DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
September 24-25, 1998

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The Recombinant DNA Advisory Committee (RAC) was convened for its seventy-second meeting at 9:00 a.m. on September 24, 1998, 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 September 24 from 9:00 a.m. until 5:00 p.m., and September 25 from 8:30 until 3:30 p.m. The following were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Childrens Hospital
Dale G. Ando, Cell Genesys, Inc.
Louise T. Chow, University of Alabama, Birmingham
Jon W. Gordon, Mt. Sinai School of Medicine
Jay J. Greenblatt, National Institutes of Health
Eric T. Juengst, Case Western Reserve University
Nancy M. P. King, University of North Carolina at Chapel Hill
Sue L. Levi-Pearl, Tourette 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

Executive Secretary:

Debra W. Knorr, National Institutes of Health

A committee roster is attached (Attachment I).

Non-Voting Representatives/Liaison Representatives:

Daniel Jones, National Endowment for the Humanities
Andra Miller, Food and Drug Administration (Alt)
Philip Noguchi, Food and Drug Administration

National Institutes of Health staff:

Agnes Adams, OD
Barry Bowman, OD
Sarah Carr, OD
Jan Casadei, NCI
Christine Ireland, OD
Julie Kaneshiro, OD
Jennifer Kostiuk, OD
Becky Lawson, OD
Allan Lock, NICHD
Catherine McKeon, NIDDK
Gene Rosenthal, OD
Margaret Ruiz, NIAID
Thomas Shih, OD
Lana Skirboll, OD
David Wilde, NCRR

Others:

Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Elizabeth Arnold, Institute of Science, Technology, & Public Policy
Bruce Aronow, Children's Hospital - Cincinnati
John Bishop, Food and Drug Administration
Judy Buckalew, Senator Lauch Faircloth
Rebecca Buckley, Duke University Medical Center
Parris Burd, Food and Drug Administration
Daniel Burineau, Department of Veterans Affairs
Jeff Carey, Genetic Therapy, Inc.
Alan Cohen, Children's Hospital of Philadelphia
Jennifer Couzin, Science Magazine
Albert Deisseroth, Yale University

Anne Dunne, Strategic Results
Thomas Eggerman, Food and Drug Administration
Suzanne Epstein, Food and Drug Administration
Donald Gay, Chiron Corporation
Diane Gianelli, AM News
Allan Glass, Congressional Fellow
Tina Grasso, GenVec
Sarina Grosswald, SJ Grosswald Association
Peter Hartogs, CNN
Rich Hayes, University of California, Berkeley
Mei-Mei Huang, University of Southern California
Deborah Hurst, Chiron Corporation
Susan Jenks, Journal of the NCI
Dorothy Jessop, Public
Evelyn Karson, Columbia Hospital for Women
Rachel King, Genetic Therapy, Inc.
Earl Lane, Newsday
LaVonne Lang, Parke-Davis
Timothy LeShan, The American Society for Cell Biology
J. Tyler Martin, Systemix, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Wendy McGoodwin, Council for Responsible Genetics
Claire Nater, Council for Responsible Genetics
Patricia Novak, Collateral Therapeutics
Amy Patterson, Food and Drug Administration
Anne Pilaro, Food and Drug Administration
Andrew Quon, American Medical Association
Abdur Razzaque, Food and Drug Administration
Joseph Rokovich, Pangaea, Inc.
Tomiko Shimada, Ambiance Awareness International, Inc.
Tomoko Shinoda, Japan/Public
Sanyin Siang, American Association for the Advancement of Science
Stephanie Simek, Food and Drug Administration
Edward Sheridan, Georgetown University Medical Center
Lorna Speid, GeneMedicine, Inc.
Rebecca Spieler, The Blue Sheet
Jean Starr, Private Practice
Mika Sugiura, The Sankei Shimbun
LeRoy Walters, Georgetown University
Rick Weiss, The Washington Post
Rhea Williams, Schering-Plough Corporation
Bonnie Wu, University of Southern California
Doris Zallen, Virginia Polytech Institute
Esmail Zanjani, Veterans Hospital, Reno
Yi Zhao, University of Southern California
Kathy Zoon, Food and Drug Administration

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

Dr. Claudia A. Mickelson, Chair of the Recombinant DNA Advisory Committee (RAC), called the meeting

to order at 9:00 a.m. on September 24, 1998. The notice of the meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* of September 2, 1998 (63 FR 46801).

Dr. Mickelson introduced the new RAC members: (1) Louise T. Chow, Ph.D., Professor of Biochemistry and Molecular Medicine, University of Alabama at Birmingham, Birmingham, Alabama; (2) Nancy M. P. King, J.D., Associate Professor, Department of Social Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and (3) Sue L. Levi-Pearl, Director, Medical and Scientific Programs, Tourette Syndrome Association, Inc., Bayside, New York. Two additional new members were absent from the meeting: (1) Xandra O. Breakefield, Ph.D., Geneticist, Neurology Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, Massachusetts; and (2) Theodore Friedmann, M.D., Professor, Department of Pediatrics, University of California at San Diego, San Diego, California.

As background, Dr. Mickelson noted that on July 31, 1998, W. French Anderson, M.D., University of Southern California, and Esmail Zanjani, Veterns Administration Hospital, Reno, Nevada, submitted two "pre-protocols" for *in utero* gene transfer. These two proposals were submitted for the purpose of stimulating debate on this topic and as a "first step" in identifying the scientific, safety, ethical, legal, and societal issues raised by these novel applications of gene transfer research. These are preliminary proposals intended to provide a context for discussion, therefore, there will be no votes during the meeting.

Dr. Mickelson noted that a number of *ad hoc* experts were invited to participate in this discussion. She introduced the following experts: (1) Rebecca Buckley, M.D., Professor of Pediatrics and Immunology, Duke University, Durham, North Carolina; (2) Alan R. Cohen, M.D., Chief, Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; (3) Albert Deisseroth, M.D., Ph.D., Chief of Medical Oncology, Yale University, New Haven, Connecticut; (4) Evelyn Karson, Ph.D., M.D., Director, Division of Reproductive Genetics, Columbia Hospital for Women, Washington, D.C.; (5) Leroy Walters, Ph.D., Director, Kennedy Institute of Ethics, Georgetown University, Washington, D.C. (and a former RAC Chair); and (6) Doris T. Zallen, Ph.D., Professor of Science & Technology Studies and Humanities, Virginia Polytech Institute, Blacksburg, Virginia (and a former RAC member). The following two *ad hoc* consultants submitted written reviews but were unable to attend today's meeting: (1) Lori Andrews, J.D., Professor of Law, Chicago-Kent College of Law, Chicago, Illinois; and (2) Robertson Parkman, M.D., Head, Immunology/Bone Marrow Transplantation, Children's Hospital of Los Angeles, Los Angeles, California.

II. Introductory Remarks/Dr. Lana Skirboll, Associate Director for Science Policy

Dr. Lana Skirboll, NIH Associate Director for Science Policy, introduced Amy P. Patterson, M.D., as the new Director, Office of Recombinant DNA Activities (ORDA). Dr. Skirboll announced that Dr. Patterson will assume her new position on October 11, 1998. Dr. Patterson is a Harvard graduate and received her M.D. degree from Albert Einstein College of Medicine. Dr. Patterson completed her internship and residency training in internal medicine at the Memorial Sloan Kettering Cancer Center and New York Hospital-Cornell Medical Center, where she subsequently served as Assistant Chief Resident. Dr. Patterson completed medical staff fellowships in adult and pediatric endocrinology and metabolism at the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Child Health and Human Development, NIH. She completed a senior staff fellowship in molecular biology and genetic testing at the National Heart, Lung, and Blood Institute (NHLBI), NIH. She subsequently joined the staff of the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), where she currently serves as a Medical Officer in the Division of Clinical Trial Design and Analysis and as interim Deputy Director, Division of Cellular and Gene Therapies, CBER, FDA. Since 1993, Dr.

Patterson has served as a Clinical Staff Physician with the NHLBI. Her clinical responsibilities include the evaluation of patients with inborn errors of lipoprotein metabolism. Dr. Patterson directs a laboratory unit for research in developmental control and hormonal modulation of messenger RNA editing and apolipoprotein B gene expression and is a co-principal investigator on a research program involving targeted gene correction of genomic DNA in mammalian cells.

Dr. Skirboll noted that Dr. Patterson brings valuable clinical experience, understanding of basic science of gene therapy, and development of the national xenotransplantation policy to her new position. Dr. Skirboll stated that Ms. Knorr who has been Acting Director, ORDA, since the former director, Dr. NelsonWivel left NIH in June 1996, will become the Deputy Director, of ORDA. Dr. Skirboll applauded Ms. Knorr's professional stewardship of ORDA through an intense period of change during the last two years.

Dr. Skirboll noted that U. S. Senate appropriations language strongly recommends that theNIH restore the RAC's mandate to approve or disapprove human gene transfer protocols. Prior to making the decision to relinquish such approval authority solely to the FDA, the NIH Director established two *ad hoc* review bodies to evaluate the state of the field and the activities of the RAC. The recommendations of these two committees were predicated on two principles: (1) the RAC must continue to serve as a forum for public discussion of novel gene therapy issues and applications, and (2) ORDA will continue to serve as a public repository of historical information and scientific archives related to recombinant DNA research, including maintenance of a publicly accessible database of human gene transfer clinical trials. The NIH Director's proposals underwent a lengthy process of public notice and comment over a period of 18 months. In the end, theNIH Director proposed two substantive changes to the RAC: (1) implementation of Gene Therapy Policy Conferences (GTPCs) in order to enhance the depth and value of public discussion relevant to scientific, safety, social, and ethical implications of gene therapy research, and (2) the RAC would no longer be required to make a recommendation to theNIH Director regarding the approval or disapproval of human gene transfer protocols.

Dr. Skirboll stated that theNIH has been carefully monitoring the RAC's activities subsequent to implementing these changes. The GTPCs have proved to be enormously successful promoting the identification and early deliberation of novel gene transfer technologies and ethical issues. In addition, GTPCs have the potential to serve as a mechanism to ensure the public that novel clinical trials will have the advantage of full public discussion prior to receiving FDA authorization to proceed. She acknowledged that research subjects participating in novel human gene transfer research protocols depend on the primary oversight of two bodies: (1) the FDA, and (2) the local Institutional Review Board (IRB). Together, these two entities have the responsibility and authority to prevent a protocol from beginning or to stop one once initiated, if necessary. The public, IRB, and the FDA all benefit from public discussion and subsequent RAC recommendations before patients are entered onto novel gene transfer clinical trials.

To that end, Dr. Skirboll announced that the FDA and NIH plan to initiate a process that will seek to obtain full public discussion of all novel human gene transfer protocols before any patient is entered on such trials.

III. Minutes of the June 18-19, 1998, Meeting/Macklin, Ando

The RAC approved a motion made by Dr. Gordon and seconded by Dr. Juengst to accept the minutes of the June 18-19, 1998, RAC meeting (with the incorporation of minor editorial changes) by a vote of 10 in favor, 0 opposed, and no abstentions.

IV. Chair's Statement Regarding *In Utero* Gene Transfer Discussion/Mickelson

Dr. Mickelson explained the intent and format for discussion of Dr. Anderson's "pre-protocols." On July 31, 1998, Drs. Anderson and Zanjani submitted the following two "pre-protocols" for *in utero* gene transfer entitled: (1) *In Utero Gene Transfer for the Treatment of ADA-DeficientSCID* and (2) *In Utero Gene Transfer for the Treatment of α-Thalassemia*. These two "pre-protocols" are intended to provide a context for the identification of the scientific, safety, ethical, legal, and social issues raised by *in utero* gene transfer. RAC discussion of these pre-protocols should not be considered as an endorsement of *in utero* gene transfer research, but rather, a first step in identifying substantive public policy issues. In doing so, the RAC continues to serve as a unique public forum for the discussion of science, safety, and ethics of recombinant DNA research. In his cover letter, Dr. Anderson outlined three primary questions for RAC consideration: (1) Are the proposed protocols appropriate for clinical application? (2) Are the two proposed diseases appropriate as initial candidates for *in utero* gene transfer? (3) Is the risk-to-benefit ratio appropriate for both the pregnant women and the fetus for any *in utero* gene transfer clinical protocol as this time? Dr. Anderson stated that if the RAC is not convinced that the approach is valid and justified, he will not continue with development of *in utero* clinical protocols.

Dr. Mickelson noted that the RAC discussion is to identify issues that will be further deliberated by the working groups to be established prior to the upcoming GTPC entitled: *Prenatal Gene Transfer: Scientific, Medical, and Ethical Issues*, planned for January 1999. Relevant policy issues will be discussed as a prelude to any pertinent modifications to *Appendix M. Points to consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)* of the *NIH Guidelines*. The RAC discussion will develop guidance for investigators and institutional review bodies, e.g., Institutional Biosafety Committees (IBCs), IRBs. The RAC discussion will provide a forum for public dialogue on relevant issues, and initiate and coordinate interagency discussions and policy development.

The format of the RAC discussion will start with general background issues of fetal immune system development, *in utero* hematopoietic stem cell transplantation, risk assessment, preclinical research design, clinical research design, ethical, legal, social, and informed consent issues. To be followed by a discussion of specific issues raised by the proposals.

Abstract of Proposals

Drs. Anderson and Zanjani, submitted two pre-protocols for RAC discussion:

(1) *In Utero Gene Transfer for the Treatment of ADA-DeficientSCID*

Severe combined immunodeficiency (SCID) due to deficiency of the purine metabolic enzyme adenosine deaminase (ADA) is a fatal childhood disease. Immune reconstitution by transplantation with human leukocyte antigen (HLA)-identical bone marrow is the treatment of choice. Patients not candidates for bone marrow transplantation are treated with the enzyme replacement polyethylene glycol-conjugated bovine ADA (PEG-ADA). Some patients have been treated with either neonatal or postnatal somatic cell gene transfer. Although progress has been favorable for patients treated with gene transfer, they remain on PEG-ADA because the number of gene-corrected cells does not appear to be sufficient to maintain a normal immune system. However, PEG-ADA is very expensive; and the shots are painful. Therefore, improved gene transfer techniques are being investigated. If an inherited genetic defect is diagnosed early during pregnancy, it may be more beneficial to deliver correct genetic information *in vivo* during fetal development. During fetal embryogenesis, the majority of the fetal hematopoietic stem cells are in a stage of rapid cycling, and the fetal immune system is still immature. Thus, it is possible for retroviral vectors to transduce many cycling cells that are stem/progenitor cells, and it is less likely for there to be

immunological problems even when the gene is delivered.Recent research in a large animal (sheep) model has demonstrated that fetal tissue *in utero* is accessible for gene transfer using direct intraperitoneal (i.p.) injection of retroviral vector. The investigators propose to treat ADA-deficientSCID by i.p. delivery of a retroviral vector carrying a normal human ADA gene to the developing fetus at 13-15 weeks of gestation. The investigators are constructing new vectors which will contain a human ADA gene regulated by an ADA locus control region (LCR) on a self-inactivating (SIN) vector backbone (only 35 base pairs remain undeleted of the viral U3 region). After selection of the most efficacious vector by tissue culture and mouse *in vivo* studies, the vector will be tested in sheep. If sheep studies demonstrate that the new vector is safe and efficient for *in utero* gene transfer, studies in nonhuman primates will be carried out, and a formal clinical protocol will be submitted to the RAC and FDA.

(2) *In Utero Gene Transfer for the Treatment of α-Thalassemia*

Homozygous α-thalassemia (Hb Bart's hydrops fetalis) is a severe genetic disease caused by the mutation or absence of all four α-globin genes. This disease is not only lethal for the fetus *in utero*, but produces toxic symptoms in the pregnant woman. Although various types of *in utero* therapy have been attempted, none have been successful. Therefore, a new approach for developing a prenatal treatment procedure is warranted. In the fetus, most cells (including thehematopoietic stem cells) are rapidly dividing and are excellent targets for gene transfer. The vector of choice is a retrovirus because retroviral vectors integrate into the target cell genome and can remain throughout the life of the cell. Studies in large animal models (sheep and monkeys) have demonstrated that it is possible to remove a small amount of blood from the second trimester fetus, incubate the blood cells with a retroviral vector, and transplant the gene-engineered cells back into the fetus relatively safely and efficiently. Long-term follow-up has shown that marker genes can continue to be expressed for at least 2 years after birth. The investigators propose to treat homozygous α-thalassemia by removing 1-2 ml of blood from the 17-20 week fetus, incubating the cells with a retroviral vector construct containing the α-globin gene under the control of globin regulatory sequences, and transplanting the gene-engineered hematopoietic cells back into the fetus. New retroviral vectors are being developed that have various safety features to reduce risk to the fetus. The investigators would test these new vectors in sheep and nonhuman primates. If sheep studies demonstrate that the new vector is safe and efficient for *in utero* gene transfer, studies in nonhuman primates would be carried out and a formal clinical protocol submitted to the RAC and FDA.

Summary of Written Review by RAC Members

Dr. Ando

Dr. Ando stated that ADA is appropriate for consideration, but the risk/benefit ratio for targeting ADA appears to be negative, partly because PEG-ADA is effective although it is expensive and inconvenient. Researchers need more data on the effects of high doses of purified vector and the potential adverse effects, e.g., birth defects and germ-line transfer. Long term gene expression in peripheral blood appears to be low but persistent. This suggests that higher doses of purified vector may be more efficacious, but may have greater biodistribution and tissue transduction. In the case of α-thalassemia, the risk of inadvertent germ-line transfer and widespread vector biodistribution is minimal. The risk/benefit ratio for a fatal disease such as α-thalassemia appears to be positive. Therefore, the initiation of human clinical trials is appropriate.

Dr. Chow

Dr. Chow stated that the ADA studies in sheep suggest that risk to the fetus is not trivial (5 of 29 animals died prior to term, possibly due to the procedure performed). There is not enough knowledge to assess

specificity and efficacy or to assess the risks of the proposed procedures to either the fetus or pregnant woman. Furthermore, because the vectors are yet to be constructed and tested, efficacy is far from predictable. However, α-thalassemia is a good choice for *in utero* gene transfer (in theory) because there are no other existing treatments available for this fatal disease. There are several questions, both ethical and scientific, which must be satisfactorily addressed: (1) Is this what society wants, rather than helping born patients? (2) Is the timing appropriate for the proposed procedures? (3) If a partial correction results in toxicity to the pregnant woman, would there be an opportunity for an abortion? (4) What happens if a partial correction sustains gestation but cannot sustain life after birth? What will the treatment be and who will pay? (5) Could the burden on the family be far greater than aborting the fetus? (6) What is the risk of obtaining fetal blood at 17-20 weeks of gestation, and again at 24 weeks of gestation?

Dr. Gordon

Dr. Gordon stated that for both proposals, there are concerns about safety, efficacy, and ethics. For ADA, safety concerns include the risk of insertional mutagenesis leading to developmental disorders or malignant disease, possibly teratogenic effects of retrovirus gene expression, and insertion of the exogenous vector into germ cells. Efficacy questions involve efficiency of gene transfer, levels of expression, and the requirement for sustained expression. Ethics concerns include: (1) the possibility of disturbing the function of the endogenous gene and possibly causing disease; (2) the necessity of following patients throughout their lives, which may create a burden for the patient and family; (3) the unknown risk that retrovirus expression poses for the fetus; and (4) an effective treatment with known safety aspects already exists for ADA deficiency. Dr. Gordon stated that germ-line integration is a major concern: "In view of the fact that germ cell integration would lead to the presence of the retrovirus in every cell of subsequent progeny (a situation associated with increase disease risk), and the fact that the new sequences could persist for many subsequent generations, this reviewer must respectfully disagree with any doctor or patient that germ-line transmission would be a benefit [of this procedure]." There are several concerns for α-thalassemia: efficiency of expression, duration of expression, safety of retroviral insertion during fetal life, and whether gene transfer would lead to effective treatment. It is possible that gene transfer might allow fetal survival, but not be sufficient to prevent developmental anomalies; therefore, the present protocol is unsafe and unsuitable as an approach for therapy for thalassemia.

Dr. Greenblatt

Dr. Greenblatt had no objections *per se* to the proposed *in utero* gene transfer procedures. He was, however, unconvinced that these two specific diseases are the most appropriate initial candidates for *in utero* gene transfer. There are insufficient preclinical data to assess the risk/benefit ratio for the pregnant woman and the fetus. There are insufficient data to evaluate whether inadvertent gene transfer to the germ-line is a benefit or risk; the risks and benefits of *in utero* gene transfer are not sufficiently understood. For ADA, there is a question whether researchers know enough about the regulatory elements of the ADA gene to ensure that unregulated gene expression in tissues other than hematopoietic cells will not result in serious adverse effects. The α-thalassemia protocol is inappropriate because of the certain fetal fatality: "I would prefer to see *in utero* gene transfer reserved for genetic diseases in which the fetus comes to term and in which a non-functioning gene results in a disease that cannot be corrected after a child is born. I do not think it should be used to treat a fetus that would not normally come to term."

Dr. Juengst

Dr. Juengst stated that both pre-protocols raise two questions against the backdrop of *Appendix M* of the *NIH Guidelines*: (1) Because *in utero* research will always involve a pregnant woman and her fetus, who is the subject of the research? If the pregnant woman is the subject, what is her *in utero* disease or

disorder? *Appendix M* may need some new way to classify research in which pregnant women are volunteering to become involved for the sake of a fetus. (2) What does prevention mean? It is not correct for either protocol to promise "to prevent all manifestations of the disease." In diseases that are ordinarily fatal *in utero*, a partially successful intervention might not prevent suffering in the patient at all, but only make suffering possible. "If the goals of the exercise are to interrupt a family's legacy of prenatally fatal genetic disease, or to prevent suffering on the part of the victims of that legacy, it would be hard to justify the risks of gene transfer interventions over the relative certainty and efficacy of a reproductive risk reduction strategy involving genetic screening and selective implantation or abortion."

Dr. King

Dr. King stated that because *in utero* research involves both the pregnant woman and the fetus, two separate consent forms are needed. The justification for enrolling subjects who are unable to make their own decisions about research must be articulated. For both protocols, benefits and risks need to be discussed more clearly. There are risks of fetal death and premature labor from both the necessary prenatal diagnosis and the intervention. If the intent to affect the germ-line is off limits, unintended germ-line effects may be off limits. "The impact of possible germ-line effects, and the implications of making an end run around objects to direct intentional GLGT (germ-line gene therapy) by means of *in utero* gene transfer, must also be considered in the harm-benefit examination." The nature and implications of any potential germ-line effect are not clear. At this time, neither disease is clearly appropriate for *in utero* research.

Dr. Macklin

Dr. Macklin stated that from an ethical perspective, there is no reason why *in utero* gene transfer research should not proceed. The potential for inadvertent germ-line integration, however, is relatively high and presents an ethical problem: "We are left with a quandary arising from the current prohibition on intentional germ-line gene transfer research. If gene transfer to the germ-line is likely, and if it occurred would most likely be beneficial, should the proposed research go forward on the grounds that germ-line transfer is unintended, albeit foreseen?" For α-thalassemia, the comparative risks are the certainty of death *in utero* versus the possibility of an increased likelihood of cancer at some point. If the probability of a gene-engineered cell developing into a cancer cell were quite high, it would be unethical to treat α-thalassemia with *in utero* gene transfer.

Dr. Markert

Dr. Markert stated that ADA is not a good choice, α-thalassemia is potentially a good choice for *in utero* gene transfer. More data must be presented for α-thalassemia, to show that gene transfer will not convert a disease that is fatal *in utero* to a disease that is fatal in the first year of life. The investigators should provide data on several aspects of the proposed research: (1) the method and risks of prenatal diagnosis of the disorder; (2) a time frame in which *in utero* diagnosis can be attempted; (3) a time frame for discussion of *in utero* therapy with the parents after diagnosis; (4) the possibility of performing HLA typing on the fetus in case there are relatives who could act as bone marrow donors; (5) a discussion of the issue of tolerance as it relates to the particular disorder and the mutations of the patient; (6) the rationale for injecting the vector or vector producing cells versus transducing hematopoietic stem cell (HSC) *ex vivo*, and the risks of the procedure selected; and (7) the risks to the fetus and the pregnant woman if *in utero* treatment results in low expression and the infant survives to term but remains profoundly affected and dies after an indeterminate period of suffering.

Dr. McIvor

Dr. McIvor stated that for both protocols, the most formidable practical challenge will be achieving efficient gene transfer. For ADA, assuming that the gene can be efficiently introduced intoHSC *in utero*, there is the problem of achieving sufficient and long-term ADA gene expression. Even though the researchers propose a plan to restore natural regulatory elements, there is no guarantee that a vector restoring these elements will necessarily function predictably in the *in utero* setting and through the process of hematopoietic cell differentiation. Will transfer and expression of the ADA gene actually benefit the patient? It appears that there is not a high degree of risk associated with the recombinant DNA aspect of the procedure, notwithstanding broad societal considerations of inadvertent germ-line transfer. For α-thalassemia, there are a number of questions: (1) How will the cells in the blood sample be processed, and what incubation conditions will be used? (2) How many target cells are anticipated to be present in the sample? (3) How and where will the cells be reinfused into the fetus? (4) Will there be sufficient transduced stem cells in the sample to allow engraftment in the fetus? (5) What level of engraftment will be necessary for effective treatment of the disease?

Dr. Mickelson

Dr. Mickelson stated that by addressing issues raised in these pre-protocols, the RAC can begin to develop clear guidelines to assist investigators in this field. In determining the viability of *in utero* gene transfer, one criterion might be to assess the deficiencies or failure of existing treatments for life threatening disorders. "The disorder should impact fetal development and *in utero* genetic intervention should offer the most effective remedy. In addition, animal studies should present evidence of a consisten and reproducible gene-based procedure that shows therapeutic efficacy." Special concerns for *in utero* studies include: (1) effects of low level transduction efficacy, (2) potential effects of genetic intervention on the response to other treatments, (3) frequency of inadvertent transduction of other tissues, (4) potential fo insertional mutagenesis, (5) similar behavior of the vector in fetal animals and adults, and (6) potential damage from follow-up procedures. For α-thalassemia, there is no merit in attempting an *in utero* intervention if the level of efficacy is so low that it only extends the time to fetal death. In contrast, ADA-SCID, is a better first experimental model, due to the fact that PEG-ADA treatment exists and may b able to augment low transduction efficacy. Failure of the in utero procedure would be less likely tolead to a catastrophic event in ADA-SCID than in α-thalassemia.

Summary of Written Review by *Ad hoc* Reviewers

Ms. Andrews

Ms. Andrews stated that *in utero* gene transfer should require greater proof of safety and efficacy than postnatal gene transfer for several reasons: (1) society tends to value fetuses over the pregnant women, there is a chance that *in utero* treatment will be ordered over the refusal of the pregnant woman; (2) parents have the option of abortion, the benefits of *in utero* gene transfer needs to be described fully when the only other option is life with a child with a serious illness; (3) the debatable question of whether the therapy has any chance of causing sterility in the offspring; (4) it seems premature to move to an obstetrician office setting beginning with the third patient, and this setting may give the pregnant women who are potential participants the idea that this procedure is much more routine than it is. The fact that α-thalassemia is fatal *in utero* raises additional responsibilities for the researchers. This situation is similar to couples who tried experimental *in utero* surgery and have felt that they had been made worse off. Scenarios of "partial cure" should be discussed.

Dr. Zallen

Dr. Zallen stated that a central question needs to be addressed: Does the risk/benefit ratio justify proceeding with these two types of *in utero* gene transfer? Because the vectors have not yet been constructed or tested, one cannot determine the overall balance of risks and benefits; therefore, it is not appropriate to proceed with either protocol at this time. Intervention *in utero* would certainly be justified if irreversible damage to fetal development occurs during gestation, and the intent of the gene transfer is to intervene early enough to reduce or eliminate the harmful effects of the disorder. α-thalassemia meets the above criteria; however, the situation is less clear for ADA. The costs of PEG-ADA and the pain of injection are not sufficient reasons to warrant an attempt at *in utero* gene transfer.

Furthermore, potential subjects must be informed regarding the risks, benefits, burdens, requirements, and costs of the research. The existence of such a protocol should be made known during genetic counseling sessions, and the option of participating in the experiment should be broached before genetic diagnosis. The optimal consent process has to be planned with as long a lead time as possible, and there must be an identified set of neutral resource people centrally involved in any consent process. In addition to the pregnant woman's own physician, this should include an expert in fetal medicine who is free from any institutional, intellectual, or interpersonal conflict of interest.

Dr. Karson

Dr. Karson stated that *in utero* therapy offers significant relative advantages for α-thalassemia, but the reasons for *in utero* treatment for ADA are not as compelling. Regarding intended targets, the worst case scenario is that not only is there is a high potential for all other types of cells, possibly including germ cells, to be transduced coupled with very inefficient transfer of the gene into the true target. A major concern is that a higher multiply of infection would only lead to increasing the transduction of non-HSC-fetal cells and possibly the fetal germ-line, and the amount of virus that would circulate to the placenta and possibly cross back into maternal circulation. None of the published animal studies begin to predict the percentage of target HSC that will be transduced. More studies are needed on the maximal safe volume for injection at this early gestational stage. Because autopsy information is necessary, participants who do not consent to an autopsy should be excluded from participation. There is no need to conduct the injection in an operating room or intensive care unit unless the pregnant woman is to receive sedation or anesthesia to reduce fetal movement; sterility of the room is not needed. Monitoring of the fetus should be conducted in an outpatient setting with optimal ultrasound equipment.

Dr. Parkman

Dr. Parkman stated that present studies from the cord blood ADA trial indicate adequate expression of the transduced ADA gene; therefore, a new vector to obtain a selective advantage during thymic differentiation is not necessary. "Germ-line transmission would be a public health hazard; this issue needs to be addressed in greater detail including probability statements concerning the likelihood of germ-line transmission." Dr. Parkman agreed with Dr. Anderson that the experimental nature of *in utero* gene transfer dictates a new ethical framework that is different from the framework of the past three decades in which the risks and benefits were limited to the individual research subject. For α-thalassemia, the researchers do not propose to test their vectors in HSC or in erythroid progenitors from patients with this disease; this testing is very important. Central to the proposed transduction of fetal HSC is their cycling status. A clear documentation of the expected frequency of transduced HSC derived from blood is necessary. The proposed approach will not be likely to achieve clinical benefit unless there is a significant increase in the fraction of HSC transduced.

Dr. Deisseroth

Dr. Deisseroth asked why was ADA chosen as a candidate for correction instead of a genetic condition that is lethal and for which there is no established therapy? Would the proposed treatment be more successful if a transgene were introduced into the vector that produced a selective advantage to stem cells? α-thalassemia may not be the most appropriate choice for the first study of intrauterine genetic therapy because the expression of the transgene must occur in a highly regulated differentiated tissue. An alternative to α-thalassemia is the treatment of a constitutional disorder that involves a single gene, the expression of which need not be highly regulated for the therapy to be successful. Dr. Deisseroth raised several specific questions about the choice of vectors in the ADA proposal and about stem cell biology in the α-thalassemia proposal.

Dr. Cohen

Dr. Cohen, a specialist in thalassemia, focused his review primarily on medical aspects of treating this disease. Dr. Cohen stated that homozygous α-thalassemia is almost always fatal to the fetus (only five infants have survived the neonatal period), therefore, it seems to be an obvious target for gene transfer during gestation. Gene transfer *in utero* has several advantages, but it requires several critical studies to demonstrate erythroid-specific expression, long-term expression, adequate expression to correct the disease, and the absence of over expression that could result in β-thalassemia. On the negative side, the proposed time of intervention (17-19 weeks of gestation) may be too late or ineffective, and it is quite possible that "successful" *in utero* gene transfer at this stage may not alter congenital anomalies or the developmental problems. It will be necessary to identify options available to the family if gene transfer *in utero* fails. The hematologic and immunologic advantages must be weighed carefully against the disadvantages of an uncertain outcome for the fetus. The risk/benefit ratio for the fetus depends on how one characterizes the risk of stillbirth, early neonatal death, or a lifelong transfusion-dependent disease if gene transfer fails. The risk/benefit ratio for the pregnant woman raises new and difficult questions; successful gene transfer would probably correct conditions responsible for much of the maternal morbidity, but unsuccessful gene transfer might increase risks to the pregnant woman as the pregnancy progresses or labor and delivery begin. An important concern is who will enter the clinical trial and what will be conveyed to the family regarding "standard" management.

Dr. Buckley

Dr. Buckley stated that the *in utero* gene transfer may not be appropriate for ADA because the procedures appear to have more potential disadvantages than potential advantages, and more risk. The advantages in choosing ADA for *in utero* gene transfer are: (1) considerable experience in using retroviral vectors, with no observed side affects, (2) success would be easy to determine, (3) the infant would not have to receive either PEG-ADA or chemotherapy. Disadvantages in choosing ADA are: (1) ADA deficiency does not produce irreversible changes by birth, (2) postnatal T cell depleted, haplo-identical bone marrow stem cell transplantation is effective, (3) gene transfer has thus far failed in treating this disease, and (4) definite risks exist for invasive procedures *in utero*. Moreover, the difficulty of performing intraperitoneal injections in 14-15 week fetuses is unclear, and the risks to the fetus are unknown. There is no way of knowing whether it is safe to perform this procedure as an outpatient until several fetuses have been injected. It is unknown whether there is any risk to the mother. A better option than ADA would perhaps be an immunodeficiency disease for which pretransplant chemotherapy is required.

Dr. Walters

Dr. Walters raised several questions regarding the ADA-SCID pre-protocol. How will at-risk couples and fetuses be identified? How successful or unsuccessful have been the stem-cell interventions with newborns? What is the current track record of late mid-trimester bone marrow transplantation? Will a

genetic counselor with no direct connection to the research team be available to inform the subjects? Will post-intervention assays be performed to determine the outcome of retroviral vector gene transfer? Would a post-intervention abortion be recommended to the pregnant woman? How convincing are the data from the sheep study regarding germ line gene transfer? What are the best candidate diseases for *in utero* gene transfer?

Dr. Walters raised several questions with regard to the _thalassemia protocol. During what gestational time frame would this procedure be performed? Why is the *ex vivo* instead of *in vivo* gene transfer procedure used for this study? Why are stem cell transplantation procedures not completely successful? Will evidence of success after the transplantation be assayed? If the intervention is unsuccessful after the last attempt, will the pregnant woman be recommended to terminate the pregnancy? Is there any animal model for α-thalassemia? Is there any successful approach with any type ofthalassemia? Will a neutral genetic counselor available to advice the subjects? Why is α-thalassemia an appropriate candidate for *in utero* gene transfer?

Ms. Levi-Pearl

Ms. Levi-Pearl stated that the social and political climate today coupled with sensational stories reported in the news media provide a number of misleading, incorrect, and frightening stories about germ line intervention on animals and plants. She was concerned about the potential reactions of the public and lawmakers about the proposed gene transfer on human fetuses. She noted the importance of a concerted effort to educate the public before any attempt at *in utero* gene transfer trial.

Food and Drug Administration

FDA provided extensive written comments on the two pre-protocols. Comments were made and questions were raised in several aspects of the pre-protocols, i.e., manufacturing and quality control issues, preclinical issues of safety, rationale, and efficacy, and clinical issues.

V. Development of the Human Fetal Immune System -- Overview/Buckley

Dr. Buckley provided an overview of the development of the human fetal immune system. The human fetus is immunocompetent at a very early age of gestation from data obtained from babies infected with syphilis during the first trimester. In the sheep animal model at Day 35 gestation age (gestational period is 150 days), there is evidence of immune system function, i.e., antibody response to antigens shortly after that there is skin graft rejection. Both antibody and T-cell responses in the fetal lamb develop very early. In humans, at 2 ½ to 3 weeks of gestationhematopoietic stem cells begin to migrate from the yolk sac to the fetal liver. By 10 to 12 weeksof gestation, the primary immunologic organs including the bone marrow and the thymus have matured. There is seeding of stem cells from fetal liver to the thymus, spleen, lymph node, and bone marrow by 8 to 11 weeks. At the end of the first trimester, the primary lymphoid organs are fully developed.

Dr. Buckley concluded that by the end of the first trimester of pregnancy, there is evidence of maturation of the immune system. She noted from her experience of treating aSCID baby by transplantation of the fetal liver from a fetus of 10 week gestation, there was evidence of graft-versus-host disease, an indication of T-cell function. Similar graft-versus-host disease was noted by transplanting the thymus from a fetus of 14 weeks of gestation.

With respect to B-cell development, Dr. Buckley noted that by the 7th week of gestation, there have been

pre-B cells found in the fetal liver; and by the 10th week, there have been IgM positive B-cells in abortuses. *In vitro* studies of immunoglobulin synthesis by fetuses as young as 10 to 12 weeks showed production of IgM and IgE. Both antigen-independent and antigen-dependent B-cell development occur in the first trimester. Dr. Buckley showed data on timing of the development of various types of immunoglobulins.

Dr. Buckley noted that there is trafficking of maternal cells andimmunoglobulins across the placenta to the fetus. SCID babies due to the lack of immune rejection are oftenchimeric with maternal T-cells as early as the beginning of the second trimester.

VI. *In Utero* Hematopoietic Stem Cell Transplantation - Experience to Date/Buckley

The Outcome of most clinical trials has not been completely successful. There are only one or two survivors over the past 20 to 30 years. Recent trials in Italy and Chicago have had more success by using the father as the donor of the CD34+ cells forSCID babies diagnosed *in utero*. Two children are still alive. There still is disagreement about the advantage of prenatal vs. postnatalhematopoietic stem cell transplantation.

Dr. Gordon asked that if the fetus is immune competent at a very early age of gestation, it may not be possible to achieve tolerance to exogenously administered antigens. Dr. Buckley said there is very little human data on this question.

Dr. Ando asked if there is evidence of antibody responses to chronic viral infection in the fetus, e.g., Human Immuniodeficency Virus and hepatitis. Dr. Buckley said there is little data on this question.

Dr. Karson asked if there has been any attempt at stem cell transplantation with α-thalassemia babies. Dr. Cohen responded that there were three attempts of*in utero* transplantation for α-thalassemia, and none of these attempts were successful.

Dr. Markert asked about the potential risk of graft-versus-host disease inSCID infants. Dr. Buckley noted there is known chimerism with maternal cells; there is no ready test to determine*in utero* if there is graft rejection to father's cells.

Dr. Bruce Aronow, (Children's Hospital, Cincinnati, Ohio) asked in cases of *in utero* stem cell transplantation if the grafted T-cells have been verified to be of donor origin. Dr. Buckley responded that in the Chicago baby the T-cells were verified to be originated from the father.

VII. Risk Assessment - General/Ando

With regard to risk/benefit assessment, Dr. Ando outlined three issues: (1) risk assessment based on science and disease mechanism, (2) preclinical toxicological studies in tissue culture and animal models, and (3) Phase I clinical trial safety studies with dose-escalation levels.

Some of the issues to be considered under any assessment based on science includes the specificity of DNA integration and potential adverse consequences related to rearrangement, recombination, random integration, and replication-competent viruses. Regulation of gene expression in the developing fetus and immune response to the vectors are important issues. He noted that the proposed retroviral vectors employ a new kind of LCR promoter fortransgene expression; developmental regulation of the transgene expression is a novel issue.

With respect to toxicological studies, the issue is the appropriate animal model to assess the maximum tolerated dose and target organs of toxicity.

Data from human dose-escalation trial are important to assess the risk/benefit since animal data are not adequate.

Other RAC Comments

Dr. Gordon noted the potential for adventitious germ-line integration in the ADA-SCID protocol due to administration of the retroviral vector by intraperitoneal injection; there is little risk for germ-line integration in the α-thalassemia protocol. He suggested that the ADA-SCID *in utero* gene correction should be conducted by the less risky *ex vivo* gene transduction. Dr. Gordon noted the difficulty of assessing germ-line risk caused by intraperitoneal injection of retroviral vectors. Insertional mutagenesis may occur at a transcriptionally active region of chromatin, and it may have a significant impact on host gene expression.

Dr. Aguilar-Cordova cautioned that assessment of potential insertional mutagenesis in animal models may not adequately predict its impact on tissue specific stem cells and tissue specific gene expression in a developing fetus.

Dr. Mickelson noted that the proposed vector technology is of the 1980's vintage. Retroviruses are employed to insert genes at random positions of chromosomes without the precision of specific replacement of given genes by homologous recombination, a special concern for potential germ-line alteration.

Dr. Noguchi noted that a major concern is the unknown effect of retroviral insertion on a developing fetus; it could interrupt the normal cascade of gene expression during development. A recent animal experiment performed by an FDA scientist showed that even with precise homologous replacement of genes the replaced gene appeared to be turned off and had no expression.

Dr. Gordon would like to observe the data on how long-term gene expression by retroviral vector would be improved by the proposed use of the novel LCR regulatory elements. He noted again the difficulty of assessing the risk to children caused by insertional mutagenesis *in utero*.

Dr. Karson noted her experience of genetic counseling for pregnant women who participated in clinical trials of new drugs. The classic example is birth defects caused by thalidomide where the association is so striking that it can be concluded from the first 20 to 30 patients. A small effect would be difficult to measure without enrolling a large number of subjects. Dr. Karson noted that in the normal process of meiosis, there are tremendous numbers of errors in chromosome replication; about 20% of pregnancies will spontaneously miscarry as a natural means to eliminate faulty fetuses. It will be difficult to assess if any effect is caused by insertional mutagenesis. She suggested that experiments should be conducted on a large number of mice to determine if there is any higher incidence of birth defects or leukemia caused by retroviral vectors. Dr. Aguilar-Cordova agreed that a large mouse study may shed some light on the adverse effects of insertional mutagenesis.

Dr. Deisseroth said one approach to assess the risk of retroviral insertional mutagenesis is to observe whether there is any increase in the miscarriage rate above the normal rate due to natural error elimination. The same protective process may counter the adverse effects of retroviral insertion by eliminating the abnormal fetuses.

Dr. Karson said that she does not have a number for the error occurrences during meiosis. The loss rate of miscarriage for pregnancies that are clinically recognized is 20%; for all conceptions, the loss rate may be as high as 95 to 99%.

Dr. Greenblatt inquired about the outcome of a rodent study to assessinsertional mutagenesis caused by retroviruses conducted 7 years ago by the National Toxicology Program of the National Institute of Environmental Health Sciences (NIEHS), NIH. Dr. Anderson responded that this study was never initiated due to funding considerations.

Regarding the embryo loss rate, Dr. Gordon noted that in the*in vitro* fertilization procedures, the conception rate for embryo transfer is pretty high. The overall low conception that Dr.Karson referred to may result from low fertilization rate.

Dr. Noguchi noted that there is a report of a disease caused byinsertional mutagenesis from insertion of a natural transposible element into a specific gene.

Dr. Karson said the concern about insertional mutagenesis is not limited to the germ-line cells. Due to the potential long life span of the treated fetus,insertional mutagenesis of other target cells caused by intraperitoneal injection of the retroviral vectors is another concern.

Dr. Aguilar-Cordova noted that insertional mutagenesis caused by transposons is a rare event. Theoretically, it is of more concern using a high titer of retroviral vectors. Studies with small animals to obtain information of a quantitative risk is useful to provide the subjects with more definitive information to make a decision regarding a trial.

Dr. Markert said that the risk to the pregnant woman should be considered. The virus injected into the fetus is likely to transfer into the pregnant woman, and her immune response to the vector is of concern.

Public Comments

Ms. Wendy L. McGoodwin, Executive Director, Council for Responsible Genetics (Cambridge, Massachusetts), in her September 18, 1998, letter toORDA expressed her concern about the RAC considering the pre-protocols for*in utero* gene transfer that could result in alteration of the human germ-line. She cited that the current*NIH Guidelines* discourages the RAC from considering protocols involving germ-line modification. ORDA received a total of 80 letters objecting to Dr. Anderson's proposals; only 2 letters were in support of*in utero* gene transfer.

Dr. Mickelson called on individuals in the audience to present their comments.

Dr. Elizabeth R. Arnold (Institute of Science, Technology and Public Policy, Silver Spring, Maryland) presented comments developed by Drs. John S. Hagelin, Director, and John Fagan, Associate Director of her organization. She cited Dr. Anderson statement that "the likelihood is high that at least some of the germ line cells will be affected." She stated that such germ-line manipulations on humans, whether they are the primary purpose of an experiment or merely a probable consequence of an experiment, are illegal in most European countries. Similarly, the RAC has stated that it will not consider germ-line research at this time. The technology is risky to germ-line, and these protocols should be considered as germ-line intervention. The safety concerns posed by Dr. Anderson's proposals would take a moratorium of at least 10 years to resolve the issues of long-term safety concerns.

Dr. Sarina Grosswald (Natural Law Party of America, Fairfield, Indiana) encouraged the RAC to uphold

the policy against entertaining any germ-line human gene transfer research. In agricultural products for example transgenic technology has a danger of unexpected side effects. In humans, no one can predict how newly introduced genetic material will interact with other genes and influence cellular functioning. Present data are insufficient to make any definitive determination about this intervention's effect on the germ-line. Dr. Grosswald encouraged a moratorium of any pre-protocols for *in utero* gene transfer until further research can demonstrate the long-term effects and safety of this approach.

Investigator Response – Dr. Anderson

Dr. Anderson responded that the reason to come forth with the pre-protocols at this time is to raise the issue of a potential inadvertent germ-line transfer for public discussion. The discussion would benefit the investigators interested in fetal gene therapy. It is understandable that acknowledgment of a potential for a low level germ-line gene transfer should cause considerable concern. Dr. Anderson said that the only way to really face the issue is to have a public discussion well before any subjects are enrolled in the study, which is at least 2 to 3 years away.

Dr. Anderson explained that the Council for Responsible Genetics misunderstood his proposals as an intentional attempt at germ-line gene transfer toward the goal of designer babies. He said that his proposals are not for germ-line gene transfer *per se* although the potential risks for inadvertent germ-line alteration should be addressed. He wanted to have these issues discussed in public. Dr. Anderson stated that if the RAC determines that the level of potential risk for a germ-line effect is not acceptable to the public, he will not go forward with the proposed clinical trials.

Dr. Anderson stated that the National Toxicology Program of NIEHS has conducted pilot studies to determine insertional mutagenesis risk of retroviruses in mice. The rate of insertional mutagenesis in mice was found to be too low to be able to provide any measure of quantitative risk to the germ-line. So far many clinical trials involving large number of patients have found no side effects due to retroviral vectors; subsequently, the priority for funding of the NIEHS mouse studies have since dropped lower.

VIII. Preclinical Sheep Studies / Zanjani

Dr. Karson asked whether animal studies were conducted to determine if any vector or transduced cells were transferred from the fetus to the placenta or to the mother. Dr. Esmail Zanjani responded that three sheep studies involving 40 to 50 animals were performed. Among 20 animals examined, three sheep were found to have low levels of vector DNA present in the blood.

Dr. Zanjani said that some of the animals were re-bred. They and their offspring all appear to be normal as of today; the oldest animal was treated *in utero* about six years ago. Transgene expression is still detectable in these animals.

Dr. Anderson said once the new vectors with the LCR regulatory elements are developed, thorough toxicological studies will be performed. The vectors will first be tested in rodents and then in sheep and primates to obtain quantitative information about their transfer into the placenta and mother.

Dr. Karson asked whether the monkeys previously used in animal studies of retroviral vectors are still alive. Dr. Anderson said that he has lost track of those monkeys used in his experiments in 1990 while he was at NIH. However, some of the data obtained were published from those monkeys five years after gene transfer; none had detectable replication-competent retroviruses at the end of the five year observation period. Dr. Anderson planned to conduct thorough primate studies for the new generation of vectors. Dr. Karson said that it is worthwhile to track down the monkeys used in previous studies to

observe if there is any long-term side effects of retroviral gene transfer.

Dr. Noguchi said for *in utero* gene transfer partial success is unacceptable. He asked if brain functions, such as intellectual development and physical coordination have been examined in the sheep. Dr. Anderson responded it is difficult to perform such study in sheep. Dr. Aguilar-Cordova said some of the neurological functions can be studied in the mouse experiments.

As a point of clarification, Dr. Anderson said that direct vector injection into the fetus proposed for the ADA-SCID protocol has a potential risk of germ-line transfer; however, the *ex vivo* procedure proposed for the α-thalassemia protocol should not have this concern. Dr. Anderson emphasized that his proposals of these two candidate diseases are for the purpose of focusing the discussion of the *in utero* gene transfer issues. His commitment is to develop treatments for lethal genetic diseases not to be limited to only these two diseases.

Responding to public comments Dr. Gordon stated that he does not agree with the proposal of a ten year moratorium on *in utero* gene transfer. There are potential for beneficial outcomes *in utero* gene therapy, and the RAC needs to evaluate the science and the preclinical study in this area of research. There is nothing to be lost in the self-education process; the public discussion does not compel the RAC to agree that the pre-protocols need to go forward.

Dr. Gordon said that even direct exposure of embryos to retroviruses, does not yield a significant number of germ-line transfer unless the titer of the retrovirus is extremely high. The need is to develop more quantitative information of germ-line risks.

Responding to the comments of the impact of reproductive technology on human evolution, Dr. Gordon said that the biggest threat to human evolution is the normal reproductive process rather than any of the attempts of gene transfer on a few thousand children. The comparison to various agricultural genetic engineering experiments is misleading. Human population is genetically highly polymorphic and is distinct from some plant species developed by selective breeding; genetic changes introduced to a limited number of members of humans have little impact on the species as a whole.

IX. Preclinical Research Design Issues - General/Chow

Presentation - Dr. Chow

Dr. Chow noted that the proposals involved the use of safety-engineered "SIN" retroviral vectors in which the entire U3 sequence except for the first 35bp in the 3' long terminal repeat (LTR) of the murine retroviral vector was deleted. Thus, LTR promoter interference with internal promoter should be eliminated. New LCR sequences are used for regulating transgene expression. Efficiency of gene expression and safety issues, e.g., potential insertional mutagenesis, of these novel retroviral vectors should be assessed in animal models. How effective are the LCR regulatory elements in controlling tissue specific expression of the ADA gene? She said that the well-known phenomenon observed in retroviral vectors of gene shut-down after their administration to animals should be addressed; this phenomenon is pertinent to the present applications *in utero*. She suggested that the ADA-knockout mice be used as a model for testing the ADA-vector.

Other Comments

Dr. Mickelson noted that the ADA knockout mice is the only model for this disorder, but there are reported abnormalities of those knockout mice. Dr. Buckley noted another Jack3 knockout mouse model for SCID.

Dr. Gordon noted that for ADA-SCID protocol, the issue of tissue specific expression is not critical since systemic enzyme replacement therapy (without any tissue specificity) is effective in the newborn. A far more important concern is attenuation of expression using retroviral vectors; he would like to see data showing that the new strategy of vector design will overcome this obstacle. In the α-thalassemia protocol, the target red blood cells are highly specific and tissue specificity of gene expression is not a major issue.

Dr. Aguilar-Cordova noted that most studies of gene shut-down of retroviral vectors have been conducted in mice. Large animal models are needed for extrapolating the data to humans.

Dr. Karson suggested that long-term colony assay with human stem cells is useful for assessing long-term expression of the new vectors. Dr. Aguilar-Cordova said that Dr. Donald Kohn (Children's Hospital of Los Angeles, California) found that long-term expression in tissue culture did not correlate with expression *in vivo*. Dr. Karson said long-term cell culture assay can be used as a first step to screen the constructs.

Dr. Mickelson asked the investigators to explain what level of transduction efficiency is needed for these two pre-protocols, and what would be the outcome for these patient populations if sufficient gene transfer is not achieved.

Dr. Chow asked the investigators to address the gene dosage issue. There are four copies of globin genes in humans; what would be the outcome if only one of the four copies is corrected.

Dr. Mickelson asked if other human data are available to support moving to fetal gene transfer, e.g., studies using adults with heterozygous thalassemia who can give informed consent.

Dr. Buckley inquired about the data showing that stem cells in the fetus are more rapidly dividing than stem cells at birth. Dr. Karson said that her studies with cord blood cells performed at Dr. Anderson's former laboratory at NIH showed that in term of transduction by retroviruses, there is a difference of at least one order of magnitude between cord blood cells at birth and a fetus at 20 gestational weeks.

Investigator Response - Dr. Anderson

Dr. Anderson stated that he would address issues except specificity and efficiency of the vectors which his co-investigator will present later on. Dr. Anderson agreed with the suggestion for the use of long-term colony culture of human stem cells to investigate gene expression with the new vector constructs. He noted Dr. Zanjani's studies with sheep showed that the abundance of dividing stem cells in the fetus will improve the transduction efficiency.

Dr. Anderson responded to the question of transduction efficiency needed for efficacy, by relating the knowledge gained to the ADA-SCID protocols. In the ADA-SCID patients, all cells in the body are defective. Normally ADA is expressed in many different cell types, i.e., T cells, epithelial cells of the upper intestinal tract, tongue, esophagus, gastric mucosa, and small intestine. He found that one major problem in previous ADA-SCID protocols has been inadequate transduction of a sufficient number of cells to produce enough ADA to obviate the need for supplementary PEG-ADA. In Dr. Donald Kohn's neonatal studies with cord blood, three babies are doing well; PEG-ADA was withdrawn for a short period of time in one of the babies but was re-administered due to worsening of immune function. These shortcomings from the ongoing ADA-SCID studies prompted Dr. Anderson to consider the alternative of *in utero* gene transfer in order to improve transduction efficiency.

To evaluate whether the new generation of vectors employing the LCR promoters will improve long term

gene expression, Dr. Anderson said he will perform the appropriate animal studies.

For the ADA-SCID protocol, Dr. Anderson said any partial correction is beneficial; over-expression does not pose a risk.

Dr. Buckley noted that the outcome of gene transfer for ADA-SCID patients may have been adversely affected by PEG-ADA administration; PEG-ADA neutralized the survival advantage of gene-corrected T cells of the patients. Dr. Anderson agreed, and he explained that when the RAC approved the first ADA-SCID protocol, it required the investigators to administer PEG-ADA as a safety measure.

Regarding the α-thalassemia pre-protocol, Dr. Anderson stated that partial correction will have serious consequences. Homozygous α-thalassemia fetuses with defects in all four copies of α-globin genes normally die *in utero*, or the fetus is aborted at 24 weeks of gestation due to pre-eclampsia to the mother. Dr. Anderson said the major concern for partial correction is that it will allow the fetus to be borne with major health problems, and it might die within a short time after birth. Dr. Anderson stated that if he is allowed to proceed with the protocol, the first fetus would be carefully selected to minimize the outcome of partial correction. He would select a fetus that was diagnosed with α-thalassemia very early and was due to receive a blood transfusion starting at 12 weeks to ensure normal development. The gene transfer procedure would then be performed in addition to the transfusion therapy.

Before proceeding with the α-thalassemia protocol, Dr. Anderson said that the vector will be evaluated to determine the transduction efficiency in sheep and monkeys. However, there are no large animal models for α-thalassemia.

Responding to the question of whether correction of a single copy of the α-globin gene would be effective, Dr. Anderson said probably yes since hemoglobin H disease, where only one copy of the α-globin gene is functional, is not a serious clinical condition. One α-globin gene expressed in a sufficient number of cells should be able to "heal" to the extent that it would be beneficial for the patient.

Regarding the question of fetal stem cells, Dr. Zanjani said that all types of studies in sheep including cell cycle analysis, *in vivo* engraftment potential, cord blood vs. adult blood stem cells, suggested a higher percentage of stem cells in fetal blood compared to postnatal blood. In sheep, there is a wide window of opportunity for stem cell engraftment *in utero*, i.e., about 1 ½ to 2 weeks during 58 to 65 days of gestation. A similar window of opportunity exists in monkeys.

Dr. Zanjani explained that the reason for the presence of abundant stem cells in the fetal blood circulation is because of the natural ontogeny of hematopoiesis in the mammalian fetuses. Stem cells are believed to be migrating from the yolk sac to fetal liver, spleen, and finally to bone marrow via the general circulation. During this window of opportunity, stem cells are more primed for engraftment and gene transfer. Dr. Buckley noted a great deal of interest in using human cord blood as a source of stem cells. She asked if there is any quantitative estimate of the number of stem cells during the window of opportunity versus full term animals or humans. Dr. Zanjani responded that he has employed a human-sheep xenograft model to assay human hematopoietic stem cells in sheep. Human stem cells were taken from human fetal liver, cord blood, and adult bone marrow; and they were engrafted in sheep. The data showed higher level of engraftment of stem cells from fetal liver than from cord blood or adult bone marrow.

X. Clinical Research Design Issues - General / Markert

Presentation - Dr. Markert

Dr. Markert outlined the rationale for selection of candidate diseases with regard to the natural history, assessment for clinical efficacy, and ethical issues. The natural history is different depending on whether the diagnosis is made at birth or *in utero* at 3 to 9 months of pregnancy. A child, known to have SCID at birth usually from a prior child having been affected with the same disorder, may be placed in isolation to prevent opportunistic infections and to receive other medications; they usually have better prognosis.

Dr. Markert listed a series of diseases for which alternative therapies exist or the initial gene therapy experiments could be performed postnatally. For these disorders, *in utero* gene transfer should be considered after enough experience is obtained from postnatal gene transfer studies. These disorders are SCID including ADA-SCID, combined immunodeficiency due to purine nucleoside phosphorylase deficiency, Chediak Higashi syndrome, Wiskott Aldrich syndrome, chronic granulomatous disease, X-linked agammaglobulinemia, and osteopetrosis. Bone marrow transplantation is effective with ADA-SCID.

Dr. Markert listed a series of disorders that could be considered for initial *in utero* gene transfer. These are diseases that affect the fetus *in utero* such that treatment at birth is too late. These disorders include leukocyte adhesion deficiency Type I, Tay-Sachs disease, Type II Gaucher disease, infantile Krabbes disease (globoid-cell leukodystrophy), metachromatic leukodystrophy (infantile form), Type A Niemann-Pick disease, and α-thalassemia major.

In addition there is a group of diseases that could be treated at birth if there were a way to get the gene into the relevant cell. Due to this technical difficulty of the gene transfer procedure, *in utero* gene transfer may be necessary in order to get the gene into relevant cells, e.g., more abundant target cells in cell cycling in the fetus. These disorders include Lesch-Nyhan syndrome, Hurler syndrome, Niemann-Pick disease Type B and C, phenylketonuria, glycogen storage disease (GSD) Type I, and Pompe disease (GSD Type II).

Dr. Markert said that there are some diseases where there is a need to correct the fertilized zygote *in vitro*. In this situation, the germ-line would be affected. These disorders include Fanconi anemia and ataxia telangiectasia.

Dr. Markert said that the candidate disease chosen for study should be a serious disorder so that clinical efficacy can be assessed. The clinical design should enable the investigators to draw a definitive conclusion as to wether the gene intervention worked.

Dr. Markert pointed out three factors relevant for ethical consideration: (1) Should the disease be fatal *in utero* in order for it to be considered for *in utero* gene transfer? (2) Should the family be allowed to have the option of pre-implantation diagnosis and to select the normal zygote for implantation, or to opt for gene therapy *in utero*? (3) Each family has different degree of willingness to accept the anticipated risks and benefits for both the pregnant woman and the fetus. It is difficult for any third party to make such a decisio for the family.

Other Comments

Dr. Gordon said another less expensive option is chorionic villus sampling or amniocentesis followed by abortion if the fetus is affected. Dr.Markert agreed. Dr. Buckley noted that there are families that object to the option of abortion, for religious reasons to terminate the pregnancy. Dr. Gordon noted that social psyche is influenced very much by what is portrayed in the mass media or characterized by politicians; the discussion involving abortion issue belongs to this category. Abortion is a cost effective means to avoid giving birth to a child with a serious genetic disorder. A responsible physician should be able to

present to the patient this cost effective option, and the patient should have the right to accept or refuse the option.

Dr. Macklin agreed with Dr. Gordon's statement. She asked Dr. Markert to elaborate on what is considered as a serious disease for *in utero* gene transfer, i.e., about the distinction between dying, dying soon, dying later, or living with a horrible life condition. Dr. Markert said that there are many people alive now who are suffering from these diseases, and one should firstpreform gene therapy studies with people with postnatal therapies. Dr. Markert noted that diagnosis of most of the disorders at birth is a costly proposition although the technology is improving; most people find out they may have an affected child based upon their family history.

Dr. Noguchi noted Dr. Patterson outlined several ethical considerations relative to *in utero* somatic cell and gene therapies in her presentation at the December 1-2, 1994, RAC meeting. Dr. Noguchi said that *in utero* stem cell transplantation as well as gene transfer are highly invasive procedures. Very often the outcome of the interventions cannot be determined until the child is born. Only diseases that have demonstrated fetal damage *in utero* should be considered.

Dr. Karson noted that about 80% of children born with cystic fibrosis are with families who did not know they were at risk; it is valuable to have pre-natal screening tests. On the other hand, most people with Tay-Sachs disease are aware of the risk and that prenatal testing is available. In the case ofTay-Sachs disease, there is high chance of partial correction via *in utero* gene transfer leading to prolonged suffering, an outcome some regard as ethically unacceptable. A similar concern exists for the α-thalassemia pre-protocol.

Dr. Buckley noted that there is a screening test forSCID that costs $41 and can detect all newborns with SCID; it is feasible to screen forSCID babies in order to receive early treatment.

Dr. Patterson pointed out additional clinical trial design issues raised by the RAC in its 1995 discussion of *in utero* stem cell transplantation. What types of clinical endpoints (pre and postnatal) should be selected? What are the implications and limitations of assessing prenatal endpoints? She noted the limitations of data obtained *in utero* for guiding the clinician regarding the need for either booster stem cell dosages or booster vector injections.

Dr. Gordon said partial correction in some disease may be beneficial, e.g., in cystic fibrosis patients partia correction of lung epithelial cells may relieve the major symptom of the disease.

Dr. Cohen noted that for α-thalassemia, a simple screening test is available to identify a couple at risk before their first pregnancy. For the less severe β-thalassemia, a screening test can identify a child with the disease at the time of birth but well in advance of the phenotypic manifestations of the disease.

Dr. Ando noted that there are limited preclinical studies about the functioning of theLCR expression cassettes; it is prudent to first try these novel vectors in a postnatal gene transfer study before attempting any *in utero* application.

XI. Ethical, Legal, and Social Issues (ELSI) in Prenatal Gene Transfer - Overview/Walters

Dr. Walters said that the field of ethics can be most helpful to raise questions. His remarks were presented in six catergories.

(1) The precedent of 1987. In April of 1987, Dr. Anderson brought his preclinical data to the RAC for

somatic gene therapy for ADA-SCID . The RAC had several meetings in 1987 to deliberate about this protocol. It was almost 3 years later that the first gene therapy study began in 1990. Dr. Anderson has again brought his *in utero* gene transfer pre-protocols to the RAC well in advance of any clinical trial; Dr. Walters predicted it could take 3 to 5 years before any attempt is made at *in utero* gene transfer.

(2) Language or terminology. Dr. Walters said that more neutral and less emotional language is preferred when referring to gene transfer studies. He suggested that the more appropriate neutral terms are: *pregnant woman* not *mother*, *gene transfer* not *gene therapy*, *cell transplantation* not *cell therapy*, *subjects* not *patients*, *experimental procedure* or *intervention* not *treatment*.

(3) Long-term strategy. What is the best long-term strategy for the treatment of genetic disorders, e.g., ADA-SCID and α-thalassemia? There are three stages in which intervention is feasible, i.e., neonatal, prenatal (post-implantation and *in utero*), and preimplantation (diagnosis and selective implantation or selective discard; gene transfer). For most diseases, neonatal orpreimplantation are better choices for the stage at which to intervene; prenatal intervention should be considered as a backup measure. Particularly for α-thalassemia, preimplantation is the best stage to intervene not only to diagnose and select or discard the fetus but for potential gene transfer in the future.

(4) The role of genetic counselor. There should be a genetic counselor or a primary physician to discuss *in utero* gene transfer with the family. This neutral counselor, not directly involved with the study, can better assist the family in making the strategic decisions to participate in the clinical trial. And discuss other options such as preimplantation intervention, post-implantation and prenatal intervention, neonatal intervention, and other nonmedical options, (adoption or not having children)

(5) Germ-line effects on the fetus. As indicated from public responses to the pre-protocols, a potential germ-line effect is a lightning rod issue. Distinction can be made between intentional germ-line modification and germ-line modification as an unintentional or unintended side effect. Within the field of bioethics, there is a respectable minority viewpoint. In principle, that deliberate germ-line intervention to prevent disease is an ethically acceptable intervention. For this intervention to be acceptable, a better technique for targeted genetic intervention should be developed, e.g., homologous recombination to replace a defective gene with a normal gene. There is a distinction between intentional vs. unintended side effects on germ-line. Chemotherapy and radiation therapy have unintended side effects on the germ-line, and they are accepted as a price when undertaking these therapies. There should not be any difference in principle if it is *in utero* gene transfer rather than chemotherapy or radiation therapy. There is a need for calm rational analyses of various types of potential harm. The minimal kind of germ-line effect is that a silent gene has been added to the fetus treated *in utero* and there are no adverse effects of the added gene to any offspring; such an unintended germ-line effect should pose no serious problem. At the other extreme are second generation infants that have multiple congenital anomalies directly traceable to germ-line effects from *in utero* gene transfer. The preclinical studies in animals should address the question of whether any unintentional germ-line effects can adversely affect the offspring.

(6) The question of whether clinical trials of *in utero* gene transfer are appropriate now. Most of the reviews and public comments indicate that it is too early at present to attempt any *in utero* gene transfer in humans.

Other Comments

Dr. Macklin noted that the unintended germ-line effects can be a benefit, risk, or neutral to the offspring. Furthermore, some of the unintended side effects may be acceptable. She asked if there are no arguments against intended interventions to affect the germ-line in principle since the intended

interventions would be only aiming at good effects, assuming the technology is safe and effective.

Dr. Walters responded that the logic of the two arguments is different. The chance of an unintended side effect, should not be cause to stop a clinical study. In the case of intended germ-line repairs, it is a forward positive argument to state that germ-line intervention is a good goal if it is aimed at preventing disease. Dr. Walters said that it takes additional arguments to justify a positive attempt because of the concern that once the technology is developed for preventing a disease such a technology might be used to enhance certain qualities of human embryos.

Dr. Karson noted the current shortage of an adequate technology to repair a specific gene at a precise chromosomal location, to effect a genetic alteration in subsequent generations of offspring. Random insertion of genes into the germ-line will be lost by motion. Dr. Walters asked if the unintended germ-line effects is not likely to create harm in the descendants of the patient. Dr. Karson responded that it is a separate issue.

Dr. Karson noted the parallel of the unintended side effects of chemotherapy to the gene transfer proposed with the unintended germ-line effect. Dr. Walters said another example is intracytoplasmic sperm injection.

Dr. Noguchi noted that radiation and chemotherapy effects are predictable based upon dose-escalation. In contrast, some effects of biologics are completely unpredictable; a rare event can be very serious. Guillain-Barr's syndrome is a result of childhood vaccination.

Dr. Anne Pillaro (FDA) said as opposed to chemotherapy or radiation, biologics frequently act by cascade mechanism that could magnify a particular effect. During development of an organism there is a differential expression of genes; one question is what are the effects of the vector on the development of the embryo or vice versa. Dr. Walters inquired if there are any biologics that are known to have germ-line effects. Dr. Pilaro said that vaccines or protein therapeutics have no known germ-line effects

Dr. Macklin noted the analogy of the germ-line side effects of *in utero* gene transfer to chemotherapy or radiation therapy. In principle, it is difficult to reject *in utero* gene transfer on the basis that it is uniquely new or different from other applications. Dr. Walters agreed. Dr. Macklin noted that an analogy with birth defects caused by thalidomide is not a valid analogy because its current use is to benefit a woman who is not now pregnant and who has a serious disease. The intervention is designed at benefitting the woman who has the option of not becoming pregnant or having an abortion if she does become pregnant. The *in utero* gene transfer is to benefit the fetus, and it involves a two patient problem; the risk consideration is different from the thalidomide analogy. Dr. Karson noted the high probability of the side effects of thalidomide to the fetus versus the low probability of germ-line effects of *in utero* gene transfer; she considered the analogy with thalidomide is still valid.

Dr. Aguilar-Cordova said that he can see the analogy with side effects of other therapies, e.g., chemotherapy or radiation therapy. He asked what would be unique for *in utero* gene transfer? Dr. Noguchi responded that the ability to predict the severity and incidence of a biological adverse event is much more difficult than chemotherapy or radiation therapy where applicable models are available. He cited the unpredicted severe inflammatory responses after adenoviral vector administration in a cystic fibrosis patient of an early study.

Dr. Juengst noted the difference in the predictability of adverse effects due to conventional therapy that *in utero* gene transfer. He asked if one has higher obligation to prevent those side effects that one can predict or a higher obligation to avoid the unknown risks. Dr. Walters responded that the investigators

have to do their best to minimize the likelihood of harming the patients; it is always a balance against the severity of the disorder.

Dr. Gordon noted a distinction between unintended germ-line gene insertion vs. intended germ-line insertion. It is not always possible to assess the risk of unintended germ-line insertion since the offspring may not have any phenotypic consequences until several generations later when certain allelic combinations occur. These unpredictable risks should be explained to the potential subjects. For intentional germ-line gene insertion if an acceptable technology, homologous recombination, is developed, it would be very difficult to argue against using it to ameliorate a disease.

With regard to the role of a genetic counselor, Dr. Macklin said that the genetic counselor should provide the pregnant woman with available options and should be independent of the investigators. In addition, a genetic counselor should be involved with the recruitment process prior to any discussion with the investigator. She asked about the sequence of events that would lead the pregnant woman to the investigators. Dr. Karson responded that she has been a primary genetic counselor for nine years. If th family already has an affected child, they understand that for a recessive disorder there is one in four chance of having another affected child. A genetic counselor should be involved in this early stage of discussion. Dr. Karson noted that the community of practitioners who deal with children with ADA- SC very small; therefore pregnant women learn about the research protocol because their physicians refer them to the investigators who conduct the study. Dr. Macklin was concerned that the potential pregnant women might be given misplaced hope from the investigators without considering the other available options.

Dr. King agreed with Dr. Walters that the terminology used to describe the research is very important in order to properly inform the potential subjects. She agreed with the neutral terms, i.e.*pregnant woman* not *mother*, *gene transfer* not *gene therapy*, *cell transplantation* not *cell therapy*, *subjects* not *patients*, *experimental procedure* or *intervention* not *treatment*.

XII. Informed Consent Issues/ Zallen , Macklin, Juen

Fetal-child issues/ Zalle

Dr. Zallen noted that any approved protocol receives scrutiny from many sources, i.e., the FDA, fundin agency, and other researchers. However, the informed consent process goes on without any oversight. The Informed Consent document is the only window into the process of what the potential study participants have heard. For this reason it is an important document.

Dr. Zallen addressed the fetal-child issues in 5 categories

(1) What procedures should be used to identify and enroll candidate families? A sufficient amount of time has to be allowed if women are to make an informed decision, considering the time required for genetic testing and to be informed about potential options; ideally the process should start before a pregnancy is established. A genetic counselor serves an important role in this process. Is there any need for a fetal advocate?

(2) What terminology or language should be used to properly describe the procedures without misleading desperate people into beliefs of cures? "Experimental procedure" not "treatment" is a better choice of terminology. She noted that Dr. Walters mentioned several preferredterminologies for this purpose

(3) What are the risks and how can they be properly explained? There are many levels of risks, e.g., risks

related to the initial procedures (drawing blood, injecting viruses, etc.), risks related to prenatal development and insertional mutagenesis, risks related to long term health problems and partia corrections, and risks related to potential future infertility of the fetus and germ line contamination for offspring. How can these risks be identified, explained, and quantified? What is the proper terminology to describe the quantitative measures of the risks that can be used in Appendix M of the *NIH Guidelin*?

(4) What are acceptable arrangements for the long-term follow-up or partial correction? The costs of partial correction could be substantial. How can these costs be estimated and who should pay for them? The RAC did not succeed in addressing the cost issue in its past deliberations.

(5) What are the pregnant women's responsibilities? What are the fetus-child's responsibilities? How much intrusion into the individual's life is permissible as the investigators are trying to gather valid scientific information? A valid infrastructure or registry should be established to guide the long term follow-up of subjects who have received *in uter*gene transfer. Autopsy is critical to gather information regarding the possible spread of the genetic material to the gonads. Record-keeping, e.g., videotaping, of the informed consent procedure is important to assure that a proper process has been followed.

Other Comments

Dr. Aguilar-Cordova said that better information from preclinical studies is needed to provide the subjects with quantitative information regarding potential germ line risks. Dr. Macklin said that the Informed Consent document should clearly state that the risk is unknown if there is no quantitative information.

Dr. Macklin said that the fetal advocate is a bad idea; it should be entirely the woman's or the couple's decision. She questioned how would such an advocate be chosen, what would be the tasks of the advocate, and whether the advocate should have the authority to override the decision of the pregnant woman? Dr. Zallen said she shared the same concerns

Regarding communicating probabilities and risks to the subjects, Dr. Juengst said an analogy to other experiences, e.g., potential germ line effects of chemotherapy, is more useful than any quantitative description of the risks.

Dr. Cohen suggested conducting a model study of the informed consent process by setting up an experimental situation where subjects, genetic counselors, and pediatricians or obstetricians are engaged in the informed consent process.

Regarding quantifying the risks, Ms. King said "risk of harm" and "chance of benefit" are better terminologies since risk and benefit are not coequal terms. Regarding modeling the informed consent process, Ms. King noted it is difficult to develop a model that is applicable to differentfamilies an investigators.

Dr. Markert noted that close communication exists between subjects and pediatricians in the rare disea community; frequently the pediatricians refer the subjects to the proper protocols. Dr. Karson noted tha some situations the primary care physicians in their best judgment may not want to refer their patients to a particular protocol.

In terms of potential germ line risks, Dr. Gordon noted that there is no existing animal data to allow the physicians, investigators, or the patients to have a quantitative discussion of these risks.

Dr. Walters noted that Phase classification of clinical trials is a useful description of the protocols for the

patients; all Phase I studies are simply toxicity and safety studies without the likelihood of benefit to the patients.

Dr. Noguchi noted a distinction between the viruses and the vectors derived from them (much more is known about the adverse effects of the viruses than the vectors) for gene transfer, and such distinction is frequently not made in the Informed Consent document to describe the risks.

Dr. Aguilar-Cordova noted that the investigators, due to a vested interest in the clinical trial, very often are not impartial when informing potential subjects.

Maternal issues/Macklin

Dr. Macklin outlined maternal issues for discussion, i.e., maternal risk/benefit, maternal follow-up, statement regarding provision of necessary medical care and related costs, unbiased genetic counselors, and privacy/confidentiality issues.

Regarding the assessment of maternal risks and benefits, Dr. Macklin noted before describing such an assessment to the potential subjects, the assessment has already been made by the investigators and reviewed by IRB and FDA, or has been discussed by the RAC. What the potential subjects need is to t such information to decide on their willingness to participate in the clinical trials based on the risk/benefit consideration. Dr. Macklin noted that frequently the potential subjects tend to trust their doctor's invitations to participate in the study and misunderstand the experimental nature of the study as a therapeutic intervention for their condition; a similar conclusion was reached by deliberation of an Advisory Committee on Human Radiation Experiments that looked into the informed consent process. The Advisory Committee also noted the importance of the terminology. People tend to have a negative connotation of the protocol if it is presented as an "experiment." "Research," or "investigation" convey to them a positive view of the study. Dr. Macklin noted the term "study" is frequently being understood as only laboratory tests rather than conveying the meaning of a research endeavor. Dr. Macklin noted that the risks should include psychological, social, and legal risks. A bad outcome that results in a birth of an afflicted child is a psychological burden to the woman. In conclusion, Dr. Macklin said that risk/benefit is a complex issue and it should be discussed in the context of a particular protocol.

Dr. Macklin noted that maternal follow-up is burdensome and time consuming process lastingperhaps t the lifetime of the treated woman; this issue must be fully disclosed to the pregnant woman as part of the informed consent process.

Dr. Macklin noted a disagreement with regard to whether there is an obligation to provide care and treatment for any injury resulting from participation in the research protocol. However, the disclosure has to be made in the Informed Consent document. The Informed Consent document should also include a statement regarding the option of an unbiased genetic counselor.

Finally in terms of privacy and confidentiality, Dr. Macklin said the pregnant woman may want her participation in *in uter*research to remain confidential from either the father-to-be or an insurance company or both. She said the pregnant woman's wish should be honored.

Other Comments

Dr. Cohen stated that in theα- thalassemia pre-protocol any need of post-birth long-term transfusion fo babies born with partial corrections is a novel issue for an insurance provider.

Dr. Gordon noted that any medical attention, e.g., post-birth blood drawing needed after a child of *in uter* gene therapy is born, should be stated in the Informed Consent document. Dr. Macklin said any potential psychological risks to the child should also be stated. Dr. Markert said the statement of long ter follow-up is reassuring to the families.

Responding to questions on the insurance coverage, Dr. Anderson said that he would open a dialogue with the insurance providers before any trials are initiated. From his estimation, the insurance providers may be very reluctant to provide care for - thalassemia babies, which would be fatal *in uter* without treatment.

Paternal and Societal Issues/ Juengs

Paternal Issues

Dr. Juengst noted that the two pre-protocols primarily involve the pregnant women and informed conse from the pregnant women is sufficient; however, it is prudent to strive to obtain informed consent from both parents.

Societal Issues

Dr. Juengst pointed out 3 categories of societal issues for RAC public dialogue

(1) Issues beyond the RA's purview. How much of the research budget should be allocated to gene transfer research given the priorities and needs for other biomedical research? What is society's basic stance towards *in uter* gene transfer. Some of the public considered that the *in uter* gene transfer could have a negative social consequence such as the development of an attitude that all disabilities are unacceptable and that intervention will decrease the tolerance for people with disabilities. Dr. Juengst noted that society has not yet taken a basic stance on such issues and it is not useful for the RAC to debate them.

(2) Issues for RAC consideration. The pre-protocols involve two subjects, i.e., the pregnant woman and the fetus. Which of the subjects is expected as the beneficiary of *in uter* gene transfer? Is it a health problem for the family to have a partially corrected infant rather than death in *utef?* If the primary reason is to address the family's health problem, then there may be other safer and cheaper alternatives such as selective abortion. These are options for the family but not for the fetus. It should be clarified who is the beneficiary of this research, the fetus, the pregnant woman, or the family? Another question is what if the unintended germ line effects turn out to be beneficial?

(3) Issues about the RAC itself. What is the best forum for public dialogue and education? What is the best regulatory apparatus to oversee *in uter* gene transfer research? What is the proper role for the RAC on these issues?

Other Comments

Ms. Levi-Pearl noted that the public responses indicated a need to take a cautious approach to *in uter* gene transfer research. She noted the social and political climate today coupled with sensational stories reported in the news media provide a number of misleading, incorrect, and frightening stories about germ line intervention on animals and plants. It is not difficult to envision the reactions of the public and lawmakers to the appropriation of taxpayer dollars in support of such studies in humans. She said that a massive proactive public education effort should be undertaken at the same of time of the announcement

of any provision of funding for the research. When the researchers are unsure of potential risks and harms that could emerge from initiating a particular study, the government spokespersons must be forthcoming in their explanations to the public of the inherent risks and benefits of the initiative in question. Without such a concerted effort to educate the public, it is not difficult to envision the eventual legislation of a permanent moratorium on such studies in humans. Ms. Levi-Pearl was concerned about the potential use of these therapies for cosmetic enhancement. She favored that the RAC provide input in crafting legislative options to protect the public against any misuse of the science while not stifling the scientific progress in combating life threatening disorders.

Dr. Cohen said that in the α- thalassemia pre-protocol the decision to have a new born baby is a famil decision and the father-to-be should have a role. Should a consent from both parents be sought before undertaking *in uter*gene transfer forα- thalassemia ? Does the father-to-be agree to take the risk of hav a partially corrected child? Drs. Juengst and Macklin responded that the fath decision is not sufficient and the primary decision maker should be the pregnant woman. Drs. Gordon and Noguchi said that the father has an interest in the outcome of the treatment; the investigators would face a difficult situation when there is a conflict between the parents. Dr. Gordon said that the therapist should not perform an intervention without informed consent from both parents if the therapist considers it to be essential. Dr. Macklin noted that the relationship of a therapist or physician to the patient is different than the researcher-subject relationship. A physician should not abandon his or her patient in the middle of the treatment process, but a researcher can refuse to enroll onto a research protocol if there is a serious disagreement between the parents.

Dr. Karson said the requirement for an autopsy should be an entrance criterion. Dr. Macklinagreed consent to an autopsy should be an inclusion criterion; however, if people change their mind, their refusal to permit an autopsy may not be overridden. Dr. Aguilar-Cordova agreed. Dr. Karson said that th subjects have an obligation to fulfill their responsibilities under the clinical protocol. Dr. Aguilar-Cordova said that one can strongly suggest and request an autopsy but can not make it a mandatory requirement. Ms. Knorr noted that the RAC has had extensive discussion of the autopsy issue in the past; a request not a requirement for an autopsy could be clearly stated at the beginning of the Informed Consent document. Dr. Deisseroth noted from the standpoint of an investigator it is very difficult to enforce a autopsy requirement. Ms. King noted that an important dimension of autopsy discussion is whether the research subjects are considered as "public servants" having certain responsibilities when entering publicly supported clinical trials. Dr. Macklin said that the Informed Consent document may state either the subject or the investigator may withdraw any subject from a research protocol if certain obligations are not fulfilled; she saw the merit of having the statement of a consent to an autopsy as an entrance criterion.

Dr. Gordon inquired about the distinction between advocacy for the fetus and for the mother or family. Dr. Juengst explained that the distinction he made was for whose benefit is the research being conducted. is on behalf of the parents, then there are other reproductive options to reach their goal; if it is on behalf of the future child, then the focus of the research should be on the welfare of the future child.

Dr. Walters was concerned that prolonging survival of the fetus may be deleterious to the health of the pregnant woman in the α- thalassemia pre-protocol. Dr. Cohen agreed that the delay to terminat pregnancy by therapeutic abortion may pose more risks to the pregnant woman, e.g., preeclampsia severe hypertension, and bleeding following delivery that requires transfusion. The literature states that if one identifies a fetus with homozygous α- thalassemia that therapeutic abortion is suggested to protect pregnant woman's health. Dr. Cohen noted that risk from prolonged gestation could result from either an unsuccessful *in uter*gene transfer that delays abortion or from a successful intervention allowing the fetus to be carried to term. Dr. Markert inquired about the mechanism of preeclampsia . Dr. Co responded that the mechanism is not well understood.

Dr. Gordon was concerned about the development of a mosaic hematopoietic system whe hemoglobinopathies are treated with gene transfer, i.e., the physiological consequence of a significan proportion of diseased bone marrow cells co-existing with a few percentage of treated cells.

Dr. Walters asked if the *in uter* gene transfer procedure would adversely affect the pregnant woman. He said the pregnant woman would have to make a heroic effort to take a substantial level of risk in entering the α- thalassemia protocol. Dr. Noguchi noted that for this reason the father-to-be has an interest i preserving the family. Dr. Macklin agreed that the father-to-be has an interest but not a right to make the decision; the Informed Consent document should clearly explain to the subjects the risk and benefit of the intervention.

Dr. Aguilar-Cordova asked the investigators how they will handle the paternal/maternal issue. Dr. Anderson said that because of the many unknowns of the *in uter* intervention, the InformedConsent document should be very honest. There should be solid family support to participate in the clinical trial, and the pediatricians should be supportive. He said that for these protocols the informed consent process will be videotaped.

XIII. Data Management Update/ Greenblat

Protocol Registration

To date, 265 human gene transfer protocols have been registered with ORDA including 30 gene marki protocols, 231 gene therapy protocols, and 2 non-therapeutic protocols. Two protocols are to be resubmitted. Therapeutic protocols include 25 for infectious diseases (all HIV-1), 34 for monogenic diseases, 162 for cancer, and 10 for other diseases/disorders (rheumatoid arthritis, coronary and peripheral artery diseases, arterial restenosis , and cubital tunnel syndrome). Since the June 18-19, RAC meeting, the following 18 protocols have been recommended for sole FDA review (no protocol was recommended for full RAC discussion):

9802-233

Dreicer , Robert; the University of Iowa Hospitals and Clinics, Iowa City, Iowa; Seigler , Hilliard; D University Medical Center, Durham, North Carolina; Rubin, Joseph; Mayo Clinic, Rochester, Minnesota; DeConti , Robert; H. Lee Moffitt Cancer Center, Tampa, Florida; and Gonzalez, Rene; the University o Colorado Cancer Center, Denver Colorado; *Phase II Study of Direct Gene Transfer of HLA-B7 Plasmid DNA/ DMRIE /DOPE Lipid Complex (Allovectin-7) as an Immunotherapeutic Agent in Patients with Stag III or IV Melanoma with No Treatment Alternatives* Sponsor: Vical , Inc

NIH / ORDA Receipt Date: 2S0le FDA Review Recommended by NIH / OR8-28-98

9802-234

Thompson, John A.; University of Washington, Seattle, Washington; Dreicer , Robert; the University o Iowa Hospitals and Clinics, Iowa City, Iowa; Seigler , Hilliard; Duke University Medical Center, Durham North Carolina; Galanis , Evanthia ; Mayo Clinic, Rochester, Minnesota; and DeConti , Robert; H Moffitt Cancer Center, Tampa, Florida; *A Controlled, Randomized Phase III Trial Comparing the Response to Dacarbazine with and without Allovectin-7 in Patients with Metastatic Melan* Sponsor: Vical , Inc

NIH / ORDA Receipt Date: 2-9 Sole FDA Review Recommended by NIH / ORDA : 7-20-98

9804-248

Schuchter , Lynn; University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; *Phase I Trial of Therapeutic Cancer Vaccine Using Intratumoral Injections of B7-1 (H5.030CMVhB7) in Patients with Metastatic Melanoma or Metastatic Breast Ca*

NIH / ORDA Receipt Date: 4-23 Sole FDA Review Recommended by NIH / ORDA : 5-13

9804-249

Junghans , Richard Paul; Beth Israel Deaconess Medical Center, Boston, Massachusetts; *Phase I Study of T Cells Modified with Chimeric Anti- CEA Immunoglobulin-T Cell Receptors (IgTC Adenocarcinom.*

NIH / ORDA Receipt Date: 4-28 Sole FDA Review Recommended by NIH / ORDA : 5-18

9804-250

Swisher, Steven; University of Texas M.D. Anderson Cancer Center/Texas Heart Institute, Houston, Texas; *An Efficacy Study of Adenoviral Vector Expressing Wildtype p53 (Ad5CMV-p53) Administere Intralesionally as an Adjunct to Radiation Therapy in Patients with Non-Small Cell Lung Cancer* Sponsor: Gencell (Division of Rhone- Poulenc Ror

NIH / ORDA Receipt Date: 4-28 Sole FDA Review Recommended by NIH / ORDA : 5-18

9805-251

Figlin , Robert; University of California at Los Angeles, Los Angeles, California; *Phase I/II Trial of Antigen-Specific Immunotherapy in MUC-1 Positive Patients with Adenocarcinoma of the Prostate Usir Vaccinia Virus-MUC1-IL2 (TG 103* Sponsor: Transgene , S.

NIH / ORDA Receipt Date: 5-1 Sole FDA Review Recommended by NIH / ORDA : 5-22

9805-252

Sobol , Robert E.; Sidney Kimmel Cancer Center, San Diego, California; *A Phase I Study of Allogeneic Tumor Cells Genetically Modified to Express B7.1 (CD80) Mixed with Allogeneic Fibroblasts Geneticall Modified to Secrete IL-2 in Patients with Colorectal Carcinoma.*

NIH / ORDA Receipt Date: 5-7 Sole FDA Review Recommended by NIH / ORDA : 5-27

9805-252

Sobol , Robert E.; Sidney Kimmel Cancer Center, San Diego, California; *A Phase I Study of Allogeneic Tumor Cells Genetically Modified to Express B7.1 (CD80) Mixed with Allogeneic Fibroblasts Geneticall Modified to Secrete IL-2 in Patients with Colorectal Carcinoma.*

NIH / ORDA Receipt Date: 5-27 Sole FDA Review Recommended by NIH / ORDA : 5-27

9805-253

Scadden , David T.; Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston Massachusetts; Mitsuyasu , Ronald; University of California, Los Angeles, Los Angeles, California; an Deeks , Steven; University of California, San Francisco, San Francisco, California *A Phase II Study of Autologous CD4-Zeta Gene-Modified T Cells in HIV Infected Patients with Undetectable Plasma Vire on Highly Active Anti-Retroviral Drug Therapy* Sponsor: Cell Genesys , Inc

NIH / ORDA Receipt Date: 5-14 Sole FDA Review Recommended by NIH / ORDA : 6-3

9805-254

Rosenberg, Steven A.; National Institutes of Health, Bethesda, Maryland; *Immunization of Patients with Metastatic Melanoma Using DNA Encoding the GP100 Melanoma Antige* Sponsor: National Cancer Institute - Cancer Therapy Evaluation Program (NCI- CTEP

NIH / ORDA Receipt Date: 6-4 Sole FDA Review Recommended by NIH / ORDA : 6-24

9806-255

Muller, Carolyn Y.; University of Texas Southwestern Medical School, Dallas, Texas; *Phase I Trial of Intraperitoneal Adenoviral p53 Gene Therapy in Patients with Advanced Recurrent or Persistent Ovaria Cancer*. Sponsor: National Cancer Institute - Cancer Therapy Evaluation Program (NCI- CTEP

NIH / ORDA Receipt Date: 6-2 Sole FDA Review Recommended by NIH / ORDA : 6-22

9806-256

Suzuki, Tsuneo; University of Kansas Medical Center, Kansas City, Kansas; *Autologous , Irradiated Melanoma Cells Transduced Ex Vivo with an Adenovirus Vector (Adv/GM- CSF) Express Granulocyte-Macrophage Colony Stimulating Factor Gene*.

NIH / ORDA Receipt Date: 6-3 Sole FDA Review Recommended by NIH / ORDA : 6-23

9806-257

Suzuki, Tsuneo; University of Kansas Medical Center, Kansas City, Kansas; *Autologous , Irradiated Cancer Cells (Breast Cancer, Colon Cancer, Head and Neck Cancer, and Soft Tissue Sarcoma) Transduced Ex Vivo with an Adenovirus Vector (Adv/GM- CSF) Expressing Granulocyte-Macroph Colony Stimulating Factor Gene.*

NIH / ORDA Receipt Date: 6-3 Sole FDA Review Recommended by NIH / ORDA : 6-23

9806-257

Suzuki, Tsuneo; University of Kansas Medical Center, Kansas City, Kansas; *Autologous , Irradiated Cancer Cells (Breast Cancer, Colon Cancer, Head and Neck Cancer, and Soft Tissue Sarcoma)*

Transduced Ex Vivo with an Adenovirus Vector (Adv/GM- CSF) Expressing Granulocyte-Macroph Colony Stimulating Factor Gene.

NIH / ORDA Receipt Date: 6-3 Sole FDA Review Recommended by NIH / ORDA : 6-23

9806-258

Crystal, Ronald G.; Cornell University Medical College, New York, New York; *Phase I Study of Direct Administration of a Replication Deficient Adenovirus Vector (Ad_{GV}VEGF121.10) Containing the VEGF121 cDNA to the Ischemic Myocardium of Individuals with Diffuse Coronary Artery Disease Vi Minimally Invasive Surgery.* Sponsor: GenVec , Inc

NIH / ORDA Receipt Date: 6-3 Sole FDA Review Recommended by NIH / ORDA : 8-13

9806-259

Figlin , Robert; University of California at Los Angeles, Los Angeles, California; Thompson, John, A. University of Washington, Seattle, Washington; and Galanis , Evanthia ; Mayo Clinic, Rochest Minnesota; *Phase II Study of Direct Gene Transfer of IL-2 Plasmid DNA/ DMRIE /DOPE Lipid Comple (Leuvectin) as an Immunotherapeutic Regimen in Patients with Metastatic Renal Cell Carcin* Sponsor: Vical , Inc

NIH / ORDA Receipt Date: 6-15 Sole FDA Review Recommended by NIH / ORDA : 7-6

9806-260

Hersh , Evan; Arizona Cancer Center, Tucson, Arizona; *Phase I Study of HLA-B7/b2M Plasmid DNA/ DMRIE /DOPE Lipid Complex (Allovectin-7) by Direct Gene Transfer with Concurrent Low-Dos Subcutaneous IL-2 Protein Therapy as an Immunotherapeutic Regimen in Malignant Melanoma.*

NIH / ORDA Receipt Date: 6-26 Sole FDA Review Recommended by NIH / ORDA : 7-16

9806-261

Amado , Rafael G.; University of California at Los Angeles, Los Angeles, California; and Yuen, Alan R. Stanford University Medical Center; Stanford, California; *A Phase I/II Study of the Safety and Feasibility of RevM10 or RevM10/ Antisense Pol 1 Transduced Hematopoietic StemCells (HSC) in Non-Hodgkin's Lymphoma Patients Already Being Treated with High Dose Chemotherapy and Peripheral Blood Stem Cell Support*. Sponsor: Systemix , Inc

NIH / ORDA Receipt Date: 6-30 Sole FDA Review Recommended by NIH / ORDA : 7-20

9807-262

Wolf, Judith K.; The University of Texas MD Anderson Cancer Center, Houston, Texas; *A Phase I Study of Ad-p53 (NSC #683550) for Patients with Platinum- and Paclitaxel -Resistant Epithelial Ovarian Ca*

NIH / ORDA Receipt Date: 7-24 Sole FDA Review Recommended by NIH / ORDA : 8-13

9808-263

Lang, Frederick F., Jr. and Yung, W. K. Alfred; The University of Texas MD Anderson Cancer Center, Houston, Texas; *Phase I Trial of Adenovirus-Mediated Wild Type p53 Gene Therapy for Malignant Gliomas*Sponsor: NCI-Cancer Therapy Evaluation Program (NCI- CTEP

NIH / ORDA Receipt Date: 8-13-98. Sole FDA Review Recommended by NIH / ORDA :

Protocol Amendments

There were 22 protocol amendments since the June RAC meeting. The majority of the amendments were very minor either adding investigators, new sites, or changing investigators. Other minor amendments included changing pre-study screening tests, altering the transduction procedure, changing inclusion and exclusion criteria, switching from the World Health Organization (WHO) toxicity grading to the National Cancer Institute (NCI) Common Toxicity Criteria, minor editorial changes, blood drawing timing changes, and changing viral titer from plaque forming units (pfu) to particle units

Protocol Updates

Protocol 9406-081 entitled: *IL-12 Gene Therapy Using Direct Injection of Tumor with Genetically Engineered Autologous Fibroblasts*.29 patients were treated for a total of 39 treatment cycles. The maximum tolerated dose (MTD) was determined to be 7,000 ng /24 hours. The protocol was amend treat pediatric patients.

Protocol 9804-247 entitled: *A Phase I Safety and Dose Escalation Trial of Autologous Transfect Human Fibroblasts Producing Human Factor VIII in Patients with Hemophilia A*. Several modifications were made to the protocol: the number of cohorts was increased from 3 to 4 and the number of patients from 9 to 12; the protocol title was modified from "dose escalation trial" to "study;" an interim safety analysis was added after the first 6 patients had been treated; the minimum age was changed from 13 to older than 15 years of age; the inclusion criteria were changed to state that patients must have received conventional factor VIII replacement therapy for at least 50 days; a protocol stopping rule was added so that the protocol will be temporarily stopped if two or more patients develop antibodies to factor VIII equal to or greater than 10 Bethesda units

Protocol 9403-069 entitled: *A Phase I/II Pilot Study of the Safely of the Adoptive Transfer of Syngeneic Gene Modified Cytotoxic T Lymphocytes in HIV Infected Identical Twins*.Update forPeriod 3 involve administration of 3 infusions of gene-modified CD4 and CD8 cells at 2 week intervals.

Safety Reports

The following are safety reports submitted since the June RAC meeting:

Protocol 9303-038 entitled: *Administration of Neomycin Resistance Gene Marked EBV Specific Cytot T Lymphocytes to Recipients of Mismatched Related or Phenotypically Similar Unrelated Donor Marro Grafts*. A death was due to relapsed secondary acute myelogenous leukemia

Protocol 951-130 entitled: *Administration of Neomycin Resistance Gene Marked EBV Specific Cytoto T Lymphocytes to Patients with Relapsed EBV Positive Hodgk's Disease. A* death was reported due to progressive disease

Protocol 9709-214 entitled: *A Phase II Multi Center Open Label Randomized Study to Evaluate Effectiveness and Safety of Two Treatment Regimen of Ad5CMV -P53 Administered by Intra Tumoral Injections in 78 Patients with Recurrent Squamous Cell Carcinoma of the Head and Ne*(a) A patient experienced vomiting, chills, and fever one hour after receiving the first dose. It was possibly related to the treatment. (b) One patient was hospitalized for dehydration, renal failure, and gastroenteritis. A follow-up report stated that the patient was hospitalized for over 3 weeks. While hospitalized the patient went into cardiac arrest. This event was not related to the study drug

In a related trial in Europe (protocol 9709-214) there were two adverse events. (a) Fever, hypotension, and Grade 4 local pain and possible septicemia were reported. On follow-up the patient was given antibiotics and fever resolved. Blood cultures were negative. The investigator considered the original event was due to injections and were not related to the study drug. (b) A second patient had swelling at the injection site at the left cheek on Day 15 following treatment. Two days later the patient developed dyspnea and a tracheostomy was performed. On follow-up, the investigator considered the adverse not related to the study drug. They believed that the event was caused by either the injection itself or tumor progression.

Protocol 9712-226 entitled: *A Phase II Multi Center Open Label Randomized Study to Evaluate Effectiveness and Safety of Ad5CMV -p53 Administered by Intra Tumoral Injections in 39 Patients wit Recurrent Squamous Cell Carcinoma of the Head and Nec*One day after the first injection of the second course of treatment the patient experienced an increase in tumor size that was considered to be due to disease progression and not swelling from the injection. The patient was removed from the study. The patient was hospitalized for intravenous antibiotics administration. Cultures were positive for *Staphylococcus aureu*. The Infection was considered possibly related to the treatment.

Protocol 9403-069 entitled: *A Phase I/II Pilot Study of the Safety of the Adoptive Transfer of Syngeneic Gene Modified Cytotoxic T Lymphocytes in HIV Infected Identical Twins*:eport of death due to rectal lymphoma; it was considered remotely related to the study.

A motion was made by Dr. Markert and seconded by Dr. Macklin to accept the Data Management Rep by a vote of 8 in favor, 0 opposed, and no abstentions. The first day meeting was closed at 4:50 p.m.

XIV. Chair Opening Remarks for September 25, 1998, Discussion/Mickelson

Dr. Mickelson called the meeting to order at 8:35 a.m. on September 25, 1998. Dr. Mickelson summarized the issues discussed yesterday. These general issues were: science and mechanisms of genetic diseases, appropriate animal models, dose-escalation studies, insertional mutagenesis, potential for ge line gene transfer, homologous recombination, natural mutagenesis occurring during meiosis, and differences in the effects of biologics and drugs. Specific recommendations should be made to the investigators in today's discussion. These recommendations should touch upon, but not be limited to, the following areas. What is expected from the animal studies? What models are appropriate? How does one deal with the effects of partial correction? What are the clinical endpoints for partial correction?

XV. Pre-Protocol Candidate Diseases – Clinical Overview/Buckley, Cohen

Presentation on ADA- SCID – Buckley

Dr. Buckley introduced the subject of human SCID as a prototypic model*in uter*gene therapy. SCID is a rare and fatal syndrome of diverse genetic origin characterized by an absence of T and B cell function. The mean age of clinical presentation and diagnosis is 6.5 months. When the children get sick

they begin to fail to thrive. SCID children frequently have chronic diarrhea and experience recurren infections such as oral candidiasis , parainfluenza 3, adenovirus, cytomegalovirus, Epstein-Barr viru Even a benign childhood disease such as chicken pox is potentailly fatal for SCID children. Most of SCID children are boys (83.6%). X-linked SCID is due to mutations in a gene encoding the γ_c chain of the T cell growth factor receptor, which is shared by many other receptors for interleukin-4 (IL-4), IL-7, IL-9, and IL15. It is the deficiency of this major component of the receptor that makes the babies unable to develop T and B cells. Molecular defects of other types of SCID are: Janus kinase 3 (Jak3) defic ADA deficiency, IL-7R alpha chain deficiency, and recombinase activating gene 1 (RAG1) and RAG deficiencies. At Duke University, 46% of SCID infants are due to deficiency of the common (γ_c) chain of the T cell receptor, 15% are due to ADA deficiency, and 7% due to Jak3 deficiency. All types of SCID c be successfully treated by bone marrow transplantation without the need for pre-transplant chemotherapy because the SCID infants do not have T and B cell function to reject the graft

Until 17 years ago bone marrow transplantation required strict HLA identity between the donor an recipient in order to avoid lethal graft-versus-host disease (GVHD). Now it is possible to avoid GVHD cell depletion which allows omission of GVHD prophylactic drugs, e.g., cyclosporin and steroids. Buckley described the technique she has used to deplete the T cells from bone marrow in detail. Dr. Buckley noted that the technique is very successful with a survival rate of 81% (89 SCID infants treate Duke University). She obtained 100% survival for HLA -identical transplants and 78% fo HLA-haploidentical transplants. Survival rates according to genetic types are: 100% for Jak3 deficiency 100% for IL-7Rα deficiency, and 85%for ADA-deficiency.

Dr. Buckley described 15 SCID infants who were transplanted between 8 and 24 days of life. In these 1 infants, SCID was due to γ_c deficiency (10 infants), ADA deficiency (2 infants), Jak3 deficiency (1 infant), and autosomal recessive disorders of unknown causes (2 infants). No pre-transplant conditioning an post-transplant GVHD prophylaxis were needed. Two received T cell depleted bone marrow fro HLA -identical sibling, and 13 received T cell depleted marrow from HLA-haploidentical parental ma The mean number of nucleated marrow cells given was 4.68 x 10^8/kg infant body weight. Only one baby was lost in this group of 15 SCID infants. To date, a total of 22 SCID infants were transplanted in th 3.5 months of life and with only one loss so far.

Dr. Buckley noted that there is a simple test known as an absolute lymphocyte count (costing $41) that can effectively identify SCID infants before they are sick. In conclusion, Dr. Buckley stated that all form SCID can be diagnosed at birth if the absolute lymphocyte counts were included in newborn screening the neonatal period, both HLA -identical and haploidentical T cell depleted stem cell transplants are effective in reconstituting all forms of SCID . In these neonates, pre-transplant conditioning and GV prophylaxis are not necessary.

Regarding the ADA- SCID pre-protocol, Dr. Buckley noted that there are advantages and disadvantage of selecting this disease for *in uter* gene transfer. Advantages include the prior clinical experience of gene transfer studies, easily determined success outcome, no need for chemotherapy, and no need for PEG-ADA. Disadvantages include no irreversible prenatal changes in ADA- SCID , available effectiv post-natal bone marrow transplantation, limited success of post-natal gene transfer so far, invasive procedure for *in uter* gene transfer, pre-natal diagnosis needed, and unknown risks to the pregnant women. Dr. Buckley said that for SCID in which pre-transplant chemotherapy is required, one might b able to justify risky *in uter* gene transfer.

Dr. Buckley presented a survey of world wide experience with immunodeficiency transplants. She noted for certain diseases the success rate was poor, e.g., Wiskott -Aldrich Syndrome; in this group of disorde there is some T cell function and myeloablation is needed. She considered that this kind of disease is

stronger candidate for *in uter* gene transfer.

Other Comments

Dr. Karson asked if the T cell depletion procedure can be adapted for cord blood cells. Dr. Buckle responded that it is possible but more difficult since there are more T cells in cord blood than in bone marrow and her procedure does not work well for frozen blood cells.

Dr. Gordon asked if all candidate diseases require cell specific gene correction. Dr. Buckley responded that all these diseases can be corrected by stem cell transplantation and should be able to be corrected by gene transfer.

Presentation on α- Thalassemia -- Dr. Coh

Before addressing the issues of the α- thalassemia pre-protocol, Dr. Cohen gave some backgroun information on thalassemia . He noted that homozygo α- thalassemia is a fatal disea *in uter* and there is not a large population of infants born alive with this disorder. He described globin gene developmen and its implication for gene therapy.

Dr. Cohen said that thalassemia and other hemoglobinopathies are prevalent in the Mediterranean, states, southern Asia including Thailand, Cambodia, Vietnam, and the southern part of China. In North America most of the patient population are immigrants from these areas. First generation immigrants may have language and cultural barriers, this consideration is relevant to the informed consent process.

Thalassemia is a disorder about quantitative abnormalities in globin production; ot hemoglobinopathies , e.g., sickle cell anemia, are qualitative abnormalities in the globin chains of hemoglobin molecules. In humans, the globins are present as clusters comprised of several genes that are developmentally expressed. There is a switch at about six to ten weeks of gestation from the embryonic to the fetal form and another switch occurs at about the time of birth from the fetal to adult form of hemoglobin. The α- globin chain is a common component of both the fetal and adult forms o hemoglobin. A deficiency in α- globin production, as in the candidate conditio α- thalassemia) *in uter* gene transfer, is therefore, incompatible with life after gestation. Without α- globin production, feta development is impaired starting from 8 to 10 weeks of gestation when α- globin becomes the essentia component of hemoglobin molecules.

β- Thalassemia , in contrast, is a more common form of the disease in North America. It is compatible wi fetal development since the β- globin chain is required at the later stages of fetal and into adult life β- Thalassemia can be recognized at birth by newborn hemoglobin screening, and there are clinica manifestations by about 6 months to a year post-birth.

Most forms of α- thalassemia result from deletions of the duplicate globin genes (a total of four gen copies). The most common form in the U. S. is the Southeast Asian variant which involves deletion of all 4 copies of the α- globin gene

Clinical severity of the disease varies with the number of copies of the α- globin gene deleted. A loss of single copy of the α- globin gene is clinically unrecognizable; a loss of two copies results in a carrier sta called Alpha Zero Thalassemia . If 3 copies are missing hemoglobin H disease occurs. If all 4 copies ar non-functional, the result is hydrops fetalis , the most severe form of the disorder. Dr. Cohen showe blood counts of the various forms of α- thalassemia , and noted that partial correction of the homozygou α- thalassemia will have symptoms similar to the less severe forms of the disease

Dr. Cohen described the maternal and fetal outcome of homozygous α- thalassemia in 46 cases from 19 to 1983 reported in Hong Kong. Maternal and fetal complications include anemia (65%), polyhydramnios (59%), hypertension (61%), antepartum hemorrhage (6.5%), placenta previa (4.3%), postpartum an (46%), postpartum transfusion (33%), and fetal anomalies (17%).

Dr. Cohen described 4 babies with homozygous α- thalassemia who have survived gestationwithout specific intervention. These surviving babies provide cases for consideration of the issue of what would be the standard of care for a fetus affected with homozygous α- thalassemia . Three of the babies wer delivered by Caesarean section and one by vaginal delivery. All 4 babies received post-delivery blood transfusions. The follow-up ranges from 10 months to 6 years. The first baby at 6 years has had a moderate delay in speech and hearing; the second at 5 years has experienced a normal development; the third at 10 months has had mild gross motor delay; and the fourth at 2 years has spastic quadriplegia and profound neurodevelopmental delay

There were 2 babies who had been diagnosed *in uter* and had been rescued by blood transfusion *in uter*. The first baby was diagnosed at 25 weeks of gestation and was given an intrauterine transfusion. The baby has experienced congenital anomalies at 2 years and cognitive and motor delay. The second baby was diagnosed at 32 weeks of gestation and was given transfusion at that time. This baby at 3 years of age had a normal neurologic assessment

Dr. Cohen pointed out that all of these 6 babies survived via a chronic transfusion program, and have became transfusion-dependent. They belong to the category of thalassemia major that requires regula blood transfusion every 3 or 4 weeks and ultimately results in iron overload. If they have HLA compatib siblings, they would be candidates for bone marrow transplantation.

Dr. Cohen described 3 homozygous α- thalassemia babies identified during fetal life and who receive *in uter* stem cell transplants. The first baby received T cell depleted maternal marrow at 18 weeks of gestation. Engraftment by umbilical cord sampling at 24 weeks was not evident although there were some donor cells found at autopsy. The second baby received cryopreserved liver cells at 15 weeks o gestation. There was no evidence of engraftment. The third baby received CD34-enriched paternal marrow. There was a trace PCR signal for β globin gene sequences in the cord blood and marrow. Thi baby was transfusion dependent after birth.

Dr. Cohen mentioned 5 possible outcomes of *in uter* gene transfer for α- thalassemia . (1) Successfu gene transfer. The child does not have severe anemia and is not transfusion dependent. (2) Alive but transfusion dependent. The infant is born with a condition similar to thalassemia major. (3) Alive bu transfusion dependent with anomalies. This situation raises a question as to whether the neurodevelopmental problems might precede the intervention or might occur irrespective of th intervention. (4) The fetus is aborted due to unsuccessful gene transfer. (5) Spontaneous abortion.

Dr. Cohen raised several issues that should be discussed. (1) What is the standard of care? What are the possibilities/alternatives to gene transfer? Should nature be allowed to take its course? Is *in uter* transfusion an option? (2) How is failure handled if one finds at 24 weeks that the attempted gene therapy has been unsuccessful? Is it the parent's choice, the investigator's choice, or both to proceed with a transfusion or abortion? (3) Whose call is it in the family, mother or father or both, as how to handle the situation of an unsuccessful attempt?

Dr. Cohen noted strong arguments for both sides of the debate as to whether α- thalassemia is a goo candidate for *in uter* gene transfer: (1) It is either the best candidate because it is fatal *In uter*, (2) Or, it is

the worst candidate because it is fatal.

Other Comments

Dr. Anderson asked if absence of α- globin expression α- thalassemia fetus prolong ζ globin (th embryonic form) expression during early fetal development. Dr. Cohen responded that in one of the surviving infants without transfusion therapy there was a high level (20%) of a hemoglobin form (Portland) suggesting an extended period of ζ- globin expression

Dr. Karson inquired about the outcome of bone marrow transplantation in babies w β- thalassemia wit respect to whether there is destruction of maternal cells by the fetal immune system. Dr. Cohen noted that in a large number of bone marrow transplants (over 700) performed in Pisaro , Italy, the outcome i extraordinarily good, i.e., 92 to 95% alive and free of thalassemia . Those bone marrow transplantation required myeloablation . Total correction of the bone marrow did not have to occur in order to have satisfactory peripheral blood correction. The children were supported with red cell transfusion when they received the bone marrow transplant; the transfusion apparently did not affect the engraftment of bone marrow.

Dr. Ando asked how the pregnancy with thalassemia comes to the attention of the physician, and how decision is made for an abortion, i.e., based on maternal distress, fetal distress, or both? Dr. Cohen responded that most thalassemia cases came to the physic's attention because of several scenarios: (1) previous history of an affected infant with hydrops , (2) carrier status of both parents as manifested b small red blood cells, and (3) ultrasound examination of hydrops or polyhydramnios fetus dur pregnancy. A concern for the mother's health is a major consideration for abortion.

Dr. Karson asked what is the homing mechanism to the bone marrow of cells administere intraperitoneally . Dr. Buckley explained that there are homing receptors that guide the stem cells to th target site. As a point of clarification, Dr. Anderson said that the direct intraperitoneal injection of th retroviral vector is proposed for the ADA- SCID pre-protocol; β- thalassemia pre-protocol will employ *ex vivo* transduction procedure.

Dr. Gordon noted a variation in the outcomes of infants who survived α- thalassemia ; he asked if such variation will hamper prediction of the outcome of *in uter* gene transfer. Dr. Cohen agreed that there is a limited value of predicting *in uter* gene transfer from such variable data; proper animal models are needed.

Dr. Juengst asked if there are other manifestations besides anemia in cases where there are large deletions of the α- globin gene cluster. Dr. Cohen responded that there are too few cases of survivin α- thalassemia infants to allow evaluation of the clinical manifestations

Dr. Macklin said that the term, "the standard of care," is not appropriate for a rare disorder that has no accepted therapy; such terminology properly refers to legal liability for the investigators for failing to provide "the standard of care." She said that alternative therapies should be clearly presented to the subjects. Dr. Cohen responded that there is no real alternative therapy for α- thalassemia ; in his institutio *in uter* transfusion is considered as an experimental procedure that requires IRB approval

Dr. Gordon said that partially corrected cells mixed with uncorrected cells would create bone marrow chimerism in β- thalassemia fetus; there is no information as to what degree of chimerism is nee confer correction of the phenotype.

Dr. King noted the complicated nature of the terminology of the standard of care and alternative therapies. She asked whether *in utero* transfusion is considered as the standard of care or an alternative therapy. Dr Cohen agreed that it is a complex issue.

Dr. Karson noted a parallel between transfusion treatment of Rh incompatibility thalassemia ; both require early diagnosis and early treatment at 16 weeks of gestation.

Dr. Greenblatt noted that the vector involves the use of the new LCR regulatory elements; he asked there is any risk of over production of α- globin by this vector. Dr. Cohen noted that the pathophysiolog thalassemia is an imbalance of globin production; imbalance favoring over β- globin results i β- thalassemia and vice versa Thalassemia patients with excess α- globin may have hemolyti anemia instead of microcytic anemia

Dr. Chow asked whether the levels of ζ- globin expression will change the clinical symptoms o α- thalassemia , and whether the *in utero* transfusion will complicate the diagnosis of the disorder. Dr. Cohen said both issues need further study.

Dr. Ando inquired if there is animal model for α- thalassemia . Dr. Cohen said there is a gene-knockou mouse model for this disorder.

Dr. Markert said that the subjects should be informed of the alternative therapies even though these ar experimental interventions.

Dr. Karson asked about the regulation of ζ-globin expression. Dr. Anderson said that ζ-globin expressio is not well understood, unlike regulation of α and β- globin

Dr. Chow suggested the hemoglobin H disease, which has three of four gene copies defective, as the initial candidate disease for *in utero* gene transfer. Dr. Cohen said that it may not be justifiable to perform the risky *in utero* gene transfer on the less severe hemoglobin H disease. But if it is acceptable then β- thalassemia , a mild form of the disorder, may be a better candidate disease

Dr. Anderson's proposal for a Phase Zero study

Dr. Anderson proposed an alternative "Phase Zero" approach to conduct the *in utero* gene transfer for α- thalassemia . He will submit such a proposal to the RAC after completion of preclinical studies of th new vectors in sheep and monkeys. This Phase Zero study will be a pure research protocol to enroll women who have already made a decision to abort the fetus and who are willing to give informed consent to participate in the study. The entire procedure of *ex vivo* transduction and infusion of transduced cell will be performed and the fetus aborted at 24 weeks of gestation. The aborted fetus will be evaluated to determine the success of gene transfer. There are three possible outcomes: no evidence of transduction and engraftment, partialcorrection , and successful correction. Any of these outcomes would provid valuable information for future studies. There is no benefit at all to the subject and the fetus in this proposal.

Dr. Markert said the Phase Zero proposal involves many ethical issues that need to be addressed at GTPC

Dr. Macklin noted that the proposed Phase Zero study is similar to most Phase I studies without any benefit to the subjects; she considered the proposal to be an ethically superior choice provided that the abortion decision is made prior to entrance to the study. There are precedents in obtaining fetal tissue for

research.

Dr. Aguilar-Cordova noted that the ethical and legal issues of the new proposal warrant an in-depth discussion at a GTPC . The proposed Phase Zero study may be carried out to evaluate marker gen expression and vector distribution in normal subjects.

Dr. Anderson said he did not favor carrying out a protocol simply designed to obtain fetal tissue or one that simply uses any non-therapeutic marker gene(s) *in vitro* *uter* studies.

Dr. Noguchi noted that conceptually the RAC has discussed therapeutic protocols where, as part of the protocols, any removed tissue was examined for potential gene transfer, e.g., removal of a lung after alpha-1 antitrypsin gene transfer (9403-070), removal of a knuckle joint after gene transduction fo rheumatoid arthritis (9406-074), and limb amputation after gene transfer for vascular endothelial growth factor (VEGF) (9409-088)

Dr. Noguchi said that another issue of importance for RAC discussion is how to measure the success of *in uter* gene transfer as pointed out by Dr. Patterson during her 1994 RAC presentation. Dr. Patterson noted that one of the outcomes mentioned by Dr. Cohen is to offer the option for abortion if the therapy is unsuccessful. She noted limitations of assessing prenatal endpoints for making the abortion decision. For example, are prenatal measurements of engraftment a reliable predictor at the ultimate percent chimeris achieved?

Dr. Markert said that the delayed abortion at the 24 weeks of gestation proposed for the Phase Zero st poses additional risk for the pregnant woman. She noted that the study on the aborted fetus poses a serious societal issue; it is different than obtaining organs or tissues from adult patients in protocols mentioned by Dr. Noguchi.

Dr. Mickelson said that the RAC should focus the present discussion on the two pre-protocols.

Dr. Gordon agreed that there is a significant risk to the pregnant woman to postpone abortion until 24 weeks of gestation. The RAC should not discuss this complex issue at the present meeting. Dr. Aguilar-Cordova said that the Phase Zero proposal may be considered as an alternative approach to the α- thalassemia pre-protocol

Ms. Levi-Pearl said that the Phase Zero concept should be discussed by a RAC subcommittee; the discussion should keep in mind the public sensitivity to this issue. With regard to the ADA- SCID pre-protocol she questioned whether ADA- SCID is a proper candidate disease because there is a effective alternative therapy. For the α- thalassemia pre-protocol she was concerned about the consequ outcome of a partial correction and the potential harm to the pregnant woman.

XVI. Preclinical Research Design Issues -- Specific Clinical Indications/McIvor

Presentation – Dr. McIvor

With regard to preclinical research design Dr. McIvor raised three major questions for the investigators to address:

(1) Efficiency of gene transfer. He noted the most compelling data are from the sheep studies, and asked whether the efficiency is high enough for human applications. For the ADA- SCID pre-protocol th expression level is not critical, but adequate and properly regulated expression is needed for

α- thalassemia gene transfer. Gene transfer into mouse hematopoietic cells in tissue culture has b performed, but the data is lacking on *in uter* gene transfer in mice. He noted that large animal data are also needed.

(2) Regulation of gene expression. Regulation of transgene expression by the LCR natural express cassette is a good idea. Regarding testing gene expression in model systems, Dr. McIvor said that it should be first tested *in vitro* in murine hematopoietic stem cells and *in vivo* with a move towards immunodeficient mouse system transplanted with the human hematopoietic cells. A more difficult ta to test the vectors in long term human hematopoietic stem cells in tissue culture

(3) Efficacy of transgene expression in correcting pathophysiology . A good ADA- SCID mouse m not available for conducting preclinical studies. Dr. McIvor noted a model developed by Claudio Bordignon (Milano , Italy) may be useful. In this model the ADA-deficient hematopoietic cells, e transduced or non- transduced , were transplanted into immunodeficient mice, and the immuno of these mice can be evaluated to determine whether gene transfer is able to correct the pathophysiolog There is a good gene knockout α- thalassemia mouse model for evaluating gene transfer *in uter* and regulation of gene expression.

Other Comments

Dr. Karson suggested switching the mode of gene transfer in these two protocols, i.e. *ex vivo* for the ADA- SCID and *in vivo* direct vector injection for α- thalassemia . In the ADA- SCID pre-protocol, dir intraperitoneal injection of the vector may transduce a large number of cells in the peritoneum whic not the intended target cells. A mouse model can be used to assess the background transduction of peritoneal cells versus the intended target hematopoietic stem cells. For treatment of ADA- SCID *in vivo* transduction of the hematopoietic cells will increase the efficiency of gene transfer. Dr. Karson sugg the direct vector injection mode for α- thalassemia gene transfer since the number of stem cells availabl from the 18 week fetal blood is limited. She noted that the α- thalassemia mouse model is useful i evaluating gene transduction by direct vector injection. She agreed that long term human hematopoietic cellculture is an useful model for ADA- SCID preclinical study. For vector injection at a very ea gestational age (10 to 12 post menstrual weeks) the yolk sac may be considered as an injection site. Animal data should be obtained to assess whether it is more beneficial to switch the mode of gene transfer in these two pre-protocols.

Dr. Juengst noted that treatin α- thalassemia might unmask other genetic problems that are not cause by α- globin gene deletions

Dr. Chow suggested as a first step, to test the new types of vectors in human ADA- SCID patients vi post-natal gene transfer. For the α- thalassemia pre-protocol she suggested that infants with hemoglobi disease and who are transfusion dependent may be a better patient population to test the new vectors.

Dr. Aguilar-Cordova noted that the ADA- SCID patients usually survive gestation. In this pre-protocol it advantageous to have some animal data that compare the efficiency of gene transfer *in uter* versus post-natal gene transfer. Similarly for direct vector injection, animal studies should be performed to compare transduction efficiency of injection into yolk sac, or peritoneum, or by *ex vivo* gene transduction.

Dr. Gordon noted the need for more animal data on potential germ line integration. For ADA- SCID ther an alternative therapy and there is a need for more animal data to assess the risk of intraperitoneal vec injection in the fetus. For α- thalassemia , the correction of a small subset of hematopoietic stem cells the fetus would produce a genetic mosaic in the bone marrow which is populated predominantly by

α- thalassemic cells; pathophysiological consequences particularly to the pregnant woman of the mo cell pattern are unknown.

Dr. Markert noted that the regulatory elements used for ADA gene expression in the proposed vector is major improvement over the vector used in previous studies; the new vector may allow proper gene expression in the thymus. She noted that the transplant mouse model for ADA- SCID , suggested by Dr McIvor, has limited value due to its lack of relevance to human application, e.g., the lack of T cells in human patients is not reflected in the mouse model. In the sheep studies the number of stem cells that are transduced should be determined. The data from the human studies with cord blood is useful in assess stem cell transduction efficiency. For the α- thalassemia pre-protocol the issue of tolerance is significant terms of how the fetus with a normal immune system will tolerate hematopoietic cells transduced w gene that is not expressed in the fetus.

Dr. Buckley said that it is useful to develop a gene knockout mouse model for the ADA- SCID in whic there is no background level of ADA.

Dr. Ando noted the efficiency of gene transfer found in animals is not predictive for the efficiency in the human fetus; it is a new area to validate the developmental gene regulation of the vectors using the LCR elements.

Dr. Mickelson raised several questions for the investigators to address. What are the variations in vector constructs that are to be tested in tissue culture and in animals? What are the types ofassays o therapeutic endpoints to evaluate *in uter*gene transfer in animal models? What are the appropriate animal models to evaluate long term effects of random integration? What would be predicted for the efficiency of the retroviral vectors in the human fetus? A large animal model is needed to address the risk/benefit to the fetus and the pregnant woman. The risk of inadvertent germ line gene transfer needs to be addressed. Dr. Mickelson said her evaluation of the risk/benefit does not favor the use of*in uter*gene transfer for a disease that is normally fatal*in uter.*

Investigators' Response - Drs. Anderson, Aronow , Huang, and

Dr. Anderson and his co-investigators, Drs. Bruce Aronow , Mei-Mei Huang, and Bonnie Liu, provi responses to RAC questions and presented the data of the proposed vectors to be used for*in uter*gene transfer.

Dr. Aronow said that the vector is designed to have proper developmental regulation of the transgene Aronow initiated his study of the ADA gene and its regulation in 1986. The function of the regulator elements of the ADA gene was studied in a series of transgenic animal experiments. The LCR of the A gene is contained within a DNA fragment of 2.3 kb. The LCR with an enhancer core in the middle of th DNA fragment allows formation of a transcriptionally active region of the chromatin that can be regulate by transcriptional factors. Several vectors containing the LCR expression cassette were constructe based on the murine leukemia virus backbone. Dr. Aronow described a series of constructs and ide the one that gave the best gene expression in his transgenic mouse experiments.

Dr. Huang described the construction of a SIN retroviral vector expressing the ADA gene. The vector called GTL102 is based on the pG1 murine retroviral backbone. She noted 3 differences between th GTL102 vector and PG1: (1) a CMV promoter replacing the 3' LTR , (2) a shortened packaging sign (3) of significance, deletion of 400 base pairs in the U3 region so that the viral LTR would not interfer with ADA gene expression. Preclinical studies were performed with constructs expressing the ADA gene or a reporter gene. Experiments in tissue culture are to test for viral stability, gene expression in

hematopoietic stem cells, and to establish conditions for optimal transduction. For animal model studie the vectors will be first tested in the murine model and then the sheep model

Dr. Anderson said the experiments planned for the next one and half years will address the issue of long-term gene expression of the vectors. The plan would be to finish construction of the vectors and test them in *in vitro* systems, (primary tissue culture) and the in small animal models such as mice. The data will be presented to the RAC. After obtaining input from the RAC, large animal studies including sheep and primates will be carried out to address the issue of potential germ line gene transfer, transduction efficiency in stem cells, and long term gene expression.

Dr. Wu described the vector construct expressing the α- globin gene. She said that the entir globin ge cluster is localized at the tip of Chromosome 16. Its major regulatory element has been identified as HS-40; the core sequence of HS-40, about 350 base pairs, is sufficient to confer tissue specific expression of the α- globin gene. Dr. Wu described th globin gene expression cassette including th HS-40 core sequence, CpG islands, and mRNA stabilityelements . The expression cassette will inserted into the SIN vector with the deleted U3 sequences of the viral LTR . The vector will be tested i the mouse α- thalassemia model to evaluate tissue specificity and long-term expression of th globin gene.

Other Comments

Dr. Aguilar-Cordova asked if the vector displays expression independent of its position of integration. Dr. Wu responded that the LCR fo globin gene can confer position-independent gene expression

Dr. McIvor asked if the ADA LCR also confers position-independent expression simila t globin LC Dr. Aronow responded yes. The facilitator sequences flanking the enhancer are able to facilitate th structuring of chromatin into an active chromatin domain. Dr. Aguilar-Cordova asked whether the LCR species specific. Dr. Aronow responded that LCR sequences are highly homologous between the n and human and they are interchangeable between these two species. Dr. Chow asked if expression of the α- globin gene LCR has been evaluated in transgenic mice. Dr. Aronow said yes and the exp related to the HS-40 hypersensitive sites of the LCR

Dr. Anderson responded to additional RAC questions. The efficiency of the constructs to express the transgenes in hematopoietic stem cells will be studied. He agreed that bone marrow transplantation SCID is a reasonable alternative experimental therapy, and he will consider other candidate diseases t require chemoablation . In animal models he will work out the procedure on how to quantify the result from a fetus. Dr. Anderson will work with the FDA to conduct proper safety and toxicological studies. β- thalassemia is not proposed for the study since many other laboratories are actively studying thi disorder. Dr. Anderson agreed to consider the alternative scheme of switching vector delivery modes between these two pre-protocols or do a combination approach for both proposals. Yolk sac injection in small animals or sheep will be considered. Dr. Anderson suggested further discussion of the Phase Zero proposal at the forthcoming GTPC or at future RAC meetings. Dr. Anderson agreed that the vectors ca be first tested by post-natal gene transfer in human subjects with the less severe hemoglobin H disease. More rigorous sheep studies will be performed in collaboration with Dr. Zanjani

Dr. Anderson said that the risks to the germ line will be tested in mice and large animals by quantitative polymerase chain reaction (PCR) analysis of sperm a*in situ* analysis of gonads. However, if he is convinced that there is a substantial risk relative to any potential benefit of*in uter*gene transfer he would not proceed further with developing the clinical protocol. Drs. Karson and Noguchi suggested a study w a large number of mice to assess the risk of germ line gene transfer. Dr. Aronow said that the techniqu

for *in uter* gene transfer in mice has not yet been developed. Dr. Gordon suggested an alternative system of exposing the primordial germ cells to the vectors to test for gene transfer. Dr. Aronow said tha transgene expression may depend on which cell types are transduced . Dr. Noguchi said that consid the importance of the germ line issue the investigators should make every effort to develop animal models or design experiments to address this important issue.

Dr. Anderson agreed to the comments made by several reviewers that there is no complete " preimmune status in a fetus of 13 to 15 weeks; the issue of tolerance will be reconsidered in his future protocol.

Dr. Anderson noted that his mention of potential germ line gene transfer in the pre-protocols has caused some misunderstanding by the Council for Responsible Genetics. In his estimation that such a probability for germ line effect is extremely small; it is nonetheless a finite risk.

Dr. Buckley suggested to test the vectors first in neo-natal ADA infants. Dr. Anderson agreed.

Ms. Levi-Pearl noted that the *in uter* gene transfer proposals have provoked strong responses from many major news media. She asked whether the *in uter* gene transfer is simply an alternative therapy for the ADA- SCID families; if yes it should not be attempted considering the serious public concern. Dr Anderson said gene transfer is not simply an alternative therapy for ADA- SCID ; it is a very important st in developing a therapy for this disorder. The reason to choose ADA- SCID for the present proposal i based on his past experience with gene transfer in neo-natal and childhood ADA- SCID patients. Th present proposals include a new type of vectors using the LCR elements that may permit long term ge expression. If the approach is successful, it can be applied to other monogenic disorders.

XVII. Clinical Research Design Issues – Specific Clinical Indications/ Marker

Presentation – Dr. Marker

Dr. Markert discussed specific clinical issues with respect *in uter* gene transfer for the treatment of ADA- SCID . Regarding alternative therapies, she noted that both HLA -identical or haploidentical depleted bone marrow transplantation is effective for the treatment of several forms of SCID in newbor However, in X-linked SCID , which is deficient in the γ_c chain of the IL-2 receptor, T cell but not the B cell function is restored by this treatment. Gene therapy would be an intervention to correct both the T and B cell functions. A complicating factor for the treatment of ADA- SCID is the concomitant use of PEG-ADA which is needed for the treatment of infants who have infections. The use of PEG-ADA obliterates the growth advantage of transplanted bone marrow cells. Dr. Markert noted that it is important to perform typing for the potential candidate fetus of *in uter* gene transfer. Very often there are HLA -identica siblings or relatives available for alternative post-natal bone marrow transplantation. With respect to the selection of the vector, Dr. Markert noted that the new vectors with LCR regulatory elements significant improvements. Loss of tolerance is an issue when PEG-ADA is used in conjunction with post-natal gene transfer; without tolerance the infant will have sufficient immune response to reject both the vector and transgene . With regard *in uter* stem cell transplantation, Dr. Markert noted that the fetus does not often develop severe GVHD due to insufficient immune function; but if GVHD occurs difficult to detect *in uter*. For monitoring the patients who undergo the gene transfer for ADA- SCID , it i important to monitor the immune function in order to determine how many transduced stem cells ar needed to reconstitute the immune system.

With regard to the α- thalassemia pre-protocol, Dr. Markert noted the issue of risks and benefitsto pregnant woman. She was concerned about the adverse effect of *in uter* gene transfer to the health of the pregnant woman. In terms of the level of gene expression needed to correct the deficiency, Dr. Mark

noted that correction of 60 to 70% of red cells to the normal state is required for a successful treatment of sickle cell disease. HLA typing is important to identify HLA -identical individuals as donors for stem c or bone marrow transplantation; the extended family of the α- thalassemia subjects are likely to have HLA match. With respect to the tolerance issue, Dr. Zanjani has discussed this issue in his sh experiments. The last issue is the safe blood volume obtainable from a fetus. Dr. Markert noted that fo 17 to 19 week fetus, which weights 200 to 250 grams, the total blood volume is about 40 to 50 ml. For a 50 gram fetus the total blood volume is only 10 ml. A safe limit of blood to be withdrawn is 5% of the total volume, i.e., 0.5 ml for the 50 gram fetus. The safe amount of blood available for the *ex vivo* gene transfer protocols is a significant issue.

Other Comments

Dr. Anderson agreed that blood volume is a concern; he would prefer to perform the procedure on the larger 17-19 week fetus to obtain 1-2 ml of blood. He said that a detailed procedure for blood cell processing will be developed in the future.

Dr. Karson noted that there are devices designed to obtain cells via amniocentesis; these devices may useful for withdrawing blood cells from the fetus.

Dr. McIvor noted that detailed procedures, e.g., the blood drawing and stem cell enrichment, have yet to be worked out for the protocol. Dr. Anderson agreed that the pre-protocol is simply to present the concept, and the detailed procedures will be developed in the future.

Dr. Noguchi said that safety concerns for vector validation, e.g., replication-competent virus or trace metals, is a more serious issue for transducing the small volume of fetal blood than for other post-nata applications. Dr. Anderson noted FDA scientists provided many useful comments regarding his pre-protocols; many of those questions will be addressed when the final protocols are developed.

Dr. Anderson noted that FDA has corrected his terminology regarding a "Phase Zero" proposal; FDA considers a proposal to test a therapeutic on a fetus should be classified as a Phase I study. Dr. Noguchi noted that to gather information to assess safety is a Phase I trial.

Dr. McIvor noted that better data on gene transfer efficiency in sheep is needed for both pre-protocols. Dr. Anderson agreed that quantitative data are lacking in the present sheep studies. For α- thalassemia th transduction efficiency of 50% is needed for a successful clinical outcome. Dr. McIvor asked what would be the minimum transduction efficiency for the ADA- SCID protocol. Dr. Anderson responded that th available animal data are not sufficient for this prediction; a human trial is needed. Dr. McIvor noted that the new vectors under development are an important step to overcome, hopefully the obstacle of *in vivo* gene shut down found in many retroviral vectors. Dr. Markert noted that the new vectors may allo sufficient gene expression in the thymus, which is important for correcting the ADA- SCID . Dr. Aron agreed.

Dr. McIvor was concerned about proceeding with the *in uter* gene transfer for ADA- SCID now. He note that the ongoing ADA- SCID gene transfer trials for newborns and children are not totally successful, i.e low efficiency of stem cell gene transfer and the continuous need for PEG-ADA. There is a need to learn more about somatic cell gene transfer before moving into the more complex area of *in uter* gene transfer. Dr. Anderson said that the limited success of somatic gene transfer prompted him to propose the *in uter* approach. The new vector will have better gene regulation, and the increased abundance of dividing stem cells in the fetus will hopefully increase the efficiency of gene transfer. Dr. Karson noted that one of th shortcomings of the ongoing ADA- SCID gene transfer can be avoided by a gradual but not precipitou

withdrawal of PEG-ADA. Dr. Markert said restoring the thymus function is critical for the success o ADA- SCID trials since gene-modified T cells need to be "educated" in the thymus in order to be full functional.

XVIII. Ethical, Legal, and Social Issues (ELSI) - Specific Clinical Indications/Mackl

Presentation – Dr. Macklin

Dr. Macklin summarized the RAC discussion of the pre-protocols, and its implications for developing an ethical framework for *in uter* gene transfer research. She summarized areas of agreement and disagreement among RAC members in their general discussion of *in uter* gene transfer research and the RA's discussion of the two pre-protocols. In addition, Dr. Macklin outlined a series of unanswered general questions arising from the RA's deliberations.

Areas of agreement (for both protocols)

(1) More animal safety and efficacy data are needed.

(2) More information is needed on the probability of germ-line alteration (especially for the α- thalassemia pre-protocol).

(3) Ideally, a candidate for *in uter* gene transfer research should be one in which there is a long time period during which information can be provided for the woman/couple to make a decision.

(4) There is a need for genetic counseling to be made available to the woman/couple either prior to or as part of the recruitment process; the genetic counselor should be independent of the research team.

(5) Consent to autopsy should be an inclusion criterion for entry into these studies; however, if people change their mind their refusal to permit an autopsy may not be overridden.

Areas of disagreement (specific to each protocol)

ADA- SCID **is not** a good candidate for *in uter* gene transfer research because: (1) an alternative--successful post-birth treatment--exists for this disease, and (2) there is a reasonably high probability of inadvertent germ-line gene transfer. ADA- SCID **is** a good candidate precisely because an alternative treatment exits, so the parents have another option and theirdecision about whether t participate in the research has a greater likelihood of being fully voluntary and less "forced" by desperation.

α- Thalassemia has a **favorable** benefit-risk ratio because: (1) the disease is usually fatal to the fetus, and (2) carrying an afflicted fetus is toxic for the woman. α- Thalassemia has an **unfavorable** benefit-risk ratio because: (1) the fetus may survive but the resulting child may have severe anomalies (partial correction), and (2) the pregnant woman may turn out to be worse off than if she opts for the recommended current alternative, i.e., abortion of an affected fetus.

Additional disagreement regarding the α- thalassemia protocol focused on the role of the father-to-be Some RAC members and *ad hoc* consultants contended that the father-to-be should be involved in the decision to enroll, whereas others said the father should not be involved. All agreed that the male partner has an **interest** in the outcome, but some questioned whether that interest conferred a **right** to be a

coequal decision-maker with the woman.

General (unanswered) questions arising from the discussion

(1) Is a disease an unacceptable candidate for *in utero* gene transfer research when an effective post-birth therapy exists? Or, alternatively, does that feature make it a better candidate from the perspective of informed decision-making?

(2) Is a disease an acceptable candidate for *in utero* gene transfer research when the consequence of nontreatment is death of the fetus *in utero*?

(3) Is a disease an acceptable candidate if a consequence of in utero therapy is partial correction resul in survival leading to a worse outcome?

(4) Should a disease be lethal in order to be a candidate for *in utero* gene transfer research?

(5) In the consent process, what should be said about other alternatives that are themselves "innovative" or unproven therapies?

(6) Should the risk (how high a risk?) of inadvertent germ-line transfer make a proposed protocol for *in utero* gene transfer research unacceptable?

Other Comments

Dr. Noguchi said that the father-to-be has an interest in the outcome of the α- thalassemia protocol; th father-to-be bears part of the burden of a partially corrected baby. Dr. Macklin agreed that the male partne has an **interest** in the outcome, but she questioned whether that interest conferred a **right** to be a coequal decision-maker with the woman.

As a point of clarification, Dr. Noguchi noted that during the RAC discussion in 1995, it came to a conclusion that *in utero* gene transfer is only appropriate for a disease in which there is significant morbidity during the intrauterine stage. Dr. Aguilar-Cordova noted that this is an area of disagreement in the present discussion.

Dr. Karson noted partial correction is a concern for subjects to enter α- thalassemia protocol. She no that without *in utero* gene transfer six α- thalassemia babies were born alive but with defects. Dr. Cohe noted that the actual frequency of live birth in this disease is uncertain since the total number of α- thalassemia cases are unknown. He noted that all six babies are born with a lifelong serious disease Dr. McIvor said that the six cases illustrate the predicament of partial correction for α- thalassemia

Dr. Mickelson said that ADA- SCID is a good candidate disease for *in utero* gene transfer because it has clinical experience of the ongoing somatic gene transfer trials, and the availability of an alternative therapy if the *in utero* gene transfer fails. Dr. McIvor suggested to include post-natal stem cell transplantation as part of the future protocol for ADA- SCID

Dr. Anderson asked if it would be ethical to perform *in utero* gene transfer on an ADA- SCID fetus if ther is a matched bone marrow for transplant after birth. Dr. Markert noted it is a difficult ethical question however, the pregnant woman should be informed about any risk and benefit of gene transfer. She noted that different families have different opinions about what level of risks they consider acceptable.

Dr. Noguchi said that the two pre-protocols are to serve as the focal point of discussion; the RAC does not need to accept or endorse any particular proposal. Dr. Markert noted that there are other better candid diseases in which there are no alternative therapies. Dr. Aguilar-Cordova agreed.

Ms. King cautioned that choosing diseases which have no alternative therapies would likely have situational coercion in the informed consent process. The subjects frequently have difficulty in understanding the difference between research and treatment, and in their assessment of risk of harm and chance of benefit. In addition payment by managed care systems and compensation for research injury will complicate the issues. Dr. Markert said the much larger issues of compensation and insuranc coverage are beyond the present scope of RAC discussion. Ms. King said the investigators should understand that their mission is primarily for conducting the clinical research rather than providing treatment for patients who ordinarily have no alternative therapy. Dr. Markert said that the families shou understand the financial costs of a partial correction. Dr. Cohen noted that the care of an - thalassemic infant is about $30,000 per year for the first few years of life.

Ms. Jean Starr, Kensington, Maryland, from the audience noted that the infants once born have legal rights; however, they can not reject any *in uter* intervention that might cause them to bear an outcome of partial correction.

Ms. Levi-Pearl noted that individuals who are born with disabilities are very sensitive to the issues of *in uter* gene transfer to correct any congenital defects for fear of discrimination against persons with disabilities.

XIX. Chair's Closing Remarks/Mickelson

Dr. Mickelson emphasized that the purpose of these RAC discussions of Dr. Anderson's proposals was to begin a dialogue on the issues surrounding *in uter* gene transfer; the discussion was not for approval of the proposals.

Dr. Noguchi applauded the RAC for initiating a process that will advance *in uter* gene transfer research under an ethically and publicly responsive manner; a similar process in 1988 preceded the FDA approval of the first somatic gene transfer clinical protocol in 1990. Dr. Noguchi noted that the FDA first brought the issue of *in uter* gene transfer to the RAC in 1994 when the FDA felt the need for public discussion of reviewing a pending IND *in uter* stem cell transplantation. He said the FDA appreciates the RAC discussion of the complex issues. Having Dr. Patterson from FDA to head ORDA represents a forma commitment by the Federal Government to foster and enhance the unique public service of the RAC.

XX. Future Meeting Dates, Announcements/Mickelson

The third GTPC *Prenatal Gene Transfer: Scientific, Medical, and Ethical Issues*, is scheduled for January 7-8, 1999 at the Hyatt Regency Bethesda, and the next RAC meeting is scheduled for March 11-12, 1999, at NIH , Building 31C, Conference Room 10

XXI. Adjournment/Mickelson

Dr. Mickelson adjourned the meeting at 3:15 p.m. on September 25, 1998.

Debra W. Knor
Executive Secretary

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

Date: 09/25/98

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