DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
March 11-12, 1999

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The Recombinant DNA Advisory Committee (RAC) was convened for its 73rd meeting at 9:00 a.m. on March 11, 1999, at the National Institutes of Health (NIH), Building 31, Conference Room 10, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Claudia Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on March 11 from 9:00 a.m. until 5:00 p.m. and on March 12 from 8:30 a.m. until 4:00 p.m. A committee roster is attached (Attachment I). The following individuals were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Children's Hospital
Dale G. Ando, Cell Genesys, Inc.
Xandra O. Breakefield, Massachusetts General Hospital
Louise T. Chow, University of Alabama at Birmingham
Jon W. Gordon, Mount Sinai School of Medicine
Jay J. Greenblatt, National Institutes of Health
Nancy M.P. King, University of North Carolina at Chapel Hill

Sue L. Levi-Pearl, Tourette's Syndrome Association, Inc.
Ruth Macklin, Albert Einstein College of Medicine
M. Louise Markert, Duke University Medical Center
R. Scott McIvor, University of Minnesota
Claudia A. Mickelson, Massachusetts Institute of Technology

Ad Hoc Consultants:

Paul R. Billings, Council for Responsible Genetics
Dave Gordon, Joshua M. Gordon Foundation
Katherine A. High, Children's Hospital of Philadelphia
Christine-Lise Julou, Worldwide Regulatory Affairs
Haig H. Kazazian, Jr., University of Pennsylvania
Margaret Liu, Chiron Corporation
Roland A. Owens, National Institutes of Health
Lonnie Russell, Southern Illinois University School of Medicine

Executive Secretary:

Debra W. Knorr, National Institutes of Health
A committee roster is attached (Attachment I).

Nonvoting Agency Representatives/Liaison Representatives:

Daniel P. Jones, National Endowment for the Humanities
Andra Miller, Food and Drug Administration
Philip Noguchi, Food and Drug Administration
Clifford Scharke, Office for Protection from Research Risks

National Institutes of Health Staff:

Barry Bowman, OD
Jan Casadei, NCI
Debra Dotson, NCI
Christine Ireland, OD
David Kaslow, NIAID
Jennifer Kostiuk, OD
Becky Lawson, OD
Rebecca Link, NHLBI
Mike Miller, OD
Pearl O'Rourke, OD
Roland Owens, NIDDK
Gene Rosenthal, OD
Thomas Shih, OD
Sonia Skarlatos, NHLBI
Lana Skirboll, OD

Others:

Virginia Ackerman, Medeva
James Albright, Cystic Fibrosis Foundation
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Valder Arruda, Children's Hospital
Elizabeth A. Austin, Glaxo Wellcome, Inc.
Steve Bauer, Food and Drug Administration
Robert J. Beall, Cystic Fibrosis Foundation
Paul Billings, Council for Responsible Genetics
R. Michael Blaese, Kimeragen
Christine Boisclair, Genzyme Corporation
Nell Boyce, NewScientist
Massimo Cardinali, Food and Drug Administration
Jeffrey W. Carey, Osiris Therapeutics, Inc.
Barrie J. Carter, Targeted Genetics Corporation
A. Antonio Championsmith, Cell Genesys, Inc.
Audrey Chang, BioReliance
Robert L. Clark, Rhone-Poulenc Rorer
David E. Colburn, American Homecare Federation, Inc.
Linda Couto, Avigen
Jean-Sylvain Demelier, GenCell
Wanda deVlaminck, Avigen
Pia Delaire, GenCell
Margaret M. Dillon, Systemix, Inc.
Lynley K. Donovan, GenCell
Anne Dunne, Strategic Results
Ross Durland, Gene Medicine, Inc.
Judith E. Epstein, Naval Medical Research Center
Suzanne L. Epstein, Food and Drug Administration
Lin Fei, Chiron Corporation
Paul Fields, Food and Drug Administration
Terence R. Flotte, University of Florida
Martha French, Gene Medicine, Inc.
Joyce Frey, Food and Drug Administration
Jeffrey Friedman, Collateral Therapeutics
Terry Galvin, Food and Drug Administration
Donald Gay, Chiron Technologies
Bette Goldman, Food and Drug Administration
Mitchell Goldman, Rhone-Poulenc Rorer
Dave Gordon, Joshua M. Gordon SMA Foundation
David A. Gordon, Booz-Allen & Hamilton, Inc.
Angus Grant, Public
Tina M. Grasso, GenVec
Judith S. Greengard, Chiron Corporation
Richard Hedstrom, National Medical Research Institute
Tim Hefferon, Strategic Results
Nancy Herring, Transgene, Inc.
Roland Herzog, Food and Drug Administration
Katherine High, Children's Hospital of Philadelphia
Ayse Hisim, Capital Consulting Corporation

Pamela Hodges, Human Genome Sciences, Inc.
Stephen L. Hoffman, Naval Medical Research Center
Thomas E. Hogan, F-D-C Reports, Inc.
Joseph Hughes, University of Pennsylvania Medical Center
Deborah Hurst, Chiron Corporation
Beth Hutchins, Canji, Inc.
John D. Iuliucci, Ariad Pharmaceuticals, Inc.
Dale E. Johnson, Chiron Corporation
Douglas J. Jolly, Chiron Corporation
Christine-Lise Julou, Rhone-Poulenc Rorer
James Kaiser, Food and Drug Administration
Stephen M. Kaminsky, Cornell University Medical College
Mark A. Kay, Stanford University School of Medicine
Haig Kazazian, Jr., University of Pennsylvania
Annie Kennedy, Muscular Dystrophy Association, Inc.
Arifa Khan, Food and Drug Administration
Steven Kradjian, Vical, Inc.
Sanjai Kumer, Naval Medical Research Center
Gary J. Kurtzman, Genovo
Alex La Croix, Genovo
Peter Larson, University of Pennsylvania
Debbie Lavinge, Public
Jim Lavinge, Public
Margaret Lavinge, Muscular Dystrophy Association
Brian Ledwith, Merck Research Laboratories
Will Lee, Cato Research
Martha Leibbrandt, Chiron Corporation
Steven A. Lerman, Rhone-Poulenc Rorer
Margaret Liu, Chiron Corporation
Yahong Helen Liu, Genetic Therapy, Inc.
Zhifeng Long, Genetic Therapy, Inc.
Russette M. Lyons, Genetic Therapy, Inc.
Catherine Manno, University of Pennsylvania Medical Center
Alan McClelland, Genetic Therapy, Inc.
Valerie McDonnell, BioReliance
William K. McVicar, GenCell
Kathryn A. Miller, GenCell
James E. Morris, Genzyme Corporation
Tim Nichols, University of North Carolina
Warren W. Nichols, Merck Research Laboratories
Jon A. Norman, Vical, Inc.
Patricia L. Novak, Collateral Therapeutics
Edward Otto, Genetic Therapy, Inc.
Christine M. Pannunzio, Osiris Therapeutics, Inc.
Theresa Pierson-Sunding, Cell Genesys, Inc.
Anne Pilaro, Food and Drug Administration
Barry Polenz, Targeted Genetics Corporation
Joseph Posluszny, Berlex
Andrew Quon, Association of American Medical Colleges
Siyamak Rasty, Genovo

Abdur Razzaque, Food and Drug Administration
Thomas C. Reynolds, Targeted Genetics Corporation
Dwaine Rieves, Food and Drug Administration
Holger H. Roehl, Chiron Corporation
Lonnie Russell, Southern Illinois University School of Medicine
Carlo Russo, Merck Research Laboratories
Donna R. Savage, Intelligent Fingers
Michael C. Scaife, Systemix, Inc.
Philip Scuderi, Bayer Corporation
Mercedes Serabian, Food and Drug Administration
Tomiko Shimada, Ambience Awareness International, Inc.
David G. Shoemaker, Cato Research
Jay Siegel, Food and Drug Administration
Stephanie L. Simek, Food and Drug Administration
Richard O. Snyder, Cell Genesys, Inc.
Lorna Speid, Gene Medicine, Inc.
Rebecca Spicler, The Blue Sheet
Judith A. St. George, Genzyme Corporation
Dina Stolman, Food and Drug Administration
Dan Takefman, Food and Drug Administration
Dominick Vacante, BioReliance
Marion Valerio, Rhone Poulenc Rorer
Samuel C. Wadsworth, Genzyme Corporation
Darin J. Weber, Food and Drug Administration
Chipper Whalen, Capital Consulting Corporation
Rhea Williams, Schering Plough Corporation
Carol Wilson, Food and Drug Administration
James Wilson, University of Pennsylvania Medical Center
Nelson Wivel, University of Pennsylvania Medical Center
Antoine Yver, Rhone-Poulenc Rorer
Lou Zumstein, Introgen Therapeutics, Inc.

I. Call to Order and Opening Remarks/Dr. Mickelson

Dr. Claudia Mickelson, Chair of the Recombinant DNA Advisory Committee (RAC), called the meeting to order at 9:00 a.m. on March 11, 1999. The notice of the meeting and proposed actions under the *NIH Guidelines Involving Recombinant DNA Molecules* (*NIH Guidelines*) were published in the *Federal Register* on February 17, 1999 (64 FR7964). Issues to be discussed by the RAC at this meeting include a proposed amendment to Appendix B-I. *Risk Group 1 (RG1) Agents*; discussion of conclusions from the January 7-8, 1999, Gene Therapy Policy Conference (GTPC) titled *Prenatal Gene Transfer: Scientific, Medical, and Ethical Issues*; a presentation on gonadal biodistribution of gene transfer vectors and the potential risk of inadvertent germ-line transmission; a discussion on gene transfer vector containment; and RAC review of a human gene transfer protocol for hemophilia B using an adeno-associated virus (AAV) vector.

Dr. Mickelson introduced Dr. Roland A. Owens, Senior Investigator, NIDDK, NIH; Mr. David Gordon, President of the Joshua M. Gordon Foundation, Rockville, Maryland; and Dr. Paul Billings, Director, Council for Responsible Genetics, Cambridge, Massachusetts.

Dr. Mickelson noted that, of the 114 protocols submitted to the RAC since January 1997, the Committee

requested public review of 13 protocols—those considered by the RAC to be novel, thus requiring public review. The basis for the RAC's recommendations for full public review included the first use of new viral vectors (e.g., replication-competent viruses), a new patient population (e.g., "healthy" individuals), and new diseases (e.g., Canavan disease and hemophilia A).

II. Minutes of the September 24-25, 1998, Meeting/Drs. Greenblatt, Macklin, and Mickelson

Copies of the minutes were provided, having been reviewed and approved by a subcommittee composed of Drs. Greenblatt, Macklin, and Mickelson. A subcommittee approach was used to facilitate release of the minutes as expeditiously as possible, due to the cancellation of the December 1998 RAC meeting in lieu of the January 1999 GTPC.

III. Data Management/Dr. Greenblatt

Dr. Greenblatt summarized the 280 human gene transfer protocols received to date, including 248 gene therapy protocols, 30 gene marking protocols, and 2 nontherapeutic protocols in normal volunteers. The gene therapy protocols break down as follows: 27 for human immunodeficiency virus (HIV) infection, 36 for monogenic diseases (mostly cystic fibrosis), 173 for cancer, and 12 for other diseases or disorders.

Since the September RAC meeting, 33 amendments were received, most of which were minor and added either a new investigator or a new institution to the study. One amendment (Protocol #9806-261) was for the use of a slightly modified vector; the RAC members who reviewed this amendment believed that this change did not warrant further discussion at the full meeting.

During this reporting period, 14 safety reports were received, most of which reported toxicities not related to the study medication. Four reports indicated serious or unusual toxicities that were believed to be possibly related to the study medication:

- In Protocol #9709-212, a Phase I study of direct gene transfer of HLA-B7 plasmid Allovectin-7 with IL-2 plasmid Leuvectin as an immunotherapeutic regimen in patients with metastatic melanoma, one patient experienced mild to moderate pain at the injection site.
- In Protocol #9709-214, a Phase II multicenter, open-labeled randomized study to evaluate the effectiveness and safety of two treatment regimens of Ad5CMV-p53 administered by intratumoral injection in 78 patients with recurrent squamous cell carcinoma of the head and neck, one patient was later diagnosed with Guillain-Barre acute syndrome that was possibly related to the treatment.
- In Protocol #9712-226, a Phase II multicenter, open-labeled study to evaluate the safety of Ad5CMV-p53 administered by intratumoral injection in 39 patients with recurrent squamous cell carcinoma of the head and neck, one patient experienced local ulceration that exposed the carotid artery, resulting in death due to carotid artery rupture.
- During this reporting period, 16 new protocols were received by the Office of Recombinant DNA Activities (ORDA). While 15 were exempted from full RAC review, one protocol (#9901-279) was scheduled to receive full RAC discussion on the second day of this meeting.

IV. Proposed Amendment to Appendix D of the *NIH Guidelines* Regarding the Introduction of a Gene Coding for Ampicillin Resistance Into *Chlamydia trachomatis*/Drs. Ando and Markert

Requester: Diane Stothard (Indiana University, Indianapolis, Indiana)

Reviewers: Drs. Ando and Markert

In a facsimile dated January 27, 1999, Dr. Diane Stothard of Indiana University, Indianapolis, Indiana, requested permission to conduct experiments that involve the introduction of a gene coding for ampicillin resistance into *Chlamydia trachomatis*, a Risk Group 2 agent. According to Section III-A-1-a of the *NIH Guidelines,* experiments that involve the transfer of a drug resistance trait to a microorganism that is not known to acquire the trait naturally shall be reviewed by the RAC. Ampicillin is one of the few antibiotics accepted for the treatment of pregnant women infected with *Chlamydia trachomatis*. In a facsimile dated March 2, 1999, Dr. Stothard withdrew her request to transfer ampicillin resistance into *Chlamydia trachomatis*. However, the NIH asked Dr. Markert to summarize her review of the proposal as it provides important considerations for future proposals. Two critical issues were raised: the risk of escape from the laboratory and the scientific rationale. Dr. Markert indicated that, according to a number of experts around the country with whom she had conferred, the risk of escape is considered very minimal because *Chlamydia* is a "fastidious" organism that is difficult to grow in the laboratory. Inadvertent transmission could occur only through accidental exposure to the eye; however, an individual could be treated immediately in the event of such exposure. Based on the fact that a different antibiotic resistance can be substituted for ampicillin and that there was inadequate scientific rationale for introducing the ampicillin resistance gene into *Chlamydia*, Dr. Markert recommended against approval of Dr. Stothard's request. The request was subsequently withdrawn.

Dr. Markert noted that the general issues arising from the review of this proposal may be confronted again in the future, namely, scientifically evaluated risk vs. the public perception of risk.

Dr. Ando agreed with Dr. Markert's comments and added that the development of other plasmid systems will continue to increase and will most likely involve the use of inexpensive selection markers. The RAC should discuss the potential environmental and health risks associated with the use of such markers.

Dr. Noguchi indicated that *Chlamydia* is one of the natural contaminants in parrots, and in general, it is a good idea not to use a readily available clinical antibiotic if a negative public health consequence is possible.

V. Amendment to Appendix B-I. Risk Group 1 (RG1) Agents/Dr. Mickelson

Requester: Margarita C. Curras-Collazo (University of California, Riverside, California)
Reviewers: Drs. Mickelson, Terence Flotte (University of Florida, Gainesville, Florida), Barrie Carter (Targeted Genetics Corporation, Seattle, Washington), and Roland Owens (ad hoc) (NIDDK, Bethesda, Maryland)

On December 11, 1998, the ORDA received a facsimile from Dr. Margarita C. Curras-Collazo, University of California at Riverside, Riverside, California, requesting to lower the containment level (from Biosafety Level (BL) 2 to 1) for recombinant AAV vectors produced in the absence of helper viruses. Subsequent to this request, the ORDA received a telephone call from Ms. Brenda Wong, Biological Safety Officer, University of California at San Diego, La Jolla, California, asking that this request be reconsidered due to the potential of insertional mutagenesis. The ORDA solicited the opinion of the RAC Chair and three experts in the AAV field. It was the opinion of the RAC Chair and the three experts that the BL1 level of physical containment was appropriate. The wild-type AAV is a low-level risk agent not associated with disease in humans, and although there is a theoretical possibility of mobilization of an integrated vector, the risk is not greater than initial transduction. Dr. Mickelson summarized her review of this request: Because the wild-type AAV tends to have a preferred site of integration in the human genome and the recombinant vector does not, it is no more likely to cause insertional mutagenesis than the already-approved Moloney murine leukemia virus-based vectors that insert randomly and for which the

RAC has approved a BL1 physical containment.

Dr. Owens commented on the relative potential risk for mobilization. He added that if the AAV *rep* gene is in the vector, there may be a slightly increased risk factor because that protein appears to be involved in integration events. However, it would not create any more risk than having a wild-type AAV, which has already been designated as harmless.

Dr. Gerard Spahn's (The Salk Institute, La Jolla, California) review suggested that much of the public has gained immunity to adenoviruses, thus reducing the risk of helper activity. Dr. Noguchi commented on this aspect, stating that the experience with adenovirus vectors in cystic fibrosis patients suggests that many patients develop antibodies, as demonstrated by a positive enzyme-linked-immunosorbent assay (ELISA); however, neutralizing antibodies are not found in half of the patients.

In answer to a question from Dr. Aguilar-Cordova, Dr. Mickelson clarified that risk containment in this case was an assessment of how much risk this agent might pose to employees or technicians working in the laboratory. Dr. Owens stated that the concern is not infecting people with AAV but infecting them with what the AAV is carrying—toxins, potential oncogenes, or other genes that might have some growth regulatory effect.

Committee Motion 1

A motion to amend Appendix B-I. Risk Group 1 (RG1) Agents of the *NIH Guidelines* was made by Dr. Aguilar-Cordova, seconded by Dr. Greenblatt, and approved by a vote of 11 in favor, 0 opposed, and no abstentions. The new Appendix B-I is proposed to read:

"Risk Group 1 (RG1) Agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli* K-12 (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions), AAV types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and is produced in the absence of a helper virus.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed."

VI. Discussion Regarding Prenatal Gene Transfer Research/Drs. Mickelson, McIvor, and Markert and Ms. King

Reports: Drs. Mickelson, McIvor, Markert, and King
Participants: David Gordon (Joshua M. Gordon Foundation), Paul Billings (Council for Responsible Genetics)

On July 31, 1998, Dr. W. French Anderson, University of Southern California, Los Angeles, California, and Dr. Esmail Zanjani, Veterans Hospital, Reno, Nevada, submitted the following two "preprotocols" for *in utero* gene transfer: *In Utero* Gene Transfer for the Treatment of ADA-Deficient SCID and *In Utero Gene Transfer for the Treatment of beta-Thalassemia*. These two preprotocols provided the catalyst for the RAC's recommendation to the NIH Director (made at its September 1998 meeting) that a Gene Therapy Policy Conference (GTPC) should be held on the topic of prenatal gene transfer. On January 7-8, 1999, the NIH convened the GTPC titled *Prenatal Gene Transfer: Scientific, Medical, and Ethical Issues.* This

conference was intended to provide an open public forum for discussion of relevant policy issues among members of the scientific, biomedical, ethical, and legal communities and the public. The anticipated outcome of the GTPC was twofold: (1) development of a policy paper that will highlight the conclusions of the working groups and conference participants and (2) a comprehensive list of issues that should be further deliberated by the RAC at subsequent meetings. To achieve this goal, RAC members and *ad hoc* experts were assigned to one or more of the following working groups on the basis of their individual areas of expertise: Working Group I—Preclinical Research Issues; Working Group II—Clinical Research Issues; and Working Group III—Ethical, Legal, and Societal Issues.

Prior to the conference, working group participants were asked to deliberate a set of questions related specifically to the primary focus of their assigned working group. The questions assigned to each working group were formulated through a consultative process involving the ORDA staff and a panel of experts representing relevant NIH Institutes and Centers. Working group deliberations were facilitated by the working group chair via telephone conference calls and an electronic discussion group.

Each working group formulated its preliminary observations and conclusions to the assigned questions in advance of the conference. The working groups were asked to identify any additional questions, issues, or concerns that were raised during the course of their discussions.

At the January 7-8, 1999, GTPC, the conference cochairs invited questions from both the speakers and working group participants. The chair of each working group presented a brief overview of the working group's preliminary observations and conclusions and any outstanding issues or questions at the end of the relevant session. At the close of the conference, conference cochairs were asked to present their conclusions as follows: (1) areas of agreement, (2) areas of disagreement, (3) conclusions, and (4) additional questions raised as a result of their deliberations. Subsequently, the working groups were asked to revise their preliminary responses to the assigned questions based on the GTPC deliberations.

Working Group I--Preclinical Research Issues

(1) What is known about the emergence of human fetal immune competence?

(2) What is known about the induction of fetal immune tolerance?

(3) On the basis of preclinical and clinical studies of prenatal hematopoietic stem cell transplantation, what is known about the biology and kinetics of stem cell regarding engraftment, selection, survival, and transplacental migration?

(4) On the basis of preclinical studies on prenatal gene transfer, what is known about transgene expression, vector dissemination, fetal immune response to transgene products and/or vector, germ-line integration and gene transfer, and effect(s) of gene transfer on prenatal and postnatal development?

(5) Recognizing that there also are ethical, legal, and societal issues to consider, do currently available preclinical data support the safety and feasibility of proceeding to prenatal gene transfer clinical trials using ex vivo transduced stem cells or direct gene transfer?

Working Group II—Clinical Research Issues

(1) With regard to selecting potential clinical indications for prenatal gene transfer: (a) What are the limitations related to accuracy? (b) What are the implications of these limitations for the design and conduct of the trial? (For example, how should potential genotype/phenotype differences be addressed?)

(c) What are the key elements to consider for optimal clinical trial design and analysis?

(2) With regard to selection of clinical endpoints and analysis of clinical outcomes: (a) What are the clinical endpoints that should be selected for the prenatal period? (b) What are the implications and limitations of assessing prenatal endpoints? (For example, are prenatal measurements of engraftment a reliable predictor of the ultimate percent ofchimerism achieved?) (c) What are the clinical endpoints that should be selected for the postnatal period?

(3) On the basis of preclinical studies of prenatal gene transfer, clinical studies of prenatalhematopoietic stem cell transplantation, and clinical studies of postnatal gene transfer, what can be inferred about the potential risk to the pregnant woman and fetus?

(4) What clinical parameters should be considered for the following: (a) exclusion criteria for the pregnant woman? (b) monitoring of the pregnant woman? (c) monitoring of the fetus? (d)detection and assessment of inadvertent germ-line transmission?

Working Group III—Ethical, Legal, and Societal Issues

(1) What are the ethical issues raised by prenatal gene transfer specific to (a) the fetus? (b) gene transfer?

(2) What subject recruitment/enrollmentprocess(es) should be considered for prenatal gene transfer?

(3) With regard to the informed consent and ongoing subject counseling processes for clinical trials of prenatal gene transfer: (a) What are the components of an optimal informed consent process? (b) How should the risks and benefits be described/quantified? (c) What are the challenges in achieving nondirective decisionmaking? (d) What special issues are raised? (e) What legal paradigms need to be taken into account? (f) Is there a need for the involvement of an independent counselor?

(4) Given the potential for inadvertent germ-line integration and gene transfer: (a) What are the ethical issues that must be addressed and resolved before human prenatal gene transfer studies should proceed? (b) What are the societal issues that must be addressed and resolved before human prenatal gene transfer studies should proceed?

Working Group I Report: Preclinical Research Issues/Dr. McIvor

Dr. McIvor summarized Working GroupI's report. In general, there is very little preclinical research that would support proceeding with clinical *in utero* gene transfer research at this time, despite preliminary studies involving gene transfer by introduction of vectors into the amniotic fluid of sheep.*In utero* gene therapy may prove to be an effective alternative to conventional modes of treatment for some hereditary and acquired diseases. Several well-designed studies have demonstrated expression of transgenes and marker genes, following prenatal gene transfer in experimental animals. While the currently available results are encouraging, a substantial number of critical issues remain to be addressed. Currently, insufficient preclinical data exist to support clinical trials.

Working Group I's report was organized into four categories: efficiency of gene transfer*in utero*, expression of genes transferred*in utero*, immune response, and safety. All four considerations warrant further studies designed to assess the potential for abnormal prenatal and postnatal development in the context of gene transfer*in utero*, including characterization of pathologic and toxicologic effects. Dr. McIvor stated that many additional preclinical issues remain to be addressed, as outlined in the highlights of Working Group 1's report below; several of them are posed in his group's report to the RAC.

Efficiency of Gene Transfer *In Utero*

- In *ex vivo* gene transfer, cells are removed from the fetus, genetically manipulated, and then reintroduced into the fetus. Efficiency of gene transfer still needs further study. When the genes are reintroduced into the animal, how effectively will these cells engraft? What has been learned from gene transfer protocols on adult humans that would indicate the appropriateness of such protocols in the *in utero* setting? Working group members expressed concern about the possibility of transplacental migration of newly introduced cells and the possibility of risk to the mother.
- *In vivo* gene transfer efficiency is more problematic because of less control over which cells are exposed to the vector; it is difficult to target specific cells. Many of the issues in *ex vivo* gene transfer efficiency are inherent in *in vivo* gene transfer efficiency. How can particular cell types be targeted by vector modification? If targeting is not used, which cells are being exposed to the vector inadvertently, and what will be the outcome of such exposure? Would there be inadvertent transfer into the germ line?

Expression of Genes Transferred *In Utero*

- The level of expression is important if it is relevant for the potential application, and the gene expression would need to endure well into adulthood after introduction into fetal cells. Under some conditions, the newly introduced gene expression may need to be regulated. Applications for which gene expression might not need to be regulated may be good candidates for initial experiments on *in utero* gene transfer expression. What are appropriate model diseases for preclinical studies? Gene knockout technology makes available a number of animal models for testing effectiveness of expression, but the example of beta-thalassemia raises the clinical and ethical questions of whether to consider a disease that is likely to be fatal to the fetus.

Immune response

- One of the potential advantages of *in utero* gene transfer is that the immune response problems seen in adults may not occur in the fetus, but different problems may arise. It may be possible to effectively tolerize against a gene product so that therapy for that individual can be more effective. However, all these considerations should be addressed in preclinical studies to characterize tolerance to the gene product; immune response of the fetus to the vector or to the transgene product must also be characterized.

Safety

- Many issues are similar to the safety issues raised in adult gene transfer—the potential risk of insertional mutagenesis caused by the vectors, replication- competent viruses, and others. To what extent is fetal development changed by introducing a vector? What might be the effects of the actual surgical procedure apart from the genetic implications? Because of these serious concerns about the potential for abnormal prenatal and postnatal development, extensive studies of the pathologic and toxicologic effects of *in utero* vector administration should be undertaken using an appropriate animal model. The Working Group emphasized the issue of safety to the mother and not just to the developing fetus.

RAC Discussion of the Working Group I Report
(Public comments were provided by Mr. David Gordon and Dr. Paul Billings.)

Dr. Mickelson noted the potential for tolerization to the vector as well as tolerization to the insert. She also stated that preclinical studies should examine methods of delivery in which the risk to the mother in the gene transfer clinical trial is no greater than the risk inherent in the delivery method.

Dr. Gordon expressed his desire to include a clause about the selection of animal models for whatever aspect of immune tolerance is tested. Prior to the GTPC, Dr. Gordon believed that a fetus could be tolerized once and then would never have a reaction to the proteins *in utero*. He learned at the conference that this was untrue—the vector is administered and the protein is gone within 24 hours, but the gene product is retained as a message in translated protein for a longer period of time. He suggested that the Working Group reflect that fact in its report.

Dr. Markert offered three issues that warrant consideration: (1) Because many preclinical research design issues will be disease-specific (e.g., duration of gene expression), the summary statement should be flexible on this point; (2) diseases for which good animal models do not exist should not be excluded from future consideration; and (3) preclinical experiments involving the introduction of specific genes into normal animals and the issue of regulated gene expression should also be addressed. Dr. McIvor agreed with Dr. Markert's statements about the availability of animal models. Even though a gene is knocked out in the mouse, that action does not necessarily cause the same disease phenotype in humans.

Dr. Noguchi indicated that a neonatal model might provide useful insights into the *in utero* situation, but there are significant differences. For instance, on a mass basis alone, small or absent neonatal effects may elicit an extraordinary effect *in utero*. A statement about the effects of remodeling *in utero*—the growth and death of cells occurring simultaneously—should be included to indicate the different growth rates of various parts of the body (e.g., the gut continues to grow until late in the third trimester).

Dr. Breakefield discussed the timing of the formation of the blood-brain barrier; some vectors that cannot enter the brain in adults may be able to enter the brain during certain periods of *in utero* development.

Dr. Aguilar-Cordova indicated that much research can be done on large numbers of small-animal models that might yield genetic answers and additional questions regarding vector type, tissue, sensitivity, and distribution, using only marker genes.

Dr. Billings commented on the safety issues in this report, reemphasizing and supporting the separation of maternal issues from fetal issues. Knockout mouse models may not capture the complexity of human fetal development, particularly of the nervous system. Monitors should be developed to sensitively detect disruptions in neural development. Transfection at a certain stage might have very different effects on the disruption of messenger RNA trafficking and protein trafficking at different stages of fetal development. Another issue discussed at the January GTPC was the risk of creating chimeric individuals earlier in fetal development, whether stem cells or transfected cells from the fetus are used as cell treatment. The impact on safety has not been studied adequately enough to allow cell treatments to be compared with vector-based treatments.

Dr. Ando stated that more generic studies are needed to address safety issues in the area of reproduction teratogenic and germ-line effects, and toxicology in animal models.

Dr. Gordon indicated that one issue addressed at the January conference was that the current technology

available to extend to the fetus is relatively poorly developed; therefore, selecting which diseases to address first may be more directly related to the available technology.

Dr. Billings posed two concerns: (1) Investigators who create a knockout mouse for a specific response might not be inclined to evaluate subtle developmental problems that might arise in other organ systems, and (2) there is a question as to whether early irreversible changes in fetal development in an animal model would preclude gene therapy interventions. He then questioned how decisions are made and which parts of such decisions are scientific and which are ethical and social.

In answer to Dr. Billings' concerns, Dr. Anderson stated that the process of deciding which diseases are studied is driven by three basic factors: (1) initiator interest—an investigator has a specific interest in a disease and therefore carries forth a protocol; (2) funding initiation—the NIH or another organization issues a specific request for research in a given area and provides funding; and (3) technology initiation—the study of diseases is restricted or focused by the available technology.

Dr. Owens emphasized that *in utero* gene therapy should be restricted to diseases in which there is a clear clinical advantage over other available interventions *in utero.*

Dr. Mickelson explained that there is a tremendous amount of statistical data that can be gathered about fundamental organ function and that it might be possible to set up criteria to assess whether a particular level of secondary organ dysfunction is allowed. Using animal models may mean that researchers would miss the more subtle effects, such as secondary effects on higher neurological functions. It would be difficult to assess the social skills or intelligence of sheep, for example, so the possible secondary effects would be difficult to assess.

Dr. Breakefield indicated that, in animal models, investigators can detect gross alterations in the formation of brain structures, although the more subtle changes would be more difficult to assess.

Dr. Markert agreed that the diseases that have good animal models are the ideal ones to study; however, she reiterated the importance of not limiting even initial gene transfer trials to diseases that have perfect animal models.

Dr. Gordon said that he could not foresee a situation in which gene transfer procedures would be extended to human trials without some initial animal testing.

Dr. Macklin volunteered to keep track of the commonalities among the reports of all three working groups reports and the ensuing discussion.

Working Group II Report: Clinical Research Issues/Dr.Markert

Dr. Markert summarized Working Group II's report. The consensus of the Working Group was that gene transfer *in utero* is not appropriate at this time because of the absence of preclinical animal data and postnatal human data showing efficacy of gene transfer. The optimal design for a clinical trial involving *in utero* gene transfer would be a disease in which the diagnosis could be made with certainty early in pregnancy so that the parents have sufficient time to consider the research option. Human leukocyte antigen (HLA) typing should be performed to determine the existence of a potential HLA-matched cell donor, if applicable. The disease chosen should have an absolute correlation between the genotype and the resulting phenotype or between uterine and postnatal phenotypes. The *in utero* gene transfer should be safe, with a low likelihood of morbidity caused by insertion of the gene into cells that do not normally express the gene. Animal studies should support a level of gene expression conducive to correction of the

phenotype rather than merely a slight change. Neither the gene transfer procedures nor carrying the fetus to term should endanger the mother's life.

Consensus was reached concerning which diseases would be appropriate candidates—serious diseases and not simply traits. There should be serious morbidity/mortality risk for the fetus, either *in utero* or postnatally, and the ideal diseases would be those in which outcome is poor with postnatal therapy. However, there was no consensus that *in utero* therapy could not be conducted if postnatal therapy existed, although thorough discussion with the family would clearly be necessary. The existence of successful postnatal therapy for a disease makes it more difficult to proceed with *in utero* gene transfer, and the existence of such a therapy should be made clear to the family. Additionally, the diseases to be treated *in utero* should not be associated with severe anomalies that would not be corrected by the transferred gene, because the resultant correction of one genetic problem would not help the individual overall.

On accuracy of diagnosis, the Working Group decided that gene transfer should not be performed on a normal fetus and that the selection of a disease should depend on the ability to diagnose the fetus *in utero*. The genotype-phenotype relationship should be well established. Fetuses from across the country should not be screened randomly for mutations; however, families with infants born with the severely affected phenotype would be ideal for future fetal screenings.

Working Group II was unwilling to recommend ultrasound screening to the obstetrics field. However, if any screening has a highly positive predictive value, showing a fetus definitely affected *in utero*, then that fetus could be a subject of *in utero* gene transfer studies. How the diagnosis of the fetus is made is disease specific; however, the testing should have a very high positive predictive value that the fetus will be severely affected and that the mutation exists.

After the diagnosis is made, all options should be discussed with the parents. Postnatal treatment is available for many diseases, and HLA typing may reveal an HLA-identical sibling who could be a tissue donor postnatally. There was no Working Group consensus on whether diseases that are *in utero* should be considered; these decisions should be made on a disease-specific basis.

Three instances were cited by the Working Group in which *in utero* gene transfer could be attempted without prior experiments in postnatal infants: (1) The disease is fatal *in utero*, (2) the disease causes irreversible organ damage *in utero* and the fetus would be born but would die shortly thereafter; and (3) there are technical advantages in performing gene transfer *in utero* as opposed to postnatally. In all cases, however, the Working Group agreed that studies must be done in animals beforehand to address safety issues.

Clinical endpoints should include checking for gene expression; doing so preclinically is not a consensus issue at this time because of concerns about the safety of obtaining samples and the risk to the fetus. Postnatal testing may not have positive predictive value, so it is unclear whether it is appropriate to undergo the risk of prenatal testing if a predictive answer is not ensured. Postnatal tests should demonstrate that the clinical phenotype has been reversed by measurements of gene expression (in terms of ribonucleic acid [RNA] or protein expression) for accessible tissues only. Such tests should also monitor for unintentional integration whenever possible—the mother's blood and the placenta should be tested for evidence of the virus, and permission for autopsy should be sought for both the fetus and the mother.

The major risk for the pregnant woman is infection caused by the gene transfer procedures. She should be fully informed of these risks, and the informed consent process should be thorough and thoughtful.

Using the word "experiment" (as opposed to "research" or "study") may assist women in understanding that *in uter*gene transfer is currently experimental and that the chance of success is small. Societal pressure on a pregnant woman to be a "good mother" and to do everything possible for the fetus should be considered carefully; the woman should not be pressured to agree to*in uter*gene transfer. The pregnant woman must be able to comply with the various sampling and experimental procedures.

Working Group II did not come to consensus on the necessity for the father to give consent to*in uter* gene transfer.

With respect to the fetus, the diagnosis*in uter*must be clear. Monitoring the fetus using fetal blood sampling depends on safety issues; invasive procedures should have a clearly defined goal. Postnatal testing should be conducted.

RAC Discussion of the Working Group II Report

In answer to Dr. Mickelson's question regarding the phenotype, Dr. Markert stated that the Working Gr concluded that correction of the phenotype must be demonstrated. Assessing clinically that improvement has occurred need not be accomplished by examining RNA or protein of the transduced tissue, or b brain biopsy, but it must be demonstrated.

In answer to Dr. Breakefield's question about the aspects of Lesch-Nyhan disease that might therapeutically addressed, Dr. Markert explained that Lesch-Nyhan is a devastating disease for wh there is currently no effective treatment. Technology in animal models is needed totransduce the corre tissues and fix the appropriate gene before the disease is a candidate for gene transfer.

Dr. Noguchi asked Dr. Markert to clarify whether the Working Group was recommending that any disea be treated *in uter*first without prior postnatal trial. Dr. Markert said no, and in case that was not clear, agreed to add to Working Group's report a statement that no disease should be treated*in uter*as a first step. An exception would be a disease that is fatal*in uter.*

Dr. Breakefield stated her concern about addressing the possibility of uterine infections, contracted in a attempt to save the fetus, that would interfere with future reproductive fitness of the mother. Dr. Markert stated that infection limiting future reproduction is the biggest risk to the mother; this concern was expressed by the obstetric physicians who spoke at the January 1999 GTPC

Dr. Gordon discussed the example of beta- thalassemia ; a woman might be in a position to safel terminate her pregnancy but decide to forgo that option to try gene therapy. She may then find herself more than 24 weeks pregnant but with the therapy having been ineffective. Ultimately, gene therapy is for the pregnant woman; it is she who would bear another child if therapy fails or raise the child if therapy succeeds. This statement was emphasized at the January 1999 GTPC

Dr. Billings agreed that random screening is not effective and that screening can be narrowed to families with an *a priori* risk of having a second affected child. He also laid out a decision matrix, stating that 90 to 95 percent of pregnant women will want to proceed with the pregnancy no matter what happens. Additionally, pregnant women may choose either prenatal diagnosis and selective termination or preimplantation diagnosis and selective implantation, depending on how the information is offered t them.

Dr. Mickelson commented on whether a disease should be considered for*in uter*gene transfer without prior experiments in postnatal infants. The statement needs to be clarified so as not to give the impressior

that it is acceptable to conduct *in utero* gene transfer without prior experiments in postnatal infants (with the possible exception of diseases that are fatal *in utero*. Dr. Noguchi stated that we need to have clear animal data on safety and efficacy before any such experiment is deemed acceptable. Ms. Levi-Pearl cautioned that discussion about these gene therapies and related research may lead the public to believe that gene transfer "cures" are "just around the corner," which they are not.

Working Group III Report: Ethical, Legal, and Societal Issues/Ms. King

Ms. King noted two important issues that are not currently raised in the Working Group's report: (1) To move the field of gene transfer research forward, questions should be asked about research interests, investigators, funding sources, and the development of technology, and (2) preclinical, clinical, ethical, legal, and societal issues should not be viewed in isolation but considered thoughtfully by scientists in the field.

Ms. King presented Working Group II's report. The Working Group attempted to separate the ethical, legal, and societal issues unique to *in utero* gene transfer research. Decisions regarding the ethical permissibility of embarking on *in utero* gene transfer research must include a discussion of animal models and their limitations. The complexity and uncertainty of the risk-benefit assessment include the specific gene transfer as well as ancillary procedures. Subjects of *in utero* gene transfer research will likely undergo an enormous amount of scrutiny, a fact that should be made clear to the pregnant woman. Germ-line integration should be viewed as undesirable until proven otherwise and should be minimized.

Working Group II's criteria for selecting diseases to be targeted for *in utero* gene transfer research rule out diseases that are fatal *in utero* because of the high likelihood of partial correction—a highly undesirable effect because it could result in the long-term survival and suffering of a fetus/child. When a truly effective postbirth therapy exists, *in utero* gene transfer should generally not be utilized, but the definition of "truly effective" may make such a determination disease specific.

In establishing inclusion/exclusion criteria, Working Group III agreed with Working Group II that inclusion of both partners in the consent discussion is optimal but should not be required. Maintaining subject involvement in ongoing trials includes informing subjects of the need for follow-up and forecasting requirements to subjects; on the other hand, subjects always maintain the option to withdraw from a study. Close examination must be made of the elements of appropriate follow-up to minimize the scope of procedures.

The timing of and preparation for decisionmaking are crucial because of the limited window of opportunity in which to offer research participation. Working Group III—as well as the participants of the GTPC in January—believes that forgoing abortion cannot be an inclusion criterion for research participation. A subject should understand that she will be free to choose abortion even after her participation in the research. She must clearly understand and be able to weigh both the risks and potential benefits of participation. She should have supportive people who can help her sort out the potential risks vs. benefits.

An article by J. Moreno and colleagues (*Journal of the American Medical Association* 280:1951-1958, 1998) about protections for human subjects involved in research recommended that all consent forms for Phase I studies include the following statement in boldface type at the beginning of the form: "This medical research project is not expected to benefit you." Working Group III thought that this language is very appropriate for gene transfer research studies.

Ms. King concluded her presentation with concerns related to justice, most of which are still in the form of questions:

- How can the public be involved in discussions of this kind, and is current public involvement adequate?
- Is the regulatory mechanism that takes over once studies are under way adequate to address public concerns?
- Are there or should there be policy processes to address whether it is appropriate to expend resources in one place rather than in another?
- If this research goes forward and produces therapeutic applications, how can equitable availability be ensured?

Areas of Agreement of Working Group Reports/Dr. Macklin

Dr. Macklin presented her summary of the areas of agreement among the three working groups, which were as follows:

Informed Consent and Decisionmakin

- All options, including early-stage abortion, should be discussed with the prospective parents.
- The consent process should be thorough and complete.
- Pressure may arise from the "be a good mother" argument.
- The prospective parents need adequate time to consider all options; the definition of "adequate time" is likely to be disease specific.
- The male partner may not authorize the woman's enrollment in gene transfer research without her consent. It was agreed that the male partner has an interest in the outcome, but there was no consensus on whether the male partner has a right to be a coequal decisionmaker. The likely area of agreement is that the male partner's participation in decisionmaking is desirable but no mandatory.

Inclusion/Exclusion Criteria for the Pregnant Woman

- The pregnant woman should agree to autopsies for herself and the fetus if either of them dies.
- The pregnant woman should agree to long-term follow-up for herself and the child, with the understanding that she cannot be forced to continue in the research project.

Risk-Benefit Analysis

- The risk-benefit ratio must be favorable, and the risks to the woman and fetus must be minimized.
- Risks and benefits to the fetus and to the pregnant woman should be assessed separately.
- More attention should be paid to risk levels and risk thresholds.

Which Diseases?

- Working Group II: Only serious diseases with risks to the fetus and poor outcome with postnatal therapy should be considered; there should be no association with serious abnormalities; no consensus was reached on diseases fatal *in uter*.
- Working Group III: The best diseases for research are those not normally fatal *in uter* and for which *in uter* effects could potentially be ameliorated by *in uter* treatment. Diseases that are not good candidates are those that are normally fatal *in uter* or that produce neurologic effect *in uter* and those for which an effective postbirth therapy exist

RAC Discussion on the Areas of Working Group Consensus

Dr. Billings queried what constitutes a "fetal" disease; for example, is monosomy considered a feta disease? In an exchange with Dr. Macklin, it was agreed that terminology—whether the term used is "disease" or "condition"—may be relevant to the research or policies established for it.

Dr. Noguchi commented that the "desirable vs. acceptable" question of disease candidates would be more useful if categorized as "acceptable vs. nonacceptable." On the definition of a fetal disease, Dr Noguchi commented that, for some diseases, the preponderance of its effects are on the fetus or infant; however, in many other diseases, there is a lifelong accumulation of consequences that might justify prevention at an early age or *in uter.*

Dr. Breakefield indicated her preference to choose vectors and tissues for which there was som demonstration of effectiveness, for example, in muscle or hematopoietic systems with demonstrable ef in adults. In animal model experiments, even if the target is not the nervous system, results must be evaluated carefully as to the effects on the nervous system. Gross malformations of the brain should be examined; they may occur even if the fetus is treated hematopoietically because the vectors may cros into the fetal brain (although those same vectors might not cross into an adult brain).

Dr. Gordon suggested that any malformation, gross or not, should be deemed unacceptable within the nervous system and that gross malformations in other systems found in animal studies are also unacceptable. However, he was concerned that the RAC should not accept certain malformations in an animal study without believing that those malformations were "safe" ones.

Dr. Breakefield returned to the subject of the male partner's consent and voiced the concern that the male partner may have some significant financial responsibility for the fetus, should it be born. Dr. Macklin reiterated that the two potential research subjects are the woman and the fetus; the woman consents on her behalf and on behalf of the fetus. The male partner can only authorize the intervention with the fetus but not with the woman.

Dr. Gordon reiterated that it is difficult to find grounds for the male partner restricting the activity of the woman with regard to her pregnancy. Although it would be ideal for the father to have input, the woman's body is sovereign, and the male partner cannot force her to do or not do anything with it.

Dr. Scharke introduced the importance of verifying the subject's ability to comprehend the consent process. Building in verifying feedback responses would give the investigator an accurate assessment of the extent to which the subject understands the potential positive and negative consequences of what she is about to undertake.

Dr. Noguchi expressed concern about the ability to obtain truly informed consent; parents will be faced with making extremely serious decisions that will have lifelong impact within a very short time frame. Before any experiments are begun, the community should be educated about the difficulties and hurdles of gene transfer research. Dr. Macklin requested that Dr. Noguchi provide some information about the feedback received by the FDA regarding community consultation.

Community Involvement/Dr. Noguchi

Dr. Noguchi responded to Dr. Macklin's question about community involvement. Several years ago, the concern arose about studies being delayed because of difficulties in gaining subject consent to participate in research, especially in situations in which the subject did not have the capacity to give

consent personally. The reaction to the FDA's role has been both mixed and strong. Two years of comment on this issue produced a process that does not allow emergency consent to be sought until the local Institutional Review Board (IRB) responsible for these types of interventions has provided publi disclosure to the community. The FDA has not specified the exact requirements for this "community consultation" process but declares generally that it must exist. For areas in which the gene transfer intervention is clearly of an extremely technical nature, this is one possible model for facilitating community assent to a locally conducted procedure.

Dr. Macklin disagreed, saying that *in utero* therapy is different because it does not involve a community in the same sense as the practice of emergency medicine. A substitute for the informed consent of the individual is not necessary for *in utero* gene transfer research.

Dr. Noguchi agreed, stating that his purpose in providing this example was to indicate that an educational process for the public should be in place before something as complicated as *in utero* gene transfer research is attempted.

Dr. Owens commented that Dr. Noguchi's community consultation and public education model may be a valid consideration in some instances (e.g., area-specific religious views on the status of the fetus).

RAC Consensus Statement

Dr. Mickelson presented a *Draft Executive Summary of the NIH Recombinant DNA Advisory Committee (RAC) Findings and Recommendations on Prenatal Gene Transfer Research*. This first draft of an executive summary, which is based on Working Group reports and the January 1999 GTPC , wa provided to give the RAC an idea of how the format might look. It included the findings of the three working groups under the following headings, plus recommendations in the following areas:

- Technological Developments (14 recommendations)
- Preclinical Research (6 recommendations)
- Clinical Requirements (11 recommendations)
- Informed Consent Requirements (5 recommendations)

Dr. McIvor noted that in the preclinical research recommendations, there was an overemphasis on an animal model that recapitulates the human disease. The recommendations should be modified to reflect the understanding that there may be diseases for which no good animal model exists.

Dr. Noguchi suggested that the draft executive summary include a list of the diseases that currently have good animal models to help educate the public about such models. Dr. Gordon disagreed, stating that such a list would lengthen the report unacceptably and might preclude incorporating those diseases for which good animal models do not yet exist.

Dr. Macklin suggested that two points of consensus be included before the statement on page 2 about extraordinary potential: (1) Gene transfer *in utero* is not appropriate at this time, and (2) additional animal studies are needed before proceeding with research in humans.

Both Dr. Gordon and Dr. Greenblatt thought that many of the statements in the draft executive summa were overly dogmatic, and Dr. Greenblatt suggested that perhaps individual comments should b submitted at a later date. Ms. Knorr offered to collect all the comments and work them into a new draf executive summary that would be reviewed by the RAC at its June 1999 meeting. However, because many members of the public are looking to the RAC for some general closure, a few statements of

consensus from this meeting would be important.

Dr. Aguilar-Cordova remarked that, unless there was disagreement, it might be possible for the RAC to agree on a general statement that, at this point in time, it is not ready to consider a human *in utero* trial and that additional preclinical, toxicity, efficacy, and animal data are required before a human *in utero* trial will be considered.

Dr. Billings requested a clarification of item 3 in the draft executive summary, which states that "Prenatal gene transfer should never be performed on a healthy fetus." He wanted to know whether that means that a fetus that has inherited a disease-associated gene would be considered "not healthy." Dr. Markert explained that this item attempts to deal with false-positive genetic testing—accurate diagnoses are crucial so that *in utero* gene transfers are not conducted on fetuses that do not need the procedure. Dr. Markert suggested a wording change: "Prenatal gene transfer should never be performed on a genetic unaffected fetus."

Dr. McIvor queried whether gene transfer research might possibly be carried out for conditions other than genetic diseases, for example, on expectant mothers who are HIV positive. Ms. Knorr noted the recent published animal data demonstrating that *in utero* gene transfer was effective in correcting a congenital heart defect. Dr. Mickelson noted that this possibility had not been considered.

Dr. Gordon suggested that item 2 of the draft executive summary read that currently, gene transfer should only be considered for somatic cells. It may be possible in the future to correct a disease in the germ line, but the acceptability of that procedure can not now be assessed given the current technology. The implication of item 2 as it currently reads, according to Dr. Gordon, is that the germ line should never be approached for any reason even if somatic cell gene therapy works at some point in the future.

Ms. Knorr noted that the ultimate goal would be to work these findings and recommendations int Appendix M of the *NIH Guidelin*

Dr. Gordon agreed with Ms. Knorr that this document should be significantly shortened to a fewbullet points. Suggestions from Dr. Gordon for the bulleted items included the following: (1) More scientific data are needed, (2) more animal models are needed, (3) procedures should be piloted to show their effectiveness, and (4) disease settings should be defined.

Dr. Gordon returned to Dr. Noguchi's suggestion about creating a list of fatal genetic diseases that have mouse models, for the benefit of researchers and funding agencies and to encourage collaboration. Dr. McIvor suggested that the RAC might contract with an expert on animal models to write such a report.

Dr. Aguilar-Cordova suggested the wording for a consensus statement and asked for preliminary feedback:

"The members of the RAC continue to explore the issues raised by the possibility of *in utero* gene therapy. However, at present, the members unanimously agree that it is premature to consider any human *in utero* gene transfer experiments. The members further agree that significant additional preclinical data, including animal transduction, efficacy, distribution, and toxicity studies, are required. In addition, the RAC agrees that significant understanding of the pathophysiology of a disease and a clear advantage of *in utero* gene transfer over a postnatal approach will be required prior to considering it a candidate for *in utero* applications."

Dr. Billings questioned whether the beta- thalassemia protocol ex *vivo* gene transfer is a variant of cell

therapy; Dr. Aguilar-Cordova answered definitively that it is. Dr. Billings further questioned at what point in fetal life is cell therapy safe and effective. Many of the cautions suggested about germ-line or *in utero* gene transfer might also be appropriate for *ex vivo* gene transfer that is applied early. Dr. Noguchi agreed that the RAC needs to make explicit whether the executive summary is talking about direct injection *in vivo* or whether *ex vivo* is included; inclusion of *ex vivo* has been part of the gene therapy discussion. Dr. Aguilar-Cordova expressed a desire to insert the word *in vivo* in front of *in utero* to emphasize that the consensus reached was applicable only to *in vivo in utero*.

Dr. Billings agreed that allogeneic cell therapy would not fall under the purview of the RAC but that *in utero* injection for gene transfer or *ex vivo* gene therapy, manipulation of cells, and reinjection would. Dr. Noguchi agreed that *in utero* gene transfer research should include both *in vivo* and *ex vivo* gene transfer research.

To assist with Ms. Knox's request for some consensus statement that could be released to the public, Dr. Gordon suggested maintaining the first paragraph of the summary statement (which described what was done) and replacing the second paragraph with the following:

"On the basis of these presentations and subsequent analyses, the RAC concludes that inadequate scientific information currently exists to warrant review of proposals to perform *in utero* gene therapy. Before such procedures can be entertained, substantial progress must be made in the following areas:

- More animal models and related test systems,
- A better understanding of human development and various systems therein like the nervous system and the immune system, and
- A synthesis of that knowledge into the development of protocols where we are optimizing the protocol for the disease state we are attempting to treat."

Dr. Aguilar-Cordova reread his suggested statement:

"The members of the RAC continue to explore the issues raised by the possibility of *in utero* gene transfer. However, at present, the members unanimously agree that it is premature to consider any human *in utero* gene transfer experiments. Significant additional preclinical data, including animal transduction, efficacy, distribution, and toxicity studies are required before consideration of a gene transfer protocol is appropriate. The Committee would be willing to consider protocols with substantial preclinical data that have a strong rationale for proceeding. In addition, the Committee agrees that significant understanding of the pathophysiology of a disease and a clear advantage to *in utero* gene transfer over a postnatal approach will be required prior to considering it a candidate for *in utero* applications."

Dr. Ando remained concerned that the initial statement was too strong and could be perceived as a ban; for instance, investigators are currently working on immunodeficiency mouse models that fairly soon could yield relevant *in utero* gene transfer data.

Dr. Gordon reemphasized his willingness to review protocols that included all the necessary data, but he stated that the data—on vector efficiency and germ line risks—are not likely to be available in the near future.

Dr. Greenblatt pointed out that the concept of the RAC "considering" protocols would mean that people could conduct gene therapy protocols that the RAC never reviewed because they were not funded by the NIH. He preferred a broader statement that would apply even to protocols not funded by the NIH. Dr. Noguchi stated that, regardless of public funding, the FDA would like to use whatever statement the RAC

makes regarding its finding, and the finding of the GTPC, that it is premature to conduct clinical trials of *in utero* gene transfer.

Dr. McIvor explained that, to be considered for an *in utero* human gene transfer protocol, an investigator must provide (1) extensive animal data that address the pharmacologic, pathologic, and toxicologic issues associated with the introduction of a vector, either *in vivo* or *ex vivo*; (2) studies that were conducted with a specific vector that was proposed to be used in an animal model of that disease; and (3) results that indicate that the disease was corrected in adult animals.

Dr. Gordon suggested the addition of a sentence:

"A more thorough understanding must be attained [changed to'is needed'] of the ontogeny of human organ systems, such as the immune system and nervous system, so that the potential efficacy and risks o *in utero* gene transfer can be better defined."

Dr. McIvor queried how to determine whether enough is known about development of the brainto satisfy this requirement. Ms. Levi-Pearl added that members of the public hope and pray that this kind of science will continue and that a chill will not be sent through the scientific community by a Government body such as the RAC.

Dr. Noguchi suggested that an important educational aspect for the RAC would be for it to hear from a number of speakers what the public thinks about its endeavors. Dr. Gordon shared his experience with receiving public feedback at conferences, which is usually polarized into two positions: (1) people with family members who have the genetic disease who aggressively want to make sure that any type of research that might help them is not hindered inappropriately and (2) people who are terrified of any gene transfer research. The RAC should be sensitive to both points of view and should also set a course somewhere between those two extremes.

After additional discussion about the specific wording, Dr. Mickelson read the statement as amended:

The [removed: members of the] RAC continues to explore the issues raised by the potential of *in utero* gene transfer. However, at present the members unanimously agree that it is premature to undertake any human *in utero* gene transfer experiments. Significant additional preclinical data and relevant human experience, including [removed: but not limited to] vector transduction efficacy, biodistribution, and toxicity studies, are required before consideration of a gene transfer protocol is appropriate. The Committee would be willing to consider protocols with substantial preclinical data that have a strong rationale for proceeding. The Committee agrees that significant understanding of the pathophysiology of a disease and a clear advantage to *in utero* gene transfer over a postnatal approach will be required prior to consideration as a candidate for *in utero* application. A more thorough understanding of the ontogeny of human organ systems, such as the immune and nervous system, is needed to better define potential efficacy and risks of *in utero* gene transfer."

Committee Motion 2

A motion was made by Dr. Aguilar-Cordova, and seconded by Dr. Gordon, to accept the consensus statement as amended, allowing the ORDA to make minor editorial changes. The final version of the RAC consensus statement for release to the public, as edited by the ORDA, after consultation with the RAC, read:

The RAC continues to explore the issues raised by the potential of *in utero* gene transfer research.

However, at present, the members unanimously agree that it is premature to undertake any human *in utero* gene transfer experiments.

"Rationale:
Significant additional preclinical and clinical studies addressing vector transduction efficacy, biodistribution, and toxicity are required before a human *in utero* gene transfer protocol should proceed. In addition, a more thorough understanding of the ontogeny of human organ systems, such as the immune and nervous systems, is needed to better define the potential efficacy and risks of human *in utero* gene transfer. Prerequisites for considering any specific human *in utero* gene transfer procedure include an understanding of the pathophysiology of the candidatedisease and a demonstrable advantage to the *in utero* approach. Once the above criteria are met, the Committee would be willing to consider well-rationalized *in utero* gene transfer protocols."

The RAC unanimously accepted this position statement on prenatal gene transfer research by a vote of 11 in favor, 0 opposed, and no abstentions.

The RAC decided that the draft executive summary document would be e-mailed to the Working Group chairs who would then e-mail their groups' comments back to the ORDA. Ms. Knorr suggested that the format of the new version of the executive summary include a brief introduction, a bulleted summary of the three Working Groups' findings, and a recommendations section. Ms. King suggested that Working Group chairs choose five or six key points to highlight from their reports, from which the new executive summary would be crafted.

VII. Discussion on Gene Transfer Vector Containment/Dr. Mickelson
Presenter: Dr. Mickelson
Reviewers: Drs. Ando and Aguilar-Cordova

Dr. Mickelson summarized this discussion, which relates to the issue previously raised by Institutional Biosafety Committees (IBCs) and investigators about the need for specific guidance about physical containment levels appropriate for conducting experiments using gene transfer vectors. Containment relates to a variety of settings, including the laboratory, animals, and human subjects. Dr. Mickelson asked whether the Committee would be willing to undertake the development of such guidance, perhaps in the form of a table, and she suggested that a working group be formed for this purpose. *Ad hoc* experts could be engaged to provide the necessary data, and the working group could polish and develop that information. Dr. Mickelson volunteered to be a member of that working group.

Ms. Knorr indicated that the NIH considers development of the guidance document to be a useful exercise. On January 16, 1998, Dr. Michael N. Oxman, IBC Chair at the University of California at San Diego, La Jolla, California, wrote a letter to Dr. Varmus with regard to the IBCs need of guidance on the review of gene transfer vectors. Dr. Oxman urged the RAC to establish guidelines for appropriate containment practices and procedures for the generation and use of multiple classes of gene transfer vectors. The RAC initiated a discussion regarding the proper containment level for specific classes of vectors employed in gene transfer research. Containment relates to a variety of settings, including the laboratory, animals, plants, and human subjects. Ms. Knorr also stated that many calls are received from IBC biosafety officers, contact people, and chairs regarding this issue.

Dr. Mickelson explained the process for this type of request: The request is transferred to other members of the ORDA or to her, a quick literature search is performed to determine the appropriate experts, and the appropriate experts are contacted. She believed that it was time to move beyond this *ad hoc* method of dealing with such questions to something more formalized. The information would be kept as up to date

as possible, including new information from investigators that would impinge on the risk assessment. Once the RAC agreed on the information, it could become part of the *NIH Guidelines* so that IBCs and IRBs could refer to it.

Dr. Aguilar-Cordova indicated that it might be worthwhile to look at Appendix G and Appendix K, which could be summarized in a clear table format. People working with vector containment do not really understand what BL1, BL2, or BL3 mean, and they are especially perplexed by the meaning of, for instance, "BL2 plus." "Large scale" needs to be better defined so the difference between Appendices G and K can be more readily understood.

Dr. Breakefield expressed concern about the amount of time necessary for RAC members to accomplish this task and wondered if this might be an undertaking for Federal employees. Ms. Knorr clarified that the RAC could form a subcommittee but only one person on the RAC would be required to participate on that subcommittee. The remainder of subcommittee members could be, for instance, biosafety officers or representatives from the American Biological Safety Association. However, the recommendation must emanate ultimately from the RAC.

Dr. Ando enumerated some of the issues that should be addressed, such as requirements for BL2 containment in a clinic (vs. a laboratory) and *ex vivo* therapy and containment in a clinic.

Dr. Noguchi agreed that this issue needs the imprimatur of the RAC because it is implementing policy embodied in the Environmental Protection Act for which the IBCs are responsible, such as the use of recombinant DNA technology.

Dr. Mickelson asked whether a mechanism existed whereby experts could be commissioned to do this work, which would then be reviewed by the RAC. Ms. Knorr reiterated that only one RAC member must be on a RAC subcommittee. The ORDA could send official letters asking for nominations or recommendations of non-RAC members to serve on this subcommittee.

Committee Motion 3

A motion was made by Dr. Markert, and seconded by Dr. Greenblatt, that a RAC subcommittee be established to review the *NIH Guidelines* and recommend proposed changes to the parent committee (the RAC). The RAC accepted the motion to establish the "Gene Transfer Vector Containment Subcommittee." Because there is a need for experts in a variety of research fields, the RAC recommended that the subcommittee consist mostly of *ad hoc* experts. Dr. Mickelson volunteered to chair the Gene Transfer Vector Containment Subcommittee. The motion passed by a vote of 11 in favor, 0 opposed, and no abstentions.

VIII. Opening Remarks for Day Two/Dr. Mickelson Dr. Mickelson welcomed RAC members, *ad hoc* reviewers, and speakers. She announced that the RAC's statement on *in utero* gene transfer, approved at yesterday's session (Day One), will be posted on the ORDA Web site Dr. Mickelson then introduced people seated at the meeting table who had not been present during Day One:

- Dr. Katherine High, Associate Professor of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
- Dr. Christine-Lise Julou, Senior Director for Worldwide Regulatory Affairs, Rhone-Poulenc Rorer GenCell, Cedex, France
- Dr. Haig Kazazian, Chair, Department of Genetics, University of Pennsylvania, Philadelphia, Pennsylvania

- Dr. Margaret Liu, Vice President of Vaccines and Gene Therapy Research, Chiron Corporation, Emeryville, California
- Dr. Lonnie Russell, Professor, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Illinois

IX. Presentation on Gonadal Biodistribution of Gene Transfer Vectors and the Potential Risk of Inadvertent Germ-Line Transmission
Presenter: Food and Drug Administration

This discussion served as a followup to the December 15, 1997, and March 10, 1998, discussions between the FDA and the RAC at which the FDA representatives informed the RAC of several preclinical studies demonstrating that DNA homologous to gene transfer vectors has been found in gonadal tissue subsequent to vector administration to extra gonadal sites. On December 15, 1997, Drs. Steven Bauer and Anne Pilaro, Center for Biologics Evaluation and Research, FDA, presented an overview related to the FDA's observation that preclinical animal studies designed to assess vector biodistribution have demonstrated unexpected persistence of vector sequences in gonadal tissue. Under the limits of confidentiality, the FDA could not discuss additional specifics of the observation; therefore, the RAC recommended that the ORDA should send a letter to all principal investigators (PIs) of clinical gene transfer trials and all IBCs requesting submission of all available data related to persistence of vector sequences in gonadal tissue. The ORDA received approximately 80 responses to this request. During its March 10, 1998, meeting, the RAC discussed these responses. On the basis of the limited information available to the RAC at that time, the Committee concluded that there is a need to initiate well-designed studies to adequately evaluate the implications of finding vector sequences in gonadal tissue. A letter dated June 5, 1998, was forwarded from the ORDA to Dr. Harold Varmus, the NIH Director, with regard to the RAC recommendation to issue a Request for Applications (RFA) for animal biodistribution studies for gene transfer vectors.

Dr. Mickelson explained that this portion of the RAC meeting was presented by the FDA to discuss issues in a public forum and to begin a dialog with the research community about existing and needed data on germ-line transmission.

Dr. Noguchi introduced this session by thanking the RAC members and the public for participating in this dialog and encouraging their feedback. He reiterated that the FDA's intent is not to inhibit the field but to discuss the issue of inadvertent germ-line transmission.

Introduction/Suzanne Epstein, Ph.D., FDA

Dr. Epstein's presentation concerned preclinical testing and the possible risk of germ-line alteration as an untoward result of somatic cell gene therapy. She began by providing a history of RAC consideration of this issue. In 1989 the RAC explicitly excluded germ-line gene therapy from proposals, stating that inadvertent germ-line alteration was an undesirable consequence and requiring submission of information in proposed protocols to assess this possibility. In 1992 James Neel said that meticulous testing for unanticipated germ-line effects should have highpriority in somatic cell therapy and that data were urgently needed. In 1998 the FDA presented a discussion of vector biodistribution from animal studies, and the RAC decided to request information from PIs and IBCs, with the goal of making available to all investigators data that would permit comparison of vectors and routes of administration. At the GTPC in January 1999, it was concluded that determination of inadvertent germ-line transmission needed further study and additional data.

The spectrum of potential risks was illustrated from somatic cell therapy, organ and tissue transplants,

blood, and *ex vivo* gene therapy at the low end—interventions that contain DNA but are not perceived as risky—to retrotransposons at the high end. *In vivo* gene therapy is in the middle range, where the risk is not really known because it has not yet been quantified.

Dr. Epstein stated that, currently, germ-line alteration is to be avoided even if adverse effects are not apparent, for two reasons: (1) Some biological consequences could take decades to emerge, and (2) even in the case of a silent transgenic situation, there is no societal consensus that transgenic outcomes are acceptable. The practical problem is how to encourage the development of promising therapies but still satisfy the public that all reasonable efforts are being made to avoid inadvertently altering the human germ line.

The FDA's current approach is to request preclinical vector localization studies in animals when vectors are to be given directly to patients; then, polymerase chain reaction (PCR) analysis of gonadal extracts is performed, looking for the presence (but not necessarily vector-derived gene expression) of vector. If a persistent positive signal is seen, then in general, trials are limited to sterile individuals while further analysis is performed, including analysis of the cell type in which the vector is present (e.g., gonads and not germ cells). Risk-benefit analysis is also conducted.

The FDA's goals for this RAC meeting were to:

- Discuss publicly the question of potential germ-line alteration, putting the risk in perspective
- Invite discussion of ethical and social issues: risk to nonconsenting progeny and to the gene pool and preserving societal acceptance of somatic cell gene therapy
- Analyze scientific and technical issues
- Discuss whether the FDA's approach is appropriate (the nature and stringency of preclinical testing and decisions about clinical trials)

The FDA also would like discussion to occur about whether any gene therapies exist for which testing need not be completed preclinically and for which positive signals in the gonads, without further data, do not preclude clinical trials in fertile individuals.

Expert Presentations

Haig H. Kazazian, Jr., M.D. (University of Pennsylvania, Philadelphia, Pennsylvania)

Dr. Kazazian presented data on alterations of the human genome caused by retrotransposition, a natural process of genome alteration by transcription of deoxyribonucleic acid (DNA) into RNA,reverse transcription of RNA back to DNA, and integration of this piece of DNA at a new chromosomal site. Retrotransposons account for greater than 30 percent of the human genome. They insert at random locations in the genome and are expressed in primary spermatocytes in meiotic prophase. Retrotransposons insert at a frequency of approximately 1 event in 5 to 10 sperm.

Based on the estimated frequency of retrotransposon insertions in the human genome and the number of mutations in the database, Dr. Kazazian estimated that the fraction of mutations that are caused by insertions is 1/600. The estimated frequency of spontaneous mutations is 10^{-9} per nucleotide per year. Based on the total genome size of 3×10^9 nucleotides and a generation span of 30 years, Dr. Kazazian estimated there are 90 mutations per sperm producing an individual upon fertilization. The frequency of retrotransposon insertions is about one new insertion in every six haploid sperm. Since these insertions are random, less than 5 percent of the insertions should be deleterious.

Lonnie Russell, Ph.D. (Southern Illinois University, Carbondale, Illinois)

Dr. Russell discussed the testis, barriers to the entrance of substances into the testis, and directed attempts to transfect germ cells. One of the major differences between the male and female reproductive systems is that the testis is an ongoing dividing organ that produces approximately 10^7 sperm per day; all of the germ cells in the female have divided and entered meiosis at around the time of birth. Divisions are supposedly where stable integration of genetic material can occur into the germ cell line. So there is a major difference between the male and the female reproductive systems in terms of possible integration of vectors. Barriers that restrict or impede substances from entering the testis include vascular endothelium, interstitial space, peritubular tissue, and the Sertoli cell barrier. Sperm exist in an "immunoprivileged environment." Dr. Russell also provided a history of selected studies, beginning in 1994, on purposeful transfection of male germ cells. Germ cell transduction is a rare event. He recommended two books that address this topic: *Biology of Mammalian Germ Cell Mutagenesis* (Banbury Report 34; James W. Allen, Bryn A. Bridges, Mary F. Lyon, and Montrose J. Moses; Cold Spring Harbor Laboratory; 1990) and *Stem Cell Biology and Gene Therapy* (Peter J. Quesenberry, Gary S. Stein, and Bernard Forget; Wiley-Liss; 1998).

Nancy M.P. King, J.D. (University of North Carolina at Chapel Hill, Chapel Hill, North Carolina)

Ms. King discussed the ethical and social implications of inadvertent germ-line transmission. Drs. Kazazian and Russell presented information that the likelihood of germ-line alteration is extremely low, and that therefore, the risk is also low. Risk of harm involves the nature of the risk, the likelihood of its occurrence, and the magnitude of the risk. Although the likelihood is extremely low, the nature and magnitude of potential changes to the gene pool are unknown.

Ms. King explained that the issues of risk of harm should be addressed on several fronts:

- Through public policy and public process by determining whether and when research with potential germ-line effects should go forward
- By investigators, through appropriate study design to minimize risks of harm and maximize the likelihood of future benefit
- By subjects, through informed decisionmaking for themselves and for future offspring

From the research she conducted on the ethical issues of inadvertent transmission, Ms. King listed the major issues as:

- Balancing risks of harm (to subjects and offspring) and chances of benefit (to subjects and/or future patients)
- Informed consent of subjects
- Proxy consent of research subjects for future offspring
- Monitoring subjects and their offspring

Ms. King enumerated some of the ethical and social issues that have been discussed for the past 8 years:

- When is it reasonable to proceed with gene transfer research that may have inadvertent germ-line effects, and when is it reasonable to proceed with gene therapy that has been proven effective in clinical trials but is also known to carry a risk of inadvertent germ-line effects?
- Which effects are considered most problematic if the germ line were altered?
- How can germ-line effects be avoided entirely?

- How can risk and benefit be balanced?
- How can improving the health of subjects vs. possibly harming future generations be balanced?
- If consequences are foreseeable though not intended, is the sense of responsibility different from spontaneous mutations?
- How do the mutagenic effects of gene therapy differ from the mutagenic effects of chemotherapy or radiation therapy?

Ms. King quoted and paraphrased several authors' discussions of the ethical and social issues surrounding germ-line effects, including Burke Zimmerman (when intentional germ-line transfer should (and should not) be carried out), Marc Lappe (difficulty of drawing the line between somatic cell and germ-line therapy), and Ray Moseley (dismissing the argument that germ-line intervention requires effective proxy consent for future offspring).

Ms. King outlined the argument that what is minimally acceptable possibly should change with the efficacy of the intervention—if adequate alternatives to gene transfer exist or if the gene transfer provides only a modest clinical effect, a lower likelihood of potentially harmful germ-line effects should be acceptable than if the clinical effects of gene transfer are potentially significant or lifesaving. The problem with that argument is that the chance of benefitting persons with serious impairments could thereby justify too much risk of harm to their offspring. The reproductive effects of gene transfer may need long-term study, and many other questions also arise: What alternative should be offered to subjects who wish to reproduce post-gene transfer, and who should pay for the alternatives? Would banking pretransfer sperm or ova be required or just strongly encouraged, and who should pay for this? Should subjects be offered *in vitro* fertilization preceded by PCR testing of gametes if they wish to reproduce after gene transfer? Or should subjects wishing to reproduce simply be told that if they have offspring after gene transfer, they will be urged to enroll the child in a registry for periodic followup?

Continued public discussion—with open consideration of scientific information—is needed in the following areas:

- The extent to which risk must be minimized.
- The kinds of risk that are present.
- The nature and magnitude of harms that are of greatest concern.
- Comparison of what researchers know now about the likelihood of germ-line effects and their potential for harm in other settings.
- Consideration of social and policy perspectives, including how risks like these are examined and discussed with research subjects and patients in other settings.
- Discussion of the duties and responsibilities of individuals, institutions, and the public; these consequences are caused by deliberate choice, which makes them conceptually different from the results of spontaneous mutations and, although infinitesimally small, the risks must still be considered.

Dr. Markert noted that insertional mutagenesis caused by gene transfer vectors appears to be less frequent than insertion by retrotransposition, which occurs in one out of six sperm. Dr. Aguilar-Cordova argued that radiation therapy or chemotherapy may cause some mutational events that would be inherited. He wondered whether there is an innate difference between causing a mutational event by insertion of a genetic element and causing one by use of radiation or chemotherapy, both of which are perhaps even more likely to be known mutagens. It is not standard practice to discuss whether an individual who has received radiation therapy ought to be allowed to reproduce.

Dr. Macklin asked whether an answer about risk might differ for the research context as opposed to the

therapeutic context. Ms. King agreed that with the increasing enrollment of women in clinical trials and the growing maturity of young women who have been successfully treated for different cancers, these issues need to be reexplored—and not exclusively in the discussion about inadvertent germ-line transmission.

Dr. Gordon made several comments: (1) Mutations can be deleterious without being fatal (e.g., male pattern baldness); (2) while the issues themselves are not different, qualitatively, the mutagenesis may be different from the point of view that there could be gain of function mutations related to gene insertion; (3) people engage in many behaviors that may increase the rate of mutagenesis in their germ line (e.g., eating or drinking patterns or exposure to mutagens at work); and (4) inadvertent germ-line transmission is millions of times less likely than an L-1 retrotransposon insertion, but this is a guess and is not derived from data.

FDA Approach to Preclinical Studies

Andra Miller, Ph.D. (FDA)

Dr. Miller presented preclinical case studies of gonadal distribution of gene transfer vectors (notof germ-line integration). She presented case studies using different classes of vectors (e.g., naked plasmid DNA, plasmid DNA in complexes with lipids, adenoviruses, and retroviruses). The route of administration influences distribution; therefore, changes in the route of administration require that new gonadal distribution studies be performed, and new studies may also be required for changes or modifications made to the vector itself. Preclinical studies must have adequate sensitivity, specificity, and duration to assess vector localization. In addition, the presence of vector rather than gene expression should be measured. A positive gonadal signal may indicate risk of a germ-line event; data are lacking and should be collected. Currently, the FDA recommends that the PCR assay be able to detect 100 or fewer copies of vector sequences per microgram of genomic DNA.

RAC members asked a few clarification questions. In answer to Dr. Gordon's question about whether the FDA cares which animal model is used, Dr. Pilaro, FDA, stated that, for the biodistribution study, a "relevant species" should be used. Relevance includes such issues as a transgene product that is active only in a certain species, looking for the risk of gonadal distribution, and toxicity. Although it is easy to use large numbers of rodents, some techniques may preclude using such a small animal (e.g., using a bronchoscope or intravascular injection). The appropriate species should be chosen relative to the expected clinical outcome.

Dr. Markert expressed interest in the legal ramifications of requiring patients to be sterile to participate in a clinical trial, comparing that to the legal inability to exclude fertile women from workplaces in which they are exposed to chemicals that could have effects on the germ line. Dr. Markert also queried as to why women who use implants for contraception are not considered in the same category as infertile women, since the chance of pregnancy is greater for a woman with a tubal ligation than it is for a woman with, for instance, a Norplant implant. Dr. Mickelson noted that the circumstances are different between workplace exposure and participation in gene transfer trials; the former is for a livelihood, while the latter is for participation in a clinical trial, which may not provide any benefit (Phase I studies).

Dr. Noguchi responded to Dr. Chow's concerns about inclusion of fertile women and men in studies after Phase I. The FDA's intent is not to restrict patient entry into studies but to ensure that the process and the reasoning behind it makes sense to everyone. Questions arise such as, Should the FDA allow nonsterile patients to participate in studies of a life-threatening disease in which there is a likelihood of gonadal transfer? What about a serious but not life-threatening disease? What if the disease is "only" arthritis? These are the kinds of questions on which the FDA wants feedback.

Steven Bauer, Ph.D. (FDA)

Dr. Bauer presented the current FDA approach for preclinical vector biodistribution studies. The origin of the FDA's concern was preclinical data indicating unexpected persistence in gonads and inadequate preclinical data—a PCR signal in gonads that was not necessarily in the germ cells or integrated. Technical sensitivity and limitation of PCR analysis were discussed; FDA current recommendations for Phase I are:

- Three samples must be taken per tissue, and the samples should be of sufficient size toyield 1 microgram of genomic DNA each (or, if necessary, sufficient replicates to sample a total of 3 micrograms).
- Two samples should constitute the experiment unspiked, and one sample should be run with a spike control.
- The sensitivity for PCR analysis should be fewer than 100 copies of vector per microgram of genomic DNA, which, using an estimation that 100,000 to 150,000 cells contain 1 microgram of DNA, represents approximately 1 insertion event out of 1,000 cells.

The FDA has responded to the concerns of gonadal biodistribution in several areas, the first being informed consent:

- A statement should be provided in the Informed Consent document regarding current results and the unknown risk of vector transmission to germ cells.
- Treated patients should be encouraged to use contraception temporarily.
- Autopsies should be requested in treated patients.

The FDA also has recommendations for the impact of results on clinical development; for example, if the assay is adequate, how the study should proceed:

- If the gonadal signal is not detected at all times, the clinical study may proceed, and no restrictions are placed on the patient population.
- If the gonadal signal is transiently positive, the clinical study must be reevaluated as to patient population and severity of illness. The study may proceed if benefits justify the risk. Semen analysis in treated males is requested in followup where applicable or appropriate.
- If the gonadal signal is persistently positive, the clinical study must restrict the patient population to sterile individuals. Semen analysis in treated males is requested in followup where applicable or appropriate. The source of the signal is analyzed.

The factors that led to the FDA's current stance were the observation that vector was being distributed to gonadal tissue, and in some cases persisting, but that few data were available about the level of risk entailed. A risk-benefit analysis is not helpful because the risks are not known. The risk is not only to the individual but also potentially to future generations, so an element of public health risk is contained in this analysis.

Dr. Markert noted that the chance of retrotransposon insertion (in six sperm) is far greater than the frequency of vector insertion; she considered it unwarranted to restrict the patient population to sterile individuals. Dr. Aguilar-Cordova echoed Dr. Markert's assessment.

In response to Ms. Levi-Pearl's comment that proactive discussions emanating from the NIH would be beneficial, Dr. Noguchi commented that if society has accepted a number of the mentioned risks, actual

data may not be needed to move forward. The FDA is comfortable with that stance, but that kind of feedback has not yet been heard; "absence of knowledge is not knowledge of absence," and until the studies are completed, risk levels are still hypothetical. In a followup question, Ms. Levi-Pearl expressed concern that, as the public and the FDA wait togather the data to speak with absolute accuracy about risk, important opportunities for advancement of disease prevention may be lost.

Investigational New Drug (IND) Sponsor Data

Katherine High, M.D. (Children's Hospital of Philadelphia, Philadelphia, Pennsylvania)

Dr. High described three studies for the treatment of hemophilia B, an X-linked disease, of gonadal distribution using an AAV vector. The vector was administered by intramuscular (IM) injection.

The first study was a biodistribution study in mice to discover the level of vector DNA; the gonads were affected, but it was unknown whether this translated to an effect on the germ cells. The second study analyzed semen in hemophilic dogs; the semen analysis was negative from animals treated on all doses but the highest (due to contamination). The third study, still in progress, is examining gonadal tissue and semen in sexually mature adult male rabbits. Ultrasound is used so that the IM injection does not inadvertently go into a blood vessel. Through day 56 (the latest data, from a few days prior to this meeting), semen samples have been negative for vector sequences. The conclusion so far is that when an AAV vector is introduced into IM sites under ultrasound, the level of inadvertent germ cell gene transfer represents a very low risk event—the risk of an untoward event is 1 in 3 million (when the level of detection is 1 in 30,000 gametes) or 1 in 30 million for the rabbit study (in which the level of detection is 1 in 300,000 gametes). Translating the rabbit data loosely to humans, someone who is treated with this vector would have to produce 30 million children before an untoward effect would be encountered.

Christine-Lise Julou, Ph.D. (Rhone-Poulenc Rorer GenCell, Cedex, France)

Dr. Julou presented information on preclinical testing and vector biodistribution to the gonads, a case study of a plasmid gene therapy vector. The planned clinical use is for peripheral arterial occlusive disease and coronary artery disease; planned clinical routes of administration are in skeletal muscle and the myocardium. Dr. Julou presented her conclusions with respect to detection of plasmid DNA in gonads:

- Male rats given a single IM dose: At 3 days after injection, no plasmid DNA was detected by conventional PCR.
- Male and female rats given repeat IM doses with 2 to 3 single administrations at 2-week intervals: At 2 weeks after injection, no copy was seen at low dose, and at mid- and high-dose levels using PCR, between 1 to 10 copies appeared in the testes of 2 out of 4 rats.
- Male pigs given an intramyocardial dose: Fewer than 10 copies were observed 7 days after injection, and no copy was detected 2 weeks later (21 days after injection) using real-time PCR.

Margaret Liu, M.D. (Chiron Corporation, Emeryville, California)

Dr. Liu discussed Chiron Corporation's studies with retroviral vector (RVV) gene therapy inhumans and in its hemophilia A preclinical program by various routes of administration, including the intravenous (IV) route. Preclinical efficacy results of the hemophilia A program indicate that IV administration of this vector, encoding human factor VIII gene at the tested doses, has shown long-term expression of potentially therapeutic levels of factor VIII in both dogs and rabbits. In hemophilic dogs, the whole blood-clotting time has been shortened, indicating expression of the gene.

Dr. Liu presented data on preclinical biodistribution of vector by PCR following IV administration in dogs and rabbits. The frequency analysis results from rabbit testes indicated that, with 99 percent confidence, the probability of any one cell being transduced is < 0.0000027. Therefore, the probability of integration occurring in any given gene is < 5.4×10^{-10}. In comparison, the frequency of spontaneous mutation of any given gene is 2×10^{-6}; the rate of hemophilia in the general male population is 1 in 75,000; the probability that a human sperm carries a spontaneous amino acid-altering mutation approaches 90 percent; and 1 to 100 spontaneous new mutations occur in the human genome per generation.

Dr. Liu concluded that gonadal PCR positivity does not *a priori* indicate germ cell transduction. The probability of any one cell in the testis being transduced is very low and decreases exponentially over time. The probability of integration occurring in any given gene is orders of magnitude less than the spontaneous mutation rate.

Dr. Liu pointed out that one of the vaccines that has been effective worldwide in eliminating a disease from the planet is the smallpox vaccine, a DNA virus vaccine that carried much mammalian DNA and was administered to millions of people. This vaccine is no longer administered because the disease has disappeared. No germ cell transduction has been reported for the smallpox vaccine.

RAC members asked a few clarification questions.

Afternoon Session Opening Comments/Dr. Mickelson

Dr. Mickelson introduced Dr. Jay Siegel, Director of Therapeutics Research and Review, Center for Biologics Evaluation and Research, FDA.

Public Comments

Nelson Wivel (University of Pennsylvania Medical Center and American Society of Gene Therapy, Thorofare, New Jersey)

Dr. Wivel stated that American Society of Gene Therapy membership includes approximately 2,000 individuals who represent most of the active researchers in gene therapy in academia and industry. The Society recognizes the risk of inadvertent gene transfer to gonadal tissue and is eager to work with the FDA and NIH to establish appropriate guidelines for detecting the presence of germ-line gene transfer. This relatively rare risk, even when using systemic administration of the vectors, does not constitute an absolute barrier to Phase I trials designed to study serious diseases. Since the risks must be considered in the context of other risks andbenefits as well as the nature and severity of the disease, the most rationa approach is to proceed on a case-by-case basis. Phase I trials should continue to be developed for diseases with severe morbidity and mortality without unnecessary restrictions on patient enrollment. Concerning primary needs for the future, although the use of animal models to mimic inadvertent germ-line gene transmission is a less than perfect strategy, the Society believes that it would be useful and productive to encourage the NIH and private foundations to consider funding an RFA that would support the development of novel strategies to address this problem.

Russ Lyons (Systemix/Genetic Therapy, Inc., Gaithersburg, Maryland)

Dr. Lyons presented some of the recent data that have been generated by Systemix/Genetic Therapy, Inc (S/GTI) in preclinical and clinical studies. For preclinical studies, S/GTI uses both a worst-case route and the intended clinical route. The clinical studies summarized were multiple clinical trials with single or

repeated intracerebral administration of large numbers of vector-producer cells. Interpretation of the study data indicates that systemic distribution did not occur with intracerebral or subcutaneous injection of vector-producer cells in preclinical studies. The positive tissues occasionally seen are interpreted to be trafficking positive peripheral blood lymphocytes (PBLs) that are transduced at the time of intracerebral injection. There is no evidence that germ-line gene transfer occurs; all the clinical gonadal specimens have been negative, and the single positive clinical testis sample was not duplicated either at higher doses or by the intended clinical route.

James Albright (Cystic Fibrosis Foundation, Bethesda, Maryland)

Mr. Albright is a 35-year-old who has cystic fibrosis (CF); he offered insight into the personal decisions an individual might make with regard to gene therapy trials. He began by indicating how he makes decisions about participating in CF studies and why he believes strongly that the risks involved are comprehensible and eminently reasonable. Much of his comfort with participation in gene therapy trials for CF derives from the knowledge that the CF Foundation staff believes completely in the research and is involved in the struggle over the long term. For CF sufferers, almost everything is a risk calculation, and the risk of vector implantation was always understood—the gene transfer protocol in which he participated was an experiment using a carefully designed study and rigorous protocol. Almost everyone can benefit, at some point in their lives, from a successful study, although most people with CF cannot participate in one. Mr. Albright's interest in gene therapy research is not only for his own health but also for the health of one of his sons, who also has CF; the risks he has accepted by participating in a CF gene therapy study pale in comparison to the hope he has that his son will be able to live a long and happy life.

Donald E. Colburn (National Hemophilia Foundation, New York, New York)

Mr. Colburn stated that the National Hemophilia Foundation (NHF) is the oldest and largest nonprofit organization dedicated to curing hemophilia and other related bleeding disorders. Approximately 15,000 males in the United States have moderate or severe hemophilia; Mr. Colburn is one of them, suffering from severe hemophilia A. Hemophilia is characterized by internal hemorrhaging that can cause permanent joint arthropathy and even death. Currenttherapy involves on-demand treatment that commences after bleeding starts, with repeated bleeding resulting in significant morbidity. Sufficient amounts of recombinant replacement of protein are unavailable to treat hemophilia prophylactically, and during the past 20 years, families with hemophilia have experienced indescribable hardships from viral transmissions by the lifesaving products they must infuse.

The NHF's Medical and Scientific Advisory Committee (MSAC) and its board of directors debated the issue of germ-line effects in February 1999, and Mr. Colburn offered the NHF's resolution on this matter: The NHF is in favor of continued trials for gene therapy, which should be somatic only; there should be no attempt to alter germ-line cells. The MSAC urges the RAC to allow protocols to proceed with appropriate monitoring and safeguards.

Mr. Colburn explained that dialog about the severity of condition, patient age, and reproductive potential are explosive topics for the populations these therapies can help. For example, the criterion that reads "subjects must be age 30 or greater, with moderate hemophilia, unable to reproduce" would force the moderately affected individual to have a vasectomy—to become sterile to achieve a cure. The potential for life quality and life saving offered by genetic therapies should be examined closely.

Margaret Lavigne (Muscular Dystrophy Association, Tucson, Arizona)

Ms. Lavigne represented the Muscular Dystrophy Association (MDA) and herself as an individual with

limb-girdle muscular dystrophy. Representing incredible hope for muscular dystrophy (MD) sufferers, progress has moved from identifying the gene defects that cause many of the forms of neuromuscular disease to the point at which MDA-funded scientists are on the verge of embarking on landmark gene therapy clinical trials. Without gene therapy, there will be no hope for the future generations who manifest the symptoms of MD and other neuromuscular disorders in their early years. Most individuals with Duchenne's muscular dystrophies may not even survive to be able to have families, and the individuals and families of those with MD or neuromuscular dystrophies deal with potential genetic risks every time they think about raising a family. The benefits of proceeding with somatic gene cell therapy clearly outweigh the risks. Life itself is a risk, and those individuals who participate in clinical trials are well aware of the costs and benefits associated with genetic therapy.

Terence Flotte (University of Florida, Gainesville, Florida)

Dr. Flotte is an academic physician scientist who spoke at the suggestion of some of his colleagues. The risk for germ-line transmission in gene therapy is primarily based on the biology of the vector system. AAV biology is fundamentally distinct from the biology of other vectors (such as retroviral vectors) for which there is a greater amount of data and experience. For example, endogenous retroviruses and retrotransposons are integrated into the human genome, whereas AAV is not found in the human genome. A group of about 20 AAV scientists, under the leadership of Dr. Paris Burd, FDA, will be meeting in May 1999 to design platform studies related to the biology of a vector for the gene therapy system (FDA/NIH Workshop on Nonclinical Toxicological Study Design Issues for the Development of AAV-Based Gene Therapeutics, A Platform Studies Approach, May 2-3, 1999, Bethesda, Maryland). Dr. Flotteencouraged the RAC to wait for the results of these platform studies before making any final decisions.

RAC Discussion of the Presentations

Dr. Gordon summarized the FDA presentations, which provided data suggesting that the risk of inadvertent germ-line alterations is not a serious concern. Although it was apparent that the risk of harm related to germ-line transmission is negligible, it was also clear that there is an absolute need to conduct platform studies to demonstrate definitively the absence of harm. The RAC reiterated its recommendation to Dr. Harold Varmus, NIH Director, about the need for the NIH to support such studies, a move that would significantly facilitate progress in the field. Animal studies have shown that there is still no evidence of integration into germ cells, only the presence of vector sequences.

Dr. Gordon stated his belief that fertile males should be allowed to participate in clinical gene therapy trials and safety trials. Participants should be apprised of the option of banking sperm prior to therapy but should not be forced to exercise that option, although it is a safe, easy, and reliable approach that is offered and performed for other diseases. He explained that he did not want the gene therapy discipline held back in an effort to exclude fertile males from studies, and it is not appropriate to require sterilization before a male can enter a study. Fertile females should not be barred from entering trials when retroviral and other gene transfer vectors are used; anatomical, physiological, and statistical data indicate that it is not likely that a woman's oocyte that leads to conception will have an affected gene. In addition, sterile individuals may not be a representative test population for actual patients.

Dr. Aguilar-Cordova indicated that, although much information is not known about inadvertent germ-line gene transfer, a tremendous amount of circumstantial data posits substantial evidence that germ-line transmission would be a very rare event. The benefit of the entire field of gene transfer research to society is real, and this benefit should be taken into heavy consideration when assessing the risk-benefit ratio.

Further RAC Discussion of the FDA Questions

After the presentations and discussion, Dr. Noguchi and Dr. Mickelson led a discussion on the three questions posed by the FDA to the RAC. The RAC came to closure on the first two questions but deferred the third question until the next RAC meeting.

In answering the three questions below, the RAC was asked to consider the following factors:

- Vector class (plasmid, retrovirus, adenovirus, other)
- Nature and severity of disease
- Patient age
- Reproductive potential (altered by disease, birth control)
- Male vs. female patients
- Prior experience with related vectors (changes such as inserted gene, vector size, selection markers, and transcription control elements; change in formulation such asliposomes)
- Route of administration

FDA Question 1. Are vector localization studies needed prior to initiation of Phase I clinical trials for all *in vivo* gene therapies? Do any of the factors listed above affect the necessity of such studies or their timing (prior to Phase I vs. permissible to perform concurrently with Phase I)?

Dr. Noguchi stated that the FDA appreciated public discussion of the gonadal biodistribution issues. He asked the RAC to address the FDA question of whether vector localization studies are needed for all *in vivo* gene therapies.

Dr. Mickelson expressed her concern about the lack of scientific data to assess the risk of gonadal biodistribution of gene transfer vectors.

Dr. McIvor acknowledged the need for obtaining more scientific data to address the issue. On an individual case-by-case basis, the likelihood of integration into germ cells is so low that the sponsor and the investigator should not be required to conduct vector localization studies on each protocol. Dr. Gordon concurred that the requirement for each individual case is not required; the studies to address the overarching issues can be conducted concurrently. Dr. Breakefield supported a platform studies approach to address the overarching issues. Dr. Aguilar-Cordova concurred with the position of not requiring vector localization studies prior to initiation of Phase I studies. Drs. Markert and Greenblatt agreed.

Dr. Noguchi stressed the need for more data to address the issue of inadvertent germ-line alteration. Dr. Gordon favored the idea of platform studies; gene transfer research laboratories should take new classes of vectors, use them in provocative test systems, and then study their uptake by the germ cells and their germ-line effects. In the interim, he strongly favors an organized method of completing these tests convincingly so that laboratories can rely on the results.

Dr. Ando explained that gene therapy is not very different from other drugs in terms of formulation and dose-escalating studies. Vector biodistribution or toxicity studies may not have to be repeated completely on the basis of available platform data, but some of the details of the particular applications need supporting data on an individual basis.

Dr. Aguilar-Cordova made a motion to adopt a position that vector localization studies are not needed prior to initiation of Phase I clinical trials for all *in vivo* gene therapies. Dr. Markert seconded the motion.

Dr. Breakefield asked whether the statement included all present and future vectors. Dr. Noguchi pointed out that novel vectors such as lentiviral vectors may require specific supporting data. Dr. Macklin expressed a similar concern. Dr. McIvor noted that the statement of not requiring the data for all *in vivo* gene therapies does not preclude the FDA's responsibility to evaluate the data on a vector-by-vector basis.

Committee Motion 4

Dr. Aguilar-Cordova made, and Dr. Markert seconded, a motion that the RAC recommends that vector localization studies are not needed prior to initiation of Phase I clinical trials for all *in vivo* gene therapies. The motion passed by a vote of 8 in favor, 2 opposed, and 2 abstentions.

FDA Question 2. If vector is detected in preclinical studies as a transient or persistent positive signal in the gonads and it is not yet known whether it might be in germ cells, are clinical trials in fertile individuals acceptable? Do any of the factors listed above affect the acceptability? If voluntary birth control is considered an adequate precaution in some cases, how long should it be used?

Dr. Noguchi requested that the RAC also discuss whether or not voluntary birth control is warranted, necessary, and logical. Since the RAC appears to be stating that vector or localization studies can begin concurrently, requiring a contraceptive may not be necessary or appropriate.

In response to the issue of voluntary birth control, Dr. Owens noted that it is reasonable for study participants to use some kind of barrier contraceptive because of the issue of germ-line transfer as well as the issue of shedding vector in semen; shedding into vaginal fluids also should be investigated. Dr. McIvor stated that, even when a PCR-positive signal is detected in the gonads of experimental animals, gene transfer in the germ cells is a low-frequency occurrence. Ms. King suggested that until more information is available it might be the investigator's or sponsor's responsibility to make other options (such as sperm-banking or testing) available; study participants could also avail themselves of these options on their own.

Dr. McIvor suggested a statement in response to the FDA question that reproductive potential should not be used as a criterion to exclude patients from gene transfer clinical trials but that there may be circumstances where such exclusion is justified in certain protocols. Dr. Macklin suggested adding "in principle" to the statement to allow for exception to the general statement.

Dr. Breakefield said that fertile patients should be encouraged to practice effective contraception during the course of somatic cell gene transfer clinical trials. Dr. Siegel agreed that it is quite typical of the experimental drug therapies for such precaution.

Dr. Aguilar-Cordova made a motion that, in principle, reproductive status should not be used as a criterion to exclude patient(s) from gene transfer clinical trials even if transient or persistent positive signals have been detected in the gonads in preclinical studies. Dr. Markert seconded the motion. Dr. Siegel made a friendly amendment to substitute the words "reproductive potential" for "reproductive status" in the statement.

Committee Motion 5

A motion was made by Dr. Aguilar-Cordova, and seconded by Dr. Markert, that, in principle, reproductive potential should not be used as a criterion to exclude patient(s) from gene transfer clinical trials even if transient or persistent positive signals have been detected in the gonads inpreclinical studies. The motion

passed by a vote of 11 in favor, 0 opposed, and no abstentions.

FDA Question 3. Are the FDA's current technical recommendations for preclinical vector localization studies appropriate to assess the risk of potential germ-line alteration? Are they appropriate for only some vector classes, and if so, which ones? When a positive signal is detected in the gonads, what followup studies should be performed preclinically and/or clinically?

Dr. Noguchi believed he understood the general sense of the RAC on these issues. Regarding the question of the adequacy of the FDA's current technical recommendations for preclinical vector localization studies, the sense of the RAC is that they are adequate only to address localization. The FDA would like to work further with the RAC to develop a more general strategy. Because of time considerations, Dr. Noguchi deferred further discussion and final vote on this question until a future RAC meeting.

X. Discussion on Human Gene Transfer Protocol (9901-279)—*A Phase I Safety Study in Patients With Severe Hemophilia B (Factor IX Deficiency) Using Adeno-Associated Viral Vector to Deliver the Gene for Human Factor IX to Skeletal Muscle*
PI: Catherine Manno, M.D. (University of Pennsylvania Medical Center and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania)
Reviewers: Dr. McIvor, Dr. Theodore Friedmann (not present), Ms. King, Dr. Owens (*ad hoc*)

Protocol Summary

Dr. McIvor read the summary description of this protocol:

Catherine S. Manno, M.D., proposes a Phase I study to determine the safety of intramuscular injection of an adeno-associated virus (AAV) vector engineered for expression of human clotting factor IX (AAV-hFIX) in the treatment of hemophilia B. AAV-hFIX consists of an AAV type 2 genome with protein coding sequences replaced by a factor IX expression cassette, containing the cytomegalovirus early promoter, human factor IX coding sequence with intron 1, and an SV40 polyadenylation signal. The vector will be generated by cotransfection with plasmids that provide AAV and adenovirus packaging functions. There are substantial data that the proposed vector structure is effective for high-level expression of factor IX. The protocol describes a dose-escalation study which will enroll nine patients with hemophilia B, consisting of three groups of three patients each. The patients within each group will receive the same dose of AAV-hFIX. The three doses are 1.4×10^{13}, 1.4×10^{14}, and 7.0×10^{14} vector genomes. The vector will be administered intramuscularly at multiple sites in a total injection volume of 3 to 10 mL. Patients will be monitored for toxic responses, immune reaction to the vector, expression of human FIX, and improvements in clotting function. The primary objective of the study is thus to evaluate the safety of intramuscular administration of AAV-hFIX to patients with hemophilia B, with a secondary objective to measure biologic and physiologic activity of the factor IX gene product.

RAC Discussion/Comments

Review/Dr. McIvor

Dr. McIvor stated that the investigators had already provided answers to most of his questions in their written response. The protocol is based on the effectiveness of AAV as a delivery and expression system for new sequences in muscle; an attribute of AAV seems to be that it produces high levels of expression in muscle. An advantage of AAV is that the virus has not been associated with any disease in humans. A significant amount of preclinical data exists to support the feasibility of the proposed trial. Preclinical

toxicity and efficacy studies were performed in rodents and dogs with hemophilia B. Some of the dogs in the studies, all of which demonstrated high levels of expression of factor IX, developed an immune response to factor IX.

Dr. McIvor's questions included the following:

- Factor IX is not usually produced in the muscle but in the liver. Would expression in the muscle cause some difference in reaction in posttranslational modifications to the protein that might influence its effectiveness?
- The production procedure stated that AAV and adenovirus-helper functions will be provided by cotransfecting plasmids. In the original submission, there was no indication of how AAV would be assayed. (That information has now been provided.)
- Is a replication-competent AAV assay conducted on the final product?
- What is meant by "factor IX activity"? (This question was answered in the investigators' responses.)
- The protocol plans to infuse a large bolus of factor IX protein shortly before the IM injection of the vector. How quickly is the large bolus of protein going to disappear, and will it impede the determination of gene expression in the patient? (The investigators answered this query by stating that the half-life of the protein is 18 hours.)

Dr. McIvor declared that the dose escalation study was laid out carefully and the protocol in general is designed to assess effectiveness; it is exciting to be able to determine whether the gene product is having an effect just by taking a blood sample and determining the clotting time.

Dr. McIvor's major concern with the protocol was the immune response—the possibility that antibody formation against the vector or against the gene product might prevent effective administration of the vector at a subsequent time. Patients are effectively warned in the Informed Consent document that such a reaction may prevent administration of the vector a second time; patients will be screened to exclude those who have a history or presence of an inhibitor to factor IX protein.

Another important issue for Dr. McIvor was the proposition that, if there is a substantial amount of inhibitory activity generated, the area of the muscle into which the vector was injected could be surgically resected if necessary. He wanted to know how a determination would be made whether to proceed with muscle resection and whether it would be wise to carry out a surgical resection on a patient who is prone to bleeding. Ways to prevent the bleeding were outlined in the investigators' responses, but Dr. McIvor requested some RAC discussion on this issue. In arelated concern, Dr. McIvor wondered whether the plan for muscle biopsies—to determine whether the gene is present and its longevity—is worth the risk of bleeding.

In the protocol, the goal of what might be expected in terms of the percentage of normal expression of factor IX was worded as 1 percent. Dr. McIvor noted that the informed consent stated the figure as 5 percent, and he was concerned about giving the patient more hope than is necessary.

Review/Dr. Friedmann (presented by Dr. McIvor)

Dr. McIvor then summarized Dr. Friedmann's review, in his absence. Dr. Friedmann enumerated the following concerns:

- Might there be a problem with expressing too much of this protein?
- Was the null vector that was used in preclinical studies the same except for the lack of insert? (The investigators answered in the affirmative.)

- A concern was raised about development of inhibitory antibodies. It is possible that inhibitors reduce not only the efficacy of administered proteins but also the endogenous low levels of functional factor IX in patients.
- Why were potential female patients not included in the eligibility criteria? (This concern may have been explained by the investigators, but Dr. McIvor suggested it be revisited.)
- Why are patients with a life expectancy of less than 20 years being excluded? (This concern was answered adequately by the investigators.)

Two of Dr. Friedmann's questions still needed to be answered by the investigators:

- If the patients will be hospitalized for 24 hours but vector shedding will be followed for 3 days while the patients are maintained in standard isolation conditions, how and where will the patients be kept during the first 3 days following injection?
- Is it appropriate to bar participation in this study of someone who does not want to bank sperm?

Dr. Friedmann had also suggested changing some wording in the Informed Consent document about positive or negative effect on the patient's health; the investigators responded to the suggestion in their written response.

Review/Ms. King

Ms. King indicated that the investigators had answered all of her questions and comments satisfactorily in their written response. Her primary concern was that it appeared that the description of the likelihood of a potential for direct benefit to subjects was overstated in the Informed Consent document in comparison to the statements in the protocol; the investigators have added a clarifying sentence and revised another sentence so that it is now very clear that direct benefit to subjects is unlikely. She queried why subjects would be required to supply their own extra factor IX for administration during biopsies and other procedures. Ms. King wondered whether the issue of subjects disqualifying themselves from receiving a therapeuticdose of vector in a later study (by virtue of development of an immune response) is not only a consent issue but also a justice issue. Regarding sperm-banking, Ms. King stated that requiring subjects to use the services of a sperm bank is a significantly less onerous requirement than sterility.

Review/Dr. Owens

Dr. Owens stated that, on the basis of animal studies, there is a reasonable expectation that this line of research will lead to a product with some therapeutic benefit. The choice of muscle as a target tissue provides the best combination of safety and potential efficacy. One particularly positive safety feature in the protocol is the built-in delay between treatment of successive patients. Dr. Owens' concern that there was no specific mention that the patients were required to keep a log of their factor IX concentrate injections was answered by the investigators: Such a log is standard operating procedure with all hemophilia patients. Dr. Owens was also assured by the investigators that the vector dosage would be adjusted on the basis of the weight of the patient.

Other Comments

Since a fraction of the general population is already seropositive for AAV, Dr. Chow wondered whether there was antibody against AAV protein, whether that fact would complicate analysis, and whether patients should be screened to determine who has already been exposed to AAV.

Drs. Macklin, Greenblatt, and Mickelson agreed that sperm-banking should be an informed consent issue

and not a direct requirement or a criterion for exclusion.

Dr. Manno's Response to RAC Reviews and Comments

Dr. Manno introduced Dr. Katherine High and noted the members of her laboratory: Dr. Linda Couto and Ms. Wanda deVlaminck from Avigen, Dr. Tim Nichols from the dog colony at the University of North Carolina (who had to leave earlier), and Dr. Mark Kay, Director, Program in Human Gene Therapy, Stanford University, Palo Alto, California. Drs. Couto, Manno, and High answered the questions as follows:

- A series of assays for replication-competent AAV, including the infectious center assay, is performed on the product immediately prior to packaging for administration, the timepoint at which the infectious center assay is taking place.
- Muscle biopsies should not present bleeding problems; health care workers who treat hemophilia patients are experts at stopping bleeding, especially if patients are treated prior to a procedure. In the case of muscle excision, there is a resident on staff who is experienced in dealing with surgery on hemophilic patients.
- Patients will stay in the hospital for 24 hours and will spend the following 48 hours in a hotel across the street from the hospital.
- Keeping treatment logs, a valuable tool, is not mentioned in the protocol but will be reiterated to patients. Most adult hemophiliacs have significant experience in keeping these logs.
- A large percentage of the general public possess antibodies to AAV and were likely infected in childhood; hemophiliacs possess AAV antibodies in the same percent as the general public. Patients will be prescreened for this antibody. Whether presence of the antibody will make a difference in efficiency of gene transfer is unknown, and answering this question is one of the goals of the human study. Effective modeling in animals is not possible because they are not naturally infected early in life with AAV serotype 2.
- Sperm-banking will not be a requirement, although it will be encouraged to give patients the option of having a vector-free source of semen in the event that vector sequences are subsequently found in their semen. Data indicate that sperm are unlikely to contain the vector.
- This Phase I protocol will study safety; the "5 percent of normal factor IX" level, listed as a potential benefit in the Informed Consent document, will be removed. New language will state that "the clinical manifestations of your hemophilia may be improved."

Dr. High explained that the official recommendation of the National Hemophilia Foundation is that patients with hemophilia be on prophylactic regimens in which they infuse factor proteins two or three times a week. Many people are not following that regimen, but for those who are, the vector administration would be timed with their prophylactic infusion of factor proteins.

In answer to a question from Drs. Scharke and Owens, Dr. Manno indicated that an initial concern was that injection into muscle could be myotoxic; therefore, creatine phosphokinase (CPK) and creatine will be monitored. Acute or chronic renal failure was not observed in any of the preclinical animal models.

Dr. Scharke posed some terminology questions. Because this is an experimental gene transfer protocol, reference to "patients" should be changed to "volunteers" or "human subjects." The consent language about doses indicates "low," "medium," or "high" without defining the actual amounts; Dr. Manno agreed to clarify the doses. Dr. Scharke also asked whether it would be to participants' medical disadvantage to abruptly remove themselves from the protocol instead of doing so in an orderly manner under supervision; Dr. Manno indicated that she would want to follow patients intensely for the first year and ideally for the rest of their lives, but that the disadvantage is not predictable at this point.

With regard to Dr. Scharke's question about the definition of extended followup, Dr. Manno stated that patients would be followed at their home hemophilia treatment center on a yearly basis, with special interest in their factor IX production, the expression of the transgene, and antibody formation. Dr. Manno agreed to add that blood samples specifically for the research project would be considered part of the long-term followup. Dr. Scharke suggested that someone else at Children's Hospital rather than Dr. Manno be listed as the contact for questions about research subject rights to avoid the appearance of a conflict of interest.

Dr. Breakefield queried whether a determination had been made about the actual area near the injection that will be expressing the transgene. Dr. High answered that several laboratories at the University of Pennsylvania are currently investigating AAV IM injection to determine exactly the radius from the injection point over which transduction occurs.

XI. Closing Remarks and Future Meeting Dates/Dr. Mickelson

Dr. Mickelson thanked Drs. Manno and High. She stated that the next RAC meeting will be held on June 10-11, 1999, at the National Institutes of Health, Building 31C, Conference Room 10 (sixth floor). [Note: The next meeting is now rescheduled to June 14, 1999 at the same location.]

XII. Adjournment/Dr. Mickelson

Dr. Mickelson adjourned the meeting at 4:00 p.m. on March 12, 1999.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, actions are not considered final unless approved by the NIH Director.]

Debra W. Knorr
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: March 12, 1999

Claudia A. Mickelson, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health