DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
March 6-7, 1995

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The Recombinant DNA Advisory Committee (RAC) was convened for its sixty-first meeting at 9:00 a.m. on March 6, 1995, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on March 6 from 9 a.m. until 5 p.m. and March 7 from 8:30 a.m. until 3:00 p.m. The following were present for all or part of the meeting:

Committee Members:

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

Executive Secretary:

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

Liaison Representative:

Daniel Jones, National Endowment for the Humanities
Ralph Yodaiken, Department of Labor

National Institutes of Health staff:

Bobbi Bennett, OD
Thomas Bock, NCHGR
Sandra Bridges, NIAID
Bruce Bunnell, NCHGR
Lin Chen, NCHGR
Marinee Chuah, NCHGR
Joe Gallelli, CC
John Gallin, NIAID
Barry Goldspiel, CC
Jay Greenblatt, NCI
James Higginbotham, NCHGR
Christine Ireland, OD
Jaya Jagadeesh, NCHGR
Isabelle King, NCHGR
Richard Knazek, NCHGR
Becky Lawson, OD
Harry Malech, NIAID
Catherine McKeon, NIDDK
Hiroaki Mizukami, NHLBI
Richard Morgan, NCHGR
David Nelson, NCHGR
Jay Ramsey, NCHGR
Yona Reizes, NIAID
Thomas Shih, OD
Sonia Skarlatos, NHLBI
Thierry Vandendriessche, NCHGR
Jarmo Wahlfors, NCHGR
Robert Walker, NCHGR
Debra Wilson, OD

Others:

Paul Aebersold, Food and Drug Administration
Estuardo Aguilar-Cordova, Texas Children's Hospital
W. French Anderson, University of Southern California
Robert Anderson, Food and Drug Administration
Dale Ando, Chiron Therapeutics
Greg Baigent, Chiron Therapeutics
Jennifer Barrett, Yale University
E. Jeffrey Beecham, Iowa Methodist Medical Center
Bridget Binko, Cell Genesys
John Bishop, Food and Drug Admininistration
Keith Black, University of California, Los Angeles
Helen Blau, Stanford University
Arindam Bose, Pfizer, Inc.
Andrew Braun, Harvard University
Linda Burch, Systemix Corporation

Tom Calloway, Systemix Corporation
Jeff Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
Robert Carton, U.S. Army
Michael Casey, Genetic Therapy, Inc.
Joy Cavagnaro, Food and Drug Administration
Kye Chestnut, University of Florida
Yawen Chiang, Genetic Therapy, Inc.
Larry Cohen, Somatix Corporation
Carol Conrad, Johns Hopkins University
Larry Couture, Ribozyme Pharmaceuticals, Inc.
Ken Culver, Des Moines, Iowa
Richard Daifuku, Targeted Genetics Corporation
Olivier Damos, Somatix Corporation
Karen Darcy, Magenta Corporation
Robert DeJager, PerImmune
Verna DeMartini, Applied Immune Sciences
Nathalie Dubois-Stringfellow, Chiron Corporation
Earl Dye, Food and Drug Administration
James Econmou, University of California, Los Angeles
Habib Fakhrai, University of California, Los Angeles
Ali Fattaey, Onyx Pharmaceuticals
Diane Fleming, Mid-Atlantic Biological Safety Association
Terry Flotte, Johns Hopkins University
Ilana Fogelman, Food and Drug Administration
Jeffrey Fox, Science Writer
Joyce Frey-Vasconcells, Food and Drug Administration
Bernd Gansbacher, Memorial-Sloan Kettering Cancer Center
Debbie Gessner, Viagene, Inc.
Simba Gill, Systemix Corporation
Marilyn Gosse, Tufts University
Tina Grasso, GenVec, Inc.
Helen Heslop, St. Jude Childrens Research Hospital
Warren Heston, Memorial Sloan-Kettering Cancer Center
Douglas Hickman, T. Rowe Price
Susan Hirano, University of Wisconsin
JoAnn Horowitz, Schering-Plough Corporation
Donna Hoy, Schering-Plough Corporation
John Jaugstetter, Genentech, Inc.
Susan Jenks, Journal of National Cancer Institute
Jim Kawecki, KA Corporation
Connie Kirby, Canji, Inc.
Michael Klein, Johns Hopkins University
Toshi Kotani, Genetic Therapy, Inc.
Karen Kozarsky, University of Pennsylvania
Steven Kradjian, Vical, Inc.
Alex Kuta, Food and Drug Administration
Bridget Laffler, FDC Reports
Willaim Larchain, Memorial Sloan-Kettering Cancer Center
Gloria Lee, Rhone-Poulenc Rorer, Inc.

Ronald Leonardi, R&R Registrations
John Levy, Iowa Methodist Medical Center
Charles Link, Iowa Methodist Medical Center
John Logan, Nextran
Zhifeng Long, Genetic Therapy, Inc.
Cynthia Louth, Life Technologies
Patrick Lu, Genetic Therapy, Inc.
Christopher Maack, Somatix Therapy Corporation
Tamie Malaska, Targeted Genetics Corporation
Daniel Maneval, Canji, Inc.
Phil Maples, Baxter Health Care Corporation
Tony Marcel, TMC Development
Stephen Marcus, Genetic Therapy, Inc.
Eliot Marshall, Science
Barbara Matthews, Food and Drug Administration
Alan McClelland, Genetic Therapy, Inc.
C. Bruce McCullough, Schering-Plough Research Institute
Gerard McGarrity, Genetic Therapy, Inc.
Andra Miller, Food and Drug Administration
Robert Moen, Geneic Sciences, Inc.
Karin Molling, Institute for Medical Virologie
Donald Moorman, Iowa Methodist Medical Center
William Murphy, University of Edinburgh
Andrea Neuman, Technology Catalysts
Philip Noguchi, Food and Drug Administration
Debbie Norby, Nextran
Sheryl Osborne, Viagene, Inc.
Jeffrey Ostrove, Magenta Corporation
Ed Otto, Genetic Therapy, Inc.
Robert Overell, Targeted Genetics Corporation
Carolyn Paradise, Chiron Therapeutics
John Parrott, University of Virginia
Lisa Piercey, BioWorld Today
Stephen Pijar, University of Maryland
Anne M. Pilaro, Food and Drug Administration
Doros Platika, Progenitor, Inc.
Raj Puri, Food and Drug Administration
Robert Ralston, Chiron Corporation
Urban Ramstedt, Virus Research Institute
Paul Recer, Associated Press
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
Bruce Roberts, Genzyme Corporation
Joseph Rokovich, Somatix Therapy Corporation
Russell Rother, Alexion Pharmaceuticals, Inc.
Howard Scher, Memorial Sloan-Kettering Cancer Center
Richard Scotland, Genzyme Corporation
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International
Juliet Singh, Baxter Healthcare Corporation

Margi Stuart, University of California, San Francisco
Franck Sturtz, Progenitor, Inc.
Nevin Summers, Ingenex, Inc.
Thomas Tarlow, Chiron Corporation
Mary Treuhaft, RGene Therapeutics, Inc.
Judy Ways, Glaxo, Inc.
Katherine Whartenby, Food and Drug Administration
Lisa White, The Blue Sheet
Carolyn Wilson, Food and Drug Administration
Deborah Vaz, Virus Research Institute
Alan Venook, University of California, San Francisco
Inder Verma, The Salk Institute
Aono Yuri, The Mainichi Newspapers
Robert Zimmerman, Chiron Corporation

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

Dr. Walters (Chair) called the meeting to order and stated that the notice of the meeting was published in the *Federal Register* on January 30, 1995 (60 FR 5687), and the proposed actions were published in the *Federal Register* on February 8, 1995 (60 FR 7630), 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 noted that 6 human gene therapy protocols will be reviewed at this meeting; the PI of a 7th protocol was notified prior to this meeting that his study would not be reviewed, based on insufficient preclinical data. In addition, there are several items to be discussed in this meeting: NIH and Food and Drug Administration (FDA) consolidated review of human gene transfer protocols, amendments to Appendix B regarding classification of microorganisms, a presentation by Dr. Miller of a presumptive human retrovirus, adenoviral vector toxicology, report of the NIH Director's *Ad Hoc* review committee, update on data management, report on the conference call of the subcommittee on *in utero* gene therapy, and update on the gene therapy information network. The discussion of transgenic xenotransplantation will be deferred to a future RAC meeting.

Dr. Walters announced the following items of interest: (1) The FDA will hold a meeting at 6:00 p.m. (March 6) for the purpose of discussing somatic cell and gene therapy production issues (60 FR 8662). (2) *Nature* (February 9, 1995) and *Science* (February 17, 1995) have reported that Japan recently approved its first human gene therapy trial. This trial involves the administration of a retrovirus vector encoding the gene for adenosine deaminase (ADA) for the treatment of severe combined immune deficiency (SCID) due to ADA deficiency. This trial was publicly reviewed and approved by a committee of the Japanese Ministry of Health and Welfare. (3) The Institute of Medicine of the National Academy of Sciences is proceeding towards its own detailed study on germ line genetic intervention. (4) An article entitled: *Gene transfer and expression in progeny after intravenous DNA injection into pregnant mice*, was published in *Nature Genetics, Volume 9*, pp. 243-248 (March 1995). Dr. Makoto Tsukamoto and colleagues reported in this paper that a single intravenous injection of expression plasmid/lipopolyamine complexes into pregnant mice resulted in gene transfer into the somatic cells of the offspring of the pregnant mice.

Dr. Walters welcomed the members of the RAC *Ad Hoc* Review Committee who are attending this

meeting in order to review the RAC proceedings. He welcomed their suggestions for enhancing the current review process. The first meeting of this *Ad Hoc* Review Committee was held on February 3, 1995; the second meeting will be held on March 8, 1995.

Dr. Walters provided an update on the status of the 5 human gene therapy protocols that were reviewed and recommended for approval (with contingencies) at the December 1-2, 1994, RAC meeting: (1) For the protocol entitled: *Phase I Trial of Interleukin-2 Plasmid DNA/DMRIE/DOPE Lipid Complex as an Immunotherapeutic Agent in Solid Malignant Tumors or Lymphomas by Direct Gene Transfer* (Protocol #9412-095), the PIs have met all of the contingencies, the primary reviewers have approved the submitted material, and the Office of Recombinant DNA Activities (ORDA) has forwarded the protocol to the NIH Director for approval. (2) For the protocol entitled: *Clinical Protocol for Modification of Tumor Suppressor Gene Expression in Head and Neck Squamous Cell Carcinoma (HNSCC) with an Adenovirus Vector Expressing Wild-type p53* (Protocol #9412-096), the PIs submitted material in response to their contingencies on February 22, 1995. This study is currently under review by the primary reviewers. (3) For the protocol entitled: *Adenovirus Mediated Gene Transfer for Cystic Fibrosis: Safety of a Single Administration in the Lung (aerosol administration)* (Protocol #9412-094), a telephone conference call was held on December 15, 1994, between the PIs and a subcommittee of the RAC to discuss the proposed experimental design for the additional preclinical studies stipulated by the RAC. The revised experimental design (approved by the RAC) was submitted by Genzyme on January 11, 1995, and these experiments are in progress. (4) For the protocol entitled: *Phase I Study of Adenoviral Vector Delivery of the HSV-TK Gene and the Intravenous Administration of Ganciclovir in Adults with Malignant Tumors of the Central Nervous System* (Protocol #9412-098), the primary reviewers approved the proposed experimental design for the additional preclinical studies stipulated by the RAC on February 6, 1995. The RAC approved experiments are in progress. (5) For the protocol entitled: *Gene Therapy of Primary and Metastatic Malignant Tumors of the Liver Using ACN53 Via Hepatic Artery Infusion: A Phase I Study* (Protocol #9412-097), the RAC scientific members approved the proposed experimental design (for the additional preclinical studies) on February 22, 1995. The RAC approved experiments are in progress (Attachment II--Protocol List).

II. REPORTS ON ACCELERATED REVIEW/DR. WALTERS

Dr. Walters said that there were 2 requests for *Accelerated Review* of human gene transfer protocols since the December 1-2, 1994, RAC meeting. On January 19, 1995, Dr. Michael Fetell of Columbia Presbyterian Medical Center, New York, New York, submitted the protocol entitled: *Stereotaxic Injection of Herpes Simplex Thymidine Kinase Vector Producer Cells (PA317/G1TkSvNa.7) and Intravenous Ganciclovir for the Treatment of Recurrent Malignant Glioma* (Protocol #9502-099). This protocol was reviewed and approved through the *Accelerated Review* process on February 10, 1995.

On February 15, 1995, Ms. Sheryl Osborne of Viagene, Inc., San Diego, California, submitted a Phase II multiple site protocol entitled: *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*. Viagene requests approval to initiate this study at the following sites: (1) The University of California San Diego Treatment Center, San Diego, California (Richard Haubrich, M.D.); (2) Kelly Avenue Clinic, Oregon Health Sciences University, Portland, Oregon (Mark Loveless, M.D.); (3) AIDS Treatment Center, University of Cincinnati, Cincinnati, Ohio (Peter Frame, M.D.); (4) Washington University Medical Center, St. Louis, Missouri (William Powderly, M.D.); and (5) George Washington University Medical Center, Washington, D.C. (David Parenti, M.D.). This request received contingent approval by a subgroup of the RAC on March 3, 1995. The stipulations for approval were: (1) Viagene should comply with the semiannual data reporting requirements for all sites involved in this Phase II study (including those who are exempt from RAC review and NIH approval), (2) revise the Informed Consent document to include a

statement regarding protection of subjects from the media, and (3) delete the recommendation for termination of pregnancy in the event that conception occurs during the course of the subject's participation in the study.

III. REPORT ON MINOR MODIFICATIONS/DR. WALTERS

Dr. Walters stated that one minor modification was approved since the December 1-2, 1994, RAC meeting. On January 24, 1995, Drs.Sobol and Royston received permission to substitute a new retrovirus vector, LXSN-tIL2, for the previously approved vector, LNCX-IL2 (Protocol #9312-060) (Attachment III--Minor Modification Table).

IV. MINUTES OF THE DECEMBER 1-2, 1994, MEETING/DRS. DOI AND DELEON

The RAC approved a motion made by Dr. Doi and seconded by Dr. DeLeon to accept the December 1-2, 1994, RAC minutes (with the incorporation of minor editorial changes) by a vote of 17 in favor, 0 opposed, and no abstentions.

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

Dr. Wivel provided an update on the NIH/FDA consolidated review process. Proposed actions to the *NIH Guidelines* were first published for public comment in the *Federal Register* on August 23, 1994 (59 FR 43426). These proposed actions were reviewed and approved (with modifications) by the RAC at its September 12-13, 1994, meeting. On October 26, 1994, NIH/ORDA forwarded the revised actions to the NIH Director for approval and the FDA Commissioner for concurrence. FDA legal counsel expressed concern that implementation of these actions would require amendment to the FDA Investigational New Drug (IND) Application Regulations (21 CFR Part 312) to accommodate the release of proprietary information. To resolve this concern, a waiver for release of information from the FDA to the NIH was proposed. While the *NIH Guidelines* could require such a waiver for NIH-funded investigators, it would be voluntary for others submitting proposed human gene transfer experiments to the FDA. The NIH expressed concern that failure to comply with voluntary waiver procedures may result in the loss of critical information necessary to maintain: (1) the human gene therapy database, (2) "real-time" reporting of serious adverse events, and (3) comprehensive overview (by category) by the RAC in a public forum. Public review and access to submission, review, and follow-up information is critical to the safe and focused advancement of human gene therapy research. As a result of these concerns, the NIH and FDA agreed on a compromise proposal that would accommodate the single submission format proposed at the July 18-19, 1994, meeting of the National Task Force on AIDS Drug Development, while maintaining public access to critical information and "real-time" reporting of adverse events. The compromise proposal involves simultaneous submission of human gene transfer protocols to both NIH and the FDA in a single submission format. This format includes (but is not limited to) the documentation described in Appendices M-I through M-V, of the *NIH Guidelines*. NIH/ORDA and the FDA will simultaneously evaluate the proposal regarding the necessity for RAC review. These revisions to the consolidated review process were incorporated into the *NIH Guidelines* and published in the *Federal Register* on February 8, 1995 (60 FR 7630).

Other Comments

Dr. Walters noted a letter dated February 24, 1995, from Sheryl L. Osborne (Viagene, Inc., San Diego, California). Dr. Wivel pointed out two of the issues raised in Ms. Osborne's letter that have previously

been discussed by the RAC: (1) requirement of Institutional Review Board (IRB) approval before RAC review; and (2) requirement of RAC review for a protocol progressing from a Phase I study to Phase II and III clinical trials.

Dr. Parkman favored a further discussion of the rationale of prior IRB approval. He stated that it is an appropriate procedure to review the Phase I/Phase II transition by the *Accelerated Review* process. Dr. Motulsky stated that reviewing protocols before IRB approval would streamline the process; however, the RAC must avoid the appearance of issuing directives to local IRBs regarding approval of clinical studies. Dr. Zallen said that this problem between the RAC and the IRB is avoidable if the PIs and IRBs adhere to the new guidelines regarding the informed consent issue as stated in the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)*. Dr. Chase favored the idea of RAC review prior to IRB approval but cautioned that there are two consequences the PIs and the sponsors need to consider: (1) The IRB frequently makes a judgment about the scientific merit of a proposal, a review criterion the RAC has not rigorously applied in the previous approval of many protocols; (2) Currently, the RAC frequently defers to the university legal department regarding the issue of fully indemnifying the human subjects for any risk in participating in an experiment of no therapeutic value. If the RAC functions as a super-IRB, such an indemnifying requirement may be imposed on all the protocols regardless of the legal position of the local institutions. Dr. Noguchi (FDA) said that the FDA permits concurrent IRB review but the study cannot proceed until final IRB approval. Responding to Dr. Zallen's concern regarding Informed Consent document, he suggested that if a protocol does not address all questions stated in the *Points to Consider*, the RAC does not have the obligation to review it.

Ms. Meyers asked for an explanation regarding an issue raised by Ms. Osborne in which actions by the NIH Director should be required to be completed within 15 calendar days of the RAC's recommendation. Dr. Wivel explained that the 15 day time frame is a misunderstanding of the process. A majority of protocols receive contingent approvals that require varying amounts of time to collect the necessary data, and in some cases, the PI took up to 18 months. Dr. Parkman said that 15 days would be a realistic time frame from the date of final RAC approval that all the stipulations are met. Dr. Ross added that the RAC is only advisory to the NIH Director, and it cannot decide when the NIH Director can and should approve a protocol. Dr. Walters asked what administrative steps are required for final NIH Director's approval after RAC recommendation. Dr. Wivel explained that the following clearance/approvals are required for a protocol: NIH General Counsel's Office, Acting Deputy Director of Science Policy and Technology Transfer, Deputy Director, and then the NIH Director. In many instances, the process can be accomplished within 15 days; but the NIH Director retains his/her discretion for final approval.

Dr. Miller stated that it is reasonable from FDA's standpoint to have concurrent FDA and IRB review since they are reviewing from two different aspects. For the RAC, the prior IRB review serves as a filter so that if IRB does not approve a protocol, the RAC does not have to review it. Dr. Parkman stated that local IRB has the primacy in approving a clinical trial; it is a reasonable system that IRB serves as both a starting and a finishing point of the approval process, and the RAC is a review step in that loop. Responding to a question by Dr. Dronamraju regarding inadequate IRB approval, Dr. Walters explained that in the early days of gene therapy in 1979 and 1980, the RAC took the primary responsibility of gene therapy review. At that time, there was a lack of local expertise in reviewing this nascent field of clinical studies. Responding to a question by Dr. Ross about whether the RAC will be able to see the Informed Consent document finally approved by IRB, Dr. Wivel explained that the local IRB has the final call in approving this document; frequently, the PIs will forward the revised Informed Consent document to ORDA. Regarding the local autonomy of IRB, Dr. Noguchi said that FDA and NIH regulations for IRB are overlapping but are not congruent. Some FDA regulated IRBs are not under any purview of NIH and vice versa. The concurrent FDA and IRB review is necessitated by the statutory limit of 30 days for a FDA

decision on an IND application. Dr. Noguchi disagreed with the notion that a prior screening by a local IRB would improve the quality of a protocol in terms of adherence to the requirement of *Points to Consider*. Dr. Zallen stated that if the RAC requirement as stipulated in Appendix M-I-D on Informed Consent document is communicated to the PIs through the NIH Office for Protection from Research Risks (OPRR), most concerns of the RAC regarding the Informed Consent document would be addressed. Dr. Noguchi added that FDA will convey the information to the PIs through its Office of Health Affairs to the IRBs regulated by FDA.

Committee Motion 1

A motion was made by Dr. Haselkorn and seconded by Dr. Dronamraju to retain the current requirement for obtaining IRB approval prior to RAC submission. A friendly amendment was made by Dr. Motulsky and accepted by Drs. Haselkorn and Dronamraju that ORDA should notify the Director of OPRR regarding the necessity for IRB adherence to the detailed questions contained in Appendices M-II through M-V of the *NIH Guidelines* (Informed Consent issues). The amended motion was approved by a vote of 17 in favor, 0 opposed, and 1 abstention.

Committee Motion 2

Dr. Miller made a motion that RAC should continue to review and approve a clinical trial moving from the Phase I to Phase II/III follow-up studies. The motion was seconded by Dr. Haselkorn.

Dr. Noguchi asked to clarify if the motion includes RAC review of all protocols of the expanded Phase II/III trials. Dr. Wivel explained that the Viagene Phase II trial is a precedent for reviewing this kind of study under the *Accelerated Review* process; the essence is to preserve the RAC option to review this clinical trial progression if necessary. Dr. Miller said if a protocol initially developed by a team of experts is to expand to different centers which may not have the same degree of expertise, it will require the RAC to review the expanded trial. Dr. Parkman agreed that at this point in time, the RAC would review Phase II studies under the *Accelerated Review* process; simple changes such as the number of patients can be dealt with as minor modifications. Dr. Parkman stated that appropriate data to be reviewed include summary of clinical and *in vitro* data generated in the Phase I studies indicating a forward movement of the gene therapy related science. Dr. Straus stated that an important aspect of RAC review is to understand if the science supports the large public investment and the risk inherent in the gene therapy studies. He was satisfied with the data provided by Viagene to support its Phase II study including new cytotoxic T lymphocyte (CTL) data and no immune deterioration in human immunodeficiency virus (HIV) patients participating in the Phase I study. Dr. Parkman indicated his personal interest in seeing these data.

Ms. Meyers said one of the reasons for so much time being spent on reviewing the Informed Consent document is that PIs frequently overlook the autopsy question. She was disappointed that only 7 autopsies were performed out of many deaths involved in gene therapy protocols. Very little data pertaining to the question of gene transfer has been obtained; these are data required to support the expansion of the trial from the Phase I to the Phase II stage.

A motion was made by Dr. Miller and seconded by Dr. Haselkorn that the RAC should continue to review and approve Phase I follow-up studies, i.e., Phase II and Phase III trials. Such studies may be submitted through the *Accelerated Review* process; however, the RAC retains the option to require full RAC review. The motion passed by a vote of 18 in favor, 0 opposed, and no abstentions.

Ms. Osborne stated in her February 24, 1995, letter: "Institutional Biosafety Committee (IBC) review

should apply only to those institutions where actual manufacturing operations occur. The clinical use of an injectable vialed gene therapy product presents no environmental or health personnel exposure risks and should not necessitate IBC review." Dr. Miller disagreed with this statement indicating that there are risks inherent in the gene therapy products even if they are contained in vials; IBC approval is needed for the use of such products.

Committee Motion 3

Dr. Walters called the question regarding the proposed revision of the *NIH Guidelines* regarding NIH and FDA consolidated review. Dr. Smith said that such a mechanism of review is reasonable.

The RAC approved a motion made by Dr. Haselkorn and seconded by Dr. Dronamraju to approve the proposed amendments to Sections I, III, IV, V, and Appendix M of the *NIH Guidelines* regarding NIH and FDA consolidated review of human gene transfer protocols, by a vote of 18 in favor, 0 opposed, and no abstentions.

Dr. Walters stated the RAC has found a way to streamline the submission and review process for investigators and yet to preserve the public purview of all gene therapy protocols; he thanked Drs. Wivel and Noguchi for shepherding this process through its many steps of development. He summarized the three motions approved by the RAC in this session: (1) Prior IRB approval is required before submission to the RAC; (2) The RAC retains its option to review expansion of Phase I studies to Phase II/III trials; and (3) A single format but simultaneous submission to both NIH and FDA and a selective RAC review of protocols in the consolidated review process.

VI. ADDITION TO APPENDIX D OF THE ***NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I TRIAL OF IN VIVO GENE THERAPY WITH THE HERPES SIMPLEX THYMIDINE KINASE/GANCICLOVIR SYSTEM FOR THE TREATMENT OF REFRACTORY OR RECURRENT OVARIAN CANCER/DRS. LINK AND MOORMAN***

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Drs. Charles Link of the Human Gene Therapy Research Institute and Dr. Donald Moorman of Iowa Methodist Medical Center, Des Moines, Iowa. Dr. Straus stated that the investigators propose to perform a Phase I study in which PA317 vector producing cells (VPC) will be infused into the peritoneal space of up to 24 women with recurrent or refractory ovarian cancer. The VPC will carry the LTKOSN.2 retrovirus. This virus was derived from the Moloney murine retrovirus. It contains the *Herpes simplex* virus thymidine kinase (HSV-TK) type I gene and the *neo*R marker gene. Fourteen days after the single infusion of cells, patients will be treated with intravenous ganciclovir (GCV) for an additional 2 weeks.

This protocol is an attempt to offer an additional experimental therapeutic modality for ovarian cancer patients who have short life expectancy. It is anticipated that the VPC will release the HSV-TK expressing retrovirus, and that the retrovirus will preferentially infect ovarian cancer cells studding the peritoneum. GCV will be preferentially phosphorylated in cells that currently express HSV-TK, leading to their death. It is assumed that TK needs only be expressed in a minority of cancer cells in order for a more generalized toxic effect of GCV metabolites to occur.

The protocol is a variation on the theme introduced in earlier RAC-approved protocols of end stage glioblastoma multiforme, where again there was reasonable expectancy that more tumor cell death would occur than would be accounted for by the proportion of infected cells. While this whole concept, including

its extension to the setting of ovarian carcinoma, is mechanistic, it is worth pursuing for proof of concept. Dr. Straus stated that the ovarian cancer model is not amenable to this type of treatment as is the brain tumor system. In the brain tumor system, it is anticipated that the tumor is relatively circumscribed and confined to such a small area that diffusion of toxic GCV derivatives would facilitate killing of bystander tumor cells. This effect is difficult to visualize in the context of ovarian cancer because the tumor is studded, both microscopically and macroscopically, across the vast surface area of the peritoneum. The vast surface area and the volume of peritoneal fluid serves to dilute the impact of the TK activity in a given cell.

Dr. Straus said that in his initial review, he raised a series of concerns about the scientific issues and a number of lesser issues about the protocol and the Informed Consent document. The written responses from the investigators have satisfied him in terms of the overall protocol and the informed consent issues. The investigators have provided additional animal model data that is encouraging. Dr. Straus found that there is precedent for this type of protocol, and there is some scientific rationale for this proposal. However, he had a serious reservation about the extremely mechanistic approach where the PIs anticipate a therapeutic effect in which VPC is diluted over a vast volume and surface area of the peritoneal space. But given the precedents and the nature of the patient population, Dr. Straus stated that the protocol is a reasonable proposal.

Review--Dr. Glorioso

Dr. Glorioso raised 3 specific questions: (1) Is there any direct evidence for the bystander effect *in vivo*? If not, it simply means that infection of ovarian cancer cells will need to be extensive to be effective; (2) If the patient is removed from the study for any reason before therapy is started, can the investigators be sure that the VPC will be eliminated from the body and not spread to other organs? Do the investigators need to use live VPC?; (3) Can more be learned from this study if the investigators evaluate anti-tumor immunity in the follow-up? Dr. Glorioso said that the investigators have provided additional data from the MC 38 intraperitoneal adenocarcinoma mouse model to estimate the *in vivo* transduction efficiency of LTKOSN.2 VPC. Approximately 47% of these cells were transduced, and approximately 50% of the animals remained tumor free. This data implies that nontransduced tumor cells are being destroyed by a bystander effect. Dr. Glorioso said that there is still a concern as to the mechanism of destruction of these tumor cells. The investigators have no data to suggest other mechanisms such as the development of antitumor immunity. Dr. Glorioso agreed with Dr. Straus' assessment that this protocol can be approved based on other precedents, but he has a serious concern as to whether the investigators will be able to evaluate the experimental outcome from the proposed study.

Review--Dr. Zallen

Dr. Zallen stated that the investigators have not provided information regarding matters of informed consent as requested in Section M-I-D of the *Points to Consider*. The investigators had revised the Informed Consent document responding to Dr. Zallen's comment. Dr. Zallen still had concern about the tone of statements used to describe this dose escalation Phase I study: The suggestion that the treatment will be therapeutic is inappropriate and needs to be stated in proper perspective for the patients. The section on the risks of the Tenckhoff catheter provides no estimate of the degree of the risk. In addition, she asked if there is any evidence of movement of the retrovirus vector to the intended target site of the ovary.

Other Comments

Dr. Parkman raised a question of persistence of the VPC. According to the preclinical data of the mouse

model, the infused VPC persisted for 3 days but were not detectable Day 7 or Day 14 post-infusion. When GCV treatment starts on Day 14, is the VPC still present in mice? The persistence of VPC in the brain tumor models is partly due to the immunologically privileged nature of the central nervous system. For GCV treatment to be effective, there must be some transduced cells, either of donor or of host origin, present during the course of the treatment. The problem of VPC persistence is more critical for other xenogeneic systems, such as the humans that are different from the mouse VPC in the mouse model. Dr. Glorioso added that in the canine studies, the VPC were lost rapidly after infusion.

Responding to a question by Dr. Dronamraju about what "mechanistic" means, Dr. Straus reiterated his concern about the amount of dilution in the infused VPC within the large peritoneal cavity. Mechanistically, there is little chance of effective transduction of tumor cells.

Dr. Walters noted that the investigators have not provided an up-to-date information in the revised Informed Consent document regarding the potential risks of VPC found in other studies using the VPC to treat brain tumors. Dr. Parkman asked if there is any evidence of gene insertion in normal tissue within the peritoneal cavity.

Investigator Response--Drs. Link and Moorman

Responding to the question of the mechanism of bystander effect, Dr. Link said such an effect has been observed in the animal models but he does not know the exact mechanism. With regard to the question of VPC dilution in peritoneum, he said that the final dose of the escalation study in humans is equivalent to the dose in the mouse models that shows efficacy, but he is uncertain if that dose level will be sufficient to transduce tumor cells in the human study. Dr. Link agreed to revise the Informed Consent document to avoid the tone of therapeutic intent of the present treatment.

Regarding the question of VPC persistence, Dr. Link said that in the canine experiment, no evidence of vector transduction of intra-abdominal tissues was found on Day 28 post-infusion. There is no available intra-abdominal tumor model of large xenogeneic animals to evaluate this question of vector transduction. In the mouse model, the VPC probably persists more than 3 days. On Day 7, some cells morphologically resembling VPC were visible under the microscope; but they cannot be recovered by cell culture. On Day 14, no evidence of VPC was present.

Dr. Parkman asked about the percentage of tumor cells dividing in the metastatic nodules in the peritoneum of ovarian cancer patients. Dr. Link responded that he did not know the answer.

Dr. Link said the risk of Tenckhoff catheter infection that lasts for more than one month is less than 1%; he will include such information in the Informed Consent document. Many chemotherapeutic drugs have been administered directly into the peritoneal cavity, and some adhesions are induced in these patients; but this complication should pose no serious problem for placing the Tenckhoff catheter in the present trial. There is no evidence of vector transduction of ovaries in mice, and such an unintended effect is not a serious concern for ovarian cancer patients since their ovaries would be removed at the time of surgery as part of their treatment.

Dr. Glorioso asked if the bystander effect has been observed in immuno-compromised animals. Dr. Link responded that such effect was observed in the partially immunodeficient nude mouse model using human ovarian cancer cells.

Dr. Miller asked whether complement present in the peritoneal fluid would lyse the VPC. Dr. Link responded that peritoneal fluid from ovarian tumor patients or from patients with ascites did not affect the

VPC. The number of VPC drops one thousand-fold after similar treatment with human sera which contain complement.

Dr. Parkman said that he would administratively approve this protocol based on precedent such as Scott Freeman's protocol (#9202-016); however, he would not scientifically approve this protocol based on the insufficient preclinical study to demonstrate persistence of the VPC and effective transduction of tumor cells. Responding to Dr. Doi's question if any scientific information will be obtained from this study, Dr. Parkman said this protocol typifies the problem of the Phase I loophole: The major purpose of this study is to determine if one can escalate to the highest cell dose without significant toxic effect, but he is unsure that significant scientific information will be obtained from the study. Dr. Miller added that, based on the available data of how fast the cell divides and how much vector need to be present, he would conclude that the experiment probably will not work. Dr. Parkman agreed that there probably will be no therapeutic response, but this protocol is a Phase I study to evaluate toxicity in which efficacy is not the primary objective. Dr. Miller said that even a Phase I trial should strive to evaluate if there is a possibility that the treatment would be effective.

Dr. Straus stated that the animal models are encouraging and similar favorable outcome may be achieved in the human patients. Since the investigators have addressed the safety issues and demonstrated their ability to monitor them, Dr. Straus did not see a reason to deny approval of this protocol. DrDeLeon said some scientific merit can be obtained from evaluating other biological endpoints by technique such as peritoneoscopy. Dr. Link said that biopsy will be performed on Day 1, and peritoneal washings on Days 3, 7, and 14. Dr. Parkman suggested a biopsy by peritoneoscopy on Days 14 and 21, right before and after GCV treatment to obtain information on what proportion of tumor cells are transduced and the morphological effects of this treatment on tumors. Dr. Link accepted Dr. Parkman's suggestion. Dr. Straus said that an earlier time point for biopsy may be valuable to determine if the VPC persist and if there is any sign of inflammation; this information can be obtained from another set of the patient population so that a given patient does not have more than 2 biopsies.

Dr. Erickson remembered that the RAC had a similar discussion during the deliberation of the Freeman's protocol (#9202-016), 4 years ago, and some RAC members voted against approving the protocol. After 3 patients were treated in the Freeman protocol, no scientific information has been obtained from this study. Dr. Haselkorn asked if there is any patient improvement based on the theory of the bystander effect from other protocols. Drs. Parkman, Erickson, and Straus recalled some anecdotal data from the brain tumor protocols. Dr. Haselkorn asked if the vector is expected to target the tumor cells in preference to the epithelial peritoneal cells. Dr. Link responded that the amphotropic virus will attach to all cells, but it will preferentially integrate and express in the dividing tumor cells.

Dr. Straus asked the investigators to clarify the dates for performing the biopsy with peritoneoscopy. Dr. Link said it will be performed on Day 1 during the same operation of placing the Tenckhoff catheter and second one on Day 7 or 14. Dr. Parkman preferred two time points: on Day 14 and another later date to assess the treatment result. Dr.Haselkorn considered the biopsy time frame an important question and would prefer to contingently approve the protocol pending development of the best experimental design mutually agreeable to the investigators and the RAC members.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr.DeLeon to accept the protocol submitted by Drs. Charles Link and Donald Moorman contingent on review and approval by Drs. DeLeon, Parkman, and Straus, of a revised experimental design which includes the time frame for biopsies. A friendly amendment was made by Dr. Zallen and accepted by Drs. Straus and DeLeon to require submission of a

revised Informed Consent document which includes clarification of the risk factors, i.e., known risk factors associated with the Tenckhoff catheter. The amended motion was approved by a vote of 17 in favor, 1 opposed, and no abstentions.

Protocol Summary: Dr. Charles Link of the Human Gene Therapy Research Institute and Dr. Donald Moorman of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct gene transfer experiments on 24 female subjects (18 years of age) with refractory or recurrent ovarian cancer. Subjects will undergo intraperitoneal delivery (via Tenckhoff catheter) of the VPC, PA317/LTKOSN.2. These VPC express the HSV-TK gene which confers sensitivity to killing by the antiviral drug, GCV. The LTKOSN.2 retrovirus vector is based on the LXSN backbone. Two weeks following intraperitoneal delivery of the VPC, subjects will receive 5 mg/kg intravenous GCV administration twice daily for 14 days. Subjects will receive between 1 x 105 and 1 x 108 VPC/kg in this dose escalation study. Subjects will be evaluated by X-ray and peritoneoscopy of the abdomen for evidence of clinical response. The objectives of this study are to determine the safety and biological efficacy of intraperitoneal VPC administration.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A PHASE I TESTING OF GENETICALLY ENGINEERED INTERLEUKIN-7 MELANOMA VACCINES/DRS. ECONOMOU, GLASPY, AND McBRIDE*

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California. This protocol is a resubmission of a study that was reviewed by the RAC at its June 1994 meeting and deferred pending submission of additional preclinical data. Dr. Motulsky stated that the investigators plan to assess the safety and immunologic effects of administering interleukin (IL)-7 producing melanoma cells as a "vaccine" to patients with metastatic melanoma. IL-7 has never been used in humans. The RAC expressed concern about the lack of toxicity studies with IL-7, and the proposal was deferred for additional data.

Mice and monkeys were given IL-7. No toxicity was observed in mice but glomerular changes not associated with blood urea nitrogen or creatinine elevations were seen in monkeys. These changes were not associated with inflammatory cellular infiltrates or evidence of immune complex deposits on the glomerular basement membrane. Furthermore, the changes were found with doses that were 2 orders of magnitude higher than the planned human doses. In view of these findings, a nephrologist will participate as a co-investigator in these studies. The investigators documented new data from experimental mice showing that lung fibrosarcoma metastases were markedly inhibited on immunization of the mice with irradiated IL-7 transduced tumor vaccines.

The investigators will add a bioethicist to the team who "will provide support to patients in deciding whether to participate...." in such studies. It is unclear how recruitment of patients for these studies will be conducted. Will the investigator as the patients' physician request participation and will the bioethicist deal with more detailed explanations, and how?

The remaining major concern of Dr. Motulsky was the renal pathology in the monkeys. Careful renal-related blood biochemistry and urinalysis will be conducted under the experimental protocol. Will there be more careful sediment analysis of the urine? At what point will the investigators do renal biopsies? Have the investigators planned to conduct the analysis in case of creatinine elevations or abnormal findings on urinalysis. In summary, Dr. Motulsky said the questions of toxicity have been answered. The investigators submitted additional efficacy results in experimental animals. Except for

some minor points, Dr. Motulsky would recommend approval.
Review--Dr. Doi

Dr. Doi provided a detailed written review of the protocol. He said that he agreed with Dr.Motulsky that the toxicity studies have been conducted, and the results are very encouraging. The only major concern is the renal toxicity at high dosage levels. Dr. Doi said that he recommends approval of the protocol.

Review--Ms. Meyers

In Ms. Meyers' initial written review, she was concerned about the use of the word vaccine. To the lay public, vaccine means an injection that prevents disease. There is no indication that the experiment intends to prevent cancer, and the word should not be used. Since Dr.Economou has not omitted vaccine from the protocol title nor the Informed Consent document, Dr.Economou must at least insert an explanation in the body of the Informed Consent document explaining that his use of the term vaccine is not the same as the dictionary: *a substance to induce immunity to a disease*. Dr. Economou should clearly state that the substance he will be injecting into the subject will not make the patient immune to cancer, nor will it prevent the patient from getting cancer in the future. The word vaccine appears on each page of the Informed Consent document 3 to 4 times. Responding to this concern, Dr.Economou has added a sentence to the Informed Consent document to state that this treatment would not make the patient immune to cancer. Ms. Meyers stated that the revised version is not a totally satisfactory solution, because the word vaccine is misleading.

Some of the subjects will be receiving their own genetically modified tumor cells, and some will be receiving cells from a former melanoma patient (presumably dead). Do the investigators have permission to use this cell line for commercial purpose? It would be unfortunate if this treatment were held up in court for years while this question was being settled.

Other Comments

Dr. Motulsky made an additional remark about the toxicity: The patients have a disease that will kill them; therefore, the minor toxicity in this group of patients might be considered differently than it would be in normal subjects.

Dr. Ross asked the investigators to explain the statement in the Informed Consent document, "I understand that if I am injured as a direct result of research procedures not done primarily for my own benefit, I will receive medical treatment at no cost."

Dr. Parkman asked two questions: (1) He asked for the interval between implanting of the tumor cells and the beginning of the therapy in the animal models. (2) Relating to the animal study, the renal toxicity was not detected by urinary sediments but by autopsy. What is the endpoint to assess renal toxicity in the human study? The Informed Consent document should request a limited autopsy in order to obtain the kidney for the purpose of examination for possible renal toxicity.

Dr. Zallen asked why the investigators insist on using the word vaccine while others have accepted the alternative word experimental material. Dr. Dronamraju asked what is the specific role of the bioethicist. Dr. Haselkorn said that the investigators have no right to call their material vaccine, since it has not been demonstrated that the material is safe and effective; the general public would regard it as a vaccine.

Dr. Miller indicated that he would abstain from voting on this protocol due to his association with the Targeted Genetics, the sponsor of the trial. He commented that the use of the word vaccine is not totally

invalid since the gene-modified cells are to stimulate specific immunity against the patient's cancer cells just like the conventional vaccines. Furthermore, not all antiviral vaccines are totally safe. Dr.Zallen said most other protocols have chosen alternative descriptions such as experimental material or immunotherapeutic. Dr. Parkman observed that melanoma is a special cancer of which there is a body of scientific evidence to indicate that anti-tumor immunity can be induced by immunization with tumor cells or extracts from these cells. This term fits the broader definition of vaccine, but the terminology should not be used generically to other types of tumors for which there is no supporting scientific rationale. Dr. Straus noted that substances such as IL-2 induce some responses, but these substances cannot be called vaccines. Dr. Chase objected to the use of the term vaccine; it is against public understanding of what vaccination means. Dr. Walters remarked that with increasing frequency vaccine has been used in a therapeutic rather than simply a prophylactic context. Dr. Straus agreed it is appropriate only for giving to people who have an infection. Dr. Walters noted that only one RAC-approved protocol has the word vaccine in the title. Dr. Secundy asked why the investigators consider it important to keep the terminology in the present protocol. Dr. Walters noted that FDA has requested two IND applications for this study; however, the investigators have asked the RAC to consider only the allogeneic arm of the study at the present time. The autologous arm of the study will be a separate protocol in the future. As a point of clarification, Dr. Parkman explained the present study involves irradiated nontransduced autologous tumor cells plus increasing doses of transduced allogeneic cells.

Investigator Response--Dr. Economou

Dr. Economou responded to the question of the role of bioethicist. A triage question was raised about how to enroll patients in different competing clinical protocols at the University of California at Los Angeles (UCLA), when the RAC reviewed his previous protocol at the June 1994 RAC meeting. After consulting with ethicists and IRB directors, a strategy has been developed. The patients will be presented with all the eligible protocols, and he/she will be asked to obtain a second opinion from an independent oncologist (usually the referring physician) as to which protocol is most appropriate for the patient. Dr. Les Rothenberg, a senior ethicist at UCLA, will participate in this protocol to conduct a prospective study about the triage system.

Regarding the toxicity question, Dr. Economou said that they have consulted with FDA officials regarding the design of all toxicology studies and inclusion of a senior nephrologist at UCLA as co-investigator of the protocol. The investigators, together with two nephrologists, have evaluated all the preclinical data and concluded that the risk of renal toxicity is remote for the human study. Nevertheless, since this human trial is of a new cytokine, the investigators plan to evaluate renal function closely, including careful examination of urinary sediments for sensitive signs of glomerular injury. The patients with any abnormality in renal function and urinalysis will be excluded from the protocol to avoid confusion with this endpoint of evaluating toxicity.

Dr. Economou agreed to remove the word vaccine from the protocol and the Informed Consent document. Regarding the question of permission to use the melanoma cell line, Dr. Economou said that the M24 cell line has been established and owned by UCLA; a joint UCLA/Targeted Genetics Intellectual Property Agreement has been signed so that there will be no legal problems about its use in the protocol.

Dr. Economou said that the statement pointed out by Dr. Ross, "I understand that if I am injured as a direct result of research procedures...", is a standard paragraph of the UCLA Informed Consent document, and he did not know its origin. Dr. Miller asked if this statement will exclude compensation if there is any therapeutic intent. Dr. Economou responded that this was not the case; there is no benefit to the patients expected in this Phase I trial.

Responding to Dr. Parkman's question about the lag period in terms of seeding the animal lungs with tumor cells, Dr. Economou said that the metastatic nodules are fast growing anaplastic tumors, and 3 days after seeding is the appropriate time to start the therapy.

Dr. Dronamraju asked for a clarification of the role of thebioethicist in informing the patients. Dr. Economou said Dr. Rothenberg is a lawyer and a professor of the Division of Human Genetics; his role is to define for the investigators a manner in which the investigators can inform patients about completing clinical trials at UCLA. He will study this model of informed consent process in a prospective manner.

Committee Motion

Dr. Motulsky made a motion to approve the revised protocol contingent on changing the word vaccine to a more appropriate term. Dr. Doi seconded the motion. Dr. Miller asked for a suggestion of the appropriate term, and Drs. Zallen and Chase indicated "immunologic treatment" is acceptable.

Dr. Motulsky said it is important to make an effort to obtain the kidney at the time of patient's death; a permission of this limited autopsy would be easier to get from the patients and their families. The autopsy would not be part of the stipulation, and he would leave this matter to the discretion of the investigators. Dr. Economou said that an autopsy will be requested in the event of a patient's death.
Dr. Walters made a friendly amendment to the motion to include a statement regarding protection of subjects' confidentiality and interest of the media.

The motion made by Dr. Motulsky and seconded by Dr. Doi to accept the protocol submitted by Drs. James Economou, John Glaspy, and William McBride, passed by a vote of 18 in favor, 0 opposed, and 1 abstention. Approval of the protocol is contingent on the review and approval of a revised Informed Consent document by the primary reviewers that includes: (1) replacement of the terms vaccine and vaccination with more appropriate terminology, i.e., immunologic treatment; (2) a statement regarding protection of subjects' confidentiality and interest of the media; and (3) replacement of the term vaccine with a more appropriate term in the title.

Dr. Miller abstained due to conflict of interest; he is associated with Targeted Genetics Corporation.

Protocol Summary: Drs. James Economou, John Glaspy, and William McBride of the University of California, Los Angeles, California, may conduct gene transfer experiments on 25 subjects (18 years of age) with metastatic melanoma. The protocol is an open label, Phase I trial to evaluate the safety and immunological effects of administering lethally irradiated autologous melanoma cells plus allogeneic cells transduced with the retroviral vector, IL-7/HyTK, which encodes the gene for human IL-7. Subjects will receive 1 x 107 irradiated unmodified autologous tumor cells in combination with escalating doses of IL-7/HyTK transduced allogeneic melanoma cells (M24 cell line). The number of M24 cells administered will be adjusted based on the level of IL-7 expression. Subjects will receive 3 biweekly subcutaneous injections of M24 cells expressing 10, 100, or 1000 nanograms of IL-7/hour *in vivo*. A final cohort of 5 subjects will receive IL-7/HyTK transduced autologous cells. Subjects will be monitored for antitumor activity by skin tests, biopsy analysis, tumor-specific antibody activity, and CTL precursor evaluation. Non-immunologic parameters will be monitored.

VIII. UPDATE ON DATA MANAGEMENT/SMITH

Dr. Smith, Chair of the Working Group on Data Management, noted that several working group members submitted responses to Ms. Wilson's (ORDA) December 27, 1994, letter regarding modifications to future data management reporting forms. Responses were received from Drs. Erickson, Ross, Smith, and

Straus. The suggested modifications will be incorporated into future reporting forms.

Dr. Smith noted that 7 safety/adverse event reports were submitted since the December 1-2, 1994, RAC meeting relating to the human gene transfer studies involving the PA317/G1TkSvNa.7 VPC (Genetic Therapy, Inc). The RAC concluded that these adverse events/safety reports were most likely to be attributable to disease progression or Ommaya reservoir placement and not related to VPC administration.

Dr. DeLeon pointed out that the word vaccine has been used in the data reporting while its usage was debated in Dr. Economou's protocol. Dr. Ross said it raises more concerns if the word is used in the Informed Consent document.

Ms. Meyers asked if all adverse events reported in the brain tumor protocols are due to disease progression. Dr. Motulsky explained in one case, the untoward effect appears to be due to the therapeutic effect of the treatment; the antitumor response had shrunk the tumor and thinned out the top of the ventricle wall that resulted in perforation of Ommaya tubing. Dr. Marcus added that this perforation caused a transient reaction and the patient made a full recovery from this episode. Dr. Noguchi said that these adverse events have been followed very closely at FDA; none of the deaths to date have been attributed to be directly related to gene therapy. A similar conclusion has been reached regarding the adverse events seen in the cystic fibrosis (CF) studies.

Dr. Smith noted that one safety/adverse event report was submitted for the Genetic Therapy, Inc., sponsored CF study (Protocol #9303-041). The subject was hospitalized for exacerbation of pulmonary symptoms and hemoptysis; these symptoms abated within 2 days following intervention. Dr. Steven Marcus (Genetic Therapy, Inc.) noted that this trial is temporarily on hold. Dr. Parkman inquired whether the adverse event could be attributable to adenovirus immunity. Dr. Marcus responded that this scenario is unlikely; chest X-ray films showed that the patient had a major preexisting pulmonary disease. The adverse event is most likely a result of disease exacerbation resulting from the subject's major preexisting cavitary lesions. The members of the RAC agreed that this event is probably not directly related to adenovirus vector administration. Dr. Walters noted that these adverse events have been promptly reported to ORDA and reviewed by RAC members.

IX. UPDATE ON GENE THERAPY INFORMATION NETWORK (GTIN)/DR. NOGUCHI AND MS. WILSON

Dr. Noguchi explained that the Gene Therapy Information Network (GTIN) has been recently approved by the FDA as a pilot project for the Submissions Management and Review Tracking System (SMART). The first phase of this prototype is anticipated to be operational for the June 1995 RAC semiannual data reporting period. The current base of information has been accumulated by Ms. Wilson and reviewed by the Working Group on Data Management. This information will be transferred into a relational database that is readily accessible by MacIntosh and IBM personal computers. Ms. Wilson stated that one of the advantages of the GTIN will be the ability to generate summary reports (unlimited variables) that readily can be disseminated to the public via the Internet.

Ms. Wilson stated that one future goal of the GTIN is to include individual patient follow-up on a "per visit" basis. This detailed follow-up information will be submitted by the primary care physician and accessed only by the FDA. Expanded demographic information will be captured for future reports. The RAC will continue to access the information that has been previously captured by the Data Management Reporting System, whereas patient-specific confidential information will not be accessible outside of the FDA. Specific security issues should be resolved within the next year. Although the current mechanism for

reporting (i.e., hard copy submission) will continue for the upcoming reporting period, disk submission is anticipated in the near future.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: PHASE I/II STUDY OF IMMUNIZATION WITH MHC CLASS I MATCHED ALLOGENEIC HUMAN PROSTATIC CARCINOMA CELLS ENGINEERED TO SECRETE INTERLEUKIN-2 AND INTERFERON-* /DR. GANSBACHER

Overview--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present an overview of the protocol submitted by Dr. Bernd Gansbacher of Memorial Sloan Kettering Cancer Center, New York, New York. Thirty patients with prostate cancer who have had at least 3 successive elevations in their prostate specific antigen (PSA) despite castrate levels of testosterone will be entered. Two dose levels of vaccine will be tested. Three patients will receive 7.5 x 106 cells; and if no toxicity observed, the remaining patients will receive 15 x 106 cells. The vaccine will consist of a human prostate carcinoma cell line, LNCaP, transduced with a retroviral vector containing both the IL-2 and interferon-gamma (IFN-) genes. The transduced cells will be irradiated to 10,000 rad before giving to patients. A minimum level of IL-2 secretion pre-irradiation has been set, and there is a requirement for class I major histocompatibility complex (MHC) molecules to be upregulated on the transduced cells compared to the parental line. There will be 4 vaccinations on Days 1, 15, 29, and 89. Primary endpoint will be toxicity. Secondary endpoints will be: (1) immune response enhancement, and (2) PSA levels and any other indications of disease response (e.g., measurable lesion shrinkage and possibly a response in detecting circulating tumor cells by PSA-polymerase chain reaction (PCR)).

Review--Dr. Smith

Dr. Smith stated that the animal model data regarding efficacy of this approach are based on: (1) the generation of an enhanced regression rate of established murine fibrosarcoma tumors when a vaccine with IL-2 plus IFN- is used as compared to vaccines with either one of the two components; and (2) the regression of prostate cancer in a Dunning rat model using cells transduced with either of these cytokines but not both. It would be useful if the experiment had been carried out with established tumors that demonstrate the dual cytokine effect. It is theoretically possible that dual cytokines could be less effective than single cytokine for the prostate cancer. The data presented in the *Blood* article (Volume 83, pp. 1289-1298, 1994) show that MHC is upregulated less than the dual cytokine transduction than with a single IFN- transduction.

Dr. Smith asked if the investigators have performed the dual cytokine experiment with the prostate cancer model. The investigators responded in writing that such an experiment has not been conducted and argued that animal models are not totally predictable for human cancer. Dr. Smith said that this experiment is straight forward and will provide useful information. Dr. Smith raised additional concerns: (1) Regardless of the outcome of the Dunning rat experiment, the investigators would proceed with the dual cytokine experiment on humans; (2) If the human trial failed to show any hint of efficacy, would the investigators plan an IL-2 only trial? The investigators have provided additional human cell data showing a proliferative response that is improved by the dual cytokine approach. Dr. Smith agreed with the investigators that the dual cytokine approach has a theoretical appeal.

In his initial review, Dr. Smith stated that no data was presented on irradiation of the transduced human cells in terms of their ability to produce cytokines and upregulate MHC. The data were subsequently provided by the investigators. Responding to Dr. Smith's initial review, the investigators outlined the

assays to be used to follow up on the immune responses. Dr. Smith asked the investigators to summarize the immunological response data obtained in the melanoma and renal cell carcinoma protocols. The Informed Consent document stated that the patient is responsible for the cost of the extra investigation-only biopsy procedure including surgery, anesthesia, and pathologic processing. Dr. Smith asked the investigators to clarify this financial cost issue. In summary, Dr. Smith said this protocol is approvable provided the above questions are clarified by the investigators.

Review--Dr. Motulsky

Dr. Motulsky said most of his concerns have been raised by Dr. Smith. It will be important to review the semi-annual data reports on the two ongoing gene therapy trials (Protocols #9206-021 and #9206-022) to observe the progress of the ongoing studies, and how they are related to the present protocol. The Informed Consent document regarding biopsy is written in technical language and needs some attention. In general, Dr. Motulsky stated that the proposal is acceptable after the questions raised by him and Dr. Smith have been satisfactorily answered by the investigators.

Review--Dr. Zallen

Dr. Zallen provided a detailed written review of the protocol. Regarding animal and preclinical studies, the investigators need to provide more data of the prostate carcinoma animal model and the effect of immunization with MAT/IL-2 on rats with prostate disease. There should be experiments with this model system to show the effect of introducing *both* cytokine genes simultaneously in animals without and with established tumors. Is there any data on established tumors in the murine fibrosarcoma model? Any *in vitro* mixed lymphocyte/tumor reaction studies with a human *prostate* cancer cell line? Do allogeneic cells persist after injection?

Regarding the experimental design, Dr. Zallen asked the investigators to clarify the distinction between the Phase I and Phase II components of this protocol: How is the cell dosage determined? What is the range of cytokine production? Will the subjects in the Phase I portion be eligible for Phase II? How will irradiation be performed with cells in the syringe? The Phase II portion is not well defined.

Regarding the informed consent issue, Dr. Zallen said there is no need for a separate Informed Consent document if the plan is similar in the Phase I and Phase II portions of the study, and if the plan is different, a separate document is needed. The revised Informed Consent document satisfied her concerns. In addition, questions on long-term follow-up and autopsy have been addressed. The Informed Consent document needs to be clarified with regard to the research costs; the cost of biopsy should not be borne by the subjects. Has the IRB approved the revised Informed Consent document?

Other Comments

Ms. Meyers would like to remove the words *vaccination* and *vaccine* from the Informed Consent document. Dr. Dronamraju asked about the number of patients. Dr.Haselkorn asked the investigators to elaborate on the data regarding IL-2 production and cell survival after irradiation.

Investigator Response--Dr. Gansbacher

Responding to Dr. Smith's question on animal model data, Dr. Gansbacher stated that the murine or rat tumor models will not predict what will happen in human cancer models. The animal models serve as examples to demonstrate the feasibility of using a vector construct *in vivo*. The fact that most animal tumors are chemically or virally induced and frequently employ the *in vitro* propagated cell lines which

grow rapidly and kill the host in a matter of weeks, makes them very different from the slow growing human tumors. Even data generated in differentmurine tumor cell lines can vary significantly from each other although the same vector is used. For these reasons, the investigators decided to focus on generating preclinical data using the vaccine cell line *in vitro* together with human leukocyte antigen (HLA)-class I matched human lymphocytes. Such data was provided by the investigators.

Dr. Gansbacher made two points: (1) Tumor cells by themselves are poor antigen presenters and CTL are unable to lyse these tumor cells. IFN- corrects this defect allowing epitopes to be presented and tumor cells are lysed. (2) Published data suggest that in patients with a high tumor burden, the lymphocytes are unable to respond normally due to defects in the signal transduction systems. The investigators have found there is a down regulation of T cell receptor and proteins associated with it in the signaling system. The investigator provided a copy of his NIH grant application to clarify the scientific rationale of the protocol. The prostate cancer cell line, LNCaP, is MHC class I negative and expresses two tissue specific antigens called PSA and PSMA. This HLA negative cell line is converted into an HLA-A1 and HLA-A2 positive cell line after transduction with the vector, N/CIFN/TIL2. The investigators demonstrated that IFN- converts these HLA class I negative cells into HLA-A1 and A2 positive cells and induced a CTL response against the tumor specific antigens. The data of the 5 day proliferation assay shows that IFN- in combination with IL-2 dramatically induces T cell proliferation and the CTL activities.

Dr. Smith asked for a clarification of the interpretation of the *in vitro* CTL data: Do the data demonstrate that combination of two cytokines is superior to either one alone? Dr.Gansbacher responded that those experiments are not always reproducible. In general, dual cytokine experiments are slightly better than IL-2 or IFN- alone. Upon longer cell culture of up to 28 days, the response becomes nonspecific. Dr. Smith asked why the dual cytokine experiment has not been performed in the Dunning rat prostate cancer model. Dr. Gansbacher responded it is because the investigator who performed that experiment has left the laboratory several months ago.

Regarding the irradiation effect on the transduced LNCaP cells, Dr. Gansbacher said after irradiating these cells in the syringe with 10,000 rads, the cells are still viable for 40 to 50 days *in vitro* and they continue to secrete IL-2 and IFN-. In fact, the levels of cytokine secretion increase due to leakage of the cell membrane. He did know how long these cells will persist *in vivo* since these are allogeneic cells matched only for one allele type. According to the results from patients of the melanoma and renal cell carcinoma protocols, these cells were not detectable 7 to 10 days after injection.

Responding to Dr. Motulsky's inquiry about the status of the ongoing trials, Dr.Gansbacher said that 12 patients have been entered onto the melanoma protocol, and 11 onto the renal cell carcinoma study. These are end stage cancer patients with a large tumor burden, and there was no anti-tumor immune response. In this protocol, patients with earlier stage cancer will be treated. In the two ongoing studies, there was a slight increase in natural killer cell activity; none of the patients had any clinical anti-tumor response.

Responding to the question of medical costs stated in the Informed Consent document, Dr.Gansbacher said that patients will only pay for standard tests, and all experimental tests will not be charged to the patients. The revised Informed Consent document has not yet been approved by the IRB. He agreed to remove the word vaccine from this document. Dr.Gansbacher said that 15 patients in the two ongoing trials are deceased, but he was unable to obtain permission for autopsy from their families. Ms. Meyers asked if there is any way to improve the situation. Dr.Gansbacher said that obtaining permission for autopsy is difficult. He was uncertain about the value of autopsy of these patients who have received irradiated cells months or a year before they die. Dr. Parkman said one important question is whether there is any transfer of the vector from the transduced cells to other host cells. Dr. Smith said at this stage

of gene therapy development, this information is valuable. In the future, autopsy may not be that critical.

With regard to the question of patient number, Dr.Gansbacher said that 3 patients in the Phase I trial will be given 7.5 x 106 cells injected on Days 1, 15, 29 and 85. If they have no toxicity, the 4th patient will go to the higher cell dose. If any of them has a toxicity, an additional 3 patients will be tested at the initial dose. It is a standard way of progressing from a Phase I to a Phase II trial. Responding to DrZallen's question of entrance criteria, Dr. Harold Scher (a co-investigator) stated that the eligibility for Phase II will allow patients of minimal tumor burden and no previous hormonal treatment to enter on the protocol; both groups will follow the same procedures. The total number of patients will be 30.

Dr. Miller remarked that the vector used in this protocol is early generation retrovirus vector that makes viral protein encoded by the *gag* gene. Dr. Parkman noted that thetransduced cells are slightly more resistant to irradiation and produce more IL-2. Dr.Gansbacher said it is a surprising result; similar phenomena have been observed in melanoma cells, and he speculated that it is due to a leaky cell membrane after irradiation.

Committee Motion

A motion was made by Dr. Smith and seconded by Dr. Motulsky to accept the protocol submitted by Dr. Bernd Gansbacher by a vote of 16 in favor, 0 opposed, and 2 abstentions. Approval of the protocol is contingent on the review and approval of a revised Informed Consent document including the following: (1) delete the term vaccine, (2) clarify the section which describes the cost for biopsies, (3) include a statement informing subjects that an autopsy will be requested, and (4) include a statement regarding the necessity for long-term follow-up.

Protocol Summary: Dr. Bernd Gansbacher of Memorial Sloan Kettering Cancer Center, New York, New York may conduct gene transfer experiments on 30 subjects (18 years of age) with progressive prostate carcinoma. Subjects will receive subcutaneous injections of the lethally irradiated class 1 HLA matched allogeneic prostate cancer cells transduced with the N2-based retrovirus vector, N/CIFN/TIL2, that encodes the genes for human IL-2 and IFN. The objective of this study is to determine the safety and biological efficacy of N/CIFN/TIL2 administration. Subjects will be monitored for: (1) PSA levels, and (2) induction of *in vivo* cellular and humoral immunity.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: GENE THERAPY FOR AIDS USING RETROVIRAL MEDIATED GENE TRANSFER TO DELIVER HIV-1 ANTISENSE TAR AND TRANSDOMINANT REV PROTEIN GENES TO SYNGENEIC LYMPHOCYTES IN HIV INFECTED IDENTICAL TWINS/DRS. MORGAN AND WALKER*

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Richard Morgan and Robert Walker of the NIH, Bethesda, Maryland. Dr. Miller said that this protocol is straight forward. These investigators propose to use retroviral vectors to transfer genes that inhibit HIV replication into T cells from HIV-seronegative identical twins, to grow the cells in tissue culture, and to study the safety, survival, and possible efficacy of infusion of these cells to HIV-infected twins. The investigators have adequately addressed the *Points to Consider*. Experiments that have been submitted for publication document inhibitory effects of the retroviral constructs against primary HIV patient isolates, although there is no way of predicting whether the inhibition seen will have any effect in humans. No animal model data was provided (SCID mice, etc.) to attempt to address issues of efficacy *in vivo*. Based on accumulating

evidence, the proposed procedures that involve recombinant DNA appear to pose minimal risk to the patient and the general public, and there are no new issues that need to be addressed. BothNIH IRB and IBC have approved the protocol. Dr. Miller recommended acceptance of the protocol as written.

Review--Dr. Haselkorn

Dr. Haselkorn said that he agrees in general with Dr. Miller's review. He provided a detailed written review. The rationale for the gene therapy trials proposed are based on the detailed molecular biology of HIV-1 infection. HIV-1 is the virus that causes acquired immunodeficiency syndrome (AIDS). It is a member of the retrovirus family, meaning that its genetic material is RNA. When HIV-1 infects a susceptible cell, its RNA is copied into single-stranded DNA. The DNA is copied to make the provirus, double-stranded DNA which in turn is inserted into the infected cell's DNA. The enzyme required for these steps include reverse transcriptase (RT) and integrase, both translated from the viral RNA. The integrated viral DNA is then transcribed, the mRNA is processed and transported out of the nucleus into the cytoplasm where it is translated into protein, yielding more RT and integrase as well as the envelope proteins that package the RNA for a new round of infection. This description is vastly oversimplified with omitting, for example, the role of viral protease that cuts the original translation product into functional pieces.

Which of these steps can be blocked specifically to prevent HIV replication? Nearly every step has been targeted in some experimental protocol. For example, the drugazidothymidine inhibits the RT. The protocol under review is based on 3 additional inhibitors of steps in the replication process. Additional details are as follows: There are 2 small virally encoded proteins called TAT and REV. Each of these proteins binds to a defined sequence in the viral RNA to enhance a specific step in replication. TAT affects transcription and REV affects transport of mRNA out of the nucleus, and perhaps other steps as well. TAT completes its function by binding to a sequence of nucleotides in HIV RNA called TAR; REV by binding to a sequence called RRE.

Molecular intervention has several forms: antisense TAT RNA to prevent the translation of TAT RNA; antisense TAR RNA to bind to the TAR sequence, preventing it from binding TAT; antisense RNA for REV; antisense for the REV target, RRE; and finally, unique to REV, the synthesis of a mutant form of REV called transdominant that forms higher order aggregates with the normal REV protein and prevents it from functioning normally.

The conventional way to evaluate these interventions is to test them on laboratory T cell lines, observing the inhibition of the replication of a laboratory strain of HIV. An important novelty in the current protocol is the use of freshly isolated CD4(+) T cells and strains of HIV freshly isolated from patients. The CD4(+) cells are the ones in which HIV replicates and are responsible for combating viral antigens in healthy individuals. The protocol involves collection of peripheral blood, isolation of CD4(+) cells from the blood, transduction of those cells with a retroviral vector expressing one or several of the TAT or REVRNAs mentioned above, amplification of the transduced cells, then administration of these "protected" T cells to a HIV-positive patient. These interventions should have several beneficial effects, e.g., the provision of a T cell class capable of combating HIV and not itself susceptible to productive infection by HIV.

The only cells suitable for this possible therapeutic approach are an individual's own T cells or those of an identical twin; others will be rejected. In the case of a HIV-positive person, the former is difficult, so this protocol uses cells from a HIV-negative identical twin. The approach is promising. It is supported by the *in vitro* studies mentioned above in which CD4(+) cells transduced with various TAT, TAR and REV constructs are protected to varying degrees against HIV infection. The protection depends to a great extent on the multiplicity of infection (MOI). Dr. Haselkorn asked the investigators to address the following

questions: (1) To what extent is the gene therapy proposed dependent upon the purification of CD4(+) cells? Has this purification been optimized adequately or is further *in vitro* work advisable before attempting the human experiments? (2) How do the numbers in the *in vitro* experiments in which MOI is varied relate to the *in vivo* situation? What MOI is expected to be encountered *in vivo*? Are the present constructs adequate or should they be improved first?

Review--Dr. Secundy

Dr. Secundy asked the investigators to clarify statements regarding autopsy in the Informed Consent document, length of time of follow-up, and what would happen if subjects terminated the study prematurely. The investigators responded appropriately and made the necessary modifications. Dr. Secundy said she was comfortable with approving the protocol.

Other Comments

Dr. Zallen noted that the investigators have not provided information required for the Informed Consent document as stated in the *Points to Consider*.

Dr. Parkman stated that this protocol is straight forward. Presumably there are 2 mechanisms by which HIV can cause the death of CD4(+) cells, i.e., direct infection and apoptosis. Will transduction of either of their genes interfere with apoptosis? Will the non-HIV infected T cells be resistant to apoptosis upon transduction of the infected cells?

Dr. Motulsky inquired how many twins are available for the present study? How often are both twins or one of the twins have HIV infection? Dr. Walters asked about the control groups of patients receiving none or some of the therapeutic genes for their treatment.

Investigator Response--Drs. Morgan and Walker

Responding to Dr. Haselkorn's question of MOI used in the *in vitro* studies and its relation to the potential *in vivo* situation, Dr. Morgan responded that in most stringent *in vitro* conditions (MOI of 1:35), about 60% protection was achieved in CD4(+) T cells transduced with RevTD or RevTD/anti-TAR vector. Based on the transduction rates following selection, it is estimated that this level of protection corresponds to the number of gene-modified cells in the population. However, the question remains which *in vitro* challenge dose of virus mimics the true *in vivo* condition that varies greatly depending on the clinical stage and anatomic location (these could range from an MOI of >10 to <0.001). The number of variables in comparison of the *in vivo* and *in vitro* settings is too great for simple answers. It is difficult to predict how a T cell will response to a high MOI (over 100) HIV infection when the T cell travels through the lymph node containing follicular dendritic cells coated with HIV. The investigators state that the proposed protocol is within the range suggested by the *in vitro* data. From this model study with twins, the investigators will be able to quickly identify which anti-HIV genes can protect cells from infection in an *in vivo* setting.

Dr. Haselkorn asked if the twins' own virus will be tested in this experiment. Dr. Morgan responded that the twins' own virus will not be tested.

Responding to Dr. Parkman's question of the mechanism of apoptosis, Dr. Morgan said it is difficult to study this question *in vitro* in order to understand the mechanism, and he does not have an answer how the transduced cells will affect the uninfected cells *in vivo*. With regard to Dr. Walters' question of control groups, Dr. Morgan said the patient will serve as his/her own internal control: Each patient will receive a control gene and at least one potentially therapeutic gene, and there is a good PCR-based assay to

distinguish cells with those 2 vectors. Dr. Morgan agreed to revise the Informed Consent document as per suggestions by Drs. Zallen and Secundy.

Responding to Dr. Motulsky's question, Dr. Morgan said there are over 150 twins available for this study, and all of them are discordant twins.

Dr. Miller asked if there is any untoward effect of T cells of the uninfected twin when transferred to the HIV-infected twin. Dr. Walker said a study has been completed in which the unmarked activated T cells from the uninfected twins were given to the infected individuals. The only untoward reaction was attributed to sensitization to fetal calf serum which could be ameliorated with antihistamines. In about one-half to two-thirds of cases, a transient elevation of plasma virus load was observed immediately after cell transfer. None of these untoward effects were observed in the ongoing study involving the T cells marked with the *neo*R gene. Dr. Miller inquired how long the marked T cells persist in the patients. Dr. Walker said the data is still incomplete; however, the cells appear to persist at least 10 to 12 weeks and perhaps up to 30 weeks.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Haselkorn to accept the protocol submitted by Drs. Richard Morgan and Robert Walker, by a vote of 16 in favor, 0 opposed, and 1 abstention.

Protocol Summary: Drs. Richard Morgan and Robert Walker of the NIH, Bethesda, Maryland, may conduct gene transfer experiments on 48 HIV seropositive subjects (18 years of age). This Phase I/II study involves identical twins (one HIV seropositive and the other HIV seronegative). CD4(+) T cells will be enriched following apheresis of the HIV seronegative twin, induced to polyclonal proliferation with anti-CD3 and recombinant IL-2, transduced with either the LNL6/NeoR or G1Na/NeoR marking vector, or transduced with up to 2 additional retroviral vectors (G1RevTdSN and/or GCRTdSN(TAR)) containing potentially therapeutic genes (antisense *TAR* and/or transdominant *rev* mutant). These T cell populations will be expanded 10 to 1,000 fold in culture for 1 to 2 weeks and reinfused into the HIV seropositive twin. Subjects will receive up to 4 cycles of treatment using identical or different combinations of control and anti-HIV retrovirus vectors. The relative survival of these transduced T cell populations will be monitored by vector-specific PCR, while the subjects' functional immune status is monitored by standard *in vitro* and *in vivo* assays.

Other Comments--48 Hours Program

Ms. Meyers raised a concern about HIV patients who disguised their identity in order to enroll into different HIV protocols as reported in the television program, *48 Hours*.

XII. PROPOSED AMENDMENT TO APPENDIX B, *CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD/DR. FLEMING*
Review--Dr. Straus

Dr. Straus noted that Appendix B of the *NIH Guidelines* is a document created in 1974. Dr. Diane Fleming (Mid-Atlantic Biological Safety Association) has conducted a timely process of updating this document. The proposed Appendix B was published in the *Federal Register*, January 30, 1995 (60 FR 5687) for public comment. Several comments have been received from: Linda B. Wolfe, Massachusetts Institute of Technology, Cambridge, Massachusetts; Karen B. Byers, Dana-Farber Cancer Institute, Boston, Massachusetts; Andrew G. Braun, Harvard University, Cambridge, Massachusetts; Joseph VanHouten, Johnson & Johnson, New Brunswick, New Jersey; and A. Lynn Harding, Chattanooga, Tennessee. Most

of the comments were in favor of the suggested revision; a few of them had suggestions. Dr. Straus summarized several issues that need to be resolved before the RAC can approve the Appendix B.

Many parts of the *NIH Guidelines* and its appendices need to be revised to accommodate the revised Appendix B. There are additional organisms suggested for incorporation into Appendix B. There are some interpretations regarding the new *risk group* classification that require clarification. The footnotes and references of Appendix B require updating. The original listing of oncogenic viruses was influenced by the prevalent concern within the scientific community in the 1970s regarding their oncogenicity in humans. Today, the role of these viruses in human cancer is better understood, and the oncogenicity is really no different from the risks of other viruses. The separate listing of oncogenic viruses has been revised in the new Appendix B. The inclusion of the Baculovirus and the vectors derived from this virus in Risk Group 1 is appropriate; however, this classification has never been formally ruled by any RAC action. There are some issues in terms of consistency of listing of the organisms within each category.

Dr. Straus stated that many of the issues that he raised have to be addressed before the RAC can adopt the new Appendix B. Dr. Straus suggested the formation of a subcommittee to finalize the proposed Appendix B presented by Dr. Fleming.

Other Comments

Dr. Miller stated that the concept of *risk group* and the classification of oncogenic viruses into Risk Groups 1 and 2 are acceptable; the revised document in general is acceptable. Dr. Straus agreed that basically the proposed Appendix B is an approvable document, but there are a large number of small irregularities that need to be addressed by a subcommittee. Dr. Wivel noted that Appendix B is primarily intended as a guidance document for local IBCs, and the comprehensive listing of etiologic organisms is to meet their needs. Dr. Wivel noted that the proper roles of the investigators and IBC in setting the biosafety level of an experiment needs to be clarified; the investigators may propose a biosafety level for a particular experiment, but the final decision rests with the IBC.

Dr. Walters inquired if the revision can be accomplished before the June RAC meeting. Dr. Straus said that a one day subcommittee meeting involving RAC members, some of the safety officers who commented on this document, and ORDA staff should be able to accomplish this task. Dr. Motulsky favored the formation of a subcommittee. Dr. Miller pointed out that the document requires periodic update. Ms. Wilson commented that the remaining Appendices K and Q have to be revised and issues of animal pathogens addressed.

Dr. Fleming stated that the Appendix B primarily lists human pathogens including some organisms that may not be studied in the United States. The restricted animal and plant pathogens should have separate listings under Appendices P and Q. She agreed to clarify this document as suggested by the reviewers and biosafety officers around the country.

Dr. French Anderson (University of Southern California) suggested the inclusion of a listing on various gene transfer vectors, such as vectors based on retroviruses and adenoviruses. Dr. Wivel noted that it is a formidable task to classify all the vectors used in the gene therapy. Dr. Miller agreed it should be a case-by-case decision by the IBC, and the risk group classification provides the basis for specifying biosafety levels according to the *NIH Guidelines*. It is a complex subject, and there is no way to deal with it by codifying it. The current system allows local adjustments responding to different local concerns.

Dr. Erickson said that the *NIH Guidelines* is a very complex document, and the new appendix should include general instructions on how to properly use the risk group classification. Dr. Ginsburg agreed that

most practicing scientists are uncertain about proper biosafety levels of their experiments; a condensed and an easy to read guideline would be useful to them. Dr. Straus agreed to the Chair to form a subcommittee for refining Appendix B for the June RAC meeting. A listing of vectors is impractical; other factors such as the inserted genes affects the safety concerns of vector constructs. Dr. Ginsburg suggested to build a database to list vectors used in different institutions. Dr. Straus agreed that such a database would be useful.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Parkman to defer approval of the proposed amendments to Appendix B, *Classification of Microorganisms on the Basis of Hazard*, pending additional revisions to the remaining appendices of the *NIH Guidelines* that are required to adequately accommodate the revised Appendix B. The motion for deferral included a recommendation that the RAC should hold a 1 day subcommittee meeting in which Dr. Straus, ORDA staff, and *ad hoc* experts could develop the required modifications. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

XIII. REPORT ON *IN UTERO GENE THERAPY CONFERENCE CALL/DR. BRINCKERHOFF AND MS. MEYERS*

Report--Ms. Meyers

Ms. Meyers said that the conference call on *in utero* gene therapy took place on February 14, 1995. The Subcommittee on *In Utero* Gene Therapy is chaired by Dr. Brinckerhoff. Its members include Dr. Chase, Ms. Meyers, Drs. Samulski, Secundy, Motulsky, Erickson, and Mr. Capron. The goal of the conference call was to identify who is knowledgeable in the area of *in utero* gene therapy so that these individuals could be invited to the RAC in order to educate the RAC about the critical issues. Key issues were targeted and experts were identified as follows: (1) What is the biology of *in utero* gene therapy? The expert identified was Dr. Mitchell Golbus of the University of California, San Francisco, California. Dr. Golbus is an obstetrician/gynecologist/medical geneticist. (2) What are the safety and technical problems associated with the process of delivering the DNA (not with the DNA itself)? The expert identified was Dr. Cathy Reed of the University of Arizona, Tucson, Arizona. Dr. Reed is an ultrasound expert on fetus imaging. (3) What are the philosophical and ethical concerns that must be addressed? How does one protect maternal versus fetal interests if there are risks to the mother for the benefit of the fetus? How can the RAC maintai a balanced position that respects the public interest in "pro-choice" and "pro-life?" There was considerable discussion about experts appropriate to address this topic. Dr. Leroy Walters (Director, Center for Bioethics, Georgetown University) was identified as an expert in this area. The subcommittee recommended that at the June RAC meeting, Drs. Golbus and Reed should be invited to make 20 minute presentations each on *in utero* gene therapy with the remainder of the time (50 minutes) open for discussion. At the end of the session, it is anticipated that the RAC will have identified specific issues to be pursued further.

Other Comments

Dr. Erickson commented that Drs. Golbus and Reed are the most appropriate experts in this area. Dr. Wivel noted that Dr. Golbus will be unavailable during the June 1995 meeting. Dr. Walters suggested a second conference call to identify additional individuals in biomedical ethics. Dr. Parkman asked to clarify if the discussion will be mainly on gene therapy or it will include cellular therapy; he noted that Dr. Golbus' expertise is in cellular therapy rather than gene therapy. Dr. Noguchi said that FDA has reviewed a request for *in utero* cell transplantation involving heterozygous cells from the father; it is a logical

extension that *in utero* gene therapy involving stem cells will be the next step. Dr. Erickson summarized the subcommittee's finding that cellular and gene therapies are inseparable, and to understand the biology it has to start with cellular therapy. Dr. Chase suggested a bioethics discussion in June 1995 and an invitation to Drs. Golbus and Reed in September. He asked if RAC members whose terms have expired are still eligible to serve on the Subcommittee. Dr. Parkman suggested Dr. Esmail Zanjani, a hematologist from the University of Nevada, Reno, Nevada, to address the issue of hematopoietic cells and gene transduction in fetuses. Dr. Zanjani has performed important studies in the last 9 years on fetal transplantation and fetal gene transfer experiments. Dr. Anderson agreed that Dr. Zanjani is an appropriate choice.

Dr. Walters suggested that scientific and bioethics experts should be invited to address the RAC at its June 1995 meeting. Dr. Parkman asked if this discussion will include the germ line issue; the RAC has to address this issue as an adventitious side effect of fetal gene therapy even if it is not intended as the primary objective. Dr. Zallen preferred to have the *in utero* gene therapy and germ line gene transfer discussed in parallel rather than linking these two issues together; they raise different kinds of ethical and societal questions. Ms. Meyers, however, considered the germ line issue urgent since it could be presented to the RAC as an untoward consequence of somatic gene therapy.

Dr. Walters noted that the technology of stem cell *in utero* therapy is very close at hand. Dr. Parkman remarked that *in utero* transplantation of stem cells have been performed for many years both within the United States and in Europe. Attempts have been made to cure either SCID or hemoglobinopathies by a research group at University of California at San Francisco and in Europe. Dr. Ginsburg agreed it is not a very new issue. Dr. Glorioso asked if the transduced stem cells used *in utero* would present a new issue. There was a discussion as to what experiments could be included in this technology. Dr. Parkman said that in Zanjani's experiments, the vector was administered intraperitoneally to the fetal sheep where he found vector sequence transmission through germ cells.

As a point of clarification, Dr. Noguchi stated that the reason the particular fetal stem cell protocol was submitted to FDA was that it involved the use of a selection device to enrich for the CD34(+) cells. Dr. Parkman noted it is a logical approach to enrich the stem cells to increase the chance of engraftment.

Dr. Haselkorn said it is a mistake to start a serious discussion of gene therapy *in utero* since this application of gene therapy technology is so far in the future. Dr. Chase disagreed with Dr. Haselkorn's conclusion; a public forum to discuss this issue is very important now since the political climate in the future may be unfavorable. Dr. Noguchi stated that the technology may be difficult but it may be easier to achieve gene transfer *in utero* than in somatic cells postnatally. Dr. Erickson noted that the *Points to Consider* was developed 5 to 6 years before the first gene transfer protocol was submitted for review; to be prepared is the modus operandi of the RAC. Dr. Dronamraju agreed with Dr. Haselkorn that the discussion is surreal; he asked about the experiment being performed at the University of Pennsylvania. Dr. DeLeon explained it involves gene transfer to spermatogonia; she preferred to have a discussion on *in utero* gene therapy now. Dr. Ross favored a deliberation of this issue, and it could be integrated with germ line discussion. Dr. Haselkorn agreed with Dr. Chase that in the future the political climate might not favor public discussion of this fetal subject.

Dr. Miller said the RAC should first address the issue of adventitious modification of germ line: the rate of normal insertion into germ line, how fast transposons move, and how it relates to gene therapy. In terms of attempted germ line modification, Dr. Walters said the Institute of Medicine of the National Academy of Sciences is preparing to address this issue in detail. Dr. Parkman said that a guideline regarding adventitious germ line modification is urgent if some level of vector insertion into germ cells is detected from the stipulated experiment involving intravenous administration of large doses of adenovirus vector to

cotton rats (Protocol #9412-097).

Dr. Walters said that a second conference call will be convened before the June 1995 RAC meeting, and the RAC will have a discussion on *in utero* gene therapy at its June meeting.

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

Dr. Wivel stated that the first *Ad Hoc* Review Committee meeting was held on February 3, 1995. The committee membership includes individuals with a broad range of expertise, including two current RAC members. Since there is some concern that the current review system may not function optimally for gene transfer research proposals, the *Ad Hoc* Review Committee plans to consider this issue in detail and develop recommendations that would allow the RAC to conduct merit reviews while maintaining overall fairness to applicants. During this early stage of human gene therapy, proposals focus primarily on safety and not efficacy; therefore, the *Ad Hoc* Review Committee stressed that the commonly presented argument that reviews should be expedited based on life-threatening circumstances is misleading. Dr. Noguchi noted that FDA reviews tend to focus on data that indicate whether safety standards have been met and not on the scientific merit underlying a Phase I clinical trial. FDA reviewers, as a rule, do not devote significant time and attention to Informed Consent documents. These differences between approaches of the RAC and the FDA review contribute to several uncertainties about ongoing clinical protocols, particularly those involving cancer. For this reason, the *Ad Hoc* Review Committee recommended an independent collective review of certain protocols that have been reviewed by the RAC, i.e., cancer trials, for the purpose of identifying the scientific criteria and endpoints that were used as the base for approval as opposed to simply the safety standards.
The next *Ad hoc* Review Committee meeting will be held on March 8, 1995, at the NIH, Building 31C, Conference Room 8, Bethesda, Maryland.

XV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: A STUDY OF THE SAFETY OF INJECTING CANCER PATIENTS WITH GENETICALLY MODIFIED TUMOR CELLS; INJECTION OF GLIOBLASTOMA PATIENTS WITH IRRADIATED AUTOLOGOUS MALIGNANT GLIOMA TUMOR CELLS GENETICALLY MODIFIED TO EXPRESS A TGF-2 ANTISENSE mRNA ALONE OR IN COMBINATION WITH INCREASING DOSES OF TUMOR CELLS WHICH HAVE BEEN GENETICALLY MODIFIED TO SECRETE INTERLEUKIN-2 (IL-2): A PHASE I STUDY/DRS. BLACK AND FAKHRAI*

Reviews--Drs. Ginsburg and Brinckerhoff (presented by Dr. Ginsburg)

Dr. Walters called on Dr. Ginsburg to present his primary review of the protocol submitted by Drs. Keith Black and Habib Fakhrai of the University of California, Los Angeles, California. Dr. Ginsburg said Dr. Brinckerhoff had raised very similar questions to his, and he would present both comments together. This proposal is designed as a Phase I gene therapy trial which will introduce two exogenous DNA expression constructs into the patient's own tumor cells forreinjection in an attempt to induce an enhanced immune response against the primary tumor. The first construct is an Epstein-Barr virus vector expressing antisense TGF-2, and the second is a retrovirus vector expressing IL-2. The investigators hypothesize tha suppression of TGF-2 by the antisense approach coupled with overexpression of IL-2 will result in enhanced immunization. The investigators presented preliminary studies in a rat 9L glioblastoma model to support this protocol.

Dr. Ginsburg stated his major concern is the lack of human cell data, and the protocol is premature; however, there are not a lot of safety issues since both vectors have been used in previously RAC approved protocols.

The protocol calls for establishment of autologous cell lines from each patient, and transducing these cells with the vectors. Can the investigators establish the cell lines from glioblastoma patients? How effectively can the cells be transduced? What are the transduction procedures to be used? Can the investigators demonstrate the desired biologic effect from IL-2 and TGF-2antisense? The investigators responded in writing that they have established 5 tumor cell lines from 5 patients. They said successful transduction was achieved with the IL-2 vector and they have not yet obtained data to demonstrate the biologic effect of TGF-2 antisense.

The investigators stated that they will use an antisense construct that represents simian TGF-. Dr. Brinckerhoff asked what is the rationale for using the simian gene on human cells? Why not use the human gene? Are the nucleotide sequences of TGF- identical between the simian and human genes? The investigators responded in writing that the nucleotide sequences are 97% homologous between these two species.

The chosen radiation dose (7,000 cGy) for the human tumor cells is based on discussion with a radiation oncologist, and no data was presented to demonstrate that this dose is sufficient to make tumor cells nonviable while not affecting gene expression.

In the 9L rat tumor model, though the antisense TGF-2 treated tumor cells appear to provide improved survival at 12 weeks, it is unclear that the addition of IL-2 adds anything advantageous. In addition, no data is shown for the Epstein-Barr virus vector alone as a control. Is it possible that the improved results with TGF- are due to the vector alone rather than a specific effect of TGF- antisense expression? Furthermore, the investigators noted that IL-2 administration is associated with significant toxicity in humans. Can inclusion of IL-2 in these studies be justified without clear evidence from the animal studies that IL-2 adds significantly to the TGF- antisense effect? The investigators explained the theoretical rationale of their approach in their written response but provided no data.

Dr. Ginsburg reiterated that his major concern is lack of preclinical data in the appropriate human cells to support the human study. He would like to review data on transduction of human cells in tissue culture, and to use the human TGF- sequence for the human cells or at least to demonstrate that the simian sequence works as well in the human cells.

Dr. Brinckerhoff's comments basically overlap with those of Dr. Ginsburg. She stated in writing: "Despite the convincing nature of some of the experimental data presented in support of the protocol, there are numerous questions still need to be answered. Some of the data with the animal models need clarification, but more importantly, essential information with the human tumors needs to be presented before approval can be given."
Review--Ms. Meyers

The title of the study used in the Informed Consent document is different from the title in the protocol. Neither of these titles will be understandable to laymen except for the word *immunization* which public understands as a vaccine that prevent disease. Since it is important that the protocol has only one title, and since the word *immunization* is misleading because it will not *prevent* cancer, Ms. Meyers suggested that the title on the Informed Consent document should be adopted and used consistently and uniformly throughout the protocol. The investigators agreed to make the suggested changes, but the revised document has not been provided.

Other Comments

Dr. Parkman stated that the major problem is lack of pertinent data of the transduced cells: The minimum level of cytokine production acceptable for human protocol was not specified, and no data was provided regarding antisense TGF- expression. Will transduction first with the antisense vector interfere with transduction and expression of the second IL-2 vector? Will the simian TGF- antisense effectively inhibit the human TGF- gene expression? Dr. Ginsburg commented that no such data on human cells was provided. Dr. Miller agreed that these are essential data for protocol approval, and he asked about the kill rate of tumor cell irradiation. Dr. Ginsburg noted the investigators have provided the irradiation data on one single cell line, and Dr. Parkman added that a clonogenic assay is needed.

Dr. Haselkorn noted that data from the 9L rat tumor model is not encouraging: The tumors grew even faster in animals receiving the vector itself than the tumors with no gene modification. Dr. Miller explained that results from 9L rat model are frequently variable; the growth behavior of these cells changes after implantation to the animal. Dr. Ginsburg said the animal experiments are not totally a simulation for the human tumors; dosage of glioblastoma cells in the animal model is small compared to advanced human brain tumors.

Investigator Response--Drs. Black and Fakhrai

Dr. Black summarized his responses in 7 major areas. (1) Establishment of human brain tumor cell lines. Dr. Black said that his laboratory has established more than 50 human brain tumor cell lines in the past 5 years. Within the last year, 7 lines were cultured from 7 patients. (2) Transduction of TGF- antisense. Dr. Black said they have capability to transduce a variety of tumor cell lines including human GT9 cells, 9L, C6 and RG2 rat glioma cells, as well as murine ovarian teratoma cells. (3) Transduction of both TGF- antisense and IL-2 vectors together in human tumor cell lines. The investigator said that they have demonstrated the dual transduction in 9L rat glioma as well as GT9 human glioma cell lines. (4) TGF- antisense expression after irradiation. Dr. Black said that the promoter is still active after irradiation, and the antisense should continue to be expressed. (5) Concern about using simian TGF- antisense in human cells. The investigator responded that the DNA sequences of the TGF- genes of monkey and human are 97% homologous between these two species. (6) Validity of animal model to human brain tumor in terms of tumor cell dosage. The investigator explained that injection of only 300 9L cells will cause a deadly brain tumor in rats and in the preclinical studies, 5,000 cells were used. In a secondary challenge with TGF- antisense, the animals remained tumor free 6 months after injection of 5 x 105 cells. In contrast, all rats died by 3 weeks after injection with 9L or IL-2 gene modified cells. Persistent *in vitro* CTL activities were observed in animals treated with the antisense vector. In this protocol, patients with minimal tumor burden will be treated. (7) Synergy of IL-2 and TGF- antisense in the animal model. Dr. Black pointed out that the first 3 patients will be treated with only the antisense vector. Although the 9L rat model does not show synergy, this phenomenon has been observed in other tumor models; the synergy will be tested in the present human study.

Dr. Black responded to additional minor questions. He noted that there are no species differences in IL-2 response. In the data presented, the vector alone offered no protection from tumor; and in contrast, TGF- antisense treated rats, 17 out of 17 animals were alive after 12 weeks. Tumor cells unlike other cell types produce an active TGF- that suppresses immunity; blocking of TGF- activity restores immune response.

Regarding the question of transduction efficiency, Dr. Black said the efficiency is about 5 to 25% using lipofection and the efficiency is expected to be higher when the electroporation technique is used in the future. A tumor cell line can be established in 4 weeks and gene-modified and expanded to 107 cells in 6 weeks for treating the patient.

In terms of safety, the IL-2 vector has been previously used in other RAC approved protocols by Dr.

Fakhrai. With regard to the question of steroid use, Dr. Black said they will treat the patients who have the lowest steroid dose since steroid is expected to suppress the immune response. Dr. Black noted that glioblastoma is a particularly effective model to use immunotherapy because of high expression of the TGF- in these patients. IL-2 production and inhibition of TGF- production of the transduced cells will be monitored before giving them to the patients.

Dr. Miller commented that the investigators have no data to support their statements, especially the data on human cells. If the data is not presented, it cannot be evaluated. Dr. Fakhrai said there is some preliminary data on GT9 human cells, and irradiation of 7,000 rads stopped its colony formation. The data of the effect of radiation on IL-2transduced GT9 cells was included in the written response. Dr. Parkman said his questions regarding a definition of minimum IL-2 production and the level of TGF- inhibition of the transduced cells for patient administration have not been answered.

Dr. Haselkorn inquired how much time it would take to establish a tumor cell line, to transduce the cells, and to verify the levels of gene products for the patient use. Dr. Black responded that it will take no more than 3 months, and it is within the time frame for the patients after they have completed other types of treatments. The patients undergoing craniotomy are asked if brain tumor tissue can be taken from them to establish a cell culture. 2 or 3 out of 10 such patients may have cell lines that meet the eligibility criteria, and these patients will be asked to participate in the study.

Dr. Ginsburg said the data of 50 cell lines that Dr. Black established was not presented in the protocol. He said the levels of TGF- expression of the cell lines change during *in vitro* cell culture. What is the rationale of treating them with antisense if they no longer produce TGF-? Dr. Ginsburg said for patients whose brain tumors do not make TGF-, immunization with unmodified cells will produce a more effective response. Dr. Black agreed with the suggestions.

Responding to Dr. Ginsburg's question of treating the first 3 patients with TGF- alone, Dr. Black responded that the design of the study originated from the observation that TGF- antisense shows promising antitumor response in the animal model. After the first cohort of TGF- alone, it will be given in combination with a dose escalation of IL-2, which is a modification of an ongoing study. Responding to Dr. Ginsburg's question of transfection efficiency, Dr. Black said 5 to 25% efficiency has been obtained in several cell lines; but no data for human glioblastoma cells established from the patients is available.

Dr. Parkman inquired that in the rat model, how much decrease of TGF- is required to show therapeutic response? Dr. Fakhrai responded that they do not have cell lines with different levels of TGF- blocking to examine this question.

Dr. Chase said that a protocol with so much deficiency of preclinical data should not be deliberated at the RAC. Dr. Ginsburg said he indicated this deficiency in his initial review. Dr. Wivel explained that the *Points to Consider*, Section IV-B-5 states that primary reviews should state whether the proposal is unacceptable in its present form; unacceptable protocols do not have to be reviewed by the full RAC.

Dr. Haselkorn asked if blocking of TGF- will result in manifestation of new antigens which will provoke an immune response. Dr. Fakhrai responded that many tumor cells including glioma produce TGF- which is an immunosuppressor. The role that TGF- is playing is immunosuppressing the responder cells by preventing the high affinity T-cell receptor from becoming active. The investigators hypothesized that blocking TGF- will allow the tumor cells to be recognized by immune surveillance as shown in the animal model. Dr. Fakhrai said that he has demonstrated his ability to gene-modify many cell lines that were used in several RAC approved protocols; it is a waste of resources to reproduce the same experiment just to support the proof of principle.

Dr. Walters called for a motion regarding this protocol. Dr. Ginsburg stated that it is clear that there is not enough data to support approval of this protocol; he asked a procedural question about whether to disapprove the protocol or to allow the investigators to come back with some additional data. Dr.Glorioso said certain data of the preclinical studies are very impressive: Getting 17 out of 17 tumor bearing animals to survive with any kind of therapy is significant. If the investigators can provide additional data to address the questions raised in the review, he would consider this protocol to be a meritorious approach. Dr. Miller said that the scientific rationale is promising; however, the investigators have no data to support it.

Committee Motion

Dr. Miller made a motion to defer the protocol pending provision of additional data on the modification of human cells and other issues raised during the RAC review. Dr. Glorioso seconded the motion.

Dr. Chase noted that Dr. Ginsburg's review of this protocol is very straight forward that there is insufficient data for the proposal to be accepted. The deferral is not a verdict on Dr.Fakhrai's individual credentials; he has participated in many protocols previously approved by the RAC. The decision of each protocol is based on a case-by-case review. The RAC frequently has long complicated discussions due to insufficient data and poorly written protocols. It would be more efficient to review and approve complete protocols with no or some stipulations. But in this case, the resubmitted protocol should be reviewed by the full RAC.

Dr. Parkman said that the protocol involves immunization with irradiated tumor cells and might potentially fit into a category of the *Accelerated Review*; therefore, it would not be resubmitted to the full RAC. Dr. Wivel explained that all deferred protocols have to be resubmitted to the full RAC.

Dr. Miller asked that, given the variability in survival of brain tumor patients, whether the investigators will be able to obtain data to evaluate antitumor response. Dr. Ginsburg agreed that from the current study design, it is impossible to evaluate the efficacy question. The primary objective of the Phase I study is to evaluate toxicity. Dr. Miller was concerned by the lack of scientific rationale to justify increased risk to the patients; the patients should be clearly informed that no benefit of treatment is expected. Dr. Ginsburg noted that the same issue has been raised for all Phase I cancer protocols. The present protocol does not have any present safety issue; the message is that the protocol is a worthwhile study if it is supported by complete preclinical data.

As a point of clarification, Ms. Wilson said that the category of *Accelerated Review* of lethally irradiated tumor cells is limited to RAC-approved vector constructs with minor modifications or with additional tumor cells. There is a precedent for a deferred protocol (#9406-078) to be resubmitted with only the additional data but not the whole protocol. Dr. Ginsburg agreed that it would be an acceptable recommendation for this protocol.

Dr. Black said that with no treatment, the survival time for the glioblastoma patients is about 12 weeks. It is possible to evaluate preliminary efficacy in the first few patients treated by this protocol. The mean survival for best standard therapies including maximum surgical resection (radiation and chemotherapy) is 38 weeks. The other alternative approach suggested by preclinical studies is antisense insulin-like growth factor.

Dr. Noguchi remarked that the present proceedings involving detailed discussion are not the best use of the committee time; there are Federal regulations to deal with this type of questions on a daily basis by the FDA staff. He would like to invite RAC members to attend the FDA IND and pre-IND meetings that

examine the proper safeguards and make sure proper data are complete. Drs.Secundy and Miller asked if Dr. Noguchi implies that the RAC should approve the protocol now, and FDA will address the outstanding questions. Dr. Noguchi responded that the level and intensity of the review is too vigorous given the fact that there are existing FDA regulations; he would prefer the RAC to address broader issues. Dr. Parkman pointed out that if the protocol involves only IL-2 gene transduction ofglioblastoma cells, then it could potentially be reviewed under the *Accelerated Review* process. But the protocol involves the new element of antisense TGF-, and the investigators have not provided data and have not made clear statements as to what degree ofTGF- inhibition will make this a functional clinical protocol. Dr. Parkman disagreed with Dr. Noguchi. The RAC should look at these issues in a new clinical protocol. As a point of clarification, Dr. Noguchi said insufficient data is a typical reason for FDA to deny a clinical trial or to put it on clinical hold. Dr. Parkman said that in Dr. Ginsburg' initial review, he suggested that the protocol was premature for RAC review. Dr. Wivel stated that if primary reviewers asked the protocol not be reviewed, it will not be placed on the RAC agenda. Dr. Noguchi said the point is well taken.

A motion was made by Dr. Miller and seconded by Dr. Glorioso to defer the protocol submitted by Drs. Keith Black and Habib Fakhrai of the University of California, Los Angeles, California, based on the lack of sufficient preclinical data. The investigators and the primary reviewers will agree on a mutually acceptable experimental design to address the scientific questions posed by the RAC members. Once these studies have been conducted, the investigators are required to submit this data to the full RAC for review and approval. The protocol was deferred by a vote of 16 in favor, 0 opposed, and no abstentions.

XVI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: GENE THERAPY FOR CHRONIC GRANULOMATOUS DISEASE/DR. MALECH*

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Harry Malech of the NIH, Bethesda, Maryland. Dr. Parkman stated that ChronicGranulomatous Disease (CGD) is a genetic disease in which affected individuals have an increased susceptibility to infection with certain bacteria and fungi due to the inability of their granulocytes to producesuperoxide and other mediators necessary to kill bacteria. The granulocytes from the patients have a normal ability to migrate to the site of infection and to phagocytize bacteria. Once the bacteria are within the cell, certain strains of bacteria (including *Staphylococcus aureus*) are not killed. Thus, these patients suffer from recurrent and sometimes life-threatening infections. The prophylactic use of antibiotics and the administration of recombinant gamma-interferon to stimulate the oxidative capacity of the defective granulocytes has led to a decreased frequency of infections in the majority of patients. Several genetic defects can produce the clinical phenotype of CGD; there are X-linked and autosomal recessive forms of CGD. The X-linked diseases are primarily due to defects in the gp90phox genes while the autosomal recessive forms are due to defects in the p47phox gene.

The investigators were the first to identify defects in the p47phox protein as the basis for the most commo form of autosomal recessive CGD . The DNA is isolated for this gene and its location on chromoson has been demonstrated. The investigators are collaborating with Dr. Richard Mulligan (Whitehead Institute, Cambridge, Massachusetts) and the investigators at Somatix Corporation (Alameda, Californi A retroviral vector has been created with an MFG backbone containing the p47phox gene. In a series of preclinical experiments, the investigators have demonstrated that the transduction of committed hematopoietic progenitors results in the generation of granulocytes that have normal oxidative capacity bulk transduction rate of approximately 40% has been achieved. It appears that the transduced cells h one retroviral sequence per cell.

Based upon these preclinical data, the investigators would like to undertake a clinical trial in 5 patients. The patients would receive granulocyte colony stimulating factor (G- CSF) to stimulate the mobilization committed hematopoietic progenitors. These hematopoietic progenitors would be collected leukapheresis on Days 5 and 6. The CD34(+) cells would be isolated from the mobilized cells an transduced for 3 days in the presence of G- CSF and a recombinant cytokine, PIXY321. PIXY321 i recombinant fusion protein containing elements of both IL-3 and granulocyte-macrophage colony stimulating factor (GM- CSF). After 3 rounds of retroviral transduction in the growth factors, the transd cells will be washed and infused on a 2 successive days into the patients. Patients will be monitored for the frequency and persistence of the transduced cells, and the expression of the transduced p47p gene. The primary endpoints of the study are the determination of whether the infusion of the transduced cells is tolerated and the frequency and persistence of the transduced hematopoietic progenit

The major question the investigators need to address is: How long will the transduced cells that hav normal enzymatic activities persist after infusion? The immature progenitor cells that have the capacity to expand is expected to persist for a long period of time. CGD patients usually take antibiotics every day protect themselves from routine infection. When the patients become seriously ill, they are given a transfusion therapy of granulocytes from normal individuals; frequently they become immunized to those cells. In the present protocol, the patients will receive their own cells corrected for enzyme deficiency and will avoid the problem of immunization. This form of therapy may prove to have some real clinical benefit for CGD patients with serious infections although it is a temporary gene therapy rather than permanen stem cell therapy.

Dr. Parkman said that the investigators have conducted efficient preclinical studies including using different cytokines to mobilize the cells; most of his questions have been responded by the investigators in writing. Dr. Parkman raised a concern regarding deleterious effects of p47phox expression on bone marrow. The one area not addressed by the investigators is whether hematopoietic stem cells may b transduced and engrafted. The experimental design suggests that the investigators consider this result a possibility. In other protocols such as studies involving Fanconi anemia and Gaucher disease, the have been presented to demonstrate if transduction of murine stem cells with the proposed vectors res in any toxicity in the bone marrow or peripheral blood of animals. Since it seems biologically likely that the transduction of some stem cells may occur, do the investigators have any data about the effect of their vector following transduction and transplantation of murine bone marrow? The investigators responde that no gene knockout mouse model for CGD is available for this kind of study. Dr. Parkman noted that study involving normal mice for a period of 4 to 6 months could provide useful information.

Dr. Parkman addressed another concern regarding experimental design. A femoral catheter will be placed to collect blood during leukapheresis . In pediatrics, there is a general position that an invasiv procedure should have some potential benefit to the patients. The investigators responded to stipulate the protocol: (a) that the first patient enrolled will be an adult; (b) that the second individual will not be enrolled until the first subject reaches Day 40 of the study without evidence of adverse effect; and (c) that every effort will be made to have all 5 subjects be adults. Dr. Parkman raised a concern of treating people under the age of 18 if the treatment is nontherapeutic . If there is evidence of persistence of transduced ce a demonstrated potential therapeutic benefit, then treatment of children with severe infection will be worthwhile. The RAC needs to address this issue.

In summary, Dr. Parkman stated that the investigators have provided efficient preclinical data on human cells. These *in vitro* human cell data are very pertinent to the protocol of a genetic disease. There is a real potential of clinical benefit with a gene therapy approach to CGD independent of the issue of whether t stem cells will be transduced . Dr. Walters noted that NIH IBC asked the investigators to conduct a p

preclinical study with transgenic animals.

Review--Dr. Erickson

Dr. Erickson complimented Dr. Parkman's thorough review. The investigators have provided the best written protocol and response he has reviewed. Dr. Erickson noted that CGD is a very lethal disease, a this protocol provides potential benefit to the patients.

Dr. Erickson said the 15 pages of Informed Consent document is very well written and complete; however it contains too much information for the patients. The document written for the *Minors* seems just about right for the *Majors*. The investigators have responded satisfactorily in writing to his concerns regarding stringency of CGD definition, and methods and specifications for virus titer and gene expression fo master and working cell banks. Dr. Erickson recommended approval of the protocol.

Review-- Mr . Capron (presented by Dr. Erickso

Mr. Capron noted that he found no description of animal model studies of the techniques for this illness. The investigators responded in writing that no animal model for p47phox CGD is available for th preclinical development phase of this protocol. A number of aspects of the protocol have already been validated with human subjects in other studies. Some animal data on the effect of p47phox gene transduction on bone marrow cells would be useful. The investigators cited similar data that have been obtained by transduction of a retrovirus vector with the ADA gene.

With regard to selection criteria for patients, Mr. Capron would recommend that every effort be made to restrict the population to subjects aged 18 years and older rather than including subjects as young as 13.

Other Comments

Dr. Miller noted that the MFG-based vector has an open reading frame upstream of the p47phox gene. Will it affect translation of the p47phox gene and does it encode a second protein molecule? Dr. Ginsburg asked if the concomitant gamma-interferon treatment can continue while the patients are on this protocol. Ms. Meyers inquired if there are adults in the patient population. Dr. Ross asked if the number of correctec cells (0.5% of total neutrophils) are enough to have any therapeutic effect, and whether it is justified t include children. Dr. Parkman explained that if the transduced cells persist more than one or two montl it is possible to front load the system with more transduced cells to obtain therapeutic efficacy. In thi Phase I trial, children with stable disease should not be included. The RAC has to weigh the risks and benefits of whether to include children with severe infection. The low percentage of corrected cells might have some beneficial effect if there is severe infection. Dr. Erickson added that this small percentage of corrected cells may have more significant effect in this disease. In the X-linked form of CGD , patients v only 5% of normal enzyme activity are asymptomatic. Dr. Ginsburg agreed that the present treatment may have beneficial effect.

Investigator Response--Dr. Malec

Dr. Malech responded to the inclusion of children in the protocol. Dr. Malech said that CGD is a with a death rate of 2% of individuals per year. Those who have more severe infections are no longer around as adults. The adults are survivors and are healthier. The children with life threatening infections usually end up in hospitals. Dr. Malech would like the committee to decide on how to resolve the issue "healthy" child. He explained that children with CGD are in constant danger of infection, and the diseas presentation is episodic. Children may go months or years without any infection, and then a life

threatening infection will kill them. Is that a stable child? If a child comes in with a severe infection that is potentially life threatening, Dr. Malech said he would not give up the opportunity to do a study on tha child, particularly if there is indication that the treatment will be beneficial.

With regard to the concerns for placement of a femoral line for the venous access needed in this treatment, Dr. Malech said that venous access in CGD is a major problem. Repeated administratio intravenous antibiotics together with repeated blood sampling takes a toll on arm veins. While obtaining small blood samples using small bore needles is not usually a major problem, insertion of large needles for administration of antibiotics or other procedures can be traumatic and painful. Dr. Malech said femo vein access is proposed as a back up and is not intended as a routine procedure; it will only be used in cases of great pain and discomfort. The greatest risk of femoral vein access is potential infection that occurs during long-term intravenous access but not for the short-term access proposed here.

With regard to Dr. Miller's question of an open reading frame present in the vector, Dr. Malech said tha open reading frame is not in frame with the p47phox, and its product is not detected in the Western blot using a probe for p47phox. Dr. Miller questioned if the open reading frame which is part of the *en*gene of the vector would encode a new antigen; however, he did not consider this problem to be a serious one for this protocol.

Regarding the issue concerning beneficial effect if only 0.5% of a patients neutrophils are transduced Malech said part of the answer was provided in comments by Dr. Erickson that individuals with th X-linked form of CGD who have 3 to 5% of the normal enzyme activities are perfectly healthy. Th investigators have published *in vitro* studies that demonstrate the small number of "normal" neutrophils would produce enough superoxide and hydrogen peroxide to act in synergy with a large number of C cells to achieve the capacity to kill microorganisms; in a sense, there is a positive bystander effect.

In response to concerns about stem cells versus progenitor cells and the effect of p47phox on the growth and function of bone marrow cells, Dr. Malech said it is a difficult question to demonstrate in an anima model. To measure the effect of replacing a significant portion of the bone marrow cells with the cells expressing p47phox requires a transgenic animal with the p47phox gene in all cells. As a surrogate model, a long-term (35 to 40 days) cell culture of CD34(+) cells transduced with the p47phox gene hav shown no effect on its growth or its colony forming capacity. The investigators are in the process of developing a gene knockout mouse model for p47phox at the suggestion of their IBC. Dr. Malech did n consider the data from that animal model to be essential for the human study.

Dr. Parkman remarked that the gene knockout model serves mostly as a therapeutic model; the question that concerns him is that the constituent expression of p47phox gene in the immature bone marrow cells might have some deleterious effect. Dr. Miller said that if there is any deleterious effect, it will kill the transduced cells which account for only a minor fraction of hematopoiesis ; the previous concern is a genes such as IL-2 gene which has a growth effect on hematopoietic cells. Dr. Ginsburg agreed with D Miller's assessment. Dr. Haselkorn was concerned that there would be a deleterious effect if th transduced cells constituently produced superoxides . Dr. Malech clarified that p47phox is just a the holoenzyme producing superoxides and by itself does not have any enzyme activity. If it is expr in the wrong cells, without the other components, nothing can happen. Furthermore, the investigators have conducted many tissue culture experiments, and no growth effect of this gene has been observed. The animal experiment to demonstrate toxicity of this protein in normal bone marrow may not provide satisfactory answers, and Dr. Malech offered the alternative of the gene knockout mouse model that w provide more meaningful results.

Regarding the question of stem cell transduction, Dr. Glorioso noted that granulocytes have very short

span; an alternative animal experiment is to transduce the peripheral blood cells and to transplant the into SCID mice. Dr. Malech commented that he has deliberately avoided using the *stem cells* in the protocol. In his opinion, no human experiment has definitively demonstrated that *stem cells* are transduced in most clinical studies. The question of long-term toxicity in marrow is important, but it is le of a problem in the present protocol since the chances of transducing the *stem cells* are very low. Dr. Glorioso asked if the stem cells are not expected to be transduced, will there be any therapeutic eff just transducing the short-lived granulocytes? Dr. Miller explained that some progenitor cells (if not th stem cells) have the capacity to develop into granulocytes for weeks or months; transduction of these progenitor cells will produce noticeable beneficial effects. Dr. Ginsburg stated that even in the worst case scenario, it will be no worse than the current treatment for these patients with allogeneic granulocyt transfusions.

Dr. John Gallin, co-investigator, stated that the NIH Clinical Center is currently following about 80 patients, one-third of them having the type of CGD proposed in this protocol. The age of these patient ranges from a few months to 30's. Dr. Malech added that there are enough patients over 21 for this stu although it will take longer time to accrue 5 adult patients. Furthermore, the younger patients: (1) have more infections, (2) are in a situation to satisfy the entrance criteria of treating someone with an active infection, and (3) will benefit directly with this procedure. Dr. Gallin proposed to limit the present protoc to children who have already been alloimmunized from receiving transfusion, who have life threatenin infection, and who would stand to benefit from this procedure.

Dr. Miller suggested that there is enough promise and little risk of the procedure to justify treating children after the first treated adult shows no untoward effect and persistence of transduced cells. Dr. Parkma preferred to have first 2 patients to be adults, and after that children who have serious infections. Dr. Ross expressed her reservation about children who are only 13 or 14 years old; she preferred to limit the study to individuals of 16 years and older.

Dr. Dronamraju asked if children will have a separate Informed Consent document. Dr. Malech saic protocol is complex, and the Informed Consent document is lengthy. There is a requirement that the patients consult with their physicians, and it is expected that the physicians will translate the essence of this study to their patients including adults and children. Dr. Zallen suggested the break up of some of t lengthy paragraphs of the Informed Consent to facilitate comprehension. If an assent form is to be prepared for the children, it could be improved by using less technical language. Dr. Malech agreed t revise the Informed Consent document. Ms. Meyers said she is comfortable with inclusion of children 13 years old.

Committee Motion

A motion was made by Ms. Meyers and seconded by Dr. Ginsburg to accept the protocol submitted by Dr. Harry Malech of the NIH, Bethesda, Maryl

Dr. Parkman made a friendly amendment to the motion to require that the first 2 patients be adult and subsequent patients be of 15 years of age. Ms. Meyers agreed to have the first 2 research subjects be adults but did not agree to limit the study to just older children. There were discussions among Drs. Miller, Malech, Parkman, and Ginsburg regarding the pros and cons of including children with life threatenin infections. Dr. Malech explained that these patients stand a very good chance of dying from infections. they come in with a deep organ infection, that is the time to give them a white blood cell transfusion or if they are alloimmunized that is the time to be given the proposed treatment. Dr. Gallin said that the l of white blood cell turnover in an infected patient are quite different from an uninfected subject. The present protocol is to observe the transduced cells 24 to 48 hours post-infusion, and interpretable data

obtainable from a 16 year old with serious infection. Dr. Ginsburg inquired what percentage of patients with life threatening infection will succumb to the infection. Dr. Malech said about 5 to 10%, and most c the patients will survive to complete the study. There is no problem in a patient population above 16 years old, but if the study shows any promise and there is a need to treat a patient below 16 years, a minor modification of the protocol would be requested in the future.

An amended motion was made by Dr. Parkman and seconded by Dr. Dronamraju to modify the tria design such that the first 2 subjects entered would be 18 years of age and that the remaining 3 subjects should be limited to individuals 18 years of age or minors 16 years of age with life threatening infections. Dr. Parkman's amended motion failed to pass by a vote of 7 in favor, 7 opposed, and 2 abstentions.

An amendment was made by Dr. Miller and accepted by Ms. Meyers to modify the protocol design such that the first 2 subjects would be 18 years of age and that the remaining 3 subjects should be limited to individuals 13 years of age with serious infections. The amended motion passed by a vote of 13 in favor, 2 opposed, and 1 abstention. Approval of the protocol is contingent on the following: (1) the first 2 subjects will be individuals 18 years of age, and (2) the remaining 3 subjects will be limited to individuals 13 years of age with serious infections.

As a point of clarification, Dr. Miller stated that the 2 negative votes were solely in regard to the age of the patients. Dr. Samulski said he abstained due to conflict of interes

Dr. Chase stated that although he voted in favor of the motion, he has reservation about treating minors; however, in this protocol, there is a slim chance of benefiting the patients. Dr. Dronamraju said he vote against approval based on his concern about the age; the decision on this protocol should not be a precedent to future protocols to include minors. Dr. Chase said that the RAC has approved other protocols involving minors. Dr. Ross noted the RAC voted to exclude the children from the protocol studying Hunter syndrome. Dr. Parkman said the RAC has excluded children in the Gaucher disease protocols, but the is legitimate rationale to include children in this study.

Protocol Summary: Dr. Harry Malech of the NIH, Bethesda, Maryland, may treat 2 CGD subje years of age (with or without concurrent serious infection), and 3 subjects 18 years of age (with or without concurrent serious infection) or CGD minors 13-17 years of age who have concurrent serious infection CGD is an inherited immune deficiency disorder in which blood neutrophils and monocytes fail t antimicrobial oxidants (p47phox mutation) resulting in recurrent life-threatening infections. Subjects will undergo CD34(+) mobilization with G- CSF. These CD34(+) cells will be transduced with the retrovi vector, MFG-S-p47phox, which encodes the gene for normal p47phox. The objectives of this study are to: (1) determine the safety of administering MFG-S-p47phox transduced CD34(+) cells, and (2) demonstr increased functional oxidase activity in circulating neutrophil

XVII. PRESENTATION ON *DETECTION OF LOW LEVELS OF A PRESUMPTIVE HUMAN RETROVIRUS IN A GENE THERAPY MARKING TRIAL/DR. MILLER*

Presentation--Dr. Miller

Dr. Miller presented his data regarding detection of low levels of a presumptive human retrovirus in cells obtained from 4 subjects entered on Dr. Friedrich Schuening's human gene marking protocol entitled *A Phase I/II Study of PIXY321, a rhGM-CSF /rhIL-3 Fusion Protein or rhG-CSF , for the Mobilization Peripheral Blood Stem Cells for Autologous Stem Cell Transplantati*(Protocol #9209-027).

Dr. Miller said during the course of testing for replication-competent retrovirus (RCR) before administer

the cells to patients, he has detected a RCR which is not related to a murine virus but rather it appe be a new human retrovirus. This new virus is present at a very low titer in the cultures of patients' cells tha have not been exposed to the retrovirus vector, and it is not detected by the S+L - assay. The virus ha been detected in 4 out of 4 patients, and its detection has prevented the investigators from re-infusing these cells back to patients in the gene-marking protocol. This finding has been reported to FDA.

Dr. Miller explained the background of the study. It is a patient population that is undergoing autologous stem cell transplantation for a malignancy following high dose chemotherapy. After chemotherapy, peripheral blood stem cells were mobilized with G- CSF ; these cells were culture *in vitro* for 5 days and were transduced with the murine retrovirus vector, LN . At the end of that culture period, the cu medium (5% of total) and cells (1% of total) were tested for RCR that might be derived from th amphotropic vector. A low level of RCR was detec

The test used to detect this virus is a marker rescue assay. Dr. Miller illustrated this assay with a slide. The rescue fibroblast cell line, *Mus du* LAPSN , was isolated from a wild mouse and transduced vector that encodes alkaline phosphatase and the neomycin resistance marker. *Mus du* LAPSN cell (106) were seeded on each 10 cm culture dish. One day later, the cells were exposed to test samples in the presence of Polybrene . After culturing the cells for at least 2 more weeks to amplify the virus, th culture medium was harvested. Two RCR tests were employed to assay the culture medium. Th standard S+L - assay required by FDA, and another by infecting naive *Mus du* cells and scoring for the presence of alkaline phosphatase activity. This new virus did not score with the S+L - assay but tes positive with the *Mus du* assay. It has been detected in 4 out of 4 patients' cells marked with the LN neo-vector, and later in a mock culture of human hematopoietic cells never exposed to the vector. In la experiments, this virus has been detected in hematopoietic cells even prior to the CD34 purificatio procedure.

Dr. Miller presented his data with a slide demonstrating that the patient virus is unrelated to standard murine virus. The interference assay was used to observe the host range of the virus. The target cell used in this interference assay were dunni , dunni /N2, dunni /N2 plus patient 1 virus, dunni /N2 2 virus, and dunni /N2 plus amphotropic murine leukemia virus. This latter target cell serves as a control for the amphotropic virus. Patient virus isolates do not interfere with infection by vectors havin amphotropic or other murine virus pseudotypes including polytropic and xenotropic

Another experiment showed that cells already infected with the murine viruses do not interfere wit infection by the patient virus isolates; therefore, these patient viruses are not related to the murine virus including the amphotropic , xenotropic , and the polytropic mink cell focus forming (MCF) virus concluded that the putative human virus is not related to standard murine viruses and thus was unlikely be the result of gene transfer.

Another slide showed that all patient virus isolates exhibit cross interference and belong to the same host range group. The experiment involved dunni /N2 target cells carrying either none, amphotropic mu leukemia virus, patient 1 virus, or patient 2 virus; these target cells were challenged with virus isolates from patient 1, 2, 3, or 4. All 4 patient isolates did not infect target cells already infected with either patient 1 or patient 2 virus; therefore, patient virus isolates cross interfere with each other. This experiment shows that all 4 patients isolates have the same host range, and that they are all unrelated to the murine amphotropic virus

Dr. Chase inquired if the patients already have the virus even without any gene transfer procedure performed on them. Dr. Miller said the mock infected cells without any exposure to the vector still will have virus detected in this experiment. Other experiments ruled out these viruses came from the cell culture

media. Dr. Miller emphasized that these human viruses are totally independent of the recombinant retroviral vector.

Dr. Miller stated that the virus titer of the human cells is very low, about one virus particle per ml. But once amplified in the *Mus dunni* cells, the virus titers can be as high as 104 to 105 per ml.

With a slide illustration, Dr. Miller pointed out that these human viruses have an interesting host range that is different from the amphotropic murine virus. Both patient 1 and 2 virus isolates infect the following lines: human HeLa, mouse NIH3T3, wild mouse *Mus dunni*, and rat NRK. But the virus isolates does not infect the cat CCC-81 cell line, a cell line used in the S+L- assay; this observation partly explains the inability of the S+L- assay to detect these viruses. In contrast, the amphotropic murine leukemia infect all cell lines mentioned above. These observations suggest that this human virus is an unique virus different from all the other retroviruses carried in Dr. Miller's laboratory.

Dr. Chase asked if the patients were treated with other unusual therapies. Dr. Miller responded that the patients have been through high dose chemotherapy and have been treated with G-CSF. Dr. Ginsburg asked about the diagnosis for these patients? Dr. Miller responded they have different diseases: 2 had multiple myeloma, 1 had breast cancer, and the other had non-Hodgkins' lymphoma. These virus is all have the same host range suggesting the virus does not induce a specific type of disease. Ms. Meyers asked if the virus is detectable in non-cancer patients. Dr. Miller said this work is still ongoing; in the preliminary study, the virus has not been detected in bone marrow from 3 normal individuals. There is a possibility that it is related to the cytotoxic chemotherapy or the G-CSF mobilization of stem cel irradiation and chemotherapeutics such as deoxyuridine have been shown to induce a xenotrop retrovirus in mice.

Dr. Miller showed a slide demonstrating that the RD114 retrovirus interferes with infection by patient retroviruses. Since the patient virus isolates do not infect the CCC-81 cat cell line, Dr. Miller suspected that they may be related to an endogenous cat virus called RD114. A G355 cat cell line was obtained from Dr. Donald Blair of NIH. The LAPSN vector pseudotyped with the patient 1 virus is able to i G355 cat cells generating a virus titer of 103. In contract, the patient 1 virus is poorly infectious to the G355 cells infected with RD114 due to interference (the virus titer generated is only 80). A similar interference by RD114 was observed with the patient 2 virus. In the control experiments, LAPSN vecto pseudotyped with RD114 as expected infects only the G355 cells but not the G355 with RD114, and th vector pseudotyped with PA317 is able to infect both cell type

Dr. Inder Verma (The Salk Institute) asked if all patients received the same kind of chemotherapy s their viruses share the same interference pattern. Dr. Miller responded they all received alkylating ager although 3 normal bone marrows are negative, the appearance of the virus could be related to G-CSF treatment. Dr. McGarrity (Genetic Therapy, Inc.) asked if the virus titer increases as the infected cells a passaged. Dr. Miller responded that the virus titer does increase

Dr. Miller presented his last slide to show that there are some variations of the infectious titers of viruses in the culture medium. Five aliquots of samples (5 ml each) were taken on Day 5 culture medium, and 5 independent assays were run on these 5 samples. Virus titers did vary among these 5 aliquots of the same patient virus. He speculated that there are genetic variations affecting infectivity among virus variants present in each patient's body, a similar type of variation due to mutations has been observed with other retroviruses. By doing limiting dilution assays, it is estimated that the virus titer is present in the patients in the range of 0.2 to 1 virus particle per ml. Dr. Ginsburg said with such a low titer it might be missed in the testing of normal subjects.

Is the virus titer amplified in the cell culture when it is growing for 5 days? Dr. Miller showed data demonstrating that there is no dramatic increase in virus titers during the 5 day period. But the titer increased after long-term culture in *Mus dunni* cells.

In summary, Dr. Miller said the patient virus is not detected by many variations of the S+L- assay. It ca grown up in *Mus dunni* cells to a titer of 104 to 105, but it still scores negative by the S+L- assays at su a high titer; it is present in patient's cells at very low titer. It is not amplified during cell cultivation. Overall, the evidence suggests it is a novel human virus either of endogenous or exogenous origin. The new virus has not been molecularly cloned. The virus is unrelated to gene transfer procedures since it is detected in mock infected cells. It can be detected in cells before CD34 purification, therefore, it is unlikely due to contamination from the monoclonal antibody column used to isolate CD34(+) cells. Similar virus was isolated from patients with 3 different diseases suggesting that it does not induce these diseases. However, it could be induced by cytotoxic chemotherapeutic treatments these patients received. As precaution, Dr. Miller said the investigators have stopped reinfusing the vector-transduced cells bac the patients because of the detection of this new virus.

Dr. Miller indicated that he is seeking FDA permission to reinfuse cells containing patient's own viru back to patients as part of the normal therapy. He asked for recommendations from the RAC on how to modify the Informed Consent document in order to inform the patients the detection of this new human virus.

Dr. Noguchi stated that Dr. Miller and scientists at Targeted Genetics Corporation (Seattle, Washington) have been in contact with FDA officials regarding the presence of the human retrovirus. So far there is no evidence of pathogenicity of this virus to humans. Dr. Noguchi said that the Informed Consent docume needs to be amended to inform the patients regarding the finding of the new virus so that the patients can make their own informed decision based on this new information.

Dr. DeLeon noted that the endogenous C-type retrovirus has been reported to be induced in embryoni cells without any treatment, and she asked Dr. Miller if tests have been performed on normal embryonic human cells. Dr. Miller responded that such studies are being planned.

Dr. Ginsburg stated that it is prudent to hold off reinfusion of patient's cells back to the patients in the g marking study until the virus is further characterized. Although the virus appears not to be greatly amplified during cell culture, there still is a risk of giving back to patients the virus which may have undergone some mutations during *in vitro* cell culture. Dr. Miller remarked that the patients, in the course of their normal therapy have been receiving their own cryopreserved cells which might contain this viru Dr. Ginsburg said even if it is a very small theoretical risk, he still has some concern about performing this procedure in the gene marking studies which do not benefit the patients. In the case of autologous bon marrow transplantation with G-CSF mobilization of stem cells, there is some patient benefit. Dr. Noguc said that FDA's concern is a fair application of the FDA regulation, and he would not favor a selective type of hold pattern for certain types of therapies. As Dr. Schuening's protocol is placed on clinical hold, i should apply to all other gene therapy protocols and all chemotherapies until the new virus is further characterized. Dr. Ginsburg clarified that what he proposed is a moratorium on gene marking studies. Dr. Miller asked whether similar criteria will be applied to a situation where the adeno-associated virus (90 of people are carrier) is detected in these patients. There will be an endless list of viruses that will be detected in the cell culture.

Dr. Samulski said a distinction should be made in this case concerning a new unknown human virus. I addition, it has been observed in Dr. Brenner's gene marking protocols that the treatment for cancer may have primed their cells to be more receptive to vector transduction; his concern is if similar receptivity will

extend to the new human retrovirus.

Dr. Ross asked if Dr. Miller would make his test for this new virus available to other investigators in the field. Dr. Miller responded that he would supply all the cell lines to any investigator who need them for the test. Dr. Miller was concerned about shutting down all gene marking protocols.

Dr. Tom Reynolds (Targeted Genetics Corporation) made a comment about putting gene marking protocols on clinical hold. In terms of risk/benefit ratio, G-CSF has been used to mobilize stem cells i allogeneic transplantation studies involving normal volunteers who are not expected to have any benef from this procedure. If it is found that G-CSF mobilization is involved in activation of this new virus, wil this kind of procedure pose an issue for normal volunteers? Dr. Ginsburg said the potential risks should be stated in the Informed Consent document.

Dr. Wivel remarked that there are mouse data suggesting that various passenger viruses tend t concentrate in tumors. He asked if the new virus replicates better in tumor cells. Dr. Miller said it is a good point, and he would look at tumor cells once a molecular probe is available.

Dr. Verma commented that characterization of the new virus is still preliminary. Molecular cloning of th virus is needed in order to determine its structure and if this virus is indeed novel. The new host range of a virus could potentially be attributed to recombination as in the generation of the MCF virus involving th *en*genes of the ecotropic and xenotropic mouse viruses. Dr. Miller said the new virus is quite diffe from all viruses he carries in his laboratory; it has been detected in two different places under very strict conditions of containment. As a point of clarification, Dr. Reynolds said the new virus has been detected in tumor patient's cells freshly isolated by leukapheresis at a blood banking facility separated from hi laboratory. Many independent assays have been performed at Targeted Genetics that confirm Dr. Miller's results; he believes the likelihood that the new virus is a laboratory contaminant is close to zero. Dr. Reynolds agreed that the most definitive data will have to wait until the virus is cloned, and its DNA sequence determined.

Dr. Miller said the RAC still has to deliberate on what should be recommended if it is a real human virus. Dr. Samulski stated that the important question now is if the same result can be validated from other g marking studies at other institutions. Anything beyond that is an extreme reaction to something that needs to be characterized.

Dr. Phil Michaels (Baxter Health Care) commented that in 1 year there are as many as 10,000 autologou transplantation procedures being performed worldwide; some use cyclophosphamide to mobilize ste cells, some use G-CSF, some use GM-CSF, and some use combination of those. There is no lack specimens to further examine the distribution of this new virus. He suggested testing a few more normal subjects who have been treated with different mobilization procedures. Dr. Miller said such a study is ongoing.

Dr. Helen Blau (Stanford University) remarked that the finding should be validated with many other ger marking studies rather than starting a new trial. Dr. Miller explained that the study is an ongoing protocol which is on clinical hold due to FDA's concern about the finding of a new virus. He said that FDA has agreed to proceed with the protocol, and he asked the RAC to provide guidance for how best to continue the trial.

Dr. McGarrity commented that Genetic Therapy, Inc. scientists have validated a vector rescue assay fo gene therapy products and for bone marrow cells. There should be information shortly regarding this new virus. He inquired if it is a qualitative or quantitative difference that the new human virus does not score or

S+L - assay. Dr. Miller responded that the assay is still negative for virus samples which have bee amplified to a titer of 104 or 105. A positive control with an amphotropic murine RCR using the procedure is amplified to a titer of 107 and scored with such a titer in the S+L - assay. Although the hun virus infects the S+L - cells as shown by a positive alkaline phosphatase transfer, it does not induce this assay.

Dr. Parkman stated that a pertinent question for the RAC relates to any incremental risk that is associated with the gene transfer aspect of the protocol. Since the virus is present before any *in vitro* cell manipulation and gene transduction, it appears to be unrelated to the gene transfer procedure. Whatever the risk is, it is a risk of the original autologous transplantation protocol and not a risk that relates to th gene transfer. According to this rationale, reinfusion of the transduced cells does not pose additiona to these patients. The purview of the RAC concerns the incremental risk associated with the gene transfer; the data so far indicate the presence of this virus does not amplify the risk to the patients regarding gene transfer. Dr. Noguchi agreed with Dr. Parkman's statement. Dr. DeLeon said she agree to this statement if it is shown that the virus is an endogenous human virus rather than a recombinant virus. Dr. Miller said there will be no concern if it is a recombinant or an assay artifact due to laboratory viruses.

Dr. Parkman commented although this new virus does not pose a recombinant DNA issue, finding of this new retrovirus may lead to a whole new field of biology of human viruses. Responding to Dr. Ross' question about other viruses present in patient's cells before reinfusion , Dr. Miller said there are alway viruses either known or unknown, present in patient's cells. He emphasized the real focus is to make sure no RCR from packaging cells is given to the patients

Dr. Glorioso asked about the threshold level of this virus that will prevent the investigators from reinfu the cells to patients. Dr. Reynolds responded, as per discussion with FDA officials, that cells will be released to patients based on the PG4 S+L - assay. Characterization of the new human virus will contir but it will not be a decision factor regarding the question of reinfusion

Dr. Samulski made a comment that it is incumbent on the investigators to develop a simple assay such PCR assay to monitor the cells before and after *in vitro* manipulation for the presence of this virus. If there is no variation or increase of the virus level, the cells should be allowed to be reinfused back to th patients. Dr. Miller said he would prefer a biological rather than a PCR assay. Dr. Verma agreed tha biological assay is more relevant. Dr. Parkman reiterated his position that based on the fact that there is no increased risk related to gene therapy that the protocol should proceed; however, if the future study shows an increase of the virus titer during *in vitro* manipulation, then the whole problem should be revisited. Dr. Miller agreed to revise the Informed Consent document to inform the patients regarding the finding of the new virus.

Dr. Walters complimented Dr. Miller for his great effort to characterize this new human retrovirus.

Summary

Dr. Miller advised the RAC that the data indicate that this virus is clearly unrelated to gene transfer and is not amplified in cell culture of patients' cells. This virus has been detected in mock cultures of patients' cells that have not been exposed to the retrovirus vector. All 4 subjects who demonstrated detectable virus were cancer patients who had undergone standard chemotherapeutic regimens and G- CSF mobilization. Whether the chemotherapy and/or G- CSF mobilization of these patients contributed to th permissive growth of this virus remains to be determined. This virus does not appear to be specific for any particular cancer type since the subjects represented multiple myeloma , breast cancer, an

non- Hodgkins ' lymphoma. The virus was not detected in 3 normal individuals. The CD34(+) purificatio process does not contribute to the introduction of this virus since the virus is detected in hematopoietic cells prior to purification.

The virus was detected by an extremely sensitive marker-rescue assay involving *Mus dc* cultivation followed by alkaline- phosphatase staining of positive foci. The standard S+L - assay failed to detect virus. Interference assays indicate that this virus is unrelated to murine retroviruses (amphotropi polytropic , or xenotropic) but is related to the RD114 cat vir

XVIII. DISCUSSION ON ADENOVIRAL VECTOR TOXICOLOGY/DR. NOGUCHI

Dr. Noguchi invited two senior toxicologists from FDA to discuss the risk assessment of adenoviral vectors: Joy A. Cavagnaro , Ph.D., and Anne M. Pilaro , Ph.D., from the Office of Therapeutics Rese and Review, Center for Biologics Evaluation and Research, FDA. Dr. Noguchi invited Ms. Margi Stuart from a patient advocacy organization, *Breast Cancer Action*, based in San Francisco, California, to present a patient perspective regarding the human gene therapy of cancer.

Presentation--Dr. Joy Cavagnar

Dr. Cavagnaro introduced herself as the Assistant Director for Pharmacology and Toxicology in the Off of Therapeutics Research and Review. She stated that she will present the rationale for preclinical studies regarding adenoviral vector toxicology. Dr. Pilaro will present the experience obtained to date and Dr. Cavagnaro will then finish up the presentation with some future challenges

It is important to discuss the rationale in the context of risks and benefits. The potential benefits of not performing preclinical safety studies include: conservation of resources including personnel and animals; ability to screen a number of early concepts more quickly in the *ultimate* species, i.e., humans; realization of providing patient benefit as early as possible; and earlier opportunity to publish clinical data in peer reviewed journals.

These potential benefits are balanced by the potential risks of not performing preclinical safety studies. There may be a missed opportunity to screen potentially toxic and/or more efficacious candidates. It may be that the approved starting dose is too low or the escalation scheme, too conservative. Ultimately, the clinical program will be more resource intensive. There is likely to be increased monitoring, because the FDA is not sure what to monitor in the clinic. There may be more patients because the FDA is not sure what subpopulations are at risk, and more long-term follow-up may be needed. Finally, serious unexpected or even expected adverse reactions may negatively impact the entire field.

Dr. Cavagnaro summarized some basic truths of regulation. Clinical investigation is different from clinic development in that gene therapy is still at the stage of early proof of concept and it is different from a traditional clinical development of pharmaceuticals. Products reaching clinical trials faster may not necessarily mean that the FDA will approve these products faster for clinical use. Often times preclinical studies are performed, but only selected data are presented to FDA. It is important to point out that FDA can only make an assessment based on the data that are submitted. And finally, the preclinical safety data submitted during the IND phase are generally confidential; knowledge or reasons may not be sha and only suspicions are generated. The public presentation to the RAC and publications of early data help advance the FDA guidance for preclinical safety evaluations.

It is important to dispel some basic myths of preclinical safety evaluation. One myth is that more data in more animals are always more useful and preferred. Dr. Cavagnaro said better designed studies are m

useful. The other myth is that unexplainable observations are better ignored, and the lack of any significant adverse findings in animals gives assurance of safety in humans. Animals showing toxicity is a good sign as shown in a previous demonstration of brain toxicity of adenoviral vectors; it is important to see toxicity in animal studies.

The goals of preclinical safety evaluation are: (1) to recommend an initial safe starting dose and dose escalation scheme in humans; (2) to identify potential target organs(s) of toxicity; (3) to identify approp parameters to monitor in the clinic trial; and (4) to identify "at risk" populations.

There are many challenges in the safety evaluation of novel biological therapies including gene therapy: (1) The unique species specificity since humans are often the only relevant species for diseases such as Hunter syndrome. (2) The use of animal models of disease for assessing both activity and safety. Cotton rats are used as a poor man's model for CF protocols. (3) Novel methods and routes of delivery such as intracranial or intrahepatic routes of delivery as opposed to the usual route of injecting directly into tum or *ex vivo* transduction. (4) Manufacturing or process changes such as improvements in products, changes in promoters of vectors, and how these changes impact on safety concerns.

There are several initial concerns in designing preclinical studies: (1) Selection of the models. Relevant species need to be identified and a physiological state needs to be selected for the study in terms of age, size, or disease of the animal models. (2) Selection of the dose. This includes a dose that will have no effect, a dose that has toxic effect, and establishing a dose limiting toxicity. Factors to be considered include route, multiple doses, and a regimen that will mimic the clinical indications. (3) Selection of the endpoint to evaluate issues of activity (efficacy) and toxicity (safety).

Presentation--Dr. Pilar

Dr. Pilaro made a presentation with slide illustrations regarding preclinical safety evaluations o adenoviral vectors and questions about where the field proceeds in the future. The discussion focused on the adenoviral vector in CF protocols involving the lung. This area is where most of the data have been published.

When testing any vector, there are issues need to be addressed in terms of selection of species. What are the indications, cancer or CF? Which is the route to administer the vector? What dose is selected? The major issues involving adenoviral vectors are: vector dissemination, vector or transgene toxicity, hos immune response, and the presence of replication competent adenovirus (RCA).

The most commonly used animal model is the cotton rat. The cotton rat, *Sigmodon hispi*is really not a rat but more like a hamster with a tail. It is a small sized animal (70 - 120 grams adult weight) that limits a lot of procedures that can be performed on it. It is a hyperactive animal easily stressed by restraints. It is a poor breeder that presents a real supply problem. The main cause for concern is that there is not a sufficient historical database for spontaneous pathology, clinical chemistry, or hematology values.

The cotton rat, however, is very useful to study several different issues in terms of gene therapy and preclinical studies. It is semi-permissive for replication of wild-type adenovirus after injection in the lung. It has a similar time course of infection to that observed in humans. The lung pathology after infection is similar to that observed in humans. The immune response after lung infection is well documented; it is a useful model of host-mediated immune effects on pathology and replication of virus.

What information have been gained from the cotton rat model? There is a database with 3 different CF transmembrane conductance regulator (CFTR) vectors and 2 different serotype vector backbones,

and Ad5, showing comparable pathology at similar doses. Analogous effects have been observed with the Ad-*gal* vectors suggesting these effects are due to vectors not the transgenes . The cotton rat provi a model to study the effect of preimmunity on vector transduction, gene expression, and pathology. Th is no evidence to date of vector rescue due to recombination or of extensive shedding of adenovirus-based vectors in a model that permits this type of analysis.

Are cotton rats always useful? It depends on what is being studied. The cotton rat most closely approximates the disease pathology and host immune response in humans after wild-type virus infection in the lung. For screening a new generation of vectors, there is now a database of toxicity of CFTR vec in the cotton rat. These data may be used to predict effects of changes in vectors and to compare the old versus new vectors for changes in pathology. It can be used to evaluate immune-mediated effect by challenging an animal with preexisting immunity. It can provide a model to study pathology and effects on viral replication. It is not useful, however, to characterize the mechanisms; there are no reagents against T cells or B cells as in the mouse model.

In terms of vector dissemination, Dr. Pilaro presented data by Oualikene et. al., published in *Journal of General Virology*, Volume 75, pages 2765-2768, 1994. In the cotton rat, after the vector is injected by intramuscular or intranasal route, the vector can be recovered in the liver, the lung, the spleen, the popliteal nodes, inguinal nodes, and nasal washing. In the nonpermissive mouse model, the vec dissemination pattern is very similar to the cotton rat although the virus is not recoverable from those sites Adenoviruses investigated in this study include the wild-type virus, E3-E1a+ and E3-E1a- adenoviral vectors.

When are other rodent models useful? Vector dissemination may be conducted in other rodent species such as the mouse. The mouse can provide a better model to study mechanisms of immune-mediated toxicity; reagents and assays are readily available and much more immunology is known in the mouse model.

What primate studies are needed or justified? Primate studies are resource intensive and the primates are not, a priori, necessarily the best model to study. They are: (1) expensive to purchase and to house; (2) difficult to handle, requiring anesthesia in most procedures; (3) less is known about primate immunology and the infectivity with the wild-type virus is not as well studied in these models; and (4) there is an unknown contribution of simian viruses that could interfere with interpretation of the data. The primates are useful to study delivery methods or devices where small animal models are not feasible; the primate models may be useful for follow-up studies of clinical adverse reactions.

The real challenge of animal studies is to see how the data can facilitate clinical development of adenoviral vectors for gene therapy. What is the safe starting dose and how can one safely escalate the dose for the human trials? Dr. Pilaro used the adverse reaction in the CF protocols to illustrate how th data from preclinical studies are used to make recommendations for the human trials in terms of vector dosage. Dose-escalation studies of CFTR vectors in cotton rats show a 2 to 10-fold difference betwee the no observable adverse effect level dose and the threshold doses for toxicity. The toxicity data are consistent between 2 different vectors and 2 serotype vector backbones. Similar studies in Rhesus monkeys and baboons show a 10-fold difference in doses between the no observable adverse effect level dose and the threshold toxic dose. In Dr. Ronald Crystal's protocol (#9212-034), the first adverse reaction occurred after a 100-fold dose escalation involving hypoxia, fever, and pneumonia. Some of the evidence of these adverse effects have been observed in primate models at high dose. Based upon these animal data, FDA officials recommended that the sponsors of the CF protocols consider dose-escalation that proceeded at a slower rate. The request involved scaling 1/2 log (3-fold) increments between cohorts. The stopping rules were tightened up and patient monitoring was extended to include these adverse effects.

How do effects in animal models compare to those observed in humans? Dr. Pilaro showed data on th no observable adverse effect level doses for AdCFTR vectors in several species of animals including t C57 BL/6 mouse, hamster, cotton rat, Rhesus monkey, baboon, and human. The no effect dose is approximately 2 x 109 infectious units (IU) of vector per square meter of total body surface area. Th dose-escalation of human studies to date is progressing up to a dose of 1 x 108 IU /m2, a level still belo the threshold toxicity dose of the animals.

Presentation--Dr. Cavagnar

Dr. Cavagnaro stated that concern about safety is universal. The application of results from safety stud from preclinical animal models to clinical trial is an iterative process: The feedback from clinical trials helps devise a more relevant animal model. Dr. Cavagnaro identified many future challenges i assessing the safety of adenoviral vector mediated therapies: (1) Identification of the most relevant species. Alternative routes of exposure are evaluated, i.e., the lung, the liver, the brain, and the peritoneal cavity. Is the cotton rat the most relevant species for the various different routes? (2) Effects of introductio of RCA. For the lung, one dose per patient appears to be safe. What are the acceptable limits for other sites? (3) Evaluation of next generation of vectors. Changes of the vectors in either the vector backbone o the expression cassette. (4) Bridging studies. To evaluate the effects of modifying the vector constructs, the nonhuman primate is a resource intensive model; it is better to pick a species that is more manageable and less expensive. (5) Index studies. The investigator should have a neutral approach to study design. The studies should all have the same route of administration, same indication, and perhaps close to the same dose range so that investigators can share their data and resources. (6) Universal studies, the indication neutral approach to study design. An investigator may have a same vector intendec for different uses, e.g., different tumors; a megastudy can be designed for this purpose without doin different studies for different diseases.

Other Comments

Dr. Parkman inquired if the investigators will move forward when they have enough data about certain route or certain vector modifications. Dr. Cavagnaro said that the challenge for facing the future; ideally the investigators can use the data generated from other studies as a bridging study to design the new study without having to reproduce all the safety data, but bridging across investigators presents many problems, e.g., willingness of investigators to share their data. Dr. Parkman noted that the confidential FDA process will hinder data sharing. Dr. Noguchi commented that public presentation at the RAC is a solution to this problem; Dr. Cavagnaro said that the concept of master files and cross referencing data another solution.

Summary

Drs. Joy Cavagnaro and Ann Pilaro provided an informational update to the RAC regarding the FD perspective regarding adenoviral vector toxicology. Specific issues that were addressed included: (1) the rationale for preclinical studies, (2) experience to date, and (3) future challenges. The following areas regarding preclinical trial design were addressed: (1) the selection of an appropriate model, i.e., species and physiological state; (2) dose selection, i.e., route, number of challenges, and regimen; and (3) endpoint selection, i.e., activity (efficacy) and toxicity (safety).

Future challenges in assessing adenovirus vector mediated therapies include: (1) the identification of the most relevant species (alternate routes of exposure), (2) the effect of introducing replication-competent adenovirus (sites other than the lung), (3) the evaluation of the next generation of vectors (changes in backbone and expression cassette), (4) bridging studies (systematic extrapolation), (5) index studies

(investigator neutral approaches), and (6) universal studies (indication neutral approach).

Presentation--Ms. Stuart

Dr. Noguchi introduced Ms. Margi Stuart to make a presentation from a patient perspective. Ms. Margi Stuart, a metastatic breast cancer patient and board member *Breast Cancer Action*, a patient advocacy organization in San Francisco, California, presented her perspectives on existing cancer treatment modalities and the advent of human gene therapy research. She noted the severe toxicities that patients and health care workers have had to endure for traditional therapies and encouraged the RAC to expedite the review and approval of studies involving breast cancer. Gene therapy is of particular interest to affected women because of the following: (1) gene therapy has the potential to cure metastatic disease (2) gene therapy offers the potential to prevent breast cancer, especially in women who have a genetic susceptibility to breast cancer, and (3) at present, gene therapy has far fewer side effects that traditional therapies. *Breast Cancer Action* supports
innovative scientifically rigorous research targeted toward curing or managing life threatening diseases.

XIX. FUTURE MEETING DATES/DR. WALTERS

The next meeting of the RAC will be on June 8-9, 1995, at NIH , Building 31C, Conference Room 6 Bethesda, Maryland.

XX. ADJOURNMENT/DR. WALTERS

Dr. Walters adjourned the meeting at 3:00 p.m. on March 7, 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