval is 3 months (range = 0-9 months). The median time from RAC review (with or without stipulation requirements is 3 months (range = 0-15 months). The median time between RAC approval and FDA approval is 7 months (range = 0-26 months). The median time between final approval (both RAC and FDA) and the date that the first subject underwent gene transfer is 3 months (range = 0- 15 months).

Status Report/Ms. Wilson:

Ms. Wilson explained that the June 1995 Data Management Report has been separated into two reports: (1) Status (investigator, trial site, accrual, and death information), and (2) Scientific (gene transfer, gene expression, biological activity, immune response, and adverse event information). The final versions of these documents are available from ORDA, Phone: 301-496-9838. 268 (approximately 45%) of all subjects who have undergone gene transfer to date have been entered in the last 6 months (329 subjects reported for December 1994 Data Management report). 168 of the 268 most recent subjects (63%) were entered on 2 HIV-1 trials sponsored by Viagene, Inc. To date, 228 subjects (38.2%) have undergone gene transfer while participating in non-industry sponsored trials, whereas 369 subjects (61.8%) have undergone gene transfer while participating in industry sponsored trials. A total of 134 subjects have died to date (either during the course of their participation in the study or during the follow-up phase). Of the 134 deaths, a total of 96 autopsies (72%) were requested by investigators. A total of 22 autopsies (16.4%) were conducted and 74 (55.2%) autopsies were requested but refused (primarily by the subjects' families). (Attachment II - Data Management Overview)

Ms. Wilson noted that the inherited genetic disorders, acquired disorders, and infectious diseases

are categorized by disease/disorder. Cancer trials are categorized by therapeutic approach. Marking studies are categorized by target cell for*ex vivo* transduction.

Summarized below are the categories of Gene Therapy protocols that have been reviewed by the RAC to date:

Category Disease/Disorder # Protocols Percentage

Acquired Disorders Total 2 (1.9%)
Peripheral Artery Disease 1 (0.9%)
Rheumatoid Arthritis 1 (0.9%)
Infectious Diseases Total 8 (7.5%)
Human Immunodeficiency Virus-1 8 (7.5%)
Inherited Genetic Disorders Monogenic 20 (18.9%)
Alpha-1-Antitrypsin 1 (0.9%)
Chronic Granulomatous Disease 1 (0.9%)
Cystic Fibrosis 11 (10.4%)
Familial Hypercholesterolemia 1 (0.9%)
Fanconi Anemia 1 (0.9%)
Gaucher Disease 3 (2.8%)
Hunter Syndrome 1 (0.9%)
SCID-ADA 1 (0.9%)

Category Therapeutic Approach # Protocols Percentage

Cancer Total 51 (49.1%)
Antisense 2 (1.9%)
Chemoprotection 4 (3.8%)
Immunotherapy/Ex Vivo 23 (21.7%)
Immunotherapy/In Vivo 7 (6.6%)
Pro-drug/HSV-TK/Ganciclovir 11 (10.4%)
Tumor Suppressor Gene 4 (3.8%)

Summarized below are the categories of Gene Marking experiments that have been reviewed by the RAC to date:

Target Cell for Ex Vivo Transduction # Protocols Percentage

Total # of Marking Protocols 25 (23.6%)
Autologous Bone Marrow and.or Peripheral Blood Lymphocytes 15 (14.2%)
Cytotoxic T Lymphocytes 1 (0.9%)
Autologous CD34+ Selected Cells 3 (2.8%)
Autologous Tumor Infiltrating Lymphocytes 4 (3.8%)
Autologous Hepatocytes 1 (0.9%)
Syngeneic Lymphocytes 1 (0.9%)

Retrovirus vectors 76 (71.7%)
Adenovirus vectors 15 (14.2%)
Adeno-associated virus vectors 1 (0.9%)
Cationic liposome complex 12 (11.3%)

Plasmid DNA 2 (1.9%)
Particle-mediated 1 (0.9%)

6 categories of delivery vehicles have been proposed to date: (1) Retrovirus vectors - 76 protocols (71.7%), (2) Adenovirus vectors - 15 protocols (14.2%), (3) Adeno-associated virus vectors - (0.9%), (4) Cationic liposome complex - 12 (11.3%), (5) Plasmid DNA - 2 (1.9%), and (6) Particle-mediated - 1 (0.9%). A total of 67 protocols (63.2%) involve the ex vivo transduction of target cells. A total of 39 protocols (36.8%) involve in vivo gene delivery.

Summarized below are the various routes of gene/vector administration (and number of associated protocols) that have been proposed to date:

Bone Marrow Transplant 21 (19.8%)
Subcutaneous Injection 21 (19.8%)
Intravenous 21 (19.8%)
Intratumoral (direct injection) 9 (8.5%)
Intranasal 8 (7.5%)
Respiratory Tract (bronchoscope) 6 (5.7%)
Intratumoral (stereotactic injection) 6 (5.7%)
Intramuscular 4 (3.8%)
Intratumoral (pulmonary catheter) 3 (2.8%)
Intrahepatic (portal vein catheter) 3 (2.8%)
Intrapleural 3 (2.8%)
Intraperitoneal 3 (2.8%)
Intratumoral (bronchoscope) 2 (1.9%)
Intraventricular 1 (0.9%)
Intratumoral (Ommaya reservoir) 1 (0.9%)
Respiratory (aerosol) 1 (0.9%)
Maxillary Sinus 1 (0.9%)
Intrajoint 1 (0.9%)
Intraarterial (angioplasty catheter) 1 (0.9%)

Vectors were either supplied or purchased from industry for 72 protocols (67.9%). Vectors were supplied by academic institutions for 32 protocols (31.1%). The vector supplier for 2 protocols has not yet been identified. A total of 34 human gene therapy protocols (32.1%) have been sponsored by industry to date.

Ms. Meyers noted that one of the Viagene sponsored protocols (FDA IND #5107 by M. Conant et al.) which did not involve NIH funding enrolled 44 men but no women. Ms. Rothenberg remarked that ethnic background and race should be included in the patient information.

Mr. Capron was disappointed that of the 72% of patient deaths on whom autopsy was requested, only 16% of the autopsies were conducted. Dr. Chase said that a higher compliance of autopsy needs cooperation from IRB and inclusion of specially trained personnel in the research team. Mr. Capron said if autopsy is essential to evaluate clinical effect of gene therapy, the autopsy rate should be increased. If the requirement to request an autopsy is not important, it should be dropped from the Informed Consent document. Dr. Smith said that local IRB has the final authority on whether autopsy should be requested. The disparate rate of autopsy among different protocols reflects the significance with which a generalized autopsy is regarded by the investigators as related to their particular studies. Dr. Ross said that the current autopsy rate of 16% is an

improvement from the last Data Management Report of 7%.

Dr. Walters noted that only one protocol (sponsored by Viagene) has not been reviewed by the RAC. It was solely reviewed by FDA (IND #5107). Ms. Meyers said that lack of woman enrollment in this HIV protocol is a deplorable situation.

Dr. Ross noted that several cystic fibrosis protocols reported detection of replication competent adenovirus and Protocol #9306-044 found replication competent retrovirus in one lot of vector preparations.
Acquired Disorders/Dr. Motulsky

The RAC has reviewed 2 protocols to date that are categorized as Acquired Disorders: (1) #9409-088 (peripheral artery disease), and (2) #9406-074 (rheumatoid arthritis). 4 subjects have undergone gene transfer on #9409-088; preliminary data suggests increased collateral artery network in 1/4 patients (increased vascularity on 4-week follow-up).

Infectious Diseases/Dr. Straus (presented by Dr. Smith):

A total of 9 protocols for human immunodeficiency virus type 1 (HIV-1) have been reviewed by the RAC to date. Two therapeutic approaches have been proposed: (1) immunotherapy, and (2) replication inhibition. Protocol #9202-017 (Greenberg): (1) reduction in quantitative HIV cultures in 2/5 subjects during CD8(+) cytotoxic T lymphocyte (CTL) administration, (2) evidence of hygromycin resistant CTL in peripheral blood, and (3) development of CD8(+) responses to epitopes derived from hygromycin-thymidine kinase in 5/6 subjects (explains transient survival of gene-modified CTL).

Protocols #9306-048, #9312-062, #9503-105, non-RAC reviewed IND #5107 (Galpin/Casciato/Merritt, Haubrich/Merritt, Parenti/Frame/Loveless/Powderly/Haubrich/Merritt, Conant/Lang/Merritt): Results pending.

Protocol #9403-069 (Walker): Preliminary results indicate gene transfer and expression in target cells.

Protocol# 9306-049 (Nabel): Preliminary results indicate 1% gene transfer in target cells by quantitative polymerase chain reaction (PCR) and evidence of gene expression by limiting dilution PCR.

Protocol #9309-057 (Wong-Staal): Study has not been initiated - pending FDA approval.

Protocol # 9503-103 (Morgan): Recently initiated - data pending.

Inherited Genetic Diseases/Dr. Motulsky

The RAC has reviewed a total of 20 protocols involving 8 monogenic diseases: (1) Alpha-1 Antitrypsin (#9403-070); (2) Chronic Granulomatous Disease (#9503-104); (3) Cystic Fibrosis (#9212-034, 9212-035, #9212-036, #9303-041, #9303-042, #9312-066, #9312-067, #9409-083, #9409-085, #9409-091, #9412-094); (4) Familial Hypercholesterolemia (#9110-012); (5) Fanconi Anemia (#9406-078); (6) Gaucher Disease (#9306-046, #9306-047, #9312-061); (7) Hunter Syndrome (#9409-087); and (8) Severe Combined Immune Deficiency due to Adenosine Deaminase Deficiency (SCID-ADA) (#9007-002).

Alpha-1-Antitrypsin (#9403-070/Brigham): Pending FDA Approval

Chronic Granulomatosis (#9503-104/Malech): Recently initiated: data pending.

Cystic Fibrosis (#9212-034/Crystal): (1) evidence of vector mediated Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) messenger ribonucleic acid (mRNA) in respiratory epithelium; level and duration of expression consistent with preclinical data (*Nature Genetics 8*: 42-51, 1994), (2) efficiency of gene transfer consistent with preclinical data (*Nature Genetics 8*: 42-51, 1994), (3) modification of nasal potential difference toward normal (not published), (4) no evidence of humoral immunity (*Nature Medicine 1*: 182-184, 1995), (5) viable adenovirus cultured from pharynx of 1 patient at 1 time point (*Nature Genetics 8*: 42-51, 1994), and (6) 2 patients demonstrated mild to moderate fatigue, fever, hypoxemia, pulmonary infiltrate, and lung function abnormalities probably related to gene transfer - all symptoms transient and patients' conditions returned to baseline levels (*Nature Genetics 8*: 42-51, 1994).

Cystic Fibrosis (#9212-035/Wilson): (1) preliminary evidence of gene transfer in 1 subject demonstrated by *in situ* hybridization of bronchial epithelial cells (Days 4 and 90).

Cystic Fibrosis (#9212-036/Welsh): (1) evidence of gene transfer and expression in nasal epithelial cells in 4/4 subjects and efficiency of transfer comparable to preclinical data (*Cell 75*: 207-216, 1993); (2) focal correction of the Cl- transport defect following a single administration in 4/4 subjects, elevated basal transepithelial voltage and normal response to cAMP agonist following treatment (*Cell 75*: 207-216), and (3) inflammatory response (mild trauma and possible ischemia) probably associated with anesthetics and vasoconstrictors used prior to vector administration (not published).

Cystic Fibrosis (#9303-041/Wilmott, Whitsett, Trapnell): (1) no evidence of gene transfer and expression demonstrated by reverse transcriptase-PCR (RT-PCR) and *in situ* CFTR mRNA, (2) viable adenovirus cultured 1 day post vector administration (nasopharyngeal and rectal swabs), and (3) 1 subject hospitalized for pulmonary exacerbation with hemoptysis (probably disease related but not related to gene transfer).

Cystic Fibrosis (#9303-042/Boucher, Knowles): preliminary evidence of gene transfer and expression by RT-PCR in 5/12 subjects and by *in situ* hybridization in 1/6 subjects, (2) minimal evidence of efficacy for correction of CFTR Cl- secretory defect as evaluated by bioelectric measures and cAMP mediated CFTR-CL- secretion in vector-treated side (minimal functional evidence of gene transfer), (3) residual viral DNA demonstrated 8 days post vector administration, (4) viable virus cultured from rectal samples 2 days post vector administration; and (5) evidence of local toxicity on vector-treated side in the highest dose cohort (nasal inflammation, myringitis, sore throat, increased nasal secretions, mandibular angle tenderness) probably related to gene transfer.

Cystic Fibrosis (#9312-067/Welsh): (1) changes in the measurement of transepithelial electric potential difference across the nasal epithelium in 2 subjects following dose #3, (2) nasal erythema in 5/6 subjects at doses 2 x 106 Infectious Units (IU) (data pending, partially blinded), (3) low levels of replication-competent adenovirus (RCA) detected in several vector lots using HeLa/A549 cell assay (RCA not detected by A549/293 cell assay for adenoviral hexon protein), (4) 1 subject experienced mild chills following 6 x 109 IU possibly related to gene transfer procedure - resolved 15 minutes post vector administration, (5) 5 subjects experienced nasal erythema (2 x 108 IU)

probably related to gene transfer, and (6) 1 subject demonstrated bilateral erythema on 2 isolated occasions and bilateral erythema in non-treated nostril on another occasion - probably related to gene transfer.

Cystic Fibrosis (#9409-091/Dorkin, Lapey): (1) low levels of RCA detected in several lots using HeLa.A549 cell assay (RCA not detected by A549/293 cell assay for adenoviral hexon protein), (2) 1 subject had chest congestion through Day 14 post vector administration, and "patchy ground glass opacities" by computer tomography scan on Day 3 (resolved by Day 28) - not determined whether related to gene transfer procedure.

Familial Hypercholesterolemia (#9110-012/Wilson): evidence of gene transfer, expression, biological activity, and immune response (*Nature Genetics 6*: 337-340, 1994).

SCID-ADA (#9007-002/Blaese): (1) Evidence of gene transfer was demonstrated in circulating T cells 3 years post gene transfer with efficiency of gene transfer comparable to Rhesus monkey marrow; (2) Gene expression was demonstrated in circulating T cells of 1 subjects by adenosine deaminase (ADA) enzyme assay 3 years post infusion; (3) Subjects receiving transduced T cells developed cellular and humoral immunity as demonstrated by increased numbers of circulating T cells (2/2 acquired normal DTH skin tests, 2/3 developed significantly increased titers of isohemagglutinins, reconstitution of interleukin (IL)-2 production to common antigens, and CTL activity against influenza and allogeneic cells); (4) Granulocyte colony stimulating factor mobilization of CD34(+) peripheral blood cells was a technical failure since the vector supernatant was cytotoxic; (5) Evidence of gene transfer and expression was demonstrated in newborns receiving transduced cord blood and placenta cells. 12-24% clonogenic myeloid progenitors transferred contained the transferred gene as measured by G418 resistance. Persistence of the transferred gene as demonstrated by semi-quantitative PCR in circulating mononuclear cells and granulocytes for 18 months (1/3000 - 1/10,000). Efficiency of gene transfer in cord blood and placenta cells was comparable to Rhesus marrow, but less than murine marrow. Preliminary data suggests that transduced lymphocytes have a selective advantage as compared to polyethylene glycol (PEG)-ADA dependent T lymphocytes. RT-PCR demonstrated vector-derived transcripts in ribonucleic acid (RNA) extracted directly from peripheral blood mononuclear cells. 2-6% of bone marrow colony forming units were G418 resistant. T lymphocyte levels have increased to normal levels, normal antigen and mitogen-induced antibody responses have developed and persisted to present. All patients are concurrently receiving PEG-ADA.

Dr. Miller stated that one should consider the question of how stringent the investigators are required to assure that there is no replication competent virus since the cost of these tests are becoming prohibitive, and how rigorous a study should be designed and conducted so that useful information will be obtained from the clinical trials. Dr Motulsky said that he would encourage more basic work devoted to the development of nonviral vectors; safety issues of vectors should be a real concern of the RAC. Responding to a question by Dr. Dronamraju about publishing the data collected by the Data Management Report, Dr. Smith said such an effort is being made to assemble the information for publication in a scientific journal. Ms. Meyers inquired about the reason why several RAC approved protocols have yet accrued any patients. Dr. Parkman said the delay is most likely pending FDA approval of the delivery systems. Dr. Smith said studies that involve *ex vivo* gene transfer or with industry sponsors appear to enroll patients with less delay.

Cancer/Drs. Erickson and Samulski:

A total of 51 gene therapy protocols for cancer have been reviewed by the RAC. Protocols are

categorized by therapeutic approach as follows: (1) antisense, 2 protocols, (2) chemoprotection, 4 protocols, (3) immunotherapy/*ex vivo* transduction, 23 protocols, (4) immunotherapy/*in vivo* transduction, 7 protocols, (4) pro-drug/HSV-TK/ganciclovir, 11 protocols, and (5) tumor suppressor gene, 4 protocols.

Dr. Samulski stated that the low level of transduction efficiency is the major concern for cancer protocols. Dr. Erickson noted that 92 subjects have undergone gene transfer on 10 cancer immunotherapy trials involving *in vitro* transduction to date. 52 of those patients have died either during or following their participation in these studies. Dr. Erickson noted that although reduction of tumor nodules has been observed in several studies, treatments such as Bacille Calmette-Guerin (BCG) injection of nodules have accomplished equivalent responses for over 20 years. Cancer immunotherapy by *in vitro* transduction with foreign genes has demonstrated little toxicity, little biological effectiveness, and minimal new knowledge on immunological responses to cancer.

Summarized below are the significant findings:

Protocol #9206-018 (Brenner): Preliminary data suggest resolution of pulmonary nodules in 1 subject, and cellular immunity was demonstrated by phenotyping and cytotoxic assays (eosinophilia 2/7 subjects, increased cytotoxic activity in 2/7 subjects, and systemic CD16/AK response in 2/7 subjects). Humoral immunity was not demonstrated. Problems were encountered in selecting transfectants from cell lines. The data were not published.

Protocol #9206-021 (Gansbacher): Preliminary data suggest stable disease in 1 subject. Evidence of gene expression for 12 months was demonstrated by enzyme-linked immunosorbent assay (ELISA) and CTL assay. The data were not published.

Protocol #9209-033/Lotze (Rubin): Preliminary data suggest activated epithelium in skin biopsies (macrophage and lymphocyte proliferation surrounding microvasculature), stable disease in 1 subject, and minor responses in 3 subjects. Cellular immunity was demonstrated by CD3(+) infiltrate at vaccination site as a function of IL-4 dose, vascular cell adhesion molecules induction, and tumor-specific CD4(+) cells (melanoma only). Fever, chills, diarrhea, and skin irritation were experienced that was related to vector administration. The data were not published.

Protocol #9303-040 (Simons): Grade 1 and 2 skin toxicities at dose level 2 -- accrual continues at this dose level.

Protocol #9306-043 (Seigler, Merritt): Preliminary data suggest enhanced major histocompatability complex (MHC) expression in transduced cells detected by immunofluorescence assays. Secretion of biologically active -interferon detected by virus inhibition assay. Enhanced cellular immunity demonstrated by Cr51-release, proliferation assays, phenotype, lymphokine profile. Enhanced humoral immunity demonstrated by ELISA, Radioimmunoassay (RIA), and serum absorption followed by ELISA and RIA. The data were not published.

Protocol #9202-013 (Nabel): Evidence of *in vivo* gene transfer, expression, biological activity, and enhanced immune response was demonstrated. Published data: (1) *Proceedings of the National Academy of Sciences 90*: 11307-11311, 1993, and (2) *Human Gene Therapy 5*: 1089-1094, 1994.

Protocol #9306-045 (Nabel): Preliminary evidence of gene transfer *in vivo* was demonstrated by PCR of biopsy tissue 1-3 days post gene transfer, evidence of *in vivo* gene expression

demonstrated by RT-PCR and immunohistochemistry, expression of recombinant protein observed using monoclonal antibodies in tissue section, and evidence of cellular immunity demonstrated by *in vitro* assays for cytokine and CTL activity in tumor biopsies of selected patients. The data were not published.

Protocol #9312-064 (Rubin): Preliminary evidence of MHC human leukocyte antigen (HLA)-B7 expression and immune response was reported. The data were not published.

Protocol #9403-072 (Hersh): Preliminary evidence of MHC HLA-B7 expression and immune response was reported. Preliminary data suggest reduction of tumor size in 6/15 subjects. Cellular immunity has been demonstrated by CTL assay. Subjects experienced severe pain on injection.

Protocol #9202-016 (Freeman): No evidence of antitumor response as demonstrated by clinical follow-up, imaging, and CA125 levels. Cytokine production data was pending. Subjects experienced Grade 1 and 2 fever, chills, nausea, and abdominal pain related to gene transfer procedure. The data were not published.

Protocol #9206-019 (Oldfield): Hemorrhage in 2 subjects related to gene transfer procedure.

Protocol #9303-037 (Van Gilder, Berger, Prados): Preliminary evidence of immune response demonstrated by Western blot of p30 (9/57 samples - 3 subjects tested). Multiple serious adverse events were determined to be result of disease progression and not related to gene transfer procedure.

Protocol #9403-031 (Roth): Preliminary data suggest tumor regression in both lesions on Day 5 post vector administration in 1/1 subject. 87% tumor regression observed 1 month post vector administration, and tumor biopsy revealed fibrosis with no evidence of viable tumor cells. The data were not published.

Gene Marking/Parkman:

Protocols #9102-004, 9105-005, 9105-006, and 9105-007 (Brenner): Significant findings have been published as a result of these marking studies regarding the origin of relapse. The following peer-reviewed publications have resulted from these trials: (1) *Lancet 342*: 1134-1137, 1993; (2) *Lancet 341*: 85-86, 1993; (3) *Blood 84*: 380-383, 1994; and (4) *Blood 83*: 3068-3075.

Protocols #9206-023 and 9206-024 (Dunbar): Long-term marking (18 months) was demonstrated in 2/6 evaluable subjects. Neomycin resistance (neoR) was detected in granulocytes and lymphoid lineages. Low transduction efficiency (*ex vivo*, 10-50% and *in vivo*, 1%). Transduction procedure modified to include plus and minus exogenous growth factors and autologous stroma.

Protocol #9206-026 (Walker): Preliminary evidence of gene transfer of CD4(+) and CD8(+) peripheral blood cells was demonstrated 20-40 weeks post transduction by neoR PCR. The data was not published.

Dr. Smith stated that limited post mortem data would be particularly valuable in the gene marking protocols. Mr. Capron said if that is the case, the RAC should make specific recommendations rather than demanding the generalized autopsy requirement for all protocols. Dr. Motulsky agreed that a general autopsy will not yield much useful information. A subcommittee of pathologists needs to carefully define what specific questions should be examined in an autopsy, e.g., the

presence of vector sequences in target and unintended organs. Dr. Smith thanked Ms. Wilson for her efforts to manage the data reporting. Dr. Smith welcomed suggestion as to what additional information should be collected in the future, and how to disseminate this information to the public at large. Dr. Walters suggested that the draft manuscript of the data reporting prepared for publication should be circulated among RAC members for comment.

VII. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *THYMIDINE KINASE (TK) TRANSDUCED DONOR LEUKOCYTE INFUSIONS AS A TREATMENT FOR PATIENTS WITH RELAPSED OR PERSISTENT MULTIPLE MYELOMA AFTER T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANT/DRS. MUNSHI AND BARLOGIE*

Review--Dr. Glorioso

Dr. Walters called on Dr. Glorioso to present his primary review of the protocol submitted by Drs. Nikhil C. Munshi and Bart Barlogie of the University of Arkansas for Medical Sciences, Little Rock, Arkansas. Dr. Glorioso stated the aims and background for this study. Allogeneic bone marrow transplantation is a possible treatment for selected patients with multiple myeloma. The curative potential for allo-transplant of donor T cells is based on the hypothesis that the T cells mediate rejection of the tumor cells. However, the adoptive transfer of the immunocompetent graft often leads to a vigorous graft versus host disease (GVHD) with accompanying severe morbidity and 50% mortality. The applicant speculates that the graft versus tumor response may precede the GVHD providing a window of opportunity for efficacy of tumor treatment before rejection of the normal host tissue. This possibility has initiated the proposal of introducing the *Herpes simplex* thymidine kinase (HS-TK) gene into the donor T cells prior to transplant in order to provide a mechanism for depletion of the donor graft if and when the GVHD begins. This method would allow the possible antitumor response to proceed with the subsequent removal of the donor graft if GVHD arises. This protocol is encouraged by: (1) the clinical findings that a second relapse or persistent tumor growth may not be managed by additional chemotherapy without a very poor prognosis, and (2) the experimental data indicating that infusion of donor leukocytes has been successfully used in the treatment of chronic myelogenous leukemia where it has produced hematologic, cytogenetic, and molecular remissions in the majority of patients relapsing after allogeneic marrow transplantation. The aims of this protocol are: (1) to determine the safety of infusing HS-TK-transduced donor lymphocytes followed by ganciclovir (GCV) into multiple myeloma patients who have undergone allogeneic bone marrow transplantation; (2) to determine the efficacy of the HS-TK/GCV method for decreasing the clinical manifestations of severe acute and chronic GVHD; (3) to determine the anti-myeloma effect of donor lymphocytes; and (4) to determine the occurrence of bone marrow hypoplasia following transduced lymphocyte infusions and the efficacy of the HS-TK/GCV approach for its prevention.

Dr. Glorioso said that he found the approach of this protocol reasonable. A minor mistaken statement in the nontechnical abstract has been corrected. This protocol is not a gene therapy for multiple myeloma but rather is to treat the secondary effects of the GVHD. Dr. Glorioso was concerned about the fact that the transduction rate is about 85% not 100%, and there may be enough non-transduced T cells capable of inducing unwanted GVHD. The investigators argued that it is particularly difficult to get 100% transduction, and they consider that the treatment may be efficacious at the present level of transduction. Finally, Dr. Glorioso asked if the infusion of HS-TK-transduced T cells would increase the risk of human cytomegalovirus (CMV) infection. The investigators responded in writing that if CMV infection occurs, it will be treated with Foscarnet. Dr. Glorioso recommended approval of the protocol.

Review--Dr. Ross

Dr. Ross stated that the protocol is relatively straight forward, and that the investigators have adequately responded to the *Points to Consider*. Dr. Ross asked about the rationale for choosing Day 21 to administer GCV; because in this particular study, timing is important. The investigators responded that in chronic myelogenous leukemia patients, the GVHD develops at a median time frame of 32 days. The two post-transplantation patients who were treated with leukocyte infusions by the investigators developed GVHD more than 21 days post donor cell infusion. Based on these observations, the investigators selected Day 21 for initial GCV administration. Dr. Ross was satisfied with the response, and she said other questions about the Informed Consent document were all satisfactorily responded by the investigators. Dr. Ross stated that she would recommend approval of the protocol.

Review--Dr. Brinckerhoff (presented by Dr. Glorioso)

Dr. Glorioso stated that Dr. Brinckerhoff had several concerns: (1) If 80 to 90% of cells are killed *in vivo* by GCV, as the cell dose escalated, the remaining cells not killed by GCV will increase. Is this a sufficient killing rate to "cure" GVHD? The investigators responded that there is fairly low number of patients developed severe GVHD, i.e., 8 out of 81 treated. If GVHD occurs and treatment with GCV is not adequate, the investigators would use a combination of immunosuppressive drugs with GCV therapy as a fall back treatment. Dr. Glorioso said the response is reasonable. (2) The investigators suggested that the "bystander" effect of cell killing of non-transduced cells does not play a major role in their system since these cells do not have cell/cell contacts that mediate the effects. Dr. Brinckerhoff asked if the investigators have performed a positive control experiment. The investigators stated in response that an experiment has been performed using the HS-TK-transduced HeLa cells as a positive control, and the bystander effect is present in these cells unlike the lymphocyte population. (3) The investigators mentioned "a desire to limit the bone marrow cytotoxicity of GCV." Dr. Brinckerhoff questioned what is the mechanism of this toxicity, how common is it, and how serious a problem is it? The investigators responded that toxicity is common with total GCV infusions of 200-300 mg/kg body weight. The study uses only 5 mg/kg, infused twice a day for 5 days and will not be a problem with a 5 day treatment plan. (4) The investigators mentioned that one consequence of immune deficiency in a T-cell depleted allogeneic bone marrow transplant is an increased risk of Epstein-Barr virus (EBV) lymphoma. Dr. Brinckerhoff asked if this is a phenomenon shared with other T-cell depleted conditions, what is the incidence, and is this a lethal complication? The investigators responded that the infusion of donor lymphocytes after a T-cell depleted allograft prevents the development of EBV lymphoma. EBV-related B-cell lymphoproliferative disease has been documented in immunodeficiency conditions and in many organ transplantations.

Other Comments

Dr. Parkman stated that donor leukocyte infusions after bone marrow transplantation is a standard therapy at the investigators' institution. The basic rationale of the protocol is sound and that there is no "bystander" effect needed in this treatment. The RAC should consider the incremental risks, if any, that are associated with the gene therapy aspects of the protocol. Dr. Parkman asked what will the treatment plan of initial GCV infusion impact on the standard GCV therapy of CMV infection? CMV infection occurs to some transplant recipients before GCV infusion on Day 21.

Dr. Zallen asked if there is any conflict of interest in obtaining informed consent from the patients since Dr. Munshi will be their primary care physician. She asked if a statement about long-term

follow-up is in the Informed Consent document.

Dr. Miller cited a recent finding by Drs. Stanley Riddell and Philip Greenberg (Protocol #9202-017) that a CTL response induced by the transduced gene in T-cells rapidly ablates the subsequently administered T-cells carrying the same antigen. This observation might complicate the interpretation of the data of T-cell ablation as to whether it is due to GCV infusion or a CTL response. Dr. Parkman remarked that such a complication is unlikely to occur in this case of T-cell depleted transplant. Dr. Glorioso asked if there are other ways to treat the cells with antibodies. Dr. Parkman said the present proposal to instantaneously kill the cells is an appealing approach. The same effect can be achieved with monoclonal antibodies, but it has the same kind of problem of repeat administration of gene-modified cells. Dr. Lai asked if the *neo*R gene selected T-cells would still induce a CTL response.

Investigator Response--Drs. Munshi and Barlogie

Responding to Dr. Glorioso's question of treating CMV infection, Dr. Munshi said it will be treated with Foscarnet which is not a nucleoside analog and will not affect HSV-TK-transduced cells. Responding to Dr. Zallen's question on conflict of interest in obtaining informed consent, Dr. Munshi said the patients will be entered by his colleagues on the transplant team, and Dr. Munshi will be responsible for following up the patients during the study. The statement of long-term follow-up was included in the revised Informed Consent document. Responding to Dr. Miller's question about the CTL response in the transduced cells, Dr. Munshi said he agrees with Dr. Parkman's opinion that the probability of developing a CTL response is low. The myeloma patients who inherently have immune problems and who are likely to develop idiolymphoma due to immunosuppression, the chance of developing a CTL response to the transduced cells is even lower. Responding to Dr. Lai's question of allo-reactivity of the *neo*R cells, Dr. Munshi said that they have data showing that the *neo* selected cells are immunologically active.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Ross to accept the protocol submitted by Drs. Nikhil C. Munshi and Bart Barlogie, University of Arkansas for Medical Sciences, Little Rock, Arkansas. The motion was approved by a vote of 20 in favor, 0 opposed, and no abstentions.

Protocol Summary: Drs. Nikhil C. Munshi and Bart Barlogie of the University of Arkansas for Medical Sciences, Little Rock, Arkansas, may conduct gene transfer experiments on 21 subjects (>18 and <65 years of age) with relapsed or persistent multiple myeloma who are undergoing T cell depleted allogeneic bone marrow transplantation. Donor peripheral blood lymphocytes will be cultured *in vitro* with interleukin-2 and anti-CD3 monoclonal antibody. T cell depleted lymphocytes will be transduced with the retroviral construct, G1Tk1SvNa.7, which encodes the *Herpes simplex* virus thymidine kinase (HSV-TK) gene. The transduced cells will be reinfused. In this dose escalation study, 3 subjects will undergo cell-mediated gene transfer per cohort (maximum of 5 cohorts) until Grade III or IVGVHD is observed. A maximum of 6 additional patients may be entered at that maximum tolerated dose. The objectives of this study are to determine: (1) the safety of transduced donor cell infusions, (2) the effectiveness of donor cell infusions in decreasing the effects of severe GVHD, (3) the effectiveness of donor cell infusions in eliminating multiple myeloma remission, and (4) the effectiveness of ganciclovir in eliminating donor cells for the purpose of preventing bone marrow hypoplasia.

VIII. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *ADOPTIVE CELLULAR THERAPY OF*

CANCER COMBINING DIRECT HLA-B7/-2 MICROGLOBULIN GENE TRANSFER WITH AUTOLOGOUS TUMOR VACCINATION FOR THE GENERATION OF VACCINE-PRIMED ANTI-CD3 ACTIVATED LYMPHOCYTES/DRS. FOX AND URBA

Review--Dr. Brinckerhoff (presented by Dr. Chase)

Dr. Walters called on Dr. Chase to present Dr.Brinckerhoff's primary review in her absence of the protocol submitted by Drs. Bernard A. Fox and Walter J.Urba, Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon. Dr. Chase stated that the objective of this proposal is to determine if modifications of an autologous tumor vaccine with lipid complex-mediated allogeneic HLA-B7/2 microglobulin gene transfer will augment the sensitization of anti-tumor effector T-cells in the lymph nodes draining the tumor vaccine injection site. The investigators have previously performed a Phase I study to analyze the toxicity and effects of direct gene transfer into tumor cell nodules, a protocol that was approved by the RAC in June 1993 (#9306-045). This trial is currently underway and no toxicity has been demonstrated. The investigators now propose 3 modifications: (1) *In vitro* lipofection of allogeneic HLA-B7 DNA into autologous tumor cells which will be used for vaccination at one of two sites not involved with tumor. The contralateral site will receive unmodified autologous tumor cells plus BCG; (2) Removal of tumor vaccine-draining lymph node 7 to 14 days after vaccination so that the T-cells obtained from the lymph nodes can be activated *in vitro* with anti-CD3 antibody and IL-2; and (3) Following activation and expansion of the tumor vaccine-draining lymph nodes, lymphocytes will be adoptively transferred to the patients along with IL-2.

It would appear that the modifications such as the *in vitro* transfection of tumor cells, are less invasive and more benign than the protocol originally approved. Although parts of this protocol are a minor modification, other changes are more substantive to warrant some discussion and explanation. In addition, parts of the protocol are confusing.

Dr. Brinckerhoff raised 5 specific questions. (1) The investigators are unclear about the similarities and dissimilarities between the present and previously approved protocol. For example, what are the differences in the numbers of cells that will be transduced and injected? Will this have an impact on the patients? (2) For the adoptive transfer, will the activation of tumor-vaccine draining lymph node cells by IL-2 and anti-CD3 have any deleterious effects on the patients? What data are available on the effects of injecting 1011 cells? Is this a standard number? (3) The protocol calls for short-term culture of the tumor cells and for their *in vitro* transfection. What data are available on the ability of tumor cells derived from different tumors to be cultured successfully? Are cells derived from some patients more refractory to culture than others? If so, how will the investigators address this problem? (4) The experimental data presented in the protocol demonstrate no enhanced efficacy with "tumor + DNA" versus "tumor + BCG + DNA." Why do the investigators propose to use BCG with all of its potential toxicities and side effects? The rationale for this portion of the protocol needs to be described. (5) The Informed Consent document is too long and complicated. The investigators have provided a written response to the above questions, and Dr. Chase said most of the questions appear to be addressed. He had a similar question regarding the protocol design involving BCG.

Review--Dr. Chase

Dr. Chase raised 5 specific concerns. (1) There is no explicit statement of how the outcome variable is to be measured under the section labeled "Statistical Considerations." (2) There is no formal statement of a null hypothesis. (3) There are too many questions being asked in a study of a

small sample. The BCG part of the trial seems to detract power from the objective of assessing the effect of the HLA-B7 "treatment" component. (4) The number of patients used at each dosage level will be too small to develop reliable estimates of a treatment effect in most cases. (5) The error probabilities are not explicitly specified. There is no discussion of Type I or Type II error probabilities. These objections could be made against most RAC approved protocols. This protocol is lacking basic principles in experimental design with highly sophisticated and complex treatment modalities for a desperate illness which does not automatically assure that useful scientific information will be obtained. In summary, Dr. Chase would unenthusiastically support approval of this protocol.

Review--Dr. Dronamraju

Dr. Dronamraju stated that the experimental design seems rather cumbersome. He asked whether too many variables raise the possibility of confounding effects. What is the point of including BCG in the study especially when BCG does not enhance efficacy? What about possible adverse effects of BCG? Is the risk worth taking? What is the risk/benefit ratio? Dr. Dronamraju was concerned about the dosing schedule. Dr. Dronamraju inclined to approve the protocol if the questions raised have been satisfactorily responded to by the investigators.

Other Comments

Dr. Parkman pointed out a table in the protocol describing the outline of protocol treatment groups in order to clarify Dr. Chase's question of the protocol design. Dr. Parkman said that the reason to include the BCG part of the study is not to deprive the patients of an established experimental therapy for melanoma using BCG plus tumor vaccine. This therapy has been incorporated in the practice of the investigators' institution. Dr. Parkman considered the experimental design to be reasonably good to accommodate an established experimental therapy with a dose escalation study of an innovative gene therapy. The issue is for non-end stage patients and the availability of other experimental therapy is a factor to be considered in the design of a gene therapy study. There is an ethical issue of depriving patients with the standard BCG treatment if the protocol design is altered. Dr. Chase said that he agrees with Dr. Parkman's general point except the inclusion of Cohort 6 patients who receive BCG on both thighs. The inclusion of this cohort has complicated the study design as pointed out by all 3 reviewers. Dr. Parkman explained that BCG therapy has been practiced for 25 years; and in melanoma patients, a certain proportion of tumors have regressed presumably due to an anti-tumor immune response induced by BCG in combination with tumor cells.

Dr. Zallen noted that the Informed Consent document stated that if a patient is ineligible to enter the present study due to an unresponsive lymph gland, he/she will be given IL-2 alone. If the IL-2 alone is another ongoing protocol, and if a patient signs up for the present study, could he/she consent to be part of other protocols? Dr. Zallen said most of her concerns about the Informed Consent document have been addressed in the revised document. She objected to the use of the term "vaccine/vaccination" in the Informed Consent document. She asked if HLA-B7 typing will be performed before entrance to the study. Ms. Meyers asked the investigators to clarify why there are two versions of the Informed Consent document. She noted that the cost of the treatment needs to be clarified, and she asked if the protocol would accept patients who have no insurance coverage. The statement of lifetime follow-up needs clarification.

Dr. Lai asked why Dr. Chase would recommend approval if he still considers the experimental design to have so many deficiencies to obtain useful information. Dr. Chase said this core issue

has confounded the RAC many times. He hoped that the investigators would benefit from RAC's advice and make some changes in their protocols. Dr. Lai said if one cannot evaluate the data, what is the point of conducting the experiment. Dr. Parkman explained a general problem of conducting a Phase I study. The primary endpoint of a Phase I study is toxicity without a requirement of obtaining any efficacy or biological information; this issue has been discussed by the *Ad Hoc* Review Committee regarding the review criteria. Ms. Meyers said that she is concerned about the scientific quality of the research and about using human beings as study subjects if no useful information is to be obtained.

Investigator Response--Drs. Fox and Urba

Dr. Fox explained the rationale of the present protocol. The study is the 4th generation adoptive immunotherapy trial following previous studies with lymphokine activated killer cells, tumor infiltrating lymphocytes (TIL), and with cells activated by a vaccine. In the latter strategy, the lymph nodes draining the inoculation sites are removed, and the cells are then activated *in vitro*, and are reinfused back to the patients. Dr. Fox said that BCG treatment is the standard care for his renal cell carcinoma and melanoma patients, and BCG treatment has shown some promise for these types of cancer. He said the present protocol is built on the ongoing experience and with the additional gene therapy component. There is a problem of performing a mouse model experiment to demonstrate the additional benefits of gene transfer to the BCG plus tumor modality since the tumor grows too rapidly in mice to allow such an experiment to be conducted. Another reason to include BCG in all cohorts of the present protocol is that BCG is a general nonspecific adjuvant, and it is hoped that BCG will stimulate the immune response in cancer patients who are anergic.

As a point of clarification, Dr. Urba said that IL-2 and BCG are standard treatments for their cancer patients. All patients will get IL-2 plus tumor vaccination and BCG in their right legs, and the gene transfer experiment is on the lymph nodes of the left legs where the experiment is trying to generate specific T cell immunity in the lymphocytes draining the left legs. The study design is based on the practical limit of available patients and is aimed at obtaining maximum scientific information. Dr. Chase was concerned that attempting to answer too many questions in an single experiment might end up with not answering any of the questions. Dr. Parkman explained that a single variable in this protocol is whether the left legs are getting nontransduced tumor cells or tumor cells that have an increasing doses of HLA-B7.

Responding to Dr. Zallen's question of IL-2 treatment, Dr. Fox said if the lymph nodes are not enlarged, those patients will just receive the standard treatment with IL-2. Dr. Fox agreed to revise the Informed Consent document to clarify questions of the technical description of lipofection procedure, the HLA-B7 testing, and the long-term follow-up of patients. Responding to the question of treatment cost, Dr. Fox said that any laboratory tests related to the DNA/liposome complex part of the study will not be charged to the patients; and for those who have no insurance coverage, the costs will be paid for by a hospital fund.

Dr. Fox explained the rationale to include the 6th cohort of BCG alone treatment in the experimental side of the legs. If a negative result is obtained from experiments in other cohorts, this BCG alone experiment of the last cohort will be informative about the immune status of those advanced cancer patients as a possible cause of treatment failure.

Dr. Fox agreed to revise the Information Consent document to substitute the term "vaccine" with "tumor inoculation," and to include a numerical denominator for the severity of infection.

Ms. Meyers asked if important information will be forthcoming from the present protocol. Dr. Parkman found that the hypothesis is reasonable and useful scientific information should be obtained from the study of the draining lymph nodes. Dr. Chase although found the study design unsatisfactory; however, if the investigators publish all the data, and the data can be reinterpreted by other investigators for example omitting the controversial data of Cohort 6, some useful information will be generated.

Committee Motion

A motion was made by Dr. Chase and seconded by Dr. Secundy to accept the protocol submitted by Drs. Bernard A. Fox and Walter J. Urba contingent on the review and approval by Dr. Secundy of a revised Informed Consent document incorporating the changes suggested by Ms. Meyers and Dr. Zallen. The motion was approved by a vote of 17 in favor, 0 opposed, and 4 abstentions.

Protocol Summary: Drs. Bernard A. Fox and Walter J. Urba of Earle A. Chiles Research Institute, Providence Medical Center, Portland, Oregon, may conduct gene transfer experiments on 18 subjects (18 years of age) with metastatic renal cell carcinoma or melanoma. Autologous tumor cells will be surgically removed, transduced *in vitro* with the cationic liposome plasmid vector, VCL-1005, which encodes HLA-B7 and beta-2 microglobulin. Subjects will receive subcutaneous injection of lethally irradiated transduced cells in one limb. The contralateral limb will be injected with lethally irradiated untransduced tumor cells in combination with BCG. Approximately 21 days following tumor cell injection, subjects will undergo lymphadenectomy for subsequent *in vitro* expansion of anti-CD3 activated lymphocytes. Activated lymphocytes will be adoptively transferred on Day 35 in combination with a 5-day course of IL-2. Approximately on Day 45, subjects will receive a second cycle of IL-2. The objectives of this study are to determine: (1) the safety of administering anti-CD3 activated antitumor effector T cells in draining lymph nodes, and (2) whether HLA-B7/-2 gene transfer augments the sensitization of anti-tumor effector T-cells in draining lymph nodes.

IX. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *TREATMENT OF PATIENTS WITH ADVANCED EPITHELIAL OVARIAN CANCER USING ANTI-CD3 STIMULATED PERIPHERAL BLOOD LYMPHOCYTES TRANSDUCED WITH A GENE ENCODING A CHIMERIC T-CELL RECEPTOR REACTIVE WITH FOLATE BINDING PROTEIN/DR. HWU*

Review--Dr. Erickson

Dr. Walters called on Dr. Erickson to present his primary review of the protocol submitted by Dr. Patrick Hwu of the National Institutes of Health, Bethesda, Maryland. Dr. Erickson stated that this cancer therapy protocol uses a strategy of transducing autologous lymphocytes with a gene which will target them to cancer cells in order to augment immunity to the cancer. Specifically, about 70% of ovarian cancers express folate-binding protein as a cell surface antigen, and a "tail" inside the cell will stimulate the cell after binding with the antigen to "fight" the tumor. In general, the protocol follows methods already approved by the RAC. Peripheral blood lymphocytes will receive mitogen stimulation by anti-CD3 antibody. The lymphocytes will be transduced with a MFG-S vector from psi-crip producer cells by immediate suspension in the retroviral containing supernatant with the anti-CD3. The retroviral supernatant will be changed regularly, perhaps each day, for 3 days. At this point, the cells will be selected in G418 media for 5 days, and then expanded with IL-2 stimulation. Preliminary studies show that about 50% of cells are successfully transduced using this protocol. These transduced cells will then be returned to patients who will be treated with very high levels of IL-2 to stimulate continued growth. This area of protocol is not

completely clear. Dr. Erickson understood that the investigators plan escalating doses, with 3 patients at each dose, at 3 x 109, 3 x 1010, and 3 to 5 x 1010 cells either intravenously (IV) or intraperitoneally (IP). When the investigators achieve the toxic dose (although the toxicity is more likely to be related to the IL-2 administration), the investigators will extend the therapy to 6 patients at the highest dose of cells tolerated. Thus, there are potentially 12 patients for the IV route and 12 patients for the IP route. In addition, the investigators plan 2 cycles in each patient; thus, there are potentially 48 infusions of cells which is quite a large number.

The preclinical studies are encouraging, but no complete eradication of tumor has been achieved. Specifically, anti-CD3 stimulated peripheral blood lymphocytes orTIL transduced with the chimeric gene lyse ovarian cancer cells *in vitro* and produce cytokines. This stimulation occurs with the chimeric gene targeted to the ovarian antigen and not when a chimeric gene to a non-related hapten is used. Further, nude mice with IP placement of human ovarian cancer which are then given mouse TIL transduced with the chimeric gene IP (3 days following the cancer cells) had increased survival rate. There were fewer lung metastases if mice bearing a sarcoma to which the folate-binding protein antigen gene was added were treated with TIL transduced with the specific chimeric gene and IL-2. However, in both situations, complete eradication of tumor did not occur. Given the high density and adherent spread of human ovarian cancer, it seems unlikely that human ovarian cancer cells placed for a mere 3 days in the nude mouse would create a very good animal model of the human cancer. As it has been previously pointed out by the RAC, ovarian cancer with its spread over the large area of the mesenteric membranes is a particularly difficult cancer as a target for cellular therapies. Dr. Erickson does not think that the particular animal models would have very good predictive value for humans.

Another weakness of the protocol is that the analysis of the results will not be very thorough. For instance, with the IV infusion oftransduced cells, the investigators state that 5 days after the first cycle, the patients may undergo transvaginal biopsy. Unless all patients undergo such treatment, no definitive data will be obtained. From the IP infusions, laparoscopy "may" be performed for each cycle. Although the investigators are reluctant to perform such invasive biopsy procedure on all patients, very little will be learned from the study without such information.

In terms of toxicity, while IV infusions of cells are likely to show any toxicity quite rapidly, it may be considerably delayed with the IP infusions. The Informed Consent document seems overly technical. In summary, Dr. Erickson said this protocol is acceptable if his questions are addressed satisfactorily.

Review--Dr. DeLeon

Dr. DeLeon commented on 4 issues: (1) Patient eligibility criteria. The investigators have indicated that the Mov18 antigen used to construct the chimeric receptor gene has been shown to be reactive in normal pancreas, salivary gland, kidney proximal and distal tubules among others. Although the investigators have stated that no significant toxicity has been observed at these sites in patients treated with this construct, the outcome might be different in patients with renal problems. Dr. DeLeon asked if it would be judicious to exclude patients with renal problems. (2) The investigators stressed the treatment aspect of the protocol as a major objective while evaluation of safety was mentioned as a secondary objective. Dr. DeLeon stated that since this protocol is a feasibility study, the study emphasis should be in the reverse order. (3) In evaluating the duration of survival of the transduced cells at the cancer site, the investigators propose to obtain tissues at approximately 1 month after each treatment for PCR and immunohistochemical studies. Why was a one-month time point selected? (4) The term "therapy" used in the Informed

Consent document should be replaced with "procedure." Long-term follow-up should be stated.

Review--Dr. Dronamraju

Dr. Dronamraju said that in its initial submission, the protocol description is very abbreviated. Upon his request, further information was provided. Dr. Dronamraju asked the investigators to explain fully the mouse experiment regarding the impact of treating mice with Mov-TIL, and explain the 4 objectives of the protocol.

Other Comments

Dr. Parkman asked 2 questions. (1) Selection of lymphocytes with G418 has technical difficulty. Have the investigators performed the G418 selection procedure with their cells; and after the time period of cell culture in G418, what percentage of the selected cells expresses the chimeric receptor? (2) In the animal model, were the data obtained from the experiments performed with the G418 selected cells? What were the effector/target cell ratios in the intraperitoneal animal model?

Dr. Miller said he routinely checks the DNA sequences submitted by the investigators for the presence of any open reading frame and the promoters that drive them. In the case of an unknown sequence, he checks its homology to other proteins particularly oncoproteins. Upon examining the present vector construct, Dr. Miller noticed a single open reading frame that starts upstream of the antibody cDNA insert and terminates after the start codon of the cDNA insert. Judging from the submitted data, this open reading frame apparently does not interfere with the cDNA expression. Dr. Lai noted that if the vector is constructed with DNA fragments generated by PCR, it should be fully sequenced to ensure that no mutations have been introduced by the PCR technique.

Dr. Ross said that the Informed Consent document should include statements that autopsy will be requested, participation is voluntary, and protection of privacy from the media.

Investigator Response--Dr. Hwu

Responding to Dr. Erickson's question of patient numbers, Dr. Hwu said there are 2 treatment arms of the study. Arm A is for patients with larger and bulkier tumors. These patients will be treated with IV therapy since they frequently have extensive adhesions in their peritoneal cavity. In the dose-escalation schedule, 3 patients will be treated at 3 x 109 cells, 3 patients at 3 x 1010, and 3 patients at 3 to 5 x 1010. If at any point grade 3 or 4 toxicity is seen, the treatment will be extended to 6 patients in order to collect the definitive data. A total of 15 patients will be treated at the maximum tolerated dose to obtain a statistically significant toxicity and cell trafficking data. Dr. Parkman asked what additional biological information will be obtained from the Arm A study? Dr. Hwu said the study will evaluate the question if the transduced cells administered via IV route will traffick to sites within the intraperitoneal cavity. On Day 5 after infusion and a certain day during follow-up, a total of 2 biopsies will be obtained. Dr. Parkman asked if the biopsy from the tumor will be compared to that from a normal tissue since the transduced cells may be present in the blood supply? Dr. Hwu responded that a skin biopsy will be obtained as a control.

Dr. Hwu presented additional data of his mouse model studies with slide illustration. Human ovarian cancer cells were implanted into nude mice intraperitoneally. On Day 3, the animals were sacrificed, and extensive growth of tumors was observed. On the same day, treatment with the transduced TIL was given to other groups of animals, and significant prolongation of survival is

observed in the treatment group. Dr. Hwu said that each group of 7 to 9 mice were treated with the transduced TIL selected by G418. In another experiment, lung metastasis was significantly reduced by treating with transduced TIL via a IV route.

Dr. Hwu agreed to revise the Informed Consent document to substitute the word "procedure" for "therapy," and to state that autopsy will be requested.

Dr. Hwu said that the MFG-S vector is the same one previously approved by the RAC, and he will check if the open reading frame is a typographical error of the submitted DNA sequences.

Dr. Dronamraju asked about patient age. Dr. Hwu responded that the median age of ovarian cancer is about 65, and the patient to be entered onto the study should be able to tolerate the IL-2 treatment.

Committee Motion

Dr. Erickson made a motion to approve the protocol with a stipulation to limit the patient number t 15 for each arm of the study with a total of 30 patients for the two arms of the protocol. Dr DeLeon seconded the motion.

Dr. Hwu said that he needs 15 patients for each of the maximum tolerated dose cohort so that the power of the study is enough. The total for each arm is 21 (3 at the first level, another 3 at the next level, and 15 at the maximum level), and the total for the protocol would be 42. Dr. Chase did not object to the higher numbers of patients requested, but both he and Dr. Parkman asked for a rationale for the number. Dr. Hwu said that his main concern is the number of patients from whom he can obtain the biopsy to evaluate the results. Dr. Parkman asked how many evaluable patients (biopsy at Day 5) is needed at the maximum tolerated dose to make the data interpretable? Dr. Hwu responded he would accept 10 evaluable patients at the maximum dose.

Dr. Erickson said he would agree to Dr. Hwu's request and stated his amended motion as the following: Approval with consent form changes, up to 21 patients in each arm of the study but with the expectation the investigators will stop when 10 evaluable patients have been accrued into the maximum tolerated dose cohort, and the protocol to be revised with inclusion of biopsy of normal tissue as a control. Dr. DeLeon seconded the amended motion.

An initial motion was made by Dr. Erickson and seconded by Dr. DeLeon to accept the protocol submitted by Dr. Patrick Hwu contingent on limiting patient accrual to 15 subjects per study arm (30 subjects total - 2 study arms). An amended motion was made by Dr. Erickson and accepted by Dr. DeLeon to accept the protocol contingent on review and approval of the following by the primary RAC reviewers: (1) limiting patient accrual to 21 subjects per study arm (42 subjects total - 2 study arms) and a maximum of 10 evaluable subjects for the cohort of maximum tolerated dose; (2) revise the protocol to include biopsy of normal tissue as a control, and (3) revised Informed Consent document incorporating the changes suggested by Ms. Meyers, Drs. Ross, Zallen, and DeLeon. The motion was approved by a vote of 19 in favor, 0 opposed, and 1 abstention.

Dr. Samulski stated that he abstained from voting due to his collaboration with the investigators.

Protocol Summary: Dr. Patrick Hwu of the National Institutes of Health, Bethesda, Maryland, may conduct gene transfer experiment on subjects (18 years of age) with advanced epithelial ovarian cancer. The number of subjects entered on the study will be limited to: (1) 21 subjects per study

arm (42 subjects total - 2 study arms), and (2) a maximum of 10 evaluable subjects for the cohort of maximum tolerated dose. Anti-CD3 stimulated autologous peripheral blood lymphocytes will be transduced *ex vivo* with the retroviral vector, MFGS-MOv--IN, which encodes a chimeric T-cell receptor reactive with folate binding protein. Subjects with a bulky tumor will receive intravenous administration of *ex vivo* expanded gene-modified cells. Subjects with a minimal tumor will receive intraperitoneal administration of *ex vivo* expanded gene-modified cells. The objectives of the study are to determine: (1) safety and maximum tolerated dose of intravenous and intraperitoneal administration of transduced lymphocytes, (2) whether intravenously administered transduced cells demonstrate tumor specificity, and (3) duration of survival of transduced lymphocytes in the systemic circulation and at the site of the tumor.

Other General Comments

Dr. Miller asked whether the FDA should be determining the maximum allowable patient number of a given protocol. It will monitor patient accrual as a protocol is progressing. There was no FDA spokesperson available to respond to Dr. Miller's question. Dr. Walters asked what is the rationale for choosing 3 patients per cohort in most dose escalation studies. Dr. Parkman said it is a common practice if there is no toxicity seen in 3 patients, the trial will go to the higher dose; if any toxicity is observed, another group of 3 patients will be treated. It is considered acceptable to have 1 out of the 6 patients having an adverse effect.
Dr. Jay Greenblatt from the National Cancer Institute remarked that based on his experience with FDA, FDA does advise investigators about the number of patients. The FDA advises investigators as to if a sufficient number of patients have been accrued onto a particular study and whether it should no longer need to enter more patients. Dr. Miller asked if it is a proper task of the RAC to approve a specific patient number. Dr. Chase said it is a pertinent discussion to determine what is an acceptable error rate for a given trial based on a statistical consideration. Dr. Straus said in addition to FDA, the IRB reviews the number of patients entered into a protocol.

X-A. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I STUDY OF AUTOLOGOUS HUMAN INTERLEUKIN-2 GENE MODIFIED TUMOR CELLS IN PATIENTS WITH LOCALLY ADVANCED OR METASTATIC PROSTATE CANCER/DRS. PAULSON AND LYERLY*

Dr. Walters stated that this protocol would not be reviewed by the full RAC, because it was approved through the *Accelerated Review* process prior to this meeting. There was a contingency to this approval in that there are requested changes in the Informed Consent document concerning patient responsibility for research-related costs.

X-B. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I STUDY OF AUTOLOGOUS HUMAN INTERLEUKIN-2 (IL-2) GENE MODIFIED TUMOR CELLS IN PATIENTS WITH REFRACTORY METASTATIC OVARIAN CANCER/DRS. BERCHUCK AND LYERLY*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of protocol submitted by Drs. Andrew Berchuck and H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina. Dr. Parkman stated that this protocol is a modification of a previous protocol submitted by Dr. Lyerly. In the previous protocol (#9403-086), the investigators received approval for the transduction of breast cancer cells with a liposome complex containing the human IL-2 cDNA. In the present protocol, the investigators propose to use the same liposome complex but to transduce autologous ovarian cells. After transduction, the cells will be irradiated and used in a

vaccination protocol. The IL-2 vector, pMP6A-IL2, is still pending FDA approval for the breast cancer study. The vector/liposome complex proposed for this study will be provided by Applied Immune Science (Santa Clara, California). The plasmid is based upon anadeno-associated virus. In preclinical studies, over 80% of primary ovarian cells in tissue culture weretransduced with a minimal production of IL-2 of 10ng/106 cells/24 hrs. 18 patients are proposed for the study: 6 will receive low doses, 6 receive medium doses, and 6 receive high doses which represents 5 x 106, 1.5 x 107 and 5 x 108 cells given as 4 monthly doses. The objective of the studies are to detect toxicity as well as any clinical response to the tumor. Clinical responses are not expected due to the advance stage of the patients' disease. In addition, laboratory analysis of the immunological responses to the ovarian cancer cells will be performed. These studies will include the presence o CTL activities forautologous tumor cells which will be determine before each injection of IL-2 modified tumors as well as 1, 3, and 6 months following all 4 injections.

Overall, this protocol is well written, and it has significant parallelism to the investigators' previous study of patients with breast cancer. There has been discussion between the investigators and their IRB concerning the minimal amount of IL-2 required for atransduced cell population. This issue seems to be clarified, and that a minimum expression of 1ng/106 cells/24 hrs of IL-2 is required. Dr. Parkman was unclear as to the time frame the IL-2 level was determined. In the protocol, the investigators stated that the ovarian cells will betransduced; and that after 24 hours, an aliquot will be irradiated with 10,000rads. After 72 hours, the supernatant will be assayed for IL-2, and the gene modified cells will consider to be successfullytransduced if the transduced cells produce IL-2 at a concentration of approximately 100 pg/106 cells/72 hours.Transduced cells reaching that level will becryopreserved for future clinical administration. What is the evidence that the IL-2 production over 72 hours is at a steady state? Since the IL-2 production is being determined as IL-2/106 cells/24 hours, this initial measurement should be performed after 24 hours unless the investigators have data that IL-2 production over each 24 hour period is the same. The IL-2 production at the steady state level would be then 30 pg/106 cells/24 hours. If 5 x 106 such cells were injected, it would represent a total production of only 150 pg/5 x 106 cells/24 hours which is significantly less than the 1ng stated as the minimal level. There was further discussion as to the minimum amount of IL-2, i.e., 1ng. The investigators have already reduced the minimal level from 10 ng to 1 ng with the rationale that this level results inimmunoprotection in mice. However, it is unclear how the level of 1ng/106 cells/24 hours is going to be reproducibly obtained. This issue needs to be clarified before final approval.

Dr. Parkman recommended approval of the protocol contingent upon clarification about the quantitation of the IL-2, including the time frame, the number of cells, and how the minimal levels o IL-2 production will be guaranteed.

Review--Dr. Doi

Dr. Doi said he has reviewed the vector system, the immunization system, and the target cells as compared to the previously approved protocol. He was satisfied with the preclinical studies, and the main difference in the present protocol is a different target cell. DrDoi asked the investigators if the patients are immunized with the primary tumor cells, will antigen heterogeneity affect immun response to a metastatic site?

Review--Ms. Meyers

Ms. Meyers raised several issues regarding the Informed Consent document and most of them have been satisfactorily addressed by the investigators except for the area on costs. Instead of

stating that costs related to this experiment are to be covered, it listed costs for surgery, doctor's visits, and laboratory studies etc. These costs and any other necessary and recommended tests will be billed to the patient's insurance company. If the insurance does not pay, the patients will be responsible. Ms. Meyers said this section of costs should be revised.

Ms. Meyers said the investigators originally requested an *Accelerated Review* of the protocol, but she found that the previous protocol has yet to accrue any patients. Ms. Meyers said it is not appropriate to approve additional experiments unless the investigators' track record and some success has been demonstrated in their previous protocol.

Review--Dr. Secundy

Dr. Secundy stated that the Informed Consent document failed to include notification about requests for autopsy. She was satisfied with the revision by the investigators regarding a statement of treatment benefit.

Other Comments

Responding to Ms. Meyers' remark regarding the previous protocol, Dr. Parkman said a protocol has to be reviewed by the data supporting that protocol, i.e., transduction rate and the relevant preliminary data. Dr. Lyerly said that the previous protocol has not yet been submitted to FDA; therefore, no patients have been entered into the study.

Dr. Chase said there are several reasons for separate applications even from the same group because each part of the research group's activity has an independent life of its own.

Dr. Samulski said at the request of the investigators, a closed session was held at the March 1994, RAC meeting to review the vector. Several concerns about the vector were raised; these concerns will be followed up by FDA in its approval of the protocol. Similar questions do not need to be readdressed in the present protocol using the same vector. Dr. Smith remarked that a prior approval by FDA should not be a criterion for *Accelerated Review* of a replicative protocol. Dr. Walters said that this protocol could be a potential candidate for an *Accelerated Review* in the future.

Investigator Response--Dr. Lyerly

Responding to Dr. Parkman's question of IL-2 production, Dr. Lyerly said the minimal level of IL-2 secretion of transduced cells is 100 pg/106 cells/72 hours. There is a typographical error of the firs level of cell dose; the correct number of cells is 5 x 106.

Responding to Dr. Doi's question of antigenic heterogeneity, Dr. Lyerly said the tumor cells to be transduced are derived from metastatic sites, and one feature of the protocol of not expanding the cells in culture and selecting out the fast growing cells is to preserve the antigen profile that exists within the tumor cell population.

Responding to a question regarding the previous breast cancer protocol, Dr. Lyerly said that many suggestions have been made in the last review of the vector, and many alterations of the original vector have been constructed. None have demonstrated adequate gene expression. These additional studies together with other data regarding endotoxin of the plasmid DNA will be submitted to FDA in the near future. These additional data, however, are not part of a stipulation

for the previous approval.

Dr. Lyerly said that the autopsy issue was debated by his IRB. He agreed to include a statement to request an autopsy from the patient's family and to revise the "cost" section of the Informed Consent document. Ms. Meyers said the revision should state clearly that the costs related to the experiment will be covered by the sponsor and any unrelated costs will have to be covered by the patient. On the autopsy question, Ms. Meyers said autopsy is very important for the science of gene therapy. Dr. Lyerly concurred with Ms. Meyers that the training and skills of personnel required in counselling patients regarding autopsy are important factors to achieve a higher autopsy rate.

Dr. Lyerly clarified Dr. Parkman's question of the minimal level of IL-2 production in the transduced cells. The level should be 240 pg/106 cells/72 hours.

A motion was made by Dr. Parkman and seconded by Dr. Doi to accept the protocol submitted by Drs. Andrew Berchuck and H. Kim Lyerly contingent on review and approval of the following by the RAC primary reviewers: (1) the protocol will be amended to include a base-line for IL-2 production (240 picograms/1 x 106 cells/72 hours), and (2) revised Informed Consent document incorporating the changes suggested by Ms. Meyers to the "Cost" section. The motion was approved by a vote of 20 in favor, 0 opposed, and no abstentions.

Protocol Summary: Drs. Andrew Berchuck and H. Kim Lyerly of Duke University Medical Center, Durham, North Carolina, may conduct gene transfer experiments on 18 subjects (18 years of age) with refractory metastatic ovarian cancer. Autologous tumor cells obtained from ascites or surgically removed tumor will be transduced with the cationic liposome plasmid DNA vector, PMP6A-IL2, which is an adeno-associated virus based vector encoding human IL-2. In this dose-escalation study, subjects will undergo 4 cycles of intradermal injections (thigh or abdomen) of *ex vivo* transduced lethally irradiated tumor cells in an attempt to induce an antitumor response. The objectives of the study are to evaluate: (1) the safety of intradermally injected transduced cells, and (2) antitumor response following therapy.

XI. DISCUSSION REGARDING RAC STIPULATIONS FOR APPROVAL OF PROTOCOL #9412-094 ENTITLED: *ADENOVIRUS MEDIATED GENE TRANSFER: SAFETY OF SINGLE AEROSOLIZED ADMINISTRATION TO THE LUNG/DRS. DORKIN AND LAPEY*

At the December 1, 1994, RAC meeting, the protocol was approved contingent on the review and approval of aerosol/nebulization experiments mutually agreed upon by the investigators and a subcommittee of RAC members. On December 15, 1994, a telephone conference call was held regarding the proposed experimental design. On May 18, 1995, the experimental results derived from these experiments were submitted to the members of the subcommittee. On May 26, 1995, Dr Straus disapproved the protocol based on the experimental results. On May 27, 1995, Dr. Ginsburg disapproved the protocol based on the experimental results. Subsequently, the investigators requested a discussion at the June 1995 RAC meeting regarding this issue.

Review--Dr. Straus

Dr. Straus said the study is an adenovirus protocol for cystic fibrosis (CF). It was originally submitted as a two part study: The first part was approved through the *Accelerated Review* process and the second part involving aerosol administration of the vector was reviewed by the full RAC. Dr. Straus briefly summarized the aerosol arm of the study. As the patients inhale, they

would trigger a device which would pulse discharge the adenovirus vector to the patient's airway. The patient would be sitting up in a containment room with filters on the nebulizer, and the room itself had its own High Efficiency Particulate Air (HEPA) filter. A clinical operator would be standing outside of this little containment chamber wearing a gown, gloves, and mask. Dr. Straus' initial concern was that aerosolization was an entirely novel means of delivering a recombinant agent to a person. There had not been any data from the ongoing adenovirus/CF trials that would suggest any clinical benefit of this treatment and that would justify initiation of another trial by the novel means of aerosolized delivery.

Dr. Straus reviewed the test results of virus release from the containment chamber. The virus was released to the chamber by short pulses of 2, 20, and 120 seconds with a dose of 2.2 x 1011 particles for each pulse. Dr. Straus was concerned that the data shows some escape of vector (102 to 103 copies of vector DNA) through the HEPA filter out into the room as a whole. Although this environment was ideal and the virus dose was far more than the proposed clinical doses, Dr. Straus was not compelled to approve the protocol considering the benefit of adenovirus vector still needed to be proven. Dr. Straus requested that this protocol be further discussed by the full RAC.

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

Dr. Ginsburg stated in writing that these studies document high levels of virus within the containment chamber and readily detectable virus at the exhaust port (103 - 104 copies) in at least one experiment. Dr. Ginsburg has a concern with the detection of 103 - 104 copies of virus in the chamber before aerosolization. This observation was dismissed by the investigators due to release of virus during the diluting of the virus and filling of the nebulizers. No description is given of steps to be taken to prevent such contamination in the future nor evidence that such steps will be effective. Though exposure to these levels of adenovirus carrying a recombinant CFTR gene probably poses little risk for a normal host, this gene therapy protocol represents a new route of administration for an infectious viral agent with significantly increased potential for contamination of bystanders and warrants a particularly high level of caution. A more stringent containment protocol along with additional experiments to document reliable containment should be required before approval.

Other Comments

Dr. Walters noted a letter by Genzyme dated June 2, 1995, responding to comments by Drs. Straus and Ginsburg.

Dr. Parkman asked the investigators to clarify if the "background" virus copies detected in the chamber is due to normal presence of adenovirus or vector contamination. The high background level complicates the interpretation of the testing data. Dr. Parkman was concerned about the 102 to 103 virus particles detected outside the containment chamber even if the data was from a worst case scenario of 120 second pulse. He was concerned about the scientific validity of the data since the background of the assay was high.

Dr. Erickson said as a member of the subcommittee, he approved this protocol based on his judgment that 120 second release is a worst case scenario unlikely to be used in a clinical setting, and the virus released by aerosol will be rapidly inactivated upon drying. The RAC should not micromanage a protocol, and the FDA will be monitoring the study. Dr. Straus disagreed with Dr. Erickson's statement.

Dr. Chase said since there is an alternative way to deliver the virus to the patients such as by bronchoscopy, those studies should be completed before attempting at the novel route of administration. Dr. Straus agreed that clinical benefit should be established by the ongoing bronchoscopy studies before initiating the aerosol study.

Dr. Dronamraju asked why adenovirus poses little risk. Dr. Straus explained that adenovirus infection is common, and healthy individuals rarely have significant symptoms due to adenovirus infection. It is a relatively safe agent. Infection due to exposure of 103 to 104 particles of the replication incompetent adenovirus vector is insignificant. Dr. Straus was concerned about creating a precedent of approval to release a recombinant agent byaerosolization without a compelling reason.

Dr. Motulsky asked what would be the worst scenario risk in this case. Dr. Miller said the additiona risk by exposing to this agent is very small sinceCFTR is a normal protein of the airway and adenovirus infection is very common. Dr. Miller said the containment of this experiment is equivalent to the Biosafety Level (BL) 2 physical containment required to handle the adenovirus in the laboratory. Dr. Straus said in this case, it is an intentional spray of large doses of virus. Mr. Capron asked to clarify the source of the risk, agents in the air, or any surface contamination outside the chamber. Dr. Straus responded that his major concern is that the agents will be carried by personnel from and around the chamber to other distant places. Dr. Parkman said there is a consent issue of health care providers who knowingly are exposed to this virus and other individuals in the surrounding who are unwittingly exposed to this agent.

Dr. Miller said there are a couple of factors to be considered. First, it would be a different situation if the study involved a serious virus that required a BL-3 containment. Second, the exposure level in the present setting is at least amillionfold lower than the vector dose that induces toxicity in the CF patients of the ongoing protocols.

Dr. Samulski asked if there is any data that would indicate escape of a viable virus from the chamber. Dr. Straus said that the investigators did a tissue culture assay of adenovirus, but they did not detect any viable virus in their samples. Dr. Samulski said the PCR assay is a highly sensitive technique, but the relevant question is if viable virus is present. A simple aerosolization procedure would inactive 50% of the virus infectivity.

Dr. Smith stated that the safety concern of this protocol is a very pertinent issue for the RAC discussion. He supported Dr. Miller's statement that the risk of this experiment is relatively small. He asked Dr. Straus if the risk warrants informed consent to be obtained from the health care workers. Dr. Straus said he is unable to answer because he is not an ethicist. He emphasized that his concern is that RAC should not let the momentum of the approval process which have approved more than 10 CF protocols to override our ability to make a sensible decision about the present protocol.

Investigator Response--Dr. Wadsworth

Dr. Samuel Wadsworth (Genzyme Corporation) responded to questions raised by RAC members with a slide presentation. Dr. Wadsworth pointed out that the vector is a replication incompetent virus which rapidly inactivated upon drying. Most of the virus particles will be quickly inactivated upon aerosolization; and if there is any contamination of surface areas, it is decontaminated by 4 different cleaning solvents used in the hospital. Dr. Wadsworth showed a slide depicting multiple

layers of containment facilities of the isolation chamber including the nebulizer, demistifier chamber, and the negative pressure room of the hospital. The patient and health care workers are all wearing gowns, gloves, and masks. The health care workers will be asked to sign an Informed Consent document. The nebulizer is activated by inhaling and will shut off after 2 seconds of aerosolization. 102 virus particles were detected at the exit port of the nebulizer after putting in 1013 virus particles, a dose above the highest limit to be used for the patients. Dr. Wadsworth concluded that the first layer of containment is highly effective.

The test results of the containment chamber showed that upon a release of 109 virus particles within the chamber for 2 seconds, only 100 particles were detected at 2 locations outside and inside of the chamber, and no viable virus was captured by a tissue culture dish. Dr. Wadsworth concluded that the containment chamber is very efficient in removing virus particles from the air within the chamber.

Dr. Wadsworth showed a PCR data to demonstrate that the sensitivity of the assay is adequate and the background level of vector sequences found in the animal room where preclinical animal experiments were conducted. The background level was mostly less than 100 copies. The assay included an internal control of a piece of competitive DNA.

A test was performed to stress the system to the point where containment was breached. 2.2 x 1011 particles were released within the containment chamber, and a maximum of 102 - 103 particles were detected outside the HEPA filter of the chamber

Responding to a question by Dr. Miller regarding virus inactivation, Dr. Wadsworth said the infectivity of the virus particles is reduced approximately 7 logs upon drying in 2.5 hours.

Ms. Meyers asked if it is necessary to test the vector on normal people. Dr. Chase asked if there is safety concerns about this aerosolization of health care workers who have asthma or are takin other steroid medications. Dr. Wadsworth responded that the containment system is effective in preventing these exposures.

Dr. Erickson noted that one adverse event that occurred in one of the CF protocols was related to the volume of virus inoculum , and no more adverse effects were observed by reducing the sam dose of virus in multiple smaller volumes. Such an adverse event may be avoided in the aerosol protocol since the virus will spread out in the entire lung. Although the current CF protocols do not appear to offer a cure for the patients, if the investigators can conclude these studies as soon as possible (by approving this study), it will permit them to move on to another more promising experiment.

Committee Motion

Dr. Erickson made a motion to approve that the RAC stipulation requirements have been satisfied. The motion was seconded by Dr. Miller.

Dr. Motulsky stated that this protocol poses such a little safety problem and has such promise he would strongly favor its approval.

Dr. Miller said if this protocol was a simple mouse experiment, he would vote for approval without any reservation. But in a clinical trial, there is the additional factor of efficacy. Ms. Meyers said in absence of efficacy in clinical trial, any risk to healthy people is a concern. Dr. Dronamraju add

the risk may be greater to certain groups of people. Dr. Miller said the risk is small since the vecto is a replication incompetent virus, and the CFTR is a normal protein of airway cells. Dr. Dav Meaker (Genzyme Corporation) remarked that he would support Dr. Miller's statement that th of large amounts of the CFTR protein in healthy people is very small since large amounts of thi protein have been given to CF patients.

Dr. Parkman said the risk/benefit analysis should be applied to both the individuals who are knowingly exposed to the agent such as the patients and the health care workers, and to society at large who do not have any consent to the exposure. Dr. Parkman stated that in absence of benefit, any risk is unacceptable. In an analogy to the environmental release issue, if there is no benefit, it is unjustified to support any release of a recombinant agent to the environment. Dr. Mille said that it is unfair to characterize this study as without any benefit; some useful scientific information may be obtained from this trial even though the patients may not directly benefit from the treatment. In his own estimation, the safety issue to the public is minimal, and the risk to other workers is no more than working in a regular virology laboratory.

Dr. Chase said he is completely convinced by the arguments made by Drs. Straus and Parkman. The principle in this case is that the findings from other CF experiments should influence how we rule on this protocol.

Dr. Meaker made additional comments on the risk/benefit ratio of the present protocol. First adenovirus vector will eventually need to be administered repetitively. Bronchoscopy is not th method of choice; the ultimate route will undoubtedly be via aerosol. In addition, bronchoscopy itself is a fairly morbid procedure for a patient. Second, the original rationale for delivery (e.g., a dose of 2 x 1010 virus particles to a small portion of the lung) will only affect a small portion of the lung in case there is any adverse effect. It is now clear that the adverse effect is related to the volume and concentration of the virus rather than to the total dose that is delivered to the lung. Dr. Meaker argued that aerosolizing this vector where you give the same dose to the whole lung wi decrease the multiplicity of infection of lung epithelial cells; thus, it is likely to be safer than delivering the vector with a bronchoscope. Finding the toxic range for aerosolization is a important question in order to define a therapeutic window. There are still many technical problems on how to evaluate efficacy in CF clinical trials.

Dr. DeLeon stated that she inclined to support Dr. Miller's position that there is minimal risk, an efficacy is not a primary objective of a Phase I clinical trial. Dr. Meaker said that they have som data demonstrating efficacy in the nasal experiment.

A motion was made by Dr. Erickson and seconded by Dr. Miller that the December 1, 1994, RAC stipulation requirements have been satisfied. The motion was approved by a vote of 11 in favor, 7 opposed, and 1 abstention.
Protocol Summary

Dr. Henry Dorkin of the New England Medical Center, Tufts 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 CFTR gene to the lung of CF patients by aerosol administration. 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 expressio

XII. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *GENE THERAPY FOR PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY/DR. MCIV*

Review--Dr. Erickson

Dr. Zallen announced that she would serve as Acting Chair in Dr. Walters' absence. Dr. Zall called on Dr. Erickson to present his primary review of the protocol submitted by Dr. R. Scott McIvor of the Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota. Dr. Erickson stated that purine nucleoside phosphorylase (PNP) deficiency causes a SCID w similarities to ADA deficiency. In contrast to ADA deficiency for which a protocol has been already approved (#9007-002), there is no PEG-ADA enzyme available for an alternative therapy. Thus, these patients have a very grim course; they are usually dead before the second decade of life. Much of this proposed human gene therapy protocol follows other retroviral-mediated protocols which the RAC has already reviewed. Dr. Erickson did not see major safety problems; however, he had questions about efficacy.

The basic scheme is that lymphocytes will be prepared from peripheral blood leukocytes (where these patients have less than 500 T lymphocytes per mm3), by lymphopheresis and differentia centrifugation. The cells will be cultured for 3.5 days in an artificial capillary cartridge with IL-2 and anti-CD3 (as a mitogen). They will be transduced with a retroviral vector of the LXSN -clas containing the PNP cDNA driven by Moloney murine leukemia virus long terminal repeat the promoter (the vector contains *ne*R gene driven by the SV40 early promoter). The data shows transgene expression and vector structure are stable for more than 30 days in culture. Studies variety of subjects has shown that one only needs 5 - 10% of normal levels of PNP for good immune function. The protocol plans to infuse cells every 2 months for a year starting with 108 cells and gradually advancing to 1010 cells.

Follow-up will be performed with blood drawn prior to the next infusion which will be assayed for pathogens and replication-competent retrovirus. The assay for vector is a competitive PCR t quantify copies of viral genome present in the patients. The investigators plan to use as a competitor an intron -containing DNA fragment which gives a 200-base pair longer fragment Because smaller PCR products are preferentially synthesized over larger ones, this quantitativ PCR is likely to provide an overestimate of the amount of vector present in the patients' cells. D Erickson would like to review data to validate this PCR assay. The investigators can easily perf mock experiments to determine the true quantitation . The patient's peripheral cells would b studied for immunological function.

The investigators have performed preclinical studies which show that a murine T-cell lymphom cell line which is PNP deficient could be transduced with the vector and gave good enzyme leve These studies involved selection with G418 (using the *ne*R gene in the vector), whereas *ne*R selection is not proposed for the clinical protocol. The vector-containing murine T-cell lymphon cells were no longer deoxyguanosine sensitive. The investigators have taken cells from PNP-deficient patient and treated them with the vector*in vitro*. The investigators have achieved 1% of normal enzyme levels which seemed to correlate with 1% of cells being transduced . This deg of transduction resulted in about 10% of normal cell immunological responses.

Dr. Erickson raised several specific questions. The goal of this protocol is to achieve 5% positive cells in the patients' peripheral blood. This goal seems quite unlikely to be achieved given that only 1% transduction achieved in the preclinical studies. Thus, the possible efficacy needs to be reconsidered. The investigators are inconsistent about the frequency of infusions. The*Points to*

Consider **makes it clear that the investigators plan every 2 month infusions. The protocol text states monthly infusions. Dr. Erickson asked the investigators to clarify this question. Finally, there is a mistake in the parental Informed Consent document where the words "you dies" should be corrected as "he/she dies." The assent form for children was not written in a language appropriate for children who are less than 10 years old. An age-appropriate assent form should be created.**

In summary, Dr. Erickson concluded that this protocol is excellent, and he would recommend approval. He would like to see more data on gene transfer efficiency and about the artificial capillary cartridge system.

Review--Dr. Chase

Dr. Chase said that he was in favor of this protocol. He asked the investigators to elaborate on the animal experiments. The animal experiments show efficacy in changing deoxyguanosine sensitivity although it is unclear whether this result represents efficacy in a clinical sense of the term. The situation is like showing a drug can reduce cholesterol level but with no evidence about the effects on the death rate from heart disease in the treated subjects. Dr. Chase asked the investigators to address the question if they have shown that mice became more healthy as a result of receiving this treatment, such as by demonstrating increased survival rates following exposure to common infectious agents. Dr. Chase pointed out since this trial is not controlled, one can only ask if the subject has improved after treatment but cannot conclude on causality. Since there is no concurrent enzyme supplement therapy similar to PEG-ADA in the ADA deficiency protocol, the protocol is a stronger one because the improvement in the immune function can be compared with historical base line data. Dr. Chase said most of his concerns have been fully addressed by the investigators' written response, and he was very satisfied with the protocol.

Review--Ms. Meyers

Ms. Meyers was excited about the present protocol for a genetic disease. Ms. Meyers would like to salute the investigators for developing this protocol without regard to projected financial returns for a marketed products (the study does not have a commercial sponsor), and for their vision to see that success of these experiments will lead to therapies for other more prevalent conditions. She was encouraged that the investigators have taken the approach of SCID -ADA protocol in a attempt to treat other genetic diseases, and she applauded the investigators for their promise to publish their data in a peer-reviewed journal.

Ms. Meyers pointed out one advantage of the present protocol is that an enzyme supplement therapy (such as PEG-ADA) will not be used in the present study so that the data of the trial can be interpreted in a more straight forward way. She deferred to her scientific colleagues to comment on whether the animal work is sufficient to justify the human experimentation.

Ms. Meyers was concerned about a statement in the Informed Consent document that the experimental treatment cannot be offered beyond the 1 year period of infusions with a 1 year follow-up as part of the clinical research trial. She said it is not appropriate to discontinue treating the child if he/she shows promise. The statement needs to be changed to state that the scientists will take every effort to secure continuation of funding for this study and to expand the trial to give parents and the child some hope that they will not be left destitute after this 1 year period. In addition, the follow-up should be life long, not just for 1 year.

Review-- Mr . Capr

Mr. Capron stated that he is pleased that the RAC was reviewing a protocol with a rare disease, but he reminded the limited nature of the intervention by treating the peripheral blood lymphocytes. Mr. Capron asked the investigators to address the efficacy question of the animal experiment.

Regarding the Informed Consent document, Mr. Capron considered the statement of the follow-up is adequate; it will be misleading to suggest that there will be continued funding after 1 year period. There were several specific suggestions about the wordings of the Informed Consent document, but the investigators have not responded to all of Mr. Capron's suggestions. In the statement that parents are requested to read the Informed Consent document and ask any questions before "agreeing" to enroll their children, the word "agreeing" should be substituted with "deciding." In the fourth paragraph of the Informed Consent document, the acronym "PNP" does not need to be further explained since the term has been used previously in earlier paragraphs. In the statement in the fourth paragraph that states the vector carries a normal gene to make the "enzyme protein," it is sufficient to use either "enzyme" or "protein" in this sentence. The original Assent document is not understandable for children of 8 to 10 years of age. In the revised Assent document, the word "new treatment" is not appropriate, it should be "experimental intervention." The statement about the major purpose of the study should be changed to state that the main purpose is to "develop a basic understanding of the technique and determine its safety." This suggestion was declined by the investigators since they considered that the goals of the study include assessment of effectiveness.

Mr. Capron said that the revised Assent document is inadequate and misleading, and additional changes should be made to the Informed Consent document.

Other Comments

Dr. Ross pointed out that in the Informed Consent document regarding complete physical examination, it states that the examination is to ensure that a child is healthy other than T-cell immune deficiency. If a child becomes sick during the course of the study, will the child still be able to continue the treatment?

Dr. Parkman asked with regard to the data of correction of deoxyguanosine toxicity i PNP-deficient mouse S49 T-lymphoma cells. He asked why the level of correction is only about half of the normal S49 cells but not 100% since the transduced cells have been selected by G41 and are all expressing the transgene ? Regarding the data of partial correction of proliferati responses by transduction of PNP-deficient T lymphocytes from patients, are these two cultures of human cells in this figure are derived from the same or from different patients, since transducibility may be different between cells from different patients? What is the transduction in these cultures? The magnitude of correction appears to be much greater than the 1% transduction rate assumed by the investigators. Regarding the data of growth of T lymphocytes using Cellco artificial capillary system, will varying transduction rates affect the growth rates? follow-up immunological analysis should be performed using the T-cell blastogenesis /antige assay as opposed to the skin test, which is more complicated to interpret the results.

Investigator Response--Dr. McIvor

Responding to Dr. Erickson's question about transduction efficiency, Dr. McIvor said that using a

higher titer vector supernatant, the investigators have achieved 1 to 2% transduction efficiency of patients' lymphocytes grown in the cartridges, a higher result than that stated in the protocol. This new cartridge system is a technique the investigators are developing and it will be used for the protocol of Hunter syndrome. The goal is to have 5% transduction efficiency since PNP deficiency patients with 5% normal enzyme activity demonstrate immunocompetence

Dr. Parkman said a persistent effect was observed in the SCID -ADA study. A study using SC mice indicates that the transduced T-cells seems to have greater persistence than th nontransduced cells. He asked the investigators if a similar mouse experiment has bee performed with PNP deficient cells. Dr. McIvor responded that such an experiment has not been performed partly because he has some reservations about the interpretation of the data of this study regarding persistence.

Related to the question of animal experiments asked by Dr. Chase, Dr. McIvor clarified that " deoxyguanosine sensitivity" refers to th *in vitro* cell culture experiment involving S49 T lymphoma cells that is deficient in PNP activities. The S49 lymphoma cell line was originally derived from a mouse, and the experiment involved an *in vitro* cell model system rather than a mouse system to evaluate the ability of gene transfer to correct the PNP deficiency.

Dr. McIvor agreed to revise the Informed Consent document as suggested by Mr. Capron. As to Mr. Capron's suggestion to limit the statement of the purpose of the study to simply "determining the safety of the procedure" but to avoid any mentioning of effectiveness, Dr. McIvor did not completely agreed with Mr. Capron. But he would take any recommendation from the RAC. Dr. McIvor stated that his intention of conducting this trial is to possibly aid these patients. Responding to questions by Dr. Chase and Mr. Capron regarding the animal experiment, Dr. McIvor clarified that there is no animal model for this disease. The model system is an *in vitro* cell culture system of the PNP-deficient S49 T-cells. Mr. Capron asked if this model is sufficient for preclinical studies. Dr. Parkman said the cell culture system is an acceptable model to perform a preclinical study to justify a clinical trial, and the model chosen is appropriate. Dr. Chase agreed that such a tissue culture model is acceptable.

Mr. Capron said he is concerned about the use of the phrase "new treatment" in the Assent document; it is a deceptive language to children. Regarding a proper statement of the Informed Consent document regarding the purpose of the study, it is just the difference in emphasis of the goals. If the so-called Phase I/Phase II study implies more than a study of safety, what is the real objective of the study in the investigators' mind? Dr. Parkman explained that transduction and transfusion of T-cells to patients have been proved to be relatively safe from several clinical trials. The principal objective in the investigators' mind is not just to determine safety; there is intention to treat the patients. Dr. McIvor agreed that it is the reason to include the statement to inform the patients and their parents that the study is to determine if there is some improvement in the patients. Ms. Meyers said there is an intention of observing for efficacy. Mr. Capron was concerne with the publicity of the promises of gene therapy. It is important not to give false hope for patients, the purpose statement of the Informed Consent document should be toned down in keeping with the state of the art that no definitive efficacy has been shown for gene therapy. Dr. Chase did not object to the use of the phrase "experimental treatment" which may or may not work. Dr. Parkman said it is reasonable for the investigators to say that they have an expectation of a therapeutic impact in this disease. Dr. Zallen found the revised Informed Consent acceptab

Dr. McIvor said he does not have a good explanation to Dr. Parkman's question why deoxyguanosine sensitivity of the S49 cells was not completely corrected by gene transfer sinc

these cells have been selected for G418. Dr. McIvor said that the data on human T-cells was obtained from cells of the same patient, and they are comparable.

Dr. McIvor said the transduction rate is about 1 to 2.5%. Dr. Parkman asked if there is any minimal transduction rate required for infusion back to the patients. Dr. McIvor responded that no minimum rate has been set.

Dr. McIvor did not have any obvious explanation for why the observed T-cell proliferative response rate of 10% is greater than the transduction rate of 1 to 2%.

Responding to a question by Dr. Parkman regarding antigen specific blastogenesis assay, Dr Filipovich , co-investigator, said that an assay will be performed for larger and older patients; fo patients of smaller statue, a less invasive skin test will be performed without causing undue risks.

Responding to Dr. Ross' question of physical examination, Dr. Filipovich said the primary conc is the safety of removing a large volume of blood needed for collecting lymphocytes. Patients should be in a healthy condition. Patients with infection will complicate the study; there is a potential of contamination of tissue culture. If during the course of the treatment the health condition of the patients become unsuitable for continuation of the protocol, the patients will be advised to terminate the treatment. Mr. Capron said it is reasonable to terminate treatment. Ms. Meyers said the decision to terminate participation should be made in the hospital with the parents and the child. Dr. Filipovich added another reason to have a complete physical examination is t provide a baseline to document if there is any improvement of the physical condition after gene transfer.

Dr. Motulsky asked how many patients with PNP deficiency are in the U.S., and how many of th might be available to the protocol. Dr. Filipovich responded that the protocol plans to enroll patients; it is a very rare disease and one patient has already been identified for the study.

Responding to Ms. Meyers' question of prolonging treatment beyond 1 year, Dr. Filipovich said investigators are planning to offer 6 treatments; and they have to be honest about what would happen after 1 year. If there is any evidence of benefit, she will try to obtain insurance coverage fo the treatment. If any source of funding can be secured including the patient's willingness to pay for the treatment, the investigators would continue the treatment beyond 1 year.

Dr. Lai asked if there is any data to indicate how long the transduced gene continues to b expressed. Dr. McIvor responded that in the S49 cells, persistent expression was observed up to 3 to 4 months, and in the human lymphocyte culture sustained for up to 45 days.

Dr. Miller said there should have some release criteria for the cells that are to be reinfused int patients. Dr. McIvor responded that there is no specific limit of gene transfer frequency as part of the release criteria. But if the transduction rate is consistently low such as 0.1 or 0.5%, the study will be discontinued. Dr. Miller asked if FDA would allow reinfusion if there is no evidence of ge transfer. Dr. Noguchi responded that FDA would require the investigators to state their intentions with different values of transduction rate but will not set a minimal criteria for release until a coupl of patients have been treated.

Committee Motion

Dr. Erickson made a motion to approve the protocol, and Dr. Miller seconded the motion.

Mr. Capron made an friendly amendment that Dr. McIvor has voluntarily agreed to revise the Informed Consent documents including the consent and assent forms as suggested by the RAC.

A motion was made by Dr. Erickson and seconded by Dr. Miller to accept the protocol submitted by Dr. R. Scott McIvor contingent on incorporation of suggested changes to the Informed Consent document as voluntarily agreed on by the investigator. The motion was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

Protocol Summary: Dr. R. Scott McIvor of the Institute of Human Genetics, University of Minnesota Minneapolis, Minnesota, may conduct gene transfer experiment on 2 children with PNP deficiency. PNP deficiency results in severe T-cell immunodeficiency, an autosomal recessive inherite disease which is usually fatal in the first decade of life. Autologous peripheral blood lymphocyt will be cultured in an artificial capillary cartridge in the presence of anti-CD3 monoclonal antibody and interleukin-2 and transduced with the retroviral vector, LPNSN-2, encoding human PNP Subjects will undergo bimonthly intravenous administration of transduced T cells for a maximu of 1 year. The objectives of the study are to determine: (1) the safety of intravenous administration of transduced T cells in children with PNP deficiency, (2) the efficiency and duration of PNP ge transfer and expression *in vivo*, and (3) the effect of PNP gene transfer on immune function.

XIII. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I TRIAL OF A POLYNUCLEOTIDE VACCINE TO HUMAN CARCINOEMBRYONIC ANTIGEN IN PATIENTS WIT METASTATIC COLORECTAL CANCER/DR. CUR*

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

Dr. Zallen called on Dr. Miller to present Dr. Ginsburg's primary review of the protocol submitte Dr. David T. Curiel of the University of Alabama, Birmingham, Alabama. Dr. Ginsburg stated i writing that this proposal is to immunize metastatic colon carcinoma patients to th carcinoembryonic antigen (CEA) by injection of recombinant expression plasmid DNA i skeletal muscle. This protocol is a resubmission initially approved by the RAC with a split vote (10:4) in June of 1994 and subsequently disapproved by the NIH Director. The RAC discussion a that time centered on the lack of sufficient preclinical data. Specifically, there was concern that human CEA might be considerably more immunogenic in the mouse; and that it would be mor appropriate to use a murine CEA or construct a mouse transgenic for human CEA . In add there was concern that immunization of mice before exposure to tumor was not an appropriate model for the treatment of patients with a pre-existing large tumor burden. It was suggested that additional data from other human trials of CEA immunization could satisfy some of thes objections.

This resubmission to the RAC includes new background data, predominantly in the form of preprints from the work of others, to support the notion that an immune response can be elicited in colon cancer patients against the self-antigen, CEA , by using either a vaccinia-CEA vecto anti- idiotype antibody. The experimental design has been changed by the addition of a expression cassette for hepatitis B surface antigen (HBsAg) to the plasmid DNA, along with th original CEA cassette. HBsAg will serve as a positive control to confirm the ability of this ap to invoke an immune response.
Dr. Ginsburg raised three specific issues: (1) Regarding the combined CEA-HBsAg dua expression plasmid, Dr. Ginsburg asked if the information to be obtained by including HBsAg control antigen was worth the potential adverse effect on CEA immunization. Is there a potentia

for complication from immune complexes resulting from long-term expression of HBsAg ? Unle the investigators can provide a more compelling rationale for including the dual expression plasmid, Dr. Ginsburg would suggest that they return to the original design with the single CEA plasmid. (2) Absence of serologic evidence for hepatitis B infection is stated as a patient selection criteria in the protocol. Does this refer to active, ongoing hepatitis B infection or will this exclude all patients with prior exposure to hepatitis B? Will this exclude patients who have received a hepatitis B vaccine in the past? How will these individuals be identified? (3) Although the protocol does not raise major safety concerns, the potential for benefit is quite remote. It seems very improbable that an anti- CEA immune response could favorably impact on the bulk tumor given results obtained from other CEA trials. With the low likelihood of clinical benefit, the statements the Informed Consent document are too optimistic and should be made more realistic.

In summary, Dr. Ginsburg stated previous concerns about the lack of evidence for immunogenicity of CEA in humans have been effectively answered by the cited studies from groups. Though Dr. Ginsburg has a low level of enthusiasm for the scientific basis of this protocol it should be noted that according to Dr. Curiel , this study was recently reviewed as a gran application entitled "Clinical Trials of Polynucleotide Tumor Vaccines" and was awarded a priority score of 145 by a NIH Study Section with funding expected in the fall of 1995. Dr. Ginsburg wou recommend approval if the investigators satisfactorily responded to his concerns.

Review--Dr. Miller

Dr. Miller said that there is more data now to support the trial. From the recombinant DNA standpoint, the risk of the proposed experiments to patients and to the public is extremely low. Dr. Miller said he is more convinced than previously by the preliminary data and the additional publications to support the trial. The human study appears to be the easiest way to obtain the relevant data. In his opinion, the previously suggested animal study with a transgenic human CE may actually be a more difficult prospect than the proposed human study. The animal model may not be totally predictive of the human disease. Given the low risk of the vector, Dr. Miller favored approval of the protocol as submitted.

Dr. Saha asked if Dr. Varmus required a transgenic mouse model study before approval of human trial. Dr. Wivel clarified that the transgenic model was simply a suggestion to develop m preclinical data, and it is not a requirement for approval.

Review--Dr. Zalle

Dr. Zallen made several specific comments: (1) Experimental design. 15 subjects are to be enro in 5 different groups. Monitoring of toxicity at 48 and 72 hours will be accomplished by phone contact with the first visit scheduled for 1 week post-injection. The escalation to the next dose level will occur after 2 weeks if no toxicity is observed, and this interval is too rapid. Dr. Zallen suggested more careful early examination of the subject in the first days following injection, and a longer period of study between dose levels would be prudent. (2) Informed Consent document. The investigators have clarified the matter of reproductive concerns. The Informed Consent document has been satisfactorily improved. Specific suggestions have been followed such as using the word "I" instead of "you", and the phrase "experimental treatment" instead of "therapy."

Other Comments

Dr. Parkman said his major concern of the previous review of this protocol was whether it was

possible in a host already bearing an CEA expressing tumor to develop an immunologica response to recombinant CEA . The supporting data can either be from a mouse model or from relevant human study. The investigators were unable to provide the data of a human study of recombinant vaccinia-CEA conducted at NIH by Drs. Hamilton and Schlom since the trial blinded study and the code had not been broken. In the present resubmission, these data together with investigators' own data of a smaller trial of the same vaccine on colorectal cancer patients suggest evidence of a cellular immune responses to CEA in some of the patients followin immunization. Dr. Parkman said these data satisfactorily responded to his concern. These data from human studies are even better than that of a mouse model.

Dr. Miller asked if these immune response to CEA are as expected and if polynucleotide- CEA be as effective as vaccinia-CEA . Dr. Parkman explained that the data shows immune response be elicited against CEA , but it does not demonstrate any clinical benefit derived from the immu response. The current data satisfies his requirement for approval.

Ms. Meyers pointed out examples from the Informed Consent document that are not understandable to patients of 8th grade level of education.

Dr. Smith agreed with Drs. Parkman and Miller in terms of the overall analysis. He asked if the proposal previously reviewed by the RAC provided the same data as the proposals reviewed by the NIH Study Section and FDA. Have the same deficiency of the protocol been noticed by thes other review bodies?

Dr. Lai noted that CEA is not really a tumor specific marker; it is expressed in normal tissues ar normal cells of the gastrointestinal tract. He was concerned about the potential toxicity to the normal tissue. The lack of toxicity in the mouse experiment does not completely address this question since mouse does not have CEA in its normal cells. Dr. Miller said the data from th human vaccinia-CEA trials would be useful
Investigator Response--Drs. Curiel and LoBug

Responding to Dr. Miller's question on DNA vaccine, Dr. Curiel said very encouraging results ha been obtained from influenza vaccination by the Merck research group using DNA vaccine in an animal experiment. The ease of intramuscular injection of naked DNA is a factor to be considered in an efficacy argument.

Regarding Dr. Smith's question of study section review, Dr. Curiel said that submission materia do include the data from the human vaccinia-CEA study. Dr. Curiel agreed to revise the Infor Consent document as suggested by the RAC. Responding to a question by Dr. Samulski regarding the vaccinia-CEA study, Dr. Curiel said the study design and the techniques are v similar to the present protocol.

Regarding the question of hepatitis testings , Dr. LoBuglio said that the protocol is study immune response to a hepatitis virus antigen, and that the protocol will exclude anyone who has evidence of either current or past hepatitis.

Regarding the scientific basis of CEA immunization to treat cancer, Dr. LoBuglio said that th are now a list of 5 to 6 human tumor rejection antigens that have been shown to induce tumor regression in patients with metastatic disease. The only end organ target toxicity of immunizati with these antigens is vitiligo in patients with melanoma in which they get patchy areas o depigmentation of their skin. The CEA molecule is expressed in normal tissue particularly

epithelial cells. There is a long history of administration of monoclonal antibodies to CEA witho inducing any end organ damage. There is no evidence of toxicity of gastrointestinal tract from trials on metastatic colon cancer patients. The potential toxicity will be monitored in the presen protocol.

Dr. LoBuglio stated the primary purpose of the study is to understand how the technology can utilized to induce immune response in patients. The actual application will be obtaining efficacy in the treatment of microscopic disease patients.

Dr. LoBuglio said from the data presented in a recent National Cancer Institute workshop on tu vaccination, the investigators' laboratory is the only one among other investigators in the field to find definitive evidence of immune response to a tumor associated antigen. Dr. Miller pointed out that all the other negative data from RAC-approved cancer immunotherapy trials pose a major concern to the RAC. Dr. LoBuglio said his institution has taken colon cancer as its major intere and the investigators have treated 250 patients with a variety of recombinant tumor antigens.

Dr. Lai asked if the trial is to develop a therapy for colon cancer since there is already evidence of immune response to CEA in other studies. Dr. LoBuglio responded that the purpose of present study is to demonstrate that this kind of vaccine strategy is nontoxic, and to observe if any immune response can be induced in humans against CEA or HBsAg . It is not expected have any realistic efficacy in patients with widespread metastatic disease

Responding to Dr. Zallen's question of the informed consent process, Dr. LoBuglio explaine the physician responsible for the trial will first discuss the protocol with the patients. The discussion will be followed up by a research nurse. The patients will be given the Informed Consent document to take home, read, and return on another occasion to sign on the study. From their experience, the patients have no difficulty in understanding the general concept. Dr. Lysaught said that shorter, simpler language of the Informed Consent document will hel understanding. Dr. LoBuglio said most patients have much more medical knowledge tha expected.

Committee Motion

Dr. Miller made a motion to approve the protocol pending modification of the Informed Consent document to be reviewed by Ms. Meyers and Dr. Zallen . The motion was seconded by Dr Parkman.

Ms. Meyers asked if the scientific members were satisfied with the toxicity issue. Dr. Miller responded yes, noting that in the current vaccinia-CEA trial, no untoward effect that might b predicted by expression of CEA has been observed. Dr. Parkman added that vitiligo has b noted in melanoma studies but no other clinically significant overt toxicity.

A motion was made by Dr. Miller and seconded by Dr. Parkman to accept the protocol submitted by Dr. David Curiel contingent on the review and approval by Dr. Zallen and Ms. Meyers o revised Informed Consent document incorporating their suggested changes. The motion was approved by a vote of 16 in favor, 1 opposed, and 0 abstentions.

Protocol Summary: Dr. David T. Curiel of the University of Alabama, Birmingham, Alabama, ma conduct gene transfer experiment of 15 subjects (18 years of age) with metastatic colorecta cancer. Subjects will receive intramuscular injection of the polynucleotide vaccine, pGT63, which

is a plasmid DNA vector expressing CEA and HBsAg . The objectives of the study are to: characterize the immune response to CEA and HBsAg following a single intramuscular injec and following 3 consecutive intramuscular injections, and (3) determine the safety of intramuscular injection of the plasmid DNA vector at doses ranging between 0.1 to 1.0 milligrams (single dose) and 0.9 to 3.0 milligrams (total multiple dose).

XIV. HUMAN GENE TRANSFER PROTOCOL ENTITLED: *INTRACELLULAR ANTIBODIES AGAINST HIV-1 ENVELOPE PROTEIN FOR AIDS GENE THERAPY/DR. MARASC*

Review--Dr. Glorios

Dr. Zallen called on Dr. Glorioso to present his primary review of the protocol submitted by Wayne A. Marasco of Dana-Farber Cancer Institute, Boston, Massachusetts. Dr. Glorioso sta that infection with HIV generally progresses from seroconversion to apparent clinical latency w later progressive immunodeficiency, opportunistic infections, malignancies, and death. During the latent period, there is a gradual reduction in CD4(+) cell counts with the eventual loss in immune function. Depletion of CD4(+) cells is the result of a direct cytopathology of HIV infection and fr inappropriate auto-immune destruction of uninfected T-cells. The HIV envelope glycoprotein, gp120, plays an important role in this progression of the disease based on its ability to form syncytia among infected cells and by the apparent binding to uninfected cells resulting in immu recognition and destruction by specific CTL . One strategy to treat the disease is to eliminate th ability of gp120 to participate in the formation of infectious virus or the release from infected cells. The investigators propose to use gene therapy to accomplish this aim through the transfer of a gene encoding a human antibody that will bind gp120 intracellularly and prevent its interactio with CD4 or participate in virus maturation. The human monoclonal antibody F105 is neutralizing by competing with the binding of gp120 to the virus receptor CD4. This antibody was further engineered to create an intrabody (sFv105) that is retained in the lumen of the endoplasmi reticulum (ER) of the CD4(+) T cells where it is capable of binding to the nascent folded envelope protein within the ER and prevents transit of the envelope-antibody complex to the cell surface. Introduction of this antibody encoding gene into a cell line capable of supporting HIV replication was shown to reduce the production of infectious HIV by 1,000 to 10,000-fold. Moreover, the cells retain its ability to express CD4 on the surface and respond to mitogenic stimulation, an otherwise behave normally. Importantly, the intracellular antibody was able to "neutralize" HIV variants that escape neutralization during extracellular treatment

The overall goal of the study is to determine whether the intracellular expression of a human single chain antibody against HIV-1 envelope glycoprotein, gp120, that blocks gp120 processing and the production of infectious virions can safely prolong the survival of CD4(+) lymphocytes HIV-1-infected subjects.

Dr. Glorioso raised 7 specific questions before the meeting and most of them were satisfactoril addressed by the investigators in writing. One remaining issue is that the number of T-cells carrying the HIV provirus in asymptomatic HIV patients is very low (on the order of 100,000 T cells) The transduction efficiency is 5 to 25%. The probability of HIV-infected cells to be transduced b the intrabody gene is very small. After expansion and reinfusion into patients, it is difficult determine the effect of transgene on the survival of these provirus carrying cells. Another poin related to the control vector is that the transduction efficiency of the control vector may be quite different from the vector carrying the intrabody gene. It will be difficult to make a comparis *vivo* of cells transduced by these two vectors

Review--Dr. Ginsburg (presented by Dr. Glorioso

Dr. Glorioso stated that Dr. Ginsburg raised 3 specific issues: (1) Is the G418 selection necessa The added potential for toxicity to the harvested lymphocytes from this added manipulation may not be worth the enrichment achieved. Only the successfully transduced cells will be assayed b PCR *in vivo*, and there is no need of this additional selection. The investigators responded in writing that this additional procedure can be deleted from the protocol. (2) The accurate quantitation of lymphocytes transduced with the sFv105 and control vectors is critical to design of this study. The investigators stated that they can detect 1:100,000 cells by PCR . Dr Ginsburg asked about reproducibility and accuracy of this PCR assay. The investigators provid additional data to address this question in their written response. (3) It should be noted that the proposed study population is restricted to otherwise healthy, HIV positive patients. Recognizing that there is still significant potential risk from the introduction of any retroviral vector, would it be possible to begin the study in a population of patients in a later phase of their illness when such risk could be more easily justified? The investigators responded in writing that the study proposes to patients with CD4(+) cell counts of at least 250 per mm3 so that an adequate number of CD4(+) cells can be harvested for transduction studies.

Dr. Glorioso raised an additional concern that there is an inherent risk in this study of patients i latent phase of HIV infection. There is some risk of provirus activation after T-cells are expanded and reinfused back to the patients

Review--Ms. Meyers

Ms. Meyers suggested that female patients should be included in this HIV study. Ms. Meyers objected to the statement in the Informed Consent document that "Although we are requesting an autopsy, it will not be required to participate in this study." She reiterated that when patients sign the Informed Consent, they should be reminded that autopsy is very important to the science of gene transfer studies, and they should express their wishes to their relatives. The statement that autopsy is not required for the study will not encourage the patients and their relatives to consent to autopsy.

Ms. Meyers stated that the acquired immune deficiency syndrome (AIDS) community is desperate for an effective treatment, but there have been too many potential therapies showing promise against HIV *ex vivo* which have proven ineffective in humans, and should animal data be available at the very least to indicate safety? She noted that the investigators stated that they intended to use the SCID / hu mouse model, but the study was not performed because of its cost. Ms. Me asked if such an animal study should be required before conducting the human experiment.

Other Comments

Dr. Parkman asked several questions: (1) Will the sFv105 intrabody recognize all different clinic isolates of HIV? Should inclusion criteria include a requirement that the HIV isolates from the patients demonstrating reactivity to the antibody? (2) Regarding the data of preclinical studies of HIV suppression, has the experiment been extended beyond Day 13 to at least Day 30? There is a trend that the virus inhibition is breaking up at this point.

Dr. Samulski said that there is some evidence in the HSV-TK studies that the intracellu transgene product is inducing immune response and some cell killing of the bystander effect h been attributed to the CTL activities against transduced cells. Would such a host mechanis

clearing the cells complicate the present experiment, i.e., by destroying the cells expressing the intrabody gene which are the very cells intended to be protected in this experiment. Dr. Glorio said he had the same concern. A study of CTL against Epstein-Barr virus transformed T-cells th carry the intrabody gene would be useful. Dr. Glorioso noted that the investigators indicated their written response that they would test the patients to observe if their HIV is reactive with the F105 antibody. Even a single amino acid residue change of gp120 might affect the neutralizing activity of the monoclonal antibody.

In evaluating the risk/benefit ratio of this study, Dr. Miller asked what is the risk of reintroduction o HIV from the patient's cells grown *ex vivo*? Since a very small fraction of CD4(+) cells to be reinfused back to the patients are transduced , will there be any likelihood of efficacy? Since risk is not zero, the question of any potential benefit to the patients is relevant. Dr. Parkman said similar potential risk exists in several other HIV protocols approved by the RAC using different strategies to protect the CD4(+) cells. If the transduced cells that are protected from HIV replica are able to persist, they will be able to function immunologically and the patients will have a be level of immune function. Dr. Miller noted that if apoptosis of CD4(+) cells are triggered by external gp120 binding to the CD4 receptor, the present strategy would not protect the T-cells from destruction. Dr. Miller asked the investigators if there is any evidence from other ongoing studies of risk of infusing activated growing T-cells back to the HIV patients. Dr. Miller asked the investigators to elaborate on the question of whether any efficacy is expected of this study if only 0.1% of T-cells are resistant to HIV replication. He was concerned that the resistant cells may not even have normal immune function. The present strategy of blocking envelope protein processing is different from the ribozyme and transdominant mutant strategies that block viral RNA from producing any viral protein. Dr. Glorioso noted that the investigators did provide some evidenc show that the transduced T-cells are functional

Investigator Response--Dr. Marasc

Responding to Dr. Glorioso's question of transducing HIV-infected T cells, Dr. Marasco s they have achieved a transduction efficiency of 5 to 42%, and the range of HIV-infected CD4(+) cells at the proposed stage of HIV patients is 1:10,000 to 1:100,000, so the investigators expect to be able to transduce a large extent of HIV infected cells. After reinfusion , most of nontransduced cells will succumb to the infection. The investigators have data showin HIV-infected T cells transduced with the sFv105 gene are protected, and the virions produce these cells are markedly less infectious. Responding to Dr. Parkman's question of transduction efficiency, Dr. Marasco said that the transduction rate is quite similar between the vector carryi the sFv105 gene and the control vector. From a mixing experiment of cells marked by these two vectors, the investigators were able to differentiate the 2 cell populations with their assays.

Dr. Marasco said that Dr. Ginsburg's question of selecting cells by G418 is complicated. On advantage of G418 selection is that it would reduce the nontransduced cells serving as a reserv for HIV infection, but Dr. Marasco said he would accept RAC's recommendation on this iss Regarding Dr. Ginsburg's question of patient selection, Dr. Marasco said the present study i modeled after the RAC-approved protocols of Drs. Wong- Staal (#9309-057) and Gary Nab (#9306-049). The patients have to be relatively healthy to recover enough of the T-cells for the study. At the initial trial, patients with a CD4 cell count greater than 200 or 250 per mm3 and having no AIDS defining illness are better candidates.

Dr. Marasco promised to make every attempt to include women in the study. He said that it is difficult issue with his IRB regarding mandatory requirement of autopsy, but he agreed to provi

language that is agreeable to the RAC and the IRB

Regarding the specificity of the monoclonal antibody F105, Dr. Marasco said that the antibod does react with the CD4 binding site of the envelope protein, does react with clinical isolates of HIV, and does have 30 to 70% of neutralizing activities toward primary virus isolates. Regarding the issue of developing escape mutants, Dr. Marasco said from the published studie these mutants mostly developed from neutralization with monoclonal antibody directed against the hypervariable loop, the V3 loop, of the gp120. Those antibodies are more strain specific an bind at a higher affinity than the F105 antibody directed against the CD4 binding site. The latter antibody has a broader neutralizing capacity. There is a natural selection against HIV having mutations at the CD4 binding site; those mutants will lose its ability to bind to the receptor. Dr. Marasco provided additional data on resistance to "escape" of envelope mutant viruses b sFv105 transduced cells. Deliberate point mutations of HIV gp120 were made at the F105 bindin site. These mutants lost greater than 98% of extracellular binding activity with F105, but th intracellular antibody derived from F105 can still inhibit virus formation more than 85% from inside of the cells. When the antibody is working from within cells, it is more effective. Dr. Parkman asked how relevant is the 1 log reduction of HIV infectivity of this data to a clinical situation. Dr. Marasc explained that the reason why this inhibition will be clinically effective is: (1) one mechanism of CD4 cell death has been attributed to envelope protein binding of CD4 in the lumen of the endoplasmic reticulum; the intrabody will inhibit this process; (2) syncytia formation will prevented because the envelope protein never gets to the cell surface; and (3) the HIV virions produced from the transduced cells is markedly less infectious. Dr. Miller said that the clinica efficacy still depends on modifying significant number of T-cells in the patients. Dr. Marasco agreed that clinical efficacy is not the primary objective of the present study.

Dr. Marasco said they have performed animal studies, but those data were not included in th protocol at the recommendation from his IRB . The study was conducted to assess the safet issue of a CTL response directed against the transduced cells. A mouse intrabody gen transduced to mouse cells, and the transduced cells were inoculated to mice. No CTL act observed in this experiment.

Dr. Marasco agreed to screen the patients isolates for its reactivity to F105. However, he was nc certain how that information is relevant to patient selection. His data show that intrabody inhibi virus formation from within cells to a degree greater than the antibody binds to CD4 extracellular i.e., even if the antibody does not affect the virus from outside it still will have effect from within cells.

Ms. Meyers reiterated that the autopsy statement in the Informed Consent document is unacceptable. Dr. Marasco said he would agree with Ms. Meyers personally, but his IRB strong reservations about insisting that patients have an autopsy. As a point of clarification, Dr. Wivel said it is beyond the purview of the RAC to dictate an IRB policy. Mr. Capron said if th considers that autopsy is important in this study, such a stipulation can be attached to the approval. Dr. Lysaught asked Dr. Marasco how the autopsy issue was initially presented to IRB ? Dr. Marasco remembered the issue presented at the IRB review was that "autop mandatory." Dr. Lysaught said the RAC only requires that autopsy be requested. Mr. Capro suggested deletion of the last sentence, "Although we are requesting an autopsy, it will not be required to participate in this study," from the section of *Request for Autopsy*. Ms. Meyers agreed to this suggestion since the statement appears to discourage autopsy. Dr. Marasco agreed t remove this sentence from the Informed Consent document. Dr. Chase said that compliance to autopsy requires investigator motivation, adequate training of the staff, and the cooperation of the

Dr. Frank Sturtz (Progenitor, Inc.) made a comment from the standpoint of a pathologist. He sai is not without any risk for a pathologist who performs the autopsy of a deceased HIV patient. If there is no compelling need for autopsy in this study, the additional risk for pathologists is a facto to be considered. Dr. Parkman said unlike other gene transfer protocols, a biopsy or a limited autopsy of the lymph nodes is very important in order to determine the persistence of transduce cells since those cells clear very rapidly from the blood stream. Dr. Ross said the specific need of autopsy should be resolved among scientific members of the RAC.

Responding to Dr. Miller's question of virus activation of reinfused cells, Dr. Marasco mentic three ongoing clinical studies involving HIV patients. In the clinical trial conducted by Dr. Clifford Lane (NIH), an increase in viremia was observed in the patients after IL-2 infusion. In two stu HIV-infected identical twins conducted by Dr. Robert Walker (NIH), increased viremia observed in patients after infusion of CTL directed against the envelope protein. Dr. Maras emphasized that these are all transient events, and there is no long-term adverse consequence. Dr. Parkman agreed that the risk is low.

Dr. Miller said his other concern is that if there is no ultimate efficacy later on, there is no point of conducting the safety trial. Dr. Marasco said the ultimate goal is to be able to transduce CD3 stem cells for long-term efficacy.

Dr. Marasco said that according to his calculation, the study should determine the relativ persistence of cells transduced by the vector with the sFv105 gene and the control vector. Dr Glorioso was concerned that the fraction of cells infected with HIV will be very small to be th target of sFv105 transgene action

Dr. Miller concluded that the protocol has limited potential for harm to the patients, and it has limited expectation of any efficacy. However, the study will assess the question of persistence of transduced cells in patients and pave the way for eventual application to stem cell therapy

Committee Motion

Dr. Glorioso made a motion to approve the protocol and Ms. Meyers proposed a friendl amendment to delete a statement in the*Section for Autopsy*. Dr. Saha seconded the motion

A motion was made by Dr. Glorioso and seconded by Dr. Saha to accept the protocol submi by Dr. Wayne Marasco contingent on removal of the following statement from the*Section for Autopsy* section of the Informed Consent document: "Although we are requesting an autopsy, it will not be required to participate in this study." The motion was approved by a vote of 16 in favor, 0 opposed, and 1 abstention.

Protocol Summary: Dr. Wayne A. Marasco of Dana-Farber Cancer Institute, Boston Massachusetts, may conduct gene transfer experiments on 6 subjects (18 and 65 years of age) with HIV-1 infection. Autologous lymphocytes from asymptomatic subjects will be transduc*ex vivo* with a retroviral vector, LNCs105, encoding the sFv105 antibody against the HIV-1 envelope protein. An identical aliquot will be simultaneously transduced with a control retroviral vecto lacking the sFv105 cassette. Transduced cells will be reinfused into patients and the differer survival of both populations of CD4(+) lymphocytes compared. The objective of the study is to determine whether the intracellular expression of a human single chain antibody against HIV-1

envelope glycoprotein, gp120, that blocks gp120 processing and the production of infectious virions can safely prolong the survival of CD4(+) lymphocytes in HIV-1-infected subjects

XV. DISCUSSION REGARDING EVOLVING STANDARDS FOR RAC REVIEW OF HUMAN GENE TRANSFER PROTOCOLS

Dr. Zallen (Acting Chair) noted that there are 3 members of the *Ad Hoc* Review Committee who are present at the RAC meeting: Drs. Parkman, Robert Desnick , and herself. Dr. Zallen requeste discussion session to address the concerns of the RAC regarding review criteria of the human gene transfer protocols.

Dr. Wivel stated that there is another panel co-chaired by Dr. Motulsky to review NIH alloc resources of gene transfer research. The *Ad Hoc* Review Committee is to review the RAC review process of human gene transfer protocols. One of the issues for discussion by the *Ad Hoc* Review Committee is the frequent invoking of precedents in the approval of subsequently submitted protocols. Dr. Wivel said the idea of a fixed threshold criterion of approval is not consistent wit the changing nature of scientific development.
Mr. Capron said that there is a difference between precedence and legislation. One may set a rule to say "when X, Y, Z happens the result is A." Then when the next case comes along, one has to examine if all the relevant facts are the same. If there are differences in the facts, the conclusion should not necessarily be the same. Some of the facts consist of what is in the protocol, and other facts consist of everything else that is ongoing with science including the experience of previously approved protocols. Therefore, if Protocol A has been approved and months or years later a similar Protocol B is submitted, the RAC does not obligate to approve Protocol B simply based on the precedence of Protocol A approval since science is changing. Mr. Capron said there is a mixed message from the NIH Director regarding the review criteria, i.e., increased scrutiny scientific quality of protocols versus routine approval of replicative protocol

Dr. Parkman questioned the wisdom of adopting a changing standard of approval by the RAC. Mr. Capron explained the standards of reasoning and proof that is required are not changed; it is simply changing the set of facts which go into reaching a judgment under those standards.

Mr. Capron further explained that the strength of the *NIH Guidelin*s that they are not a written regulation, and RAC review is guided by the *Points to Consider* of the *NIH Guidelin*The *Points to Consider* states what kind of information is needed to reach a certain judgment, and the necessary information is evolving as the science develops. Mr. Capron said it is useful to convey the consensus opinion of the RAC regarding the evolving standards of RAC review of human gene transfer protocols to the *Ad Hoc* Review Committee and to the NIH Directo

Dr. Chase stated that there are three levels of standards which the RAC have used to approve protocols: (1) a minimum safety standard that the trial will not hurt anyone in the process; (2) a higher criterion of being able to acquire some useful information from the study; and (3) the standard of the best possible experiment that can be conceived. Protocols have been approved based on various stringency of the review criteria. Dr. Chase noted a difficulty of performing a critical review in a public forum. He personally would prefer to have the science of gene therapy be vastly improved, and he was uncertain that the public discussion of the RAC is a proper forum to critically review a particular protocol. Dr. Wivel explained that the priority score method employ by NIH Initial Review Groups that ranks the proposals according to their scientific merit may no appropriate for the RAC review process since the RAC has no funding authority.

Dr. Erickson stated there is a need to create a Human Gene Therapy Study Section. He said it will be useful to have a summary statement of critiques of each RAC-reviewed protocol prepared for the benefit of the applicants and the institutional memory of the RAC. Dr. Parkman said a priority score can be assigned to a protocol to convey the message of RAC evaluation of its merit even though the RAC does not have any funding authority. Dr. Zallen asked how that rating scale wo be established. Dr. Parkman responded that each RAC member should evaluate each protocol according to his/her own scale, and the average score of the whole committee will be valid as long as each individual is internally consistent.

Dr. Miller expressed his reservation about the idea that the RAC functions like a study section. The Charter of the RAC asks the committee to review the general concern of recombinant DNA research, its impact on subjects of the experiment, the environment, and the public-at-large. Once the issues have been addressed in a particular protocol, patients have been accrued in the study, and there is no adverse effect, the RAC should then address other issues. In many instances, there is no adequate information from the ongoing studies to allow the RAC to address new issues. The RAC is not a peer review group, and it is not proper for the RAC to use the numerical scoring system.

Mr. Capron said the notion of "precedent" in a sense is referring to a protocol that is not really novel, and that the RAC does not have to reinvent the wheel on every one of these replicative protocols. These replicative protocols can be handled through the NIH /FDA consolidated rev process by exempting from RAC review and being solely reviewed by FDA. Dr. Erickson agreed that more of the replicative protocols should be expeditiously reviewe

Dr. Lai made a comment that he is a new member of the RAC, and the RAC should take a proactive role of rigorous peer review of a protocol. If the RAC finds a protocol inadequate, it should make suggestions as to how to improve the protocol.

Dr. Miller asked what is the specific charge of the RAC charter. Dr. Wivel responded that th charter's mandate is very broad. It charges the RAC to look at safety issues relative to experiments involving recombinant DNA research, and it does not have specific directives in terms of how to conduct gene therapy review. Dr. Miller stated that the RAC should try to subjectively evaluate the risk and benefit issues of gene therapy. The RAC as a public body should assess the issue of how much risk is appropriate in order to gain certain therapeutic benefit. If an experiment presents very low risk, the RAC can afford to be less aggressive about the science.

Dr. Samulski expressed that as a trained scientist, he would like to see a definitive conclusion t scientific inquiry. Most of the current clinical trials are inconclusive as judged from the reports he reviewed as a member of the Data Management Subcommittee. The FDA review will not help this situation since FDA is bound by law to assure only the safety aspect of the trial but not to obtain useful scientific information from these studies.

Dr. W. French Anderson (University of Southern California) said that from his experience of attending every RAC meeting for the past 10 years and having many of his own protocols reviewed by the RAC, he concludes that the RAC does a superb job of providing a public review and assuring public confidence in the gene therapy field. His concern as expressed in his letter to *Science* (vol. 268, page 1261, 1995) is that the RAC gets too involved in the details of routine protocols. He would encourage the RAC to devote its attention to new and innovative approaches of gene therapy.

Dr. Parkman stated that the Charter's charge to the RAC is not simply to assess the safety issue; the risk and benefit ratio assessment is an inherent factor to address the issue of what level of safety risk is acceptable. A quantitative scoring of a protocol is relevant to the assessment of risk/benefit ratio.

Dr. Chase elaborated his view of the RAC's role in gene therapy. Gene therapy has great promis and is too important to be left to the dominance of any single sector of the society. The RAC is a public body that provides an open forum for public deliberation of gene therapy. The issue of risk should no longer be the dominant consideration. The high cost of conducting gene therapy research should promote the consideration of a national strategy. If there is no public input through the RAC, then there would be three major forces driving the development of gene therapy: corporate America, patient demand, and the mass media. All these forces tend to loosen the degree of regulation, and the RAC is the only body who can have any chance of controlling or at least monitoring this process. The RAC voted to agree to have the FDA as a sole review agency for certain protocols due to the fact that society is entering a period when the corporate sector is gaining dominance. There should be a middle ground where the public, through the RAC, can continue to be able to monitor the development of this type of experimentation. Continued dialogue among all sectors of the society is vital for gene therapy to proceed with the confidence of the American people.

Dr. Miller said most of the categories of *Accelerated Review* protocols will be exempt from RAC review and to be solely reviewed by FDA under the consolidated review process. If the exemption can be granted by category, it will be simple. The problem is if the data of the application are grossly inadequate, will it still be exempted? Dr. Wivel said that decision is up to the RAC. Dr. M said his understanding of Dr. Varmus ' comment to the *Ad Hoc* Review Committee is that he wants the RAC to move in the direction of relinquishing more of the routine protocols and addressing more of the outstanding issues. Dr. Wivel said there is a precedent of relinquishing the oversigh of environmental release of recombinant DNA organisms to regulatory agencies such as U.S. Department of Agriculture and the Environmental Protection Agency.

Dr. Lai reiterated his statement that if RAC takes on a proactive role assisting investigators in improving their protocols, the RAC will aid in advancing the field of gene therapy.

Ms. Meyers stated that the Charter should be revised to reflect the role of the RAC in human gene therapy. Dr. Wivel said the Charter is renewed every 2 years. The recent amendment is to delega the authority of appointment of new RAC members to the NIH Director; previously RAC membe have to be appointed by the Secretary of the Department of Health and Human Services.

Ms. Meyers was concerned that accurate information about the data from gene therapy experiments is not getting out to the public even though the RAC has public meetings. Very few scientists publish their studies in peer reviewed journals. NIH needs a coordinated program o gene therapy. Gene therapy protocols should have high scientific quality in order to justify experimentation on human subjects. The closed FDA review will preclude public scrutiny of Informed Consent document.

Dr. Miller suggested the RAC draft a consensus statement to express RAC's viewpoint to the *Ad Hoc* Review Committee. Mr. Capron suggested to include the following points in the consensus statement: (1) to establish a gene therapy study section that operates independently of the RAC; (2) to support the view that RAC continue to work to draw conclusions from the ongoing gene therapy studies; (3) to support the NIH /FDA consolidated review process; and (4) to assure tha

the RAC strives for consistency in its review of gene transfer protocols. Mr. Capron promised to write a statement containing these four points for RAC concurrence this afternoon.

XVI. PRESENTATION TO RAC MEMBERS/DR. WIVE

Dr. Wivel presented certificates to the following members of the RAC whose terms will end July 1995: Dr. Gary Chase (Georgetown University), Dr. Patricia DeLeon (University of Delaware), Dr Krishna Dronamraju (Foundation for Genetic Research), Dr. Dusty Miller (Fred Hutchinson Canc Research Center), Dr. Robertson Parkman (Childrens Hospital of Los Angeles), and Dr. Dori Zallen (VA Polytechnic Institute) Dr. LeRoy Walters (Kennedy Institute of Ethics, Georget University) certificate will be presented at the end of the meeting. Dr. Wivel thanked the membe for their tireless support and dedication to the committee. The members will continue on the committee (after July 31) until replacements are named.

Dr. Zallen announced that the Office of Recombinant DNA Activities will be moving the week o July 10, 1995. The new address will be: 6000 Executive Boulevard, Suite 302, MSC 7010, Bethes Maryland 20892-7010.

XVII. REPORT FROM THE APPENDIX B SUBCOMMITTEE -- PROPOSED AMENDMENTS TO APPENDIX B, *CLASSIFICATION OF ETIOLOGIC AGENTS ON THE BASIS OF HAZARD, OF THE NIH GUIDELINES/DR. STRA*

Report--Dr. Straus

Dr. Straus stated that the Appendix B Subcommittee met on May 5, 1995, at NIH , and the minute of that meeting were included in the materials for the committee. The members of the subcommittee are as the followings: Dr. Stephen E. Straus (Chair), Dr. Donald Blair of National Cancer Institute, Frederick, Maryland, Dr. Andrew Braun of Harvard University, Cambridge, Massachusetts, Ms. Gwladys Caspar of Florida State University, Tallahassee, Florida, Dr. Dia Fleming of Bowie, Maryland, Dr. Joseph C. Glorioso , III, of the University of Pittsburgh, Pittsbur Pennsylvania, Linda B. Wolfe of Massachusetts Institute of Technology, Cambridge, Massachusetts, and Dr. Thomas Y. Shih of ORDA (Executive Secretary)

Dr. Straus said that Dr. Fleming has submitted a proposal for the revision of the outdated Appendix B of the *NIH Guidelin*The proposal has been reviewed by the RAC at its meetings on September 9-10, 1993, December 1-2, 1994, and March 6-7, 1995. The purpose of the subcommittee meeting on May 5, 1995, was to finalize the document in terms of its listing of pathogens and the text of *NIH Guidelin*related to the Appendix B.

Dr. Straus noted several major revisions were made to the proposed Appendix B presented at the March 6-7, 1995, RAC meeting. A more contemporary and fuller preamble to Appendix B was provided. A new section of risk assessment has been added to Section II, of the *NIH Guidelin* Section II has been renamed as *Safety Considerations* and it consists of the new Section II-A,*Risk Assessment*, and the original Section II-B,*Containment*. A major philosophical issue of the revised Appendix B is to classify the etiologic agents according to the concept of risk group instead of the containment class. This new classification is in keeping with the practices of other national and international groups. The first task of the subcommittee is to adopt a definition of risk groups of agents to be included in Appendix B. The agents are classified according to the risk they represent if an immunocompetent , healthy adult is infected with that agent intentionally o unintentionally. The definitions of risk groups are as followings: (1) Risk Group 1 (RG1) are agents that are not associated with disease in healthy adult humans; (2) Risk Group 2 (RG2) are agents

that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available; (3) Risk Group 3 (RG3) are agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available; and (4) Risk Group 4 (RG4) are agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Most of the organisms are in the same categories as the prior proposed Appendix B and in concordance with the publication entitled: *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 3rd Edition, 1993, by Centers for Disease Control and Prevention (CDC) a NIH Division of Safety. Dr. Straus noted several changes have been made by the subcommittee HIV is reclassified as a RG3 agent instead of its previous RG2 classification, a change in keeping with the serious disease caused by HIV infection. The new list provides a more comprehensive listing of human pathogens. The viruses are grouped according to virus families. The oncogenic viruses is no longer listed as a separate group. The idea of oncogenic viruses are of historica interest. Oncogenicity of viruses should be considered along with the same risk consideration other viruses; there is no evidence to indicate that the animal oncogenic viruses are in any wa oncogenic to humans. Finally, the new list is to include only human pathogens; animal and pla pathogens should not be included in Appendix B. The new list can be periodically updated through a special committee of the American Society for Microbiology (ASM) which will make it recommendation to be presented to the RAC as proposed amendments to the *NIH Guidelin*

Other Comments

Dr. Parkman said that it is logical to classify HIV as a RG3 agent. He asked why the Creutzfeldt -Jacob disease agent is not classified as a RG4 agent since it causes a serious huma disease for which a therapeutic intervention is not available. Dr. Straus said it is recommended as a RG3 agent according to the BMBL book, but the reason for this classification is unclear

Dr. Anderson requested to include in the RG1 classification of the murine leukemia and sarcom viruses, which are used in gene therapy and baculovirus often used as a gene expression vecto The Creutzfeldt -Jacob disease agent can be handled under BL-3 containment, and he agreed w its RG3 classification.

Dr. Parkman said the subcommittee has performed an outstanding job in revising the Appendix B. It is a functional document, and it is not intended to be inclusive in its listing of organisms. For other unlisted agents, the IBC can make a RG determination referring to the related organism included in Appendix B. Dr. Shih said that references are included in the *NIH Guidelin* as to where additional information can be obtained. Dr. Straus noted several updated references are now provided in the revised Section V, *Footnotes and References of Sections I-IV*.

Dr. Miller said only a few examples are given in RG1, and most of the commonly used organisms are not listed. Dr. Straus explained that the present list includes only the human pathogens; anima and plant pathogens, which do not infect humans are not included. Dr. Miller said that a murine amphotropic virus with an oncogene can infect humans and is a potential concern for huma Straus said such a virus is a construct derived from a natural virus and is not listed in Appendix B that lists only the natural viruses. Section III-C-3 of the *NIH Guidelin* provides a guidance to evaluate containment levels for such an experiment which is likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range of viral vector conditions that permit a production infection. In such case, the physical containment should be increased by at least one level.

Dr. Miller preferred to have the commonly used agents previously listed as oncogenic viruse incorporated into Appendix B. Dr. Parkman suggested to include this group of animal pathogens that are in common use and to rank them according to the risk group criteria. Dr. Miller said most of the animal pathogens would fall under the RG1 classification that represents agents not associated with disease in healthy adult humans. Dr. Samulski suggested that adeno -associ virus (AAV) should be included in the RG1 group. Dr. Straus agreed to the suggestion. Dr Parkman said there is a clear rationale to separate the listings of human and animal pathogens; Appendix B is a clean list of human pathogens. Dr. Straus noted that an alternative is to have a separate list of animal pathogens in the Appendix Q,*Physical and Biological Containment for Recombinant DNA Research Involving Animals*; but he is unsure it would assist the readers to create a separate list in Appendix Q.

Dr. Straus asked the RAC to consider if the revised Appendix B is in order, and whether to make some additional changes suggested by Dr. Miller. Regarding Dr. Dronamraju's question about t CDC list, Dr. Fleming explained that CDC's BMBL book uses agent summary statements, and th present list is generated using the BMBL information together with information from othe sources. In the future, if new organisms that need risk classification, an ASM committee o laboratory safety will evaluate the new organisms from a risk assessment standpoint.

Dr. Anderson stated that a lot of effort has been put into revising Appendix B, and the subcommittee has conducted its job well. He suggested to include the list of low-risk oncogenic viruses together with AAV in the RG1 classification, and the ASM committee can update this an annual basis. The RAC should achieve the closure on this document. Dr. Straus said Dr. Anderson's suggestion is perfectly reasonable. Dr. Parkman said that he prefers the current listing of human agents, and to create a separate Appendix B-V section of animal pathogens that are relevant to human studies. Dr. Miller said that another group of moderate risk oncogenic viruse can be included in this category.

Dr. McGarrity stated that using a retroviral vector that retains 25% of viral genome falls into sev sections of the *NIH Guidelin*: (1) if it is used in tissue culture, it is an exempt experiment under Appendix C-I; (2) if it is used in animal cells, the experiment can be performed under Biosafety Level 1 containment (Section III-C-3); and (3) if it is used in human patients, it is under Section III-A-1 for human gene transfer experiments and the containment is not specified. Dr. Straus agreed that the *NIH Guidelin*s a very complicated document, but he considered that Appendix B is not a proper place to list the vector systems derived from animal viruses. Dr. McGarrity sai that under Section III-A-1, the RAC can recommend the specific containment conditions or stipulation requirements for such experiments involving gene transfer vectors. This action will clarify some IBC concerns that these vectors should be used under Biosafety Level 2 containment. Dr. Wivel noted that Appendix B is for wild-type viruses and is for the worst cas scenario. The vector systems are more appropriate to be dealt with in the Appendix C,*Exemptions under Section III-E-6*, for experiments that do not present a significant risk to health or the environment as determined by the NIH Director with the advice of the RAC. Most of the vector would have deletion of virus genome of more than one half. Drs. Parkman and Straus agreed that Appendix C is a proper place for vectors.

Dr. Miller noted that Monkeypox virus is a RG3 agent. Dr. Straus explained that it cause symptoms similar to smallpox in humans.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Erickson to: (1) establish a subcommittee to recommend exemption of additional vector systems in Appendix C (exempt host-vector systems), and (2) accept the proposed amendments to Appendix B with the provision to develop a new Appendix B-V section of animal viruses relevant to human studies, and to list specific examples of agents under Appendix B-I, *Risk Group 1 (RG1) Agents*. The motion was approved by a vote of 17 in favor, 0 opposed, and no abstentions.

Dr. Anderson volunteered his service to the subcommittee to develop the listing of the exempted vector systems for Appendix C.

XVIII. CONTINUED DISCUSSION OF THE CONSENSUS STATEMENT TO THE *AD HOC REVIEW COMMITTEE*

Dr. Zallen presented to the RAC a consensus statement written by Mr. Capron. The RAC membe reviewed this document. After some clarification, explanation, and editorial changes of the document, Dr. Zallen asked if there is anybody who does not wish to be associated with thi document. There was no objection. The RAC unanimously adopted this document as a consensus statement to be conveyed to the *Ad Hoc* Review Committee.

The consensus statement reads as follows:

"We wish to convey to the *Ad Hoc* Review Committee our consensus that:

"1. We agree with the *Ad Hoc* Review Committee's preliminary conclusion that any gene therapy study section established by the National Institutes of Health to increase the quality of basic and clinical science should operate independently of the RAC;

"2. We also support the view that the RAC should continue to work actively to draw conclusions from the accumulating safety and efficacy data from gene therapy studies, both to aid investigators in improving the design of their protocols and to communicate to the public the actual status of research on gene therapy;

"3. In order to afford more time on its agenda for discussion of novel protocols and important issues, the RAC remains committed to avoiding review of inappropriate or unnecessary protocols through such mechanisms as: (a) the Office of Recombinant DNA Activities' preliminary screening of protocols that are not ready for RAC review, and (b) proper use of the joint process recently established with the FDA under *Appendix M-VII, Categories of Human Gene Transfer Experiments that May Be Exempt from RAC Review,* under which the FDA exercises primary oversight of protocols in designated categories, which are then exempt from RAC review; and

"4. The RAC should strive for consistency in its judgments when evaluating protocols while recognizing both that its *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects* will continue to evolve in light of experience in the field and that judgments about the acceptability of each protocol are scientifically and ethically defensible only when reached in the context of the developments in relevant scientific and other fields."

XIX. PRESENTATION ON IN UTERO GENE TRANSFER EXPERIMENTS IN SHEEP/DR. ZANJ

Dr. Wivel said that the RAC will have a series of background lectures in the area *in* *utero* gene therapy. Today's speaker is Dr. Esmail Zanjani , Veterans Administration Hospital Medical Ce Reno, Nevada, and he is going to present the data derived from *in* *utero* sheep studies involving *in* *uter* hematopoietic stem cell (HSC) transplantation *in* *utero* gene transfer.

Presentation--Dr. Zanjan

Dr. Zanjani stated that his work has been supported for the past several years by R01 grants fr the National Heart, Lung, and Blood Institute of NIH , Merit Review Program of the Department o Veterans Affairs, and by the generous support of the G. Harold and Leila Y. Mathers Charitabl Foundation. He will summarize his work in the areas of *in* *uter* HSC transplantation a *in* *uter* gene therapy of sheep studies. Some of these sheep data have been confirmed in monkeys.

In Utero Hematopoietic Stem Cell Transplanta

There are compelling reasons that one should treat certain patients early in gestation by *in* *uter* stem cell transplantation. In most cases, diagnosis and treatment can be conducted early. In addition, there could be ethical reasons for initiating treatment early in gestation. There is an emotional element of the prospect of having a normal newborn, and the cost of prenatal treatment is considerably less than treatment after birth.

Will an *in* *utero* approach be effective? There are several characteristics of hematopoiesis in th developing fetus during ontogeny that makes the young fetus an ideal host for stem cell transplantation and possibly even gene therapy. During early development of the fetus, the site of hematopoiesis naturally changes from yolk sac to liver and spleen, and finally to bone marrow One important element in this transfer of the site of hematopoiesis occurs quite early in gestati at a time when the fetus is still preimmune , so that the donor cells can be transplanted with rela ease without rejection by the host. The other advantage is to "piggy back" the stem cell to the natural migration of hematopoietic cells through blood circulation during the period of transfer the bone marrow site. One can achieve donor cell engraftment without having to use myeloablative procedures

There are many reasons that make the sheep model attractive. The sheep fetus has a long gestation period. Its immune status is known; during the preimmune period, the sheep fetus i large enough to tolerate surgery. It has two hemoglobin types for use as markers to detect donor cell engraftment: Type A donor cells can be easily distinguished from cells of Type B recipient sheep.

The procedure used for the stem cell transplantation is basically the same as that for gene transfer. The fetus can be visualized and cells can be injected intraperitoneally into the fetus There usually have twins available for the experiment, so that one of the twins can be used as a control. The animals are born often without any adverse effects due to the manipulation. The newborn will be examined for evidence of donor cell engraftment or for gene transfer.

Dr. Zanjani acknowledged several of his colleagues for the studies *in* *utero* HSC transplantation. They are: A. W. Flake, M. Tavassoli , G. D. Almeida- Porada , J. L. Ascensso R. Harrison.

The principle of *in* *utero* stem cell transplantation is different from post-natal transplantation. The post-natal transplantation is to replace defective bone marrow or to replace bone marrow after a

high dose chemotherapy. The *in utero* transplantation is to provide additional "normal" bone marrow activity.

A variety of diseases can be diagnosed early in gestation and have been shown to benefit from post natal bone marrow transplantation. These are the likely candidate diseases to benefit from *in utero* transplantation. These diseases include Fanconi anemia, thalassemia major, sickle c disease, severe combined immunodeficiency, chronic granulomatous disease, infantil agranulocytosis , infantile malignant osteopetrosis , Chedia -Higashi syndrome, Maroteau syndrome, and Hunter syndrome.

Most of the problems of bone marrow transplantation can be avoided by *in utero* transplantation since the host is immunologically naive. Other advantages *in utero* transplantation include: access to the patients prior to disease effect, no need to prepare the host by myeloablation , ne for only a small number of HSC , tolerance induction, fetus in a protected environment, and cos effectiveness.

Dr. Zanjani showed data from 3 newborn lambs after injecting HSC from a preimmune fet to unrelated recipient fetuses. The newborns are shown to be chimeric and have expression o donor cells of multilineage cell types including lymphoid, erythroid , and myeloid cells. The e has persisted over a long period of time. There are animals that are almost 6 or 7 years old that are still chimeric

The investigators asked several questions in these studies, i.e., gestation age, route of administration, sources of donor HSC , and induction of tolerance. Dr. Zanjani summarized d from these studies. The best donor cell engraftment occurs at a period in which the fetus is in the preimmune stage, i.e. 55 to 70 days of gestation. Dr. Glorioso asked if engraftment of human sheep transplantation is as efficient as the sheep to sheep experiment. Dr. Zanjani responded t the efficiency of human to sheep transplantation is much less, but it can be improved by using human specific growth factors. Dr. Zanjani said the best time for the highest engraftment in th sheep to sheep transplantation coincides with the period when the transfer of hematopoietic ce from liver to bone marrow occurs. The intraperitoneal injection is a better route of administratio than the intravenous route for the long-term effect. A probable reason is that in the process of getting cells transposed from peritoneal cavity to bloodstream, the cells pass through the lymphatic system and are educated and processed so that the cells are better tolerated by the body.

There are several sources of donor HSC , i.e , fetal liver, fetal marrow, cord blood, newborn b marrow, adult bone marrow, and adult blood. The data shows that fetal liver and marrow engraft very well and show no evidence of GVHD . The other sources except the peripheral blood are go sources for the donor cells. The engraftment efficiency of adult bone marrow is as high as 20%. Although engraftment rate is high except for cells from the fetus, all other cell sources induce GVHD . Out of 28 transplantation of cord blood cells, 15 are chimeric . 12 of the 15 chimeric have GVHD , and these animals rarely survive to term

Dr. Zanjani showed data of a xenograft model of transplanting adult human bone marrow sheep, and the sheep developed GVHD . This complication can be avoided by using antibod purified CD34(+) bone marrow cells.

For most diseases, the engraftment rate of 15 to 30% donor cell levels is therapeutically effective. These donor cell levels can be achieved by administering more cells to the recipients especially if

the cells can be purified. Another way is to administer the cells by multiple injections. An interesting observation is that there is a sudden increase in expression of donor cells about 11 to 12 months after transplantation, and it occurs not only in sheep but in monkeys and humans. In most animals, Dr. Zanjani observed donor specific tolerance. 11 of the 17 animals having dono cell levels of 10 to 15% developed tolerance to the original donor cells. In these tolerant animals, the donor cell level can be further increased by a simple post natal infusion of the original donor cells.

Dr. Zanjani summarized results from his studies *uter*sheep HSC transplantation experiment demonstrate the following: (1) intraperitoneal injection of HSC into pre-immune fetuses yie optimal donor cell engraftment, (2) HSC of pre-immune fetal donors engraft without eliciting G (3) purified or T cell-depleted post-natal donor cells engraft without eliciting GVHD , and chime lambs exhibit tolerance to donor tissue, and (5) "actively" acquired tolerance to donor HSC can utilized to achieve therapeutic levels of donor cell engraftment by "boosting" after birth.

There are several trials being performed in humans. The best time for human transplantation is 10 to 15 weeks of gestation while the best time for sheep is 6 to 9 weeks. The success rate of engraftment in humans varies. The reason for the failure appears to be that most of the fetuses were injected at a late time in gestation, and they were not completely immunoincompetent Another difficulty of human transplantation is the timing and obtaining human fetal cells of good quality suitable for transplantation use.

In Utero Gene Thera

Dr. Zanjani said that these studies of gene therapy have been carried out in collaboration wit many investigators including his group at Reno, Drs. M. Eglitis , P. W. Kantoff and W. F. And formerly of NIH , Drs. A. W. Flake and M. R. Harrison of University of California at San Francisco and Drs. R. Moen, L. Troutman and R. Lyons formerly of GTI . Two approaches have been used i these studies: the cellular approach of*ex vivo* transduction of hematopoietic cells and the vect approach of direct injection of vectors into the fetus.

In the cellular approach, the cells were taken from 100 day old fetal sheep. They were transduced with vectors carrying the *ne*R gene. After transplanting back into the fetuses, the animals after birth were examined for the presence of the marker gene. It was found that several months after the transfer, the animals continued to express the marker gene. However, the transgene was lo 30 months after the transfer. There are several shortcomings in this cellular approach of gene transfer: it involves multiple manipulations of the fetus; transduction is limited to the removed cells; and it is only applicable to older fetuses.

A simpler approach is to inject the vector directly into the fetus. Producer cells or viral supernatants in 1 to 2 ml aliquots were directly injected intraperitoneally into preimmune fe lambs. Intravenous injection did not function as well as the intraperitoneal injection. Preimmu of the recipient was important. The newborns were examined for the presence of the marker gene by G418 resistance of the transduced cells in tissue culture and by PCR analysis of the mar DNA sequences. The investigators injected 30 fetuses: 22 were born alive and others were sacrificed before birth. 14 of the 22 live births were test positive for the presence of *ne*R sequences. Interestingly, 4 mothers of the injected fetuses showed the presence of low levels of *ne*R sequences by PCR . All these 4 ewes were mothers of fetuses receiving vector produc cells; none of the mothers of fetuses receiving vector supernatants showed any *ne*R sequences The transduction efficiency was estimated from the percentage of hematopoietic cells resistant

G418, and it was about 15 to 20%. The efficiency was higher in animals receiving vector producer cells than those injected with the vector supernatants. Some marrows from sheep after 21 months of gene transfer still tested positive for the *neo*R sequences by the PCR analysis. The colonies these marrows in tissue culture demonstrated *neo*R gene expression by their resistance to G418. The marker gene could be detected in multilineage hematopoietic cells including erythr mononuclear macrophage, and primitive progenitor cells. These data suggest transduction of hematopoietic stem cells

To further demonstrate the stem cell transduction, bone marrow cells were taken from 3 animals 2.8 years after *in utero* gene transfer. The cells were transplanted into 13 normal preimmune fet lambs. Preliminary analysis of these 13 newborns by PCR showed that 9 animals were teste positive for the marker DNA sequences. The *neo*R sequences were present not only in the bone marrow and peripheral blood cells but in other tissues. In an animal 1-month old, the *neo* sequences were detected in the brain, gonads, thymus, liver, and many other places. In a 16-month old animal, not much of the marker sequences were detected in the lung and liver, but it was still positive in kidneys and testes. The present data could not distinguish whether the sequences were present in the circulating blood cells or the tissues themselves have been transduced

Sheep #182, which was *neo*R positive in the sperm, was bred to a normal ewe that gave birth to 2 lambs. One of the lambs showed *neo*R presence in the brain and many other organs by PC analysis. If the sperm was transduced , it would give rise to a transgenic animal and the level of PCR signals should be much higher. To further determine if the sperm itself was transduced sperm ejaculate from sheep #182 was sent to Dr. Martin Eglitis (NIH) for a PCR analysis. A the ejaculate still tested positive after separation of sperm from the rest of the fluid in the ejaculate the sperm in 2 different samples was found to be negative for the *neo*R sequences. Experiments are ongoing to confirm these important data.

Dr. Zanjani said that he had tested other retroviral vectors, and he fou *in utero* gene transfer using all these vectors. The conclusions from the *in utero* gene therapy studies are as follows: (1) The results demonstrate that exogenous genes can be successfully transferred into sheep by the direct injections of supernatants or vector producer cells into young fetuses without significant side effects: and (2) The long-term presence of the *neo*R marker gene in these lambs indicates tha the pluripotent stem cell may have been transduced . In addition, these studies showed t transduction can occur at high efficiency, it can be conducted in very young fetuses, and it only needs one injection.

Other Comments

Mr. Capron asked to clarify if the data shows that only semen is tested positive for the *neo*R sequence but not the sperm itself. Dr. Zanjani responded affirmatively; the positive signal from ejaculate may be due to the presence of white blood cells such as macrophages. The data now shows that germ line transduction has not occurred in these animals.

Dr. Erickson said there still has a potential danger of transducing the sperm cells since th blood-testes barrier does not completely form until later stages of fetus development. Mr. Capron commented that the experiment does not exclude the possibility of sperm transduction. Dr. Zanjani said the ejaculate samples could easily be contaminated with blood cells that contribut to the positive result. A thorough study is ongoing to address the issue of germ line transduction including examination of the original *in utero* gene transduced animal for the presence of marke

gene sequences in various organs including the sex organs. Dr. Straus agreed that the germ line transfer is an important issue that needs to be addressed. Ms. Rothenberg and Dr. Lysaught asked if the mothers of the injected fetuses showed persistent presence of the marker sequences. Dr. Zanjani responded that the mother animals have not been examined any furthe

Dr. Anderson asked how soon the *in uter*gene transfer procedure will be applicable to humans? Dr. Zanjani responded if the possibility of germ line transduction can be excluded, then th procedure should be very straight forward for the human application. Mr. Capron expressed his concern if the mother of the fetus would get the transgene . Dr. Zanjani responded that if one the vector supernatants, there is no such side effect.

Dr. Dronamraju asked if there is a possibility of a germ line gene therapy. Mr. Capron said th state-of-the-art is not at that stage. Dr. Erickson said there is a concern about insertional mutagenesis of the vectors. Mr. Capron was concerned about a maternal side effect of*in uter* gene transfer particularly if a high titer vector supernatant is used.

Dr. Zanjani noted that several animals injected more than 3½ years ago still express the trans in their bone marrows.

Dr. Walters (Chair) rejoined the meeting. He thanked Dr. Zallen for chairing the afternoon sessio of yesterday's meeting and most of today's meeting. Dr. Wivel presented to Dr. Walters hi certificate of service to the RAC, and thanked him for his tireless support and chairing of the RAC.

XX. FUTURE MEETING DATE/DR. WALTERS

The next meeting of the RAC will be September 11-12, 1995, NIH , Building 31C, Conference Roo 6.

XXI. ADJOURNMENT/DR. WALTERS

Dr. Walters adjourned the meeting at 4:05 p.m. on June 9, 1995.

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.D
Chair
Recombinant DNA Advisory Committee
National Institutes of Health