RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Symposium and Meeting

December 8-10, 1999

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTENTS

Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <http://www.nih.gov/od/oba/>.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
December 8-10, 1999

The Recombinant DNA Advisory Committee (RAC) was convened for its 76th meeting at 8:00 a.m. on December 8, 1999, at the National Institutes of Health (NIH), Building 10, Masur Auditorium, 9000 Rockville Pike, Bethesda, MD 20892. Dr. Claudia A. Mickelson (Chair) and Dr. Inder Verma (Co-Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on December 8 from 8:00 a.m. until 2:45 p.m., on December 9 from 8:30 a.m. until 3:00 p.m., and on December 10 from 8:30 a.m. until 3:30 p.m. A committee roster is attached (Attachment I). The following individuals were present for all or part of the meeting:

Committee Members:

C. Estuardo Aguilar-Cordova, Texas Children's Hospital and Baylor College of Medicine
Dale G. Ando, Cell Genesys, Inc.
Xandra O. Breakefield, Massachusetts General Hospital
Louise T. Chow, University of Alabama, Birmingham
Theodore Friedmann, University of California, San Diego
Jon W. Gordon, Mount Sinai School of Medicine
Jay J. Greenblatt, National Cancer Institute, National Institutes of Health
Eric T. Juengst, Case Western Reserve University
Nancy M.P. King, University of North Carolina, Chapel Hill
Sue L. Levi-Pearl, Tourette's Syndrome Association, Inc.
Ruth Macklin, Albert Einstein College of Medicine
M. Louise Markert, Duke University Medical Center
R. Scott McIvor, University of Minnesota
Claudia A. Mickelson, Massachusetts Institute of Technology

Executive Secretary:

Debra W. Knorr, National Institutes of Health

Ad Hoc Consultants and Speakers:

Mark Batshaw, Children's National Medical Center, University of Pennsylvania
Arthur L. Beaudet, Baylor College of Medicine
C. Thomas Caskey, Merck & Company, Inc.
Bruce A. Chabner, Harvard Medical School
Ronald G. Crystal, Cornell Medical Center
Lyndah K. Dreiling, Gencell/Rhône-Poulenc Rorer Pharmaceuticals, Inc.
Wilma Friedman, Columbia University
Linda Gooding, Emory University
Angus J. Grant, Gencell/Rhône-Poulenc Rorer Pharmaceuticals, Inc.
JoAnn Horowitz, Schering-Plough Research Institute
Marshall Horwitz, Albert Einstein College of Medicine
David H. Kirn, Onyx Pharmaceuticals, Inc.
David Magnus, Center for Bioethics
David J. Margolis, University of Pennsylvania Medical Center
David P. Meeker, Genzyme Corporation
Richard C. Mulligan, Howard Hughes Medical Institute

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Glen Nemerow, Scripps Research Institute
Amy Patterson, National Institutes of Health
Anne M. Pilaro, U.S. Food and Drug Administration
Steven Raper, University of Pennsylvania
Margaret Rick, Warren Grant Magnuson Clinical Center, National Institutes of Health
Jay P. Siegel, U.S. Food and Drug Administration
Lana Skirboll, National Institutes of Health
Mark Tuszynski, University of California, San Diego
Dinko Valerio, IntroGene
Inder Verma, Salk Institute
Robert Warren, University of California, San Francisco
Karen Weiss, U.S. Food and Drug Administration
James M. Wilson, University of Pennsylvania Health System
Kathryn Zoon, U.S. Food and Drug Administration

Nonvoting Representatives/Liaison Representatives:

Daniel W. Drell, U.S. Department of Energy
Melody H. Lin, Office for Protection from Research Risks, National Institutes of Health
Andra Miller, U.S. Food and Drug Administration
Philip Noguchi, U.S. Food and Drug Administration

National Institutes of Health Staff Members:

Levent Akyurek, NHLBI
Suresh Arya, NCI
Krysztofs S. Bankiewicz, NINDS
Ami Bhatt, NIMH
Manfred Boehm, NIH
Bryan Brewer, NHLBI
John Burklow, OD
Connie Caldwell, OD
Natasha J. Caplen, NHGRI
Henry Chang, NHLBI
Donna Chinnasamy, NHGRI
Nachimuthu Chinnasamy, NHGRI
John Chiorini, NIDCR
De-Maw Chuang, NIMH
Behor Cohn, NIAID
Ross Coppel, NLM
Anthony Craig, NIH
Lori M. Croskey, Chaplain Intern
Claudio D'Ambrosio, NIAID
Kleper N.F. De Almeida, NIAID
Maria Dinger, NHLBI
Neal D. Epstein, NHLBI
Julian Escobar, NHLBI
Wendy Fibison, NHGRI
Ruth L. Fischbach, OD
O. Ray Fitzgerald, CC
Gregory Frykman, NCI
Joseph F. Gallelli, OD
Luci Gonzalez, NIAID
Stephen Groft, OD
Jo Grossheim, Chaplain Intern
Brooke H. Haehl, NCI
Naoki Hamajima, NHGRI

Joe Harford, NCI
Changting Haudenschild, NHLBI
Julie Hedrich, NHLBI
Craig A. Higgins, NHGRI
Masaru Ichida, NHLBI
Christine Ireland, OD
John Janik, NCI
Jing Jieyu, NCI
James Kaiser, NIH
Nicholas Keiser, NHGRI
Meyoung-Kon Kim, NCI
Richard Knazek, NCRR
Sachiko Kojigaya, NHLBI
Robert Kotin, NIH
Helen Kupinsky, NHGRI
Robert Lanman, OD
Becky Lawson, OD
Rebecca Link, NHLBI
Jay Lozier, NHGRI
Mario Mautino, NHGRI
Catherine McKeon, NIDDK
Koji Miyazaki, NHLBI
Richard Morgan, NHGRI
Darrell Morris, NCI
John C. Morris, NCI
Karen Morrow, NIH
Linda M. Muul, NHGRI
Gary Nabel, VRC
Carolyn Nagler, NCI
Shriram Nallamshetty, NHLBI
Connie Noguchi, NIDDK
Samer Nuwayhid, NCI
Ray O'Neill, NCRR
Pearl O'Rourke, OSP
Makoto Otsu, NHGRI
Ryszard M. Pluta, NINDS
Robert Pratt, OOPD
Rosemary Quitley, COPR
Donald Ralbovsky, NIH
Nicholas P. Restifo, NCI
Bob Roehr, COPR
Gene Rosenthal, OD
Silvia Santamarina-Fojo, NIH
Nava Sarver, NIAID
William J. Savage, NIAID
Shepherd H. Schurman, NHGRI
Justina Schwenberger, NIAID
Magda Sgagias, NCI
Robert D. Shamburek, NHLBI
Alan Shecht, NIDDK
Susan E. Sherman, OGC/NIH
Thomas Shih, OD
Hillary Sigmon, NINR
Karen Sikes, OD
Sonia I. Skarlatos, NHLBI
Anne M. Slavotinek, NHGRI
Kumar Srinivasan, NEI

Victoria S. Statham, NIH
Howard Streicher, NCI
Masaaki Takatoku, NHLBI
Marcia L. Taylor, NIAID
Andrea True, NHLBI
Harold Varmus, OD
Robert E. Walker, NIAID
Robert P. Wersto, NIA
Judith Whalen, NICHD
Brian Wojcik, NCI
Ling Xu, NIAID
Zhiyong Yang, NIAID
Takanobu Yoshi, NIH
James A. Zwiebel, NCI

Others:

Approximately 450 individuals attended on each of the first 2 days of this 3-day RAC symposium/meeting; approximately 60 individuals attended the third day. A full list of attendees appears in Attachment II.

I. Call to Order and Day One Opening Remarks/Dr. Mickelson, Dr. Verma, and Dr. Patterson

Dr. Mickelson, RAC Chair, called the meeting to order at 8:00 a.m. on Wednesday, December 8, 1999, in the Masur Auditorium at the National Institutes of Health. The notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 18, 1999 (64 FR 63051) and December 7, 1999 (64 FR 68397), and the proposed action on November 22, 1999 (64 FR 63827). Issues to be discussed by the RAC at this symposium and meeting included a 1-day symposium on adenoviral vectors, including adenovirus biology, pathophysiology, adaptation of adenovirus for gene transfer, and safety and toxicity data from clinical trials using adenoviral vectors; discussion of a severe adverse event on Human Gene Transfer Protocol #9512-139 (a Phase I study of adenoviral vector-mediated gene transfer to liver in adults with partial ornithine transcarbamylase [OTC] deficiency); current issues in adverse event reporting and proposed action; discussion of Human Gene Transfer Protocol #9906-322—(1) nerve growth factor *ex vivo* gene therapy for Alzheimer's disease; and discussion of Protocols #9910-342 and #9910-343—respectively, a Phase I trial to evaluate the safety of an adenovirus expressing platelet derived growth factor-B (PDGF-B) for the treatment of diabetic insensate foot ulcer and a Phase I trial to evaluate the safety of the same vector, in combination with limb compression bandage, for treating venous leg ulcer.

Dr. Mickelson noted that the family of Mr. Jesse Gelsinger, who died while participating in a gene therapy trial for treatment of OTC, was present at this meeting. This symposium's purpose was to examine scientific and technical data to come up with guidelines for researchers and the RAC for clinical trials that use adenoviruses.

Dr. Verma reiterated the hallmark of the RAC, which is openness and frankness. The main purpose of this meeting is to learn, gather information, and make recommendations to the RAC to learn more.

Dr. Patterson echoed Dr. Mickelson's and Dr. Verma's statements of the major objectives of this symposium: to achieve a more thorough understanding of adenoviral gene transfer and advance the goal of ensuring the safety of patients enrolled in trials. For the first time, a patient was lost in a clinical study of adenoviral gene transfer. The sharing of adverse events data in Phase I trials for the treatment of these severe diseases, which often have a high incidence of naturally occurring adverse events, enhances the possibility that collection and pooling of these data may show patterns that enable improved understanding of the potential toxicities associated with the adenovirus vector. Variables that could influence the safety of this method of gene delivery include the type of adenoviral vector, route of vector administration, dosage, and clinical condition of the patient. The challenge is to apply knowledge and reason to more fully understand the potential toxicities associated with this method as well as to understand the cause of this individual's death. The RAC will develop a final report on adenoviral vectors, and a proposal may be issued

for public comment if the RAC's final report determines that further guidance is needed. After consideration of the public comment, the RAC will transmit its final recommendations to the NIH Director; if approved by the Director, this guidance will be incorporated into the *NIH Guidelines*.

II. Keynote: Adenovirus Biology, Pathophysiology, and Adaptation to Gene Therapy

Adenovirus Molecular Biology and Disease/Dr. Marshall Horwitz, Albert Einstein College of Medicine

Dr. Horwitz provided an overview of the adenoviruses, which belong to Adenoviridae, a family of more than 49 serotypes that infect humans. Adenoviruses were discovered at the NIH in 1953 as a cytopathic agent in the tissue culture of tonsils and adenoids; they are associated with a variety of diseases.

Diseases caused by adenoviruses, the risk groups for those diseases, and the serotypes responsible for the syndromes were enumerated by Dr. Horwitz. Adenoviruses cause much disease that has disrupted military recruitment and basic training, due to crowding and stress. Conjunctivitis, diarrhea (particularly in young children), a variety of respiratory diseases, and probably myocarditis are caused by adenoviruses. In immunosuppressed individuals, adenovirus persists in the urinary bladder, can cause a fatal hepatitis, and persists in the gastrointestinal tract. One of the hallmarks of adenoviruses—when they cause *in vivo* or *in vitro* infection of tissue—is intranuclear inclusions, which cause characteristic morphologic changes in the nuclei of infected cells.

Of the 6 subgroups of 49 serotypes, serotypes 2 and 5 of subgroup C are used most commonly in gene transfer. Dr. Horwitz explained how the adenovirus attaches to cells and how the adenovirus is transported to cell nuclei. Human liver has little of the coxsackievirus and adenovirus receptor (CAR) messenger ribonucleic acid (RNA) necessary for transportation to the nuclei, much less proportionately than in the mouse. Early viral transcripts from four regions of the viral genome encode proteins that are essential for viral replication; early transcripts from the E3 region are immunoregulatory and cytokine-regulatory functions and are not essential for viral growth *in vitro*.

Origins of deoxyribonucleic acid (DNA) replication are at each of the ends of the viral DNA. The E1A gene is a major transactivator for other adenovirus genes. E1A and E1B genes alone are sufficient to cause transformation in rodents, but advenoviruses do not cause tumors in humans. The E1A gene induces apoptosis (cell death), and several of the proteins that inhibit apoptosis are coded by E1B genes; both E1A and E1B genes are eliminated from most vectors used in human trials. E2 genes are responsible for DNA replication and have been manipulated in some of the second-generation vectors.

The E3 region codes for cytokine and immunoregulatory genes, which can affect a number of cell processes initiated by molecules that can kill the cell by binding to cell surface receptors. E3 genes are potent immunoregulatory genes that are potent enough to prevent autoimmune diabetes in mice. The E4 region affects viral messenger RNA transport; in this region, proteins can either prevent or induce cell death.

Adenovirus proteins can induce cell death, or their deletion or function can prevent cell death. Human adenoviruses are species specific for their full replication cycle. They replicate poorly or not at all in most rodents; replication in simian species is also suboptimal but is enhanced by coinfection with simian virus 40 (SV40). Adenoviruses are helper viruses for the growth of the parvovirus, adeno-associated virus (AAV), which is also used heavily in gene therapy.

Not much is known about the role of host-cell genetics in adenovirus infection. Host genetics may be important in the outcome of infection or gene therapy.

Adaptation of Adenovirus for Gene Transfer/Dr. Inder Verma, Salk Institute

Dr. Verma stated that the success of clinical trials has been limited primarily because of the difficulty of gene delivery—how to deliver genes so that they can be expressed in a sustained fashion. Adenoviruses have several advantages: They do not integrate into the host cell genome, they are easy and quick to grow, they infect dividing as well as nondividing cells, and their biology is well known. The virus is disarmed of the components necessary for its replication and then is made able to introduce the desired gene. Because of its inability to replicate itself, it does not have its own pathological consequences.

Dr. Verma showed a diagram of how adenovirus is made. What makes adenovirus so attractive is its ability to make large numbers of virus particles in a short time. One handicap of the adenoviral vector is that, although large amounts of the virus can be made efficiently, it cannot produce the required foreign protein for a sustained period of time in an immunocompetent animal, possibly due to immunological consequences. The challenge becomes how to make a vector with the least amount of immunological complications and with sustained production of the foreign protein (e.g., Factor IX for treatment of hemophilia B, in the case of Dr. Verma's laboratory).

A second problem with adenoviral vectors is that repeat administrations are not possible; a few days after infecting dogs or mice with adenovirus, researchers consistently find massive production of antibodies against the proteins as essential components of the virus. The adenoviruses are considerably problematic with regard to immunogenicity; thus, the task of most scientists working with adenoviruses is to strive to make vectors with the least amount of toxicity.

Adenoviral vectors have been extremely useful, both for biological purposes as well as for biochemical and scientific purposes, but none of the current classes of vectors is perfect. Adenoviral vectors can infect nondividing cells, but with serious immunological consequences. Scientists who work on adenoviral vectors are looking for ideal vectors that are (1) producible at high concentrations, allowing many nondividing and dividing cells to be infected; (2) convenient and reproducible in their production and use; (3) able to integrate in site-specific locations in the host chromosomes or to be successfully maintained as a stable episome; (4) able to target the desired type of cell; (5) transcriptional units that can respond to manipulation of its regulatory elements; and (6) devoid of components that elicit an immune response.

III. Examples of Adenovirus-Induced Pathophysiology
Moderators: Dr. Mickelson and Dr. Verma

Interplay Between Adenovirus and Proinflammatory Cytokines/Dr. Linda Gooding, Emory University

Dr. Gooding explained that all adenovirus serotypes can infect epithelial surfaces and that all subgroup C serotypes have the capacity to produce a long-term relationship with the host. Epidemiologic studies show that individuals can shed adenovirus episodically and asymptomatically over long periods. These viruses have the capacity, by unknown mechanisms, to maintain a long-term relationship with an immunocompetent host in the face of a variety of immunological challenges. Dr. Gooding discussed how the wild-type virus manages to accomplish this feat.

Adenoviruses suppress the immunological responses by a host, and adenoviruses contain products that block interferon, which has the capacity to inhibit virus replication. Dr. Gooding's laboratory focused on tumor necrosis factor (TNF) because it has the capacity, via interaction with specific receptors on the cell surface, to trigger apoptosis. Normal cells are not susceptible to TNF-induced cell death. Expression of some kind of adenovirus gene renders normally resistant cells sensitive to the lytic effects of TNF, which is the sole property of the adenovirus E1A proteins; expression of genes within the E3 region prevents this cytolysis.

In studying activation of an enzyme called the large cytoplasmic phospholipase A2—the primary regulated point in the production of an array of inflammatory mediators—the adenovirus prevented an inflammatory cascade. Dr. Gooding illustrated the downstream events of a worst case scenario, that is, what happens when the cytokine TNF is activated and allowed to reach significant systemic levels.

In a local inflammatory response, TNF acts on neutrophils and on vascular endothelial cells to increase vascular permeability. If, however, cells in circulation are stimulated to produce TNF at high levels, then the effects are different: TNF will trigger the production of other inflammatory mediators. Moderate quantities of TNF act to create fever and on liver to produce acute phase proteins. At higher quantities, TNF presence produces septic shock, with effects on heart, blood vessels, liver, and muscle.

Dr. Gooding concluded that cytokines have the potential, in high-dose adenovirus therapy, to have an impact on outcome and therefore should be monitored.

Receptors and Signaling Events in Adenovirus Cell Entry/Dr. Glen Nemerow, Scripps Research Clinic

Dr. Nemerow discussed the current state of knowledge of adenovirus-host cell interactions at the earliest stages. He also presented some of the gaps in knowledge of those interactions, indicated where adenoviruses may play a role in adverse reactions with host cells, and discussed ways in which specific modifications of the virus could be made to achieve more selective targeting of adenovirus-to-host cells, which might result in increased efficacy. Dr. Nemerow considers fiber protein the major structure that mediates attachment to host cells through CAR. He discussed the penton base protein, which interacts with cell surface integrins to mediate internalization of the virus particle.

Important questions that remain about CAR include the following: (1) What is the level of CAR in different tissues, and how is CAR expression regulated? (2) What is the normal function of CAR? (3) Does CAR ligation promote signaling events? and (4) Can adenovirus type 2 or type 5 use other cell receptors?

Dr. Nemerow then discussed a second receptor involved in adenovirus internalization, the integrins that contain an α_V and a β_3 subunit. Integrin questions that are being examined in multiple laboratories include the following: (1) Does α_V integrin signaling induce cytotoxic or apoptotic responses? (2) Are specific cytokines involved in these interactions? and (3) Are there other specific integrin cofactors that might be involved?

Work in other laboratories suggests that there are other potential interactions with integrins that may lead to apoptosis. Important knowledge is emerging about the precise interactions of the fiber with its receptor and $\alpha_V\beta_3$ integrins with the penton base protein, leading to internalization. Significant gaps still exist about how these interactions get the virus into the cell and lead to encoding, but there are also opportunities to understand and modify the virus to improve the selectivity of gene transfer.

Adenovirus-Induced Hepatotoxicity/Dr. Robert Warren, University of California, San Francisco

Dr. Warren presented a clinical perspective on adenovirus-induced hepatotoxicity. Adenovirus is very hepatotrophic, taken up by both benign and malignant hepatocytes. With that attractive feature of adenovirus, the problem is the potential for significant toxicity within the liver. The issue is whether this hepatotoxicity is a serious impediment for using adenovirus gene transfer in the liver.

Dr. Warren stated that most viruses (e.g., hepatitis B and C, herpes simplex) have the same effect on the liver. The characteristics of viral hepatotoxicity include a variable level of inflammatory cell infiltrates, piecemeal necrosis of the liver (if infection or host response is severe), apoptosis, fibrosis, and ultimately cirrhosis (when virus infection is chronic at a low level). Severity of an acute infection is measured through the release of enzymes specific to hepatocytes, called transaminases.

The issue that researchers seek to address is a definition of the mechanisms of adenoviral hepatotoxicity. In animal models—principally rodents—intravenous (IV) delivery of adenovirus leads to significant hepatotoxicity. This observation has led clinical investigators to be cautious about intravascular (both intravenous and intraarterial) delivery of adenovirus in humans. Strategies to diminish adenoviral hepatotoxicity include modification of the E1-deleted virus, transient immunosuppression at the time of initial virus delivery using an immunosuppressive drug, inhibition of transcription factor NFkB, coexpression of the antiapoptotic gene Bcl-2, and enhanced liver regeneration.

The question of whether hepatic toxicity limits the utility of adenovirus for gene transfer in humans is being examined at the University of California, San Francisco (UCSF), in two types of trials of treating cancer in humans: (1) direct intratumoral (IT) injection of an E1B-deleted virus for liver tumors and pancreatic tumors and (2) intraarterial injection of adenovirus to the liver for patients with metastatic colorectal cancer or primary hepatocellular carcinoma.

For the second group of studies, UCSF has been using a recombinant E1- and E3-deleted adenovirus that expresses the wild-type p53 gene under the control of a cytomegalovirus (CMV) promoter. Researchers in this second group of studies speculated that regional delivery of adenovirus might satisfy the same criteria as typical, regionally delivered chemotherapeutic agents. That is, delivery of adenovirus to the tumor (by a

catheter through the groin into the right branch of an artery leading to the liver) could be enhanced by intraarterial delivery of the virus; systemic exposure would be limited because of the high affinity of adenovirus for normal hepatocytes. At a dose of 7.5×10^{13} particles per kilogram (particles/kg), two patients showed dose-limiting toxicity—hypotension and alterations in electrocardiogram. All patients developed fever, even at the lowest levels. As dose level increased, so did adverse events, although no events were severe until the highest dose level was reached. Other adverse events included chills and slight alterations in blood pressure. A Phase II trial has begun.

Dr. Warren stated that there is much to be learned about liver toxicity. To this point, with the construct and transgene used by UCSF, liver toxicity appears to be less than expected and is not dose limiting.

Disseminated Intravascular Coagulation/Dr. Margaret Rick, Warren Grant Magnuson Clinical Center, National Institutes of Health

Dr. Rick explained that disseminated intravascular coagulation (DIC), a consequence of adenovirus infection, is an intermediary of disease that accompanies other illnesses. DIC is a widespread activation of coagulation, resulting in systemic intravascular thrombin generation, with consequent fibrin formation; the clot causes thrombosis in small and midsize vessels. This clot leads to ischemic tissue damage (because of the decreased blood supply consequent to the thrombosis) and bleeding (because of the utilization or consumption of the coagulation factors and platelets that deposit in these thrombi). DIC comes in two forms, always associated with other illnesses: (1) acute—infections (bacterial and viral), obstetrical complications, malignancies, and hepatic failure, and (2) chronic—malignancies, subacute obstetrical complications, and other conditions.

Clinical presentation of DIC is bleeding, which occurs in 70 to 90 percent of patients with this syndrome—bleeding that is cutaneous, gastrointestinal, genitourinary, or pulmonary in origin. Thrombosis occurs in 10 to 40 percent of adenovirus-treated patients, causes multiorgan dysfunction, and usually is seen in kidneys, liver, or the central nervous system (CNS).

A prime step in development of DIC is release of a clotting factor called tissue factor, which normally is present at all cell surfaces in the human body. Increased exposure of the tissue factor, or upregulation, is caused by (1) direct tissue trauma, (2) activation of endothelial cells through infection or injury, (3) monocyte tissue factor expression, and (4) production and expression of tissue factor by malignant cells. The fibrinolytic system is activated at the same time the clotting cascade begins. Endothelial cells normally express tissue factor only locally, in concentrations that do not cause a diffuse clotting problem. The exposure of cytokines, particularly interleukin-1 (IL-1) and TNF, is thought to play a major role in the activation and upregulation of tissue factor.

Diagnosis of DIC depends closely on the associated disease. Laboratory tests are needed for coagulation screening factors and platelet counts to establish a diagnosis. Screening tests are often nonspecific and can be abnormal in the presence of other diseases besides DIC, but a variety of common clotting tests will be abnormal with a prolongation of the clotting times and a decrease or consumption of the fibrinogen and platelets. Dr. Rick concluded by stating that treatment can include replacement therapy with coagulation factors, platelets, and inhibitor concentrates; interrupting the process with heparin is another treatment option, although its use is more dangerous in a patient with serious bleeding.

Toxicity Experience With Adenoviral Vectors at Baylor College of Medicine/Dr. Estuardo Aguilar-Cordova, Texas Children's Hospital and Baylor College of Medicine

Dr. Aguilar-Cordova summarized Baylor College of Medicine's many years of experience with gene therapy trials. He described the mechanics of gene therapy transfer and summarized published and more recent results, providing an analysis of the factors that might influence the toxicity of adenoviral vectors including what kind of vectors (e.g., first-generation vector or gutless vector) is being used, where it is being used (e.g., what disease or which animal species), and when it is used (e.g., patient age or health status).

Dose specifications are critical, and it is not clear that all researchers are speaking the same dose "language." Terms such as "vector particles," "infectious units," and "focus-forming units" make

comparisons of the titres or dose specifications difficult. It is also important to know the original specifications of the product used in preclinical studies and what specifications are being used in the clinics.

Dr. Aguilar-Cordova discussed the evolution of adenovirus vectors. A comparison between an E1-deleted vector and a vector with additional E2 deletion indicates a small amount of differential toxicity between these first- and second-generation vectors. Comparison between gutless (helper-dependent) vectors and first-generation vectors indicates that the gutless vector produces a more prolonged expression and, in the mouse, no elevation of liver transaminases.

Whether administration of adenoviral vectors is oral, intravenous (IV), subcutaneous, intraperitoneal (IP), or intratumoral (IT), each location has different characteristics and different toxicity profiles. Recipient immunity may have a critical effect on toxicity, and species differences may also need to be analyzed. Age-related anecdotal toxicity data indicate that older patients in brain tumor trials seem to fare a little worse than younger patients; the same data are apparent in some mouse studies. Patient health status—particularly general organ function and immune status—also affects the toxicity experience.

Standardization of dose specifications is necessary and clinical potency assurances are critical. Not all adenoviral vectors have equivalent toxicity profiles, but some generalities are possible. The additional safety of the future generation of vectors may have only a limited window of applicability. Dr. Aguilar-Cordova concluded by stating that the critical dose of susceptibility is not yet known, nor is the extent of efficacy in the clinical setting.

Helper-Dependent Adenoviral Vector—Development, Performance, and Safety/Dr. C. Thomas Caskey, Merck & Company, Inc.

Dr. Caskey observed that direct injection of adenoviral vectors into muscle, a variety of individual tissues, and the central nervous system (CNS) has provided opportunities for significant local expression. Although these various methods of administration provide enthusiasm for adenoviral vectors as a broadly useful gene therapy platform, each method of administration has different safety challenges that could significantly alter the benefit-risk ratio. This analysis is similar to the development of a new drug, in which fundamental technology has demonstrated the efficacy of the drug on the target. Determination must now be made about how adenoviral vectors are administered—by aerosol, intramuscularly, intravenously, and so forth. Studies of benefits and risks must align with the anticipated clinical usage and must take into account the severity of the disease.

Dr. Caskey's research is dedicated to improving the efficiency and efficacy of adenoviral vectors for IV use. His research strives for high capacity, deletion of adenovirus gene expression elements, production stability, production capacity, and *in vivo* long-term and high-level expression. No safety study was discussed in this presentation.

Helper-dependent adenoviruses offer promise as gene therapy vectors, in part because of overcoming the technical difficulty of their production. Dr. Caskey outlined the helper-dependent vector system. Earlier studies with ob/ob mice showed the effect of a first-generation adenovirus vector for weight loss (a vector expressing leptin); eventually, the expression is lost because the animal has immune reaction to leptin. These studies, however, demonstrated that the helper-dependent vector was better tolerated for inflammatory response, both morphologically and by enzyme-level assay. Because of substantial problems that occurred, including rearrangements observed in the vector and the inability to achieve the necessary levels of production, researchers embarked on improved methods to make this vector commercially productive. There has been no example within Merck in which helper-dependent viruses did not have a greater longevity and long-term expression—with fewer effects on hepatic dysfunction—than first-generation adenoviruses.

The goals at Merck are to (1) redesign the helper-dependent vector for stability and yield, (2) define the *cis*-acting elements for the helper-dependent vector, (3) choose the optimal backbone, (4) create a production cell line, (5) find a production process, and (6) benchmark the new helper-dependent vector against first-generation vectors and earlier versions of helper-dependent vectors.

Dr. Caskey concluded that the higher capacity of the helper-dependent vector offers an opportunity for introduction of regulatory elements. Application of the vector must be matched to the particular clinical system and how the vector is being administered. Only the benefit-risk evaluation of specific applications in gene therapy deserves extreme scrutiny before clinical trials. No single element is critical, by itself, before moving to Phase I trials.

IV. Safety and Toxicity Data From Clinical Trials Using Adenoviral Vectors
Moderators: Dr. Mickelson and Dr. Verma

Summary of Phase I Studies With Adenoviral Vectors at the University of Pennsylvania/Dr. James M. Wilson, University of Pennsylvania Health System

Dr. Wilson discussed the University of Pennsylvania's experience with regard to safety using adenoviruses, listing the trials during the past 5 years in which the university produced the vector and conducted the safety testing.

- Cystic Fibrosis (CF) (three trials)—Adenovirus vector was deleted of E1 and E3, and dosing was stopped after eight patients because of issues related to immunogenicity of E1-deleted vectors. This dosing was tolerated well, except that patients consistently developed infiltrates. The trial was stopped to generate better vectors, moving to vectors in which there were deletions of E1 and E4. Toxicity seen with E1/E4-deleted vector was referable to the respiratory tract in most patients, with grades 1 and 2 toxicities, occasional fever, and some elevation in white blood cell count. A current trial uses an E1/E4-deleted adenoviral vector administered in aerosol form. So far, only two patients are enrolled. Aerosol administration should result in less toxicity and better distribution of vector.

- Central nervous system (CNS) (two studies, one completed)—Adenoviral serotype 5, E1/E3 deleted, was used. The most frequent symptoms were headache and neuroedema at the injection site; all patients experienced fevers. Grades 1 and 2 increases in the transaminases were seen, with unclear etiology, as was a decrease in the white blood cell count. Observed electrolyte abnormality was probably not related to vector but to the fact that these patients have undergone craniotomy and resection of tumor. The other trial involves injecting the vector (E1/E3-deleted expressing interferon-β) in the tumor margins. One patient who has received this therapy had a significant spinal headache as the only adverse event.

- Melanoma and Metastatic Breast Cancer Trial—Eight patients are enrolled, with injection directly into tumor. This has been relatively well tolerated; no grade 3 toxicities have been seen.

Mesothelioma (a tumor of pleural cavity that encases the lung) (two trials)—One trial started with a protocol in which the adenoviral vector (E1/E3-deleted) was instilled into the pleural space. More than 26 patients have been enrolled, all of whom are relatively sick and whose prognoses are less than a year. Some minor (grades 1 and 2) elevations in transaminases have been observed, along with one significant but transient decline in lymphocytes. With these patients, researchers consistently see fever and chills (grades 1 and 2). The regimen is reasonably well tolerated; most patients experience pain related only to the chest tube that is already in place.

A second mesothelioma trial uses an E1/E4-deleted vector, with the hope of more prolonged gene transfer and further improvement of the safety profile. Eight patients are enrolled in this trial; experience in this group includes adverse events that consist primarily of flu-like syndromes of fever, fatigue, and chills as well as pain at the injection site.

Dr. Wilson concluded that the experience at the University of Pennsylvania with adenoviral vectors has incorporated many different applications. Ninety-five patients have been dosed, with all but 18 showcased in this presentation. [The 18 patients in the OTC study were discussed on December 9 at this meeting.] In applications other than intravascular administration, toxicity appears to be dose dependent and time limited. Evidence has been seen for systemic manifestations, indicating a broader release of cytokines because of the high fever these patients often experience.

Safety of Local Delivery of Low- and Intermediate-Dose Adenovirus Gene Transfer Vectors to Individuals With a Spectrum of Comorbid Conditions/Dr. Ronald G. Crystal, Cornell Medical Center

Dr. Crystal discussed Cornell's experience since 1993 using E1/E3-deleted adenovirus gene transfer vectors, including 3 transgenes and 90 individuals with CF, colon cancer, severe coronary artery disease, and peripheral vascular disease, plus 12 healthy subjects as controls. Dr. Crystal and his colleagues included all of this information in a manuscript that was submitted to and accepted for publication by *Human Gene Therapy* (*HGT*). With *HGT*'s permission, Dr. Crystal made copies of this manuscript available to the RAC.

Since 1993, 14 deaths have occurred in a total population of 102 (including controls): 3 individuals in the CF group, 3 in the colon cancer group, 4 in the coronary artery disease group, 1 in the same disease group undergoing minimally invasive surgery, 2 in the peripheral vascular disease group, and 1 of the 8 individuals in the control group. No dose dependency is obvious in these deaths. The overall mortality rate was within the general experience of individuals with these serious diseases.

Analysis of adverse events related to adenovirus vectors showed only 1 serious adverse event attributable to the vector, of 140 administrations of vector. This event occurred in 1993; the vector is now administered by spray (as opposed to bronchoscopic instillation), and no serious toxicities have been seen since the switchover. Other events include infrequent fevers, leukocytosis, and transaminase possibly linked to the vectors. The largest numbers of adverse events are not in the CF group but in cardiac patients who also had cardiac bypass surgery.

In terms of shedding adenovirus vectors in gene therapy trials, 1,685 samples reveal 1 replication-deficient adenovirus seen at the site of injection (the nose) and no replication-competent virus detected. For the cardiac surgery group, platelet counts are down, but this is natural for this group. The best measure of safety is how well the patients do.

Dr. Crystal concluded that these observations are consistent with the concept that local administration of low and intermediate doses of adenovirus vectors appears to be well tolerated. When supported by relevant efficacy data, it is appropriate to initiate controlled Phase II studies.

Dr. David P. Meeker, Genzyme Corporation

Dr. Meeker reviewed Genzyme's experience with gene therapy, focusing on experience with the company's adenoviral vector. Five trials have used adenovirus—three on CF (46 patients), one on metastatic melanoma (6 patients, *ex vivo*), and one on critical limb ischemia (4 patients). Preclinical toxicology trials included 5,100 mice with doses as high as 5×10^{12} particles/kg; 50 deaths occurred, some of which were attributable to the vector. Dose-dependent transient pulmonary inflammation was noted, which tended to peak by day 21 and resolve by day 28.

Primate studies included single-dose and repeat-dose studies. Thirty-three animals received a single dose, and no deaths occurred; dose-related pulmonary inflammation was noted. In repeat-dose studies, four animals received a top dose of 5×10^{12} particles/kg; there were no deaths, but at the highest dose, there was moderate alveolar inflammation. Intrapulmonary administration on nonhuman primates included three animals. Lungs appeared normal, although one animal received a dose of 1×10^{13} particles/kg and died at day 5.

Dr. Meeker provided a summary of clinical trials for CF—a nasal single-administration trial plus a repeat-administration trial with escalating doses. All patients had mild to moderate CF. Three vectors were used: E1-deleted, then E1/E4-deleted, followed by a third-generation vector. Safety in the nasal repeat-dose trial indicated 16 pulmonary exacerbations during the 1-year followup, including 2 that required hospitalization. The lung trial safety summary indicated 24 serious adverse events, only 1 of which was judged to be related to vector. The 16 pulmonary exacerbations were consistent with observations in this large, 520-patient cohort for CF. To understand the toxicity of adenovirus, a review of acute events that occurred in the first 7 days is instructive. These events included the following:

- Four of nine had fever that was transient and self-limited.

- Four of nine had evidence of infiltrations at the site of vector administration and at the site of bronchoalveolar lavage.

- At the highest dose, two of three had fever, and all three exhibited new infiltrates on their computerized tomography (CT) scans.

Since administration via aerosol produced no evidence of toxicity, bronchial administration was discontinued.

Dr. Meeker concluded by stating that Genzyme's assessment of adenovirus is that it is inefficient, producing transient, low-level gene expression. This conclusion has sent Genzyme researchers back to the laboratory to improve on the efficiency of the adenoviral vectors being used. Dose-dependent, localized lung inflammation was consistent with animal studies. Aerosol administration was well tolerated up to a dose of 1 x 10^{10}. A variable measurable immune response was present but was not clearly dose dependent. Multiple analyses of multiple sites indicated no evidence of viral shedding.

Dr. JoAnn Horowitz, Schering-Plough Research Institute

Dr. Horowitz reviewed Schering-Plough's extensive experience with Schering 58500 (SCH 58500, also known as ACN53), a recombinant adenovirus engineered to carry the human wild-type p53 gene. She focused on the safety data generated to date, specifically with intrahepatic artery (IHA) delivery. This program has been carried out exclusively in patients with advanced cancer, the majority of whom have exhausted all other available therapies.

Four toxicology routes of administration were studied—intratumoral, intravenous, intraperitoneal, and intra-arterial (carotid and hepatic). Only at the highest dose levels were hemodynamic effects seen.

SCH 58500 was administered in studies in the United States and Europe by the intratumoral route in 71 patients with advanced lung and head and neck cancers or recurrent skin tumors. The safety profile revealed primarily flu-like symptoms, which were easily manageable in all cases. No clinical effects related to coagulation changes were seen.

The initial intraperitoneal trial was conducted in three sites. SCH 58500 was administered as a single dose to 17 women with advanced ovarian cancer. One patient experienced a grade 3 elevation in alkaline phosphatase with other associated liver function changes; she died 51 days after the single administration, with the cause of death assessed as progression of disease. Forty additional women dosed at this and higher levels have not produced a repeat of this event. Additional related serious adverse events included abdominal pain, fever, and anemia.

Thirty-nine women received multiple intraperitoneal doses of SCH 58500 together with cytotoxic chemotherapy. The doses were limited by practical considerations such as volume but not by adverse events, although an increase was seen in constitutional symptoms at higher doses. Fourteen alert reports may have been related to the mechanics of the intraperitoneal catheter or the intraperitoneal delivery or to the underlying disease; relationship to treatment was possible but unclear because certainty about relationship to treatment is difficult when dealing with people with advanced cancer. The case of transient ischemic attack (TIA) mischaracterized as a stroke (appearing recently in the lay press) was reported initially by the investigator as a TIA and reported to the authorities as such; further investigation revealed that this event was a complex migraine. Dr. Horowitz pointed out the need for careful assessment of adverse events before broad public discussion takes place.

An intrahepatic artery (IHA) Phase I study was conducted with four patient groups. Safety was assessed in 30 colon cancer patients with liver metastasis using a traditional, single-dose escalation study with nine dose levels. One patient had a significant reaction (transient hypotension) at the highest dose but recovered fully. Infrequent but reported adverse events included fever, tachycardia, pain, insomnia, shortness of breath, sweating, and thrombocytopenia; all of these events were transient reactions. No clinical adverse events

were related to laboratory changes, and the only serious related adverse event was grade 4 elevated liver enzymes.

For Schering-Plough's IHA experience, the maximum tolerated dose was 7.5×10^{13} particles/kg. As part of the protocol of all studies, these advanced cancer patients are followed until death. In total, 95 deaths have been reported in the trials, none of which were believed to have a probable relationship to the vector.

Dr. Horowitz concluded that the Schering-Plough data reflect an appropriate potential benefit-risk ratio in patients with advanced cancer and that it is appropriate to continue to assess the safety and efficacy of SCH 58500 in patients with cancer with additional safety monitoring.

Ad5CMV-p53 (RPR/INGN 201)—Global Safety Assessment/Dr. Lyndah K. Dreiling, GenceII/Rhône-Poulenc Rorer Pharmaceuticals, Inc.

Dr. Dreiling described Rhône-Poulenc-Rorer's (RPR) product, patients, and database scope. RPR's product is a serotype 5 adenoviral vector with a CMV promoter carrying the human p53 tumor suppressor gene, with a backbone that is E1 deleted to render it replication incompetent. All patients have cancer and have failed previous treatment, with no therapeutic options available and an unresectable tumor. The global safety database spans 4 years and includes more than 413 patients in more than 20 studies. Throughout that 4-year period, there have been no treatment-related deaths. More than 75 percent of RPR's experience is with patients with advanced head and neck or lung cancer. The analysis presented is preliminary; a quality analysis still needs to be performed.

Patients were treated by intratumoral (IT) injection at the highest dose of 1×10^{12} viral particles. Monitored data are available for 307 treated patients, reflecting 702 cycles of therapy and 2,300 treatment days. Some patients have been treated continuously for up to 18 months.

The number of reported adverse events is almost 6,000 with these 307 patients, but this number is not unexpected with a very sick population. Only two events were related to study treatment—fever and injection site pain, both of which were reported as transient and were graded as mild to moderate. Patients who were noted to have fever and chills also were more likely to have a number of constitutional symptoms related to their treatment. Serious adverse events (events that required hospitalization or were life threatening) indicate a list that is not unexpected in this patient population. Treatment-related serious events were extremely few.

Dr. Dreiling concluded that, in this well-characterized patient population, adenovirus-p53 administered by the IT route is well tolerated. Despite exhaustive efforts, RPR found no trends suggesting safety concerns, none with adverse events, and none with laboratory parameters. This safety database, combined with product activity data, supports late-stage development.

Viral Cancer Therapy With ONYX-015/Dr. David H. Kirn, Onyx Pharmaceuticals, Inc.

Dr. Kirn reviewed the staged clinical development followed in the development of ONYX-015 in late-stage cancer patients who have no remaining therapeutic options. Data indicate that ONYX-015 has been well tolerated in more than 230 patients, including 34 patients treated intravascularly. Data also document antitumoral activity in these patients, which is worthy of further clinical development.

ONYX-015 is an adenovirus serotype 2/5 chimera that contains no transgenes but does contain an important deletion in the E1B-55KD gene region. Because of this deletion, Onyx believes this virus is able to target p53-deficient cancer cells selectively; clinical trials have shown that this virus selectively replicates in and kills tumor cells. The route of administration started as intratumoral (IT) and was changed to intraperitoneal (IP). In the interest of safety, the administration route was changed to intra-arterial (using the hepatic artery), and then to intravenous (IV). In parallel, Onyx discovered a positive interaction with chemotherapy that appeared to maximize clinical benefit to patients, so chemotherapy was woven into the trials' designs.

A total of 235 patients have been treated, with head and neck cancer representing about 111 of these patients. Dr. Kirn summarized four Phase I and II studies on head and neck cancer patients. At doses up to and including 2 x 10^{12} particles/kg, the virus has been well tolerated in these patients; no dose-limiting toxicities have been seen, and no maximum tolerated dose has emerged. The most frequent adverse events include flu-like symptoms and injection site pain; no clinical or laboratory evidence of liver toxicity has been seen, nor has any clinical evidence of blood-clotting abnormalities or disseminated intravascular coagulation (DIC) appeared.

Antitumoral activity has been observed in several trials, showing two complete responses and two partial responses (defined as greater than 50 percent tumor regression). Summary of head and neck cancer experience to date indicates a favorable safety profile in addition to antitumoral activity, particularly in combination with standard chemotherapy. A randomized Phase III trial will begin early in 2000.

Dr. Kirn then discussed Onyx's experience with intrahepatic artery (IHA) infusion. UCSF and the University of Chicago treated 19 patients with intratumoral injection of tumors within the liver with ONYX-015. That trial demonstrated that the virus was well tolerated by that route of administration in the liver at doses of up to 2 x 10^{12} particles/kg. On the basis of that safety experience, Onyx hopes to selectively perfuse multifocal tumors in the liver by this route of administration. Twenty-seven patients have been treated to date, with endpoints of safety, maximally tolerated dose, and antitumoral activity. The most frequent adverse events (grades 1 or 2) included fever, weakness, nausea, rigors, chills, and some adverse events only seen in association with chemotherapy. Liver assessments of these patients indicate no evidence of treatment-emergent clinical hepatotoxicity. Some minor changes have been observed in liver function tests—mild, transient aspartate transaminase (AST) and alanine transaminase (ALT) increases in approximately one-third of patients. Liver function test elevations in six patients were reported as adverse events; four were due to tumor progression, and two possibly were attributable to ONYX-015.

The safety summary of ONYX-015 indicates that the virus has been well tolerated, with no dose-limited toxicities or maximally tolerated doses identified. Fevers, chills, and rigors from grades 1 to 3 are common at a dose of 2 x 10^{11} particles/kg. No clinically significant liver toxicity has been seen, nor has there been clinical evidence of blood-clotting abnormalities or DIC. No deaths have occurred during this trial; six deaths have occurred due to tumor progression within the liver and elsewhere after patients have gone off study.

Dr. Kirn concluded that the ONYX-015 virus has been well tolerated in more than 230 patients, including 34 who received the virus intravascularly. Onyx has documented antitumoral activity and clinical benefit in these patients. Continued development via IHA and IV routes is warranted in advanced cancer patients at doses up to 2 x 10^{12}/kg.

Adenovirus-Mediated Expression of Human Factor IX in Rhesus Macaques and Associated Dose-Limiting Toxicity/Dr. Richard Morgan, National Human Genome Research Institute, National Institutes of Health

Dr. Morgan reported on the administration of adenoviral vectors via intravenous (IV) administration where the vector has been designed to express human clotting Factor IX.

Rhesus macaques are small nonhuman primates with a physiology much like humans, representing a useful animal model to examine toxicology and efficacy data. Data in a previous report showed no development of an immune response to the human protein in the rhesus macaque because the macaque Factor IX gene is 97 percent identical to the human Factor IX gene, making the rhesus macaque a viable animal model.

To answer the question of whether the macaque can express human Factor IX mediated by a first-generation adenovirus vector, a dose-escalation study was undertaken. Three adult rhesus macaques showed short-term gene expression that persisted for 2 weeks and declined by the third week. No activity was elicited at the lowest dose. One measure of acute phase protein response is serum interleukin-6 (IL-6) concentration, which peaked 6 hours following administration at high and medium doses; there was no response at the low dose. In the high-dose animal, measures of blood coagulation showed pronounced elevations in whole-blood clotting time and activated partial thromboplastin time, and significant changes were seen in fibrinogen levels. These changes were seen to a lesser extent in the intermediate-dose

animal, and no changes were seen in the low-dose animal, indicating a possible threshold phenomenon. Platelet count declines were observed in all animals, even at low doses.

Previous work confirmed that human protein did not elicit an immune response in macaques to human Factor IX. In the context of an adenovirus vector, researchers wondered whether there would be a response to the human Factor IX protein. All animals quickly developed high-titre antibodies to the human Factor IX protein, antibodies that were not present when the protein was administered to the macaques. The macaques also developed an antibody response that would inhibit coagulation. The administered protein has a low half-life, indicating that it is being cleared quickly from circulation.

V. Panel Discussion and Definition of Terms

Dr. Verma suggested that some presenters define terms used in their presentations, including liver function tests (LFT) and why it is important in adenoviruses, what effects for the liver and the cell are created by increases in proinflammatory cytokine levels, and the definitions of first-generation, second-generation, third-generation, and gutless vectors.

Dr. Arthur Beaudet, Baylor College of Medicine, stated that LFT refers to a group of enzymes that used to be referred to as serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) and also as aspartate transaminase (AST) and alanine transaminase (ALT). AST and ALT are the modern designations for the two most often measured liver enzymes. A rise in the blood levels of these enzymes suggests that the liver cells are being damaged to the point that their internal contents are leaking out and showing up in the bloodstream. If liver enzymes go up, the liver cells are being injured in some way.

Dr. Gooding explained what is meant by an increase in the lymphokine levels, particularly the proinflammatory cytokines like tumor necrosis factor (TNF) and interleukin-1 (IL-1) and interleukin-6 (IL-6). Each of these cytokines has the capacity to trigger more mediators. The arachidonic acid metabolites are small molecular weight compounds that have powerful biological effects to activate cells and bring them into a site of inflammation. These cells then release granule contents that can kill other cells and cause tissue damage.

Dr. Verma explained that first-generation vectors have one part (E1or E1 and E3) of the adenovirus removed, second-generation vectors are produced by attenuating a second component (e.g., temperature-sensitive mutation in E2a) of the adenovirus, and third-generation vectors is the same vector that has been debilitated even further (E1 and E4 deleted). Gutless vectors are produced by removing much of the genomic information involved in virus replication and substituting other information to prevent unwanted consequences.

Dr. Bruce A. Chabner, Harvard Medical School, asked why there have been no experiments in which an empty vector without any transgene alone is used as a control, particularly where local tumor injection is being done, given that inflammatory and immune responses to the virus are important variables. Dr. Kirn stated that no such trials are currently planned by Onyx Pharmaceuticals. Dr. Warren explained that, in the cancer field, there are few preclinical data supporting empty vectors as antineoplastic agents. Dr. Gordon noted that he had raised the same question at several previous RAC meetings.

Dr. Gordon asked about gutless and later generation vectors and whether it is true that when more parts of the adenovirus genome are deleted, it is more difficult to obtain good expression of the inserted gene. Dr. Caskey answered that it is not correct that the more virus that is deleted, the less gene is expressed.

Dr. Verma queried about the titres used with the gutless vectors and how easy they are to make.
Dr. Caskey answered that the titres necessary for a commercial product can vary significantly but that Merck & Co. expects to be able to produce viral particles in numbers high enough to make a commercially viable product.

Dr. Markert asked about the role of the CD4 and CD8 T cells in response to adenoviral vectors and whether they are needed in addition to antibodies to control infection. She also wondered what happens to CD4 and CD8 T cell responses to adenovirus after administration via different routes. If there is suppression of these

responses, are the patients being set up for potential complications by wild-type virus that they might encounter at a time when their immunity has been suppressed? Dr. Horwitz, Dr. Wilson, and Dr. Crystal responded. The fact that patients with cellular immunity deficiencies have persistent adenovirus infections would suggest that CD4 and CD8 T cells have some role in limiting the amount of virus, but there is a dearth of quantitative data; little is known about the wild-type virus because there are no good animal models to study adenovirus infection. In patients who receive vectors, virtually all cases indicate some increase that is dose dependent, along with an increase in antibody response. One exception was that, when vector was given directly into the vascular space, patients who had preexisting T cell proliferative responses, lost those responses for a few months, whereas if they did not have T cell proliferative responses, they had a typical primary response. The T cell proliferative responses do come back, and University of Pennsylvania researchers have begun to examine this depletion of T cell receptors. When looking for cellular immunity in Cornell Medical Center's patient groups, little proliferation of T cells is seen in response to the antigen. When vector is administered up to doses of 5×10^{10} in the lung of normal individuals, no adverse events are seen, and there is no immune response, either locally in the lung or systemically.

In response to Dr. Verma's query about whether a survey has been done to see which strain of adenovirus is least common, with the intent of using a vector of that type so the least number of people have antibodies to it, Dr. Dinko Valerio of IntroGene (Netherlands) indicated that his company has conducted such a survey. From a population of 400 individuals living in Europe and on both coasts of the United States, a significant percent of that population has preexisting antibodies to the majority of adenoviruses; Dr. Valerio stated unequivocally that most normal people have antibodies to most of the 49 serotypes of adenoviruses.

Dr. Aguilar-Cordova wondered whether there is evidence of efficient gene transduction following the initial inoculation. He also queried whether the same level of safety would be encountered in patients that start out with a large dose vs. those patients who receive smaller doses and are worked up to the large dose. Dr. Horowitz answered that all patients in the Schering-Plough studies had preexisting immunity and that transgene expression was confirmed by all three routes of administration. There appeared to be a dose threshold after which expression was seen, but it is unknown whether the expression would have been seen at a lower dose in the absence of antibodies. Regarding Dr. Aguilar-Cordova's second query, several researchers indicated that they do not dose-escalate within patients for the reason outlined in the question. Dr. Kirn stated that Onyx researchers could find no correlation between baseline positivity or the peak level of antibody titre and efficacy or toxicity; it is premature to state that neutralizing antibodies determine antitumoral activity or toxicity. In a number of patients, however, with repeat dosing, ongoing antitumoral activity was evident despite high levels of antibodies. Dr. James Merritt, Introgen Therapeutics, stated that Introgen Therapeutics' studies indicate no attenuation of toxicity over time; virtually all patients developed neutralizing antibodies and large spikes in antibodies after their first dose, but the same toxicity profile existed after a fifth or sixth dose, indicating no evidence of correlation between antibody response and toxicity.

Dr. Richard C. Mulligan, Howard Hughes Medical Institute, Harvard Medical School, asked whether, in the cancer trials, there is evidence in patients that the viruses are working as efficiently as they appear to work in mice or monkeys; he wondered whether it is possible to give a dose without toxicity that yields appreciable gene transfer. Dr. Horowitz answered that additional analyses are currently under way at Schering-Plough. Dr. Crystal stated that, in solid tumors such as those in the liver, gene transfer is inefficient; however, in normal tissue such as the lung and the epithelium, where levels like 1×10^{10} can be used, levels of messenger RNA have been measured in the normal or above-normal ranges.

Dr. McIvor queried whether a complex combination of reactions due to cytokine cascades and coagulation cascades requires infection of cells or if simply binding to the surface of the cell could elicit the required response. Dr. Gooding responded that the virus may not have to bind to induce cytokines, although there is scant direct evidence for this method of induction. In response to Dr. McIvor's question about whether there would be any interaction between the cytokine response and the coagulation cascade, Dr. Gooding stated that the proinflammatory cytokines at high doses induce intravascular coagulation or trigger the coagulation cascade. Dr. Beaudet added that, using the gutless vectors, the toxicity in mice in terms of platelet levels and liver function studies is a hundredfold less, with the possible conclusion that entry of the particle into the nucleus is not that toxic in that particular system.

Dr. Friedmann requested clarification from Dr. Kirn as to whether Onyx has information on the levels of preexisting antibody's effect on the efficiency of infection. Dr. Kirn responded that Onyx saw no correlation between toxicity or efficacy (measured by tumor regression) and the baseline neutralizing antibody. There is evidence that intratumoral (IT) injection is more "forgiving" of preexisting high levels of neutralizing antibody than other routes of administration. In addition, Onyx has evidence for ongoing activity and induction of replication cycles using the hepatic artery route of administration, despite the presence of neutralizing antibodies in the blood.

Dr. Friedmann asked whether it would be efficient to introduce adenovirus into the liver through the portal vein with clamped-off circulation. Dr. Warren responded that this route has been studied in delivery of drugs to tumors (not in delivery of advenovirus), with the conclusion that there is no advantage to using the portal route vs. the arterial route, especially since tumors greater than 2 millimeters derive their principal blood supply from the artery.

Dr. Breakefield raised concerns about the difficulty of evaluating toxicity studies because of the inability to compare differences in titres across studies. Dr. Aguilar-Cordova explained that adenovirus particles can be quantified and standardized among laboratories, but that "infectious units" is a qualitative term that depends on how the preparation is analyzed. Dr. Verma echoed the concerns about measuring infectivity and titres.

Dr. Beaudet questioned the doses of the OTC, Onyx, and Morgan studies, in light of the fact that the Onyx study reported no toxicities but the OTC and Morgan studies reported low platelets. Dr. Morgan stated the initial dose as 1×10^{10} plaque-forming units per kilogram (pfu/kg), an intermediate dose of 5×10^{10} pfu/kg and a high dose of 1×10^{11} pfu/kg; the viral preparation had a particle count of 1×10^{13}, and the pfu was 4×10^{11}. Dr. Wilson stated the initial range of the OTC trial went from 2×10^{9} to 6×10^{11} particles/kg, which was the maximal dose at which the death of Jesse Gelsinger occurred. There was a relationship between dose and drop in platelets, first seen in the third cohort—which had a dose of 2×10^{10}—in which a 30 to 40 percent drop occurred that returned to baseline. Dr. Kirn stated that the ONYX-015 trial, using the hepatic artery route of administration, featured an initial dose of 1×10^{7} pfu/kg per individual that escalated up to 1×10^{11} pfu/kg, with a particle-to-pfu ratio of approximately 20:1 and a particle dose range of 2×10^{8} particles up to 2×10^{12} particles. No decreased platelets have been observed with the Onyx preparation, although dose-related fevers and rigors have been observed.

Dr. Noguchi wondered whether Dr. Horowitz's or Dr. Caskey's researchers had evaluated drug-vector interactions in animal toxicology studies. Dr. Caskey responded that formal safety studies on the product candidate have not begun, but he offered to take the idea back to Merck for consideration.

Dr. Gordon asked the assembled adenovirus experts to hypothesize on the mechanisms that lead to toxicity. Particularly, if the mechanisms are intrahepatic, perhaps the liver could be protected and toxicity could be avoided when treating extrahepatic sites. Dr. Warren answered on the basis of his patients with liver metastasis of colon cancer who received intravascular delivery of the E1/E3-deleted p53 expressing virus: Liver toxicity that appeared in many of the patients was reversible in all cases, indicating no clinical sequelae. Fever was uniform in all patients. The dose-limiting toxicity in UCSF's patients was cardiac. One patient experienced, within minutes of viral injection, a dramatic reduction in the capacity of the left ventricle to pump; this reaction may be a direct effect of virus on the cardiac conduction system or the myocardium, but there have been no studies and no appropriate animal models exist. Dr. Beaudet described Baylor College of Medicine's experience with an unintentionally overdosed baboon, in which the platelet drop was related to damaged endothelial cells, which then generated other cytokine responses. Dr. Horowitz described Schering-Plough's replicating model with the mouse virus, in which the damage is through replication in the endothelial cells primarily in the central nervous system; replication in the endothelial cells at the sites of hemorrhage are clear, but the reason for this reaction is not yet understood.

Dr. Beaudet commented that he believes that toxicity is inversely related to the neutralizing antibody, and he predicted that subcutaneous administration would allow a hundredfold increase in dose compared with intravascular administration (a route more accessible to neutralizing antibody) .

VI. Public Comment/Dr. W. French Anderson, University of Southern California

Dr. Anderson made two points. Cutting-edge medical research is risky. When trying to develop treatments for deadly diseases like heart disease and cancer, bad things happen. Dr. Anderson stated that Dr. James Wilson and his colleagues at the University of Pennsylvania are outstanding clinical investigators with a long history of the highest ethical standards. If mistakes were made, they were honest mistakes made by compassionate physicians trying desperately to help their patients.

Dr. Anderson added that Mr. Jesse Gelsinger is a hero who gave his life for a cause in which he believed deeply. Development of gene therapy technology is one of the major hopes harbored by patients who are critically ill from cancer, heart disease, AIDS, and genetic diseases.

Dr. Anderson believes that gene therapy research should not be slowed or stopped. Investigators need to look at protocols and make sure they are as carefully crafted and carried out as possible. The RAC and the U.S. Food and Drug Administration (FDA) need to continue to offer public scrutiny and oversight of gene therapy protocols to maintain public confidence in gene therapy research. Investigators and regulators need to work together to develop gene therapy research as safely, effectively, and rapidly as possible.

VII. Day One Closing/Dr. Mickelson and Dr. Verma

Dr. Verma reaffirmed the purpose of this meeting—to learn, understand what might have gone wrong, and incorporate this new knowledge into the next set of clinical trials. Dr. Mickelson reiterated the deep reservoir of good will and intent that is evident on the RAC. This meeting is part of the RAC's public responsibility, with its format to be repeated in the future as similar issues arise. It was announced that the Working Group on Adenovirus Safety and Toxicity would convene at the close of this session to discuss today's events and then report back to the RAC at tomorrow's meeting.

Dr. Mickelson thanked all the participants and adjourned the first day of the December 1999 RAC meeting at 2:30 p.m. on December 8, 1999.

VIII. Day Two Opening Remarks/Dr. Mickelson

Dr. Mickelson opened the second day of the December 1999 RAC meeting at 8:30 a.m. on Thursday, December 9, 1999, in the Masur Auditorium at the NIH. She reviewed the day's agenda, which included a discussion of a severe adverse event in Human Gene Transfer Protocol #9512-139, presentation of data and information related to this Phase I study, comments from the FDA, comments and questions from the RAC, comments from the public, discussion of the deliberations of the RAC Working Group on Adverse Event Reporting, and a data management report.

Dr. Mickelson commended the investigators at the University of Pennsylvania for the promptness of their actions and their openness in presenting information publicly. She also stated that one of the main recommendations of the Working Group on Adverse Event Reporting was that the NIH, through the RAC, present on a periodic basis a systematic review of the state of the research and serious adverse events in human gene transfer trials over all vectors and all routes of administration.

IX. Discussion of Severe Adverse Event on Human Gene Transfer Protocol #9512-139: *A Phase I Study of Adenoviral Vector-Mediated Gene Transfer to Liver in Adults With Partial Ornithine Transcarbamylase Deficiency*

Clinical Aspects of Ornithine Transcarbamylase (OTC) Deficiency/Dr. Arthur L. Beaudet, Baylor College of Medicine

Dr. Beaudet provided a background of the human body's use of OTC. OTC deficiency is a disorder that involves the way the body takes on and eliminates nitrogen. Neonatal males who have complete OTC deficiency are born normal but within 24 to 72 hours become comatose because of a complete block of the ability to eliminate excess nitrogen. The OTC-deficient gene is on the X chromosome; historically, these patients usually die very young due to acutely high levels of blood ammonia. Males who survive the acute initial episode are stable (maintained on diet and medication) but are profoundly impaired. As these infants

are recognized more quickly, a more intermediate outcome results—most of these children who have only mild to moderate developmental delay are placed on a waiting list for liver transplant.

Dr. Beaudet discussed treatment options. In the acute situation, hemodialysis is used along with other intensive care regimens. Beyond the acute episode and when the patient's ammonia level is reduced, a low-protein diet is necessary, and oral drugs assist the patient in excreting nitrogen. Liver transplant and gene therapy are the other options, both carrying more uncertainty than dietary changes and drug treatment.

Late-onset OTC deficiency can affect males or females. It is generally well managed; the biggest risk is lack of awareness of the disease and subsequent risk of coma and death. Male patients with late onset may or may not have brain injury. Females with late onset are heterozygotic for OTC deficiency, and many are completely asymptomatic and mentally normal.

Dr. Beaudet then discussed the issue of who should receive gene therapy treatment. Patients with partial OTC deficiency often have the advantage of being adults who are able to give their own consent; they are at some risk for brain damage or death from their disorder but are relatively stable. It is easier for researchers to measure the effectiveness of gene therapy in the completely deficient male, whereas in partially deficient individuals, who have substantial amounts of their own enzyme activity, it is difficult for researchers to show whether the introduced gene contributed any enzymatic activity.

As a result of this adverse event, Dr. Beaudet stated his belief that the stable male patient in infancy waiting for a liver transplant may be the best patient for gene transfer therapy, assuming strong data indicating a good chance of efficacy and safety in animals.

Principal Investigator's Presentation of OTC Deficiency Protocol

Dr. James M. Wilson, University of Pennsylvania Health System

Dr. Wilson, Co-Principal Investigator, explained that, since Mr. Jesse Gelsinger's death on September 17, 1999, his research team has been singularly focused on understanding what led to the serious adverse event that resulted in Mr. Jesse Gelsinger's death. The researchers have sought input from colleagues and have worked closely with the FDA in assembling and interpreting the data and deciding how to move forward. About 250 people work at the Institute for Human Gene Therapy, about one-third of whom have been working exclusively on this issue during the past 3 months.

Dr. Mark Batshaw, Children's National Medical Center, University of Pennsylvania

Dr. Batshaw, Principal Investigator, presented his perspective on OTC deficiency as a gene therapy target and how Human Gene Transfer Protocol #9512-139 was designed. He presented an overview of why the researchers believed OTC deficiency would be a good model with which to test gene therapy.

OTC deficiency is a relatively common inborn error of metabolism, with a prevalence of 1 in 40,000 to 1 in 80,000. It is also a devastating disorder, with a first-month survival rate of only 50 percent in neonatal onset disease. Without a liver transplant, only 50 percent of those who survive the first month will live to 5 years of age; more than three-fourths of those who do survive have cerebral palsy, mental retardation, and other developmental disabilities. Even for late-onset disease, the mortality rate is about 10 percent. As coinventor of the currently used alternative pathway, in which three sodium-based products are used as "sinks" for ammonia to provide a detour around the enzymatic block, Dr. Batshaw described this therapy as incomplete at best. Although liver transplantation may hold some hope, it substitutes one disease for another—the need for lifelong immunosuppression and the not-inconsiderable rate of morbidity and mortality. Because liver transplantation seems to work, liver-directed gene therapy might offer an effective approach.

Dr. Batshaw presented an overview of the urea cycle. Ammonia accumulates in a storage form called glutamine (an amino acid). The effectiveness of gene therapy can be measured by ascertaining

(1) whether the patient's glutamine level falls, (2) whether the ammonia level falls, (3) whether the orotic acid level falls, and (4) whether the incorporation of ^{15}N of $^{15}NH_4Cl$ into urea rises.

An animal model is available—the sparse-fur (spf) mouse, which has OTC deficiency and allows an examination of metabolic parameters before and after gene therapy as well as exploration of toxicity issues. The spf mouse has a diminished lifespan of about 47 days. Studies indicate that, after gene transfer, glutamine levels in the spf mouse fall to about normal and stay that way for at least 2 months. However, the issue is how rapidly gene transfer would work—irreversible brain damage occurs in children who are in coma for more than 72 hours. If the adenovirus does not work within a day or two, it would not be effective in getting these children out of coma. Additional studies in the spf mouse provide evidence that adenoviral gene therapy not only appeared to work but also appeared to work rapidly. A series of preclinical toxicology studies in mice and primates led to the development of the clinical trial design.

To decide whom to treat, researchers met with the National Urea Cycle Disorders Foundation (NUCDF), a group of families whose children have OTC deficiency and other urea cycle disorders and with whom the researchers had a prior working relationship. A panel decided unanimously that (1) this study should go forward and (2) it should be conducted in adult, stable individuals who had partial deficiency, because these subjects could give informed consent and because they had metabolic abnormalities in which changes could be examined following gene transfer. This approach was presented to the RAC and subsequently was approved by the FDA.

The pilot study consisted of male and female adult partially deficient patients who were symptomatic and asymptomatic. The dosing was six escalating doses with half-log increments that began with 2×10^9 and ended at 6×10^{11} particles/kg. Three patients were assigned per cohort, for a total of 18. The FDA and the researchers concluded that the safest route of administration was through the hepatic artery, so that route was used. The researchers recognize now that they should have come back to the RAC to discuss this route of administration after the decision was made (and before implementation); Dr. Batshaw apologized to the RAC for not having done so. (At the December 1995, RAC meeting, the RAC approved the protocol with a stipulation to revise the protocol to administer the adenoviral vector via the intravenous route.)

Safety endpoints included clinical changes, laboratory studies (changes in ammonia levels and liver function), liver biopsy to determine liver pathology, and immune studies to examine patient response to the adenovirus. Although this was a Phase I study for safety, evaluation of efficacy was also important. The study was not stopped before the highest dose level was reached, because only partial efficacy was found at lower dose levels.

Dr. Batshaw reviewed the timetable for this OTC deficiency gene therapy program, with Mr. Jesse Gelsinger having been dosed almost 2½ years after the first patient was dosed. Four males and fourteen females were enrolled. Eleven of the eighteen patients were symptomatic, having elevations in ammonia levels and episodes of hyperammonemia. Twelve lots of vector were used for the 18 patients.

Dr. Steven Raper, University of Pennsylvania

Dr. Raper shared the clinical findings from this trial's 18 patients, focusing primarily on what has been learned about what happened to Mr. Jesse Gelsinger.

A summary of the clinical findings for the 17 patients other than Mr. Jesse Gelsinger included fever, backache, nausea, and emesis; in the laboratory findings, only low platelets and low phosphate seemed to be dose related. With regard to liver pathology, many patients had preexisting liver injury. Acute liver injury manifested as minimal to mild inflammation in some patients and occasional apoptosis. Every patient developed an antibody response to the vector. Three of the eleven symptomatic patients showed partial correction of OTC deficiency, a finding that was neither statistically significant nor dose related.

Dr. Raper reviewed the relevant toxicity findings in the six patients of cohort 1 and 2, including a transient fever spike to more than 104 degrees F, liver function test elevations, platelet counts that dropped (transiently) more progressively over the course of the cohorts, and some drop in phosphate that was dose responsive. Cohort 4 (6×10^{10} particles/kg) toxicity findings were elucidated on a patient-by-patient basis.

One error admitted to by Dr. Raper was that the researchers should have contacted the FDA between patients 013 and 014 because of a transaminase elevation of the AST level to 5.3 times the upper limits of normal; patient 014 also had transaminase elevations five to six times the upper limits of normal. According to Dr. Raper, the researchers also should have followed up their written cohort summary, which included their request to proceed to the next dose level, with an additional phone call.

Liver biopsy findings were mild. Features seen in most OTC deficiency patients occurred in the study patients as well. Inflammation was typically minimal to mild, and apoptotic cells were seen either rarely or occasionally. No relation was observed between dose and inflammation or apoptosis. Four biopsies showed evidence of obvious fibrosis, suggesting more chronic liver injury.

Because the researchers were concerned about dose-related lowering of platelet counts, in collaboration with the FDA, additional studies were added to discern whether increased consumption or decreased production was the problem. Dr. Raper offered a schematic representation of how vectors were delivered and how specimens were obtained.

Dr. Raper then discussed the case of Mr. Jesse Gelsinger, patient 019, including his past medical history and a day-by-day elucidation of his pretreatment history. At the time of enrollment, Mr. Jesse Gelsinger met all criteria. His ammonia level at the time of dosing was more elevated than at the time of enrollment (he was put on an alternate pathway therapy before dosing); future inclusion criteria will include evaluations conducted at the time of dosing in addition to those at the time of enrollment. When the protocol was first approved, the researchers were asked to include a male as the third subject in each cohort. Starting with cohort 5, researchers asked and received permission to enroll a male patient as the second subject.

Dr. Raper described Mr. Jesse Gelsinger's posttreatment course on a day-by-day basis. He was essentially comatose by the second evening after receiving treatment, although his liver function tests were actually improving by the third day, a trend that reversed subsequently. In addition to a number of organs that were failing, a neurologic examination on day 5 showed that no meaningful neurological recovery would be likely, so life support was withdrawn. The Gelsinger family allowed the researchers to perform a postmortem examination, the results of which Dr. Raper enumerated; the most unexpected finding suggested an unexplained acute insult to the bone marrow. Mr. Jesse Gelsinger's liver showed hemorrhage and necrosis of between 40 and 50 percent of the liver, consistent with a severe anoxic or oxygen deficiency process; under higher power microscope examination, his liver showed red blood cells infiltrating throughout the liver in the area of the central vein as well as necrotic cells. The endothelium was relatively spared, and there was no evidence of fibrin thrombus in the liver or any other organ; no evidence was found for significant disseminated intravascular coagulaton (DIC). The lungs were markedly edematous, with fluid buildup in the airway membranes, intense inflammatory infiltrate of white blood cells, and multiple amounts of fluid with extravasation of white blood cells in the alveolar air spaces—consistent with a diagnosis of acute respiratory distress syndrome (ARDS). The kidneys were relatively spared, with no evidence of disseminated intervascular coagulation but some red blood cell casts in the tubules compatible with a diagnosis of acute tubular necrosis (a manifestation of ischemic or shock injury). Some areas of the brain were spared, whereas other areas were severely affected, eliciting a diagnosis compatible with severe anoxic insult to the brain.

Dr. Raper summarized that this patient died of intractable ARDS. The tissue damage was most consistent with severe anoxia and/or low flow, hemorrhagic necrosis of hepatocytes (predominantly in the area of the central veins), acute tubular necrosis in the kidney, severe diffuse anoxic encephalopathy, and a completely infarcted spleen. There was little evidence of vector-induced hepatitis and a lack of evidence to support significant sequelae of DIC, and the role of the bone marrow findings remains unclear. The apparent pure red cell aplasia is unlikely to have occurred in the 4 days after treatment, which suggests that the marrow abnormality may have been preexisting, although no clinical history suggested any intercurrent or comorbid condition. Two diagnoses under consideration are an idiosyncratic reaction to medications or infection with a human parvovirus. Differential diagnosis of the respiratory failure showed ARDS secondary to a systemic inflammatory response syndrome (also characterized as an immune revolt). Bacterial pneumonia is a concern, and cardiogenic pulmonary edema and neurogenic pulmonary edema are also possible.

Dr. James M. Wilson, University of Pennsylvania

Dr. Wilson presented issues evaluating animal safety testing of the vector and some additional work that may point to what happened with Mr. Jesse Gelsinger. He reviewed past data and more recent findings with respect to evaluation of vector safety for liver-directed gene transfer, how researchers evaluated this protocol by assessing the vector, and additional studies performed in the context of this protocol.

Researchers in this trial had much experience in the performance and toxicity of adenoviral vectors administered in the circulation. Most of the studies used to support the initial RAC application for this protocol focused on second-generation vectors; however, a third-generation vector was used in this protocol. Comparison of vectors indicated that the third-generation vector had an improved safety profile compared with second- and first-generation vectors. A number of studies were performed on different strains of mice (10 studies) and primates (20 animals), and Dr. Wilson provided an overview of safety and toxicology in mice, rhesus monkeys, and baboons. One aspect of dose dependence was noted as important to consider and as somewhat problematic in developing a clinical trial: The researchers believed that the relationship between dose and toxicity is biphasic. As dose is increased, little toxicity occurs until a narrow window between doses is reached, in which a significant transient increase in the transaminase occurs. Transient decline in platelets (seen in mice and rhesus monkeys) may be an important indicator that something else is going on; however, in clinical trials, the platelet drop was insignificant. Liver failure and DIC at high dose (1×10^{13} particles/kg) were seen in rhesus monkeys; Mr. Jesse Gelsinger's dose was 6×10^{11} particles/kg.

Results of the animal studies taught researchers about the need to vigilantly measure liver damage and any index of DIC; patient monitoring in this study was based on these results. The highest dose in animals before eliciting severe consequences was 1×10^{13} particles/kg in mice and rhesus monkeys and 2×10^{12} particles/kg in baboons. Working with the FDA and based on these data, the researchers selected the highest comfortable dose for the clinical trials as 6×10^{11} particles/kg.

Dr. Wilson summarized three additional experiments that were performed in response to the initial FDA review. Regarding the change in route of administration, Dr. Wilson had thought that the procedure would be safer if the adenovirus were introduced into the hepatic artery; although this change was disclosed to the FDA, it was an oversight that it was not disclosed to the RAC. Additional studies included immune responses to vector (as a potentially significant problem in the ultimate utility of adenoviral and other vectors), systemic inflammation and serum cytokines, biodistribution of vector, and recovery of vector.

Analysis of vector-specific immunity in the OTC deficiency protocol indicated a response that has not been seen in other clinical trials nor in animal studies, i.e., lymphoproliferative response, in which patient's white blood cells are stimulated to proliferate in response to addition of the vector in an *in vitro* test.. Patients who came in with low lymphoproliferative responses (indicating no exposure in the recent past to an adenovirus type 5) all had activated T-cell. Patients whose lymphoproferative responses showed previous exposure to adenovirus actually lost their ability to respond to adenovirus. It is unknown whether this is a potential safety issue.

Mr. Jesse Gelsinger experienced fever, DIC without severe liver damage, and ARDS, all of which suggested that some aspect of the immune system was being activated early. Researchers screened serum from some of the patients as comparison. IL-6 data indicated that, as dose increased, significant increases in IL-6 occurred and then returned to baseline. IL-10 responses also increased and then came down, but these responses were delayed. The difference in these patterns is Mr. Jesse Gelsinger, whose IL-6 cytokines went up and persisted, unlike all other patients. The key questions are, What activated Mr. Jesse Gelsinger's cytokines early, and why did the cytokine levels not return to baseline?

The other critical data uniquely derived from this experience concern where vector goes when it is infused into the human hepatic artery. Measurement of the abundance of vector DNA in Mr. Jesse Gelsinger showed a significant amount of vector in the liver (as expected) but also outside the liver—in spleen, lymph node, and bone marrow. This finding bears on the issue of extrahepatic dissemination and the role of distribution of vector outside the liver. Other tests included adenoviral shedding assays and analysis of the vector lot given to Mr. Jesse Gelsinger; no problems were found, including none in animals that received

the same dose level as Mr. Jesse Gelsinger. Analyses of production lot 12, from which Mr. Jesse Gelsinger's dose was derived, indicated nothing different from the previous patient or in the seed stock that could explain the more significant response in Mr. Jesse Gelsinger's case.

Issues currently under investigation include:

- What role did OTC patient 019's underlying disease and concurrent medications contribute to the adverse outcome and interaction with the vector?
- What stimulates the initial release of cytokines?
- Did Mr. Jesse Gelsinger have a genetic predisposition to a severe response to vector?
- Why did the vector distribute beyond the liver? What role did this distribution play in pathogenesis?
- Are there markers that would predict which patients may have a severe response to vector?
- How can animal models assist in analyzing these human responses to adenovirus?
- Can modifications be made in the vector structure?
- What was the role of the abnormality in bone marrow, as found on autopsy?
- Was there a preexisting condition, such as parvovirus infection, that could have contributed to the response?

Dr. Wilson discussed the importance of the relationship of the patient community with the FDA. Expectations of this community are high, particularly for individuals and their families with lethal and disabling diseases. Initial success cannot and should not be promised. At no time during or prior to this trial did the researchers expect to see what occurred in Mr. Jesse Gelsinger. Animal studies never demonstrated pulmonary complications. By virtue of undertaking this trial, Dr. Wilson acknowledged that the researchers made a commitment to the patient community, to Mr. Jesse Gelsinger's family, and to other clinical trial participants to learn as much as possible and to share this information.

Food and Drug Administration Comments

Introduction/Dr. Kathryn Zoon, Center for Biologics Evaluation and Research (CBER), FDA

Dr. Zoon explained the role and background of the Center for Biologics Evaluation and Research (CBER). The regulation is particularly important for investigational new drug application (IND) is Title 21, Code of Federal Regulations (CFR), part 312. The regulation deals with the responsibilities of the sponsors with respect to product quality, design of their protocols, analysis of their data, the types of data that they will collect, and the oversight by FDA. Early phases of clinical development focus on product safety and adverse events. The CBER is working closely with the NIH in coordinating these activities in the area of gene therapy research.

Gene therapy is an active area in the CBER's IND studies, with currently 204 active INDs in gene therapy. In FY 1999 about 30 percent of all the gene therapy INDs are being conducted with adenoviral vectors. The FDA supports the work of the NIH and the RAC and works closely with both. Recently, the FDA made public its standard operating procedures for notifying the NIH of changes in gene therapy protocols and for receipt of averse event reports.

Toxicology/Dr. Anne M. Pilaro, CBER, FDA

Dr. Pilaro presented preclinical data that supported the FDA's decision to proceed with the intrahepatic artery (IHA) infusion. When the RAC first reviewed this protocol, investigators provided information regarding IV administration of the OTC vector to the spf mouse model, which showed that correction of the

genetic defect could be achieved. Ten safety studies with intravenous (IV) administration in the mouse indicated transient hepatitis, elevation of transaminases, and inflammation in the liver. When the researchers came to the FDA, they had done some work with both first- and second-generation vectors and demonstrated decrease in the inflammatory response with the second-generation vector. Data submitted in the original IND and seen by the RAC also involved treatment of several rhesus monkeys with the first- and second-generation vectors. Toxicities were seen in the liver, which included elevation in the transaminases, hepatitis, and inflammation in liver cells. The maximally tolerated dose by the portal vein route was 5×10^{12} particles/kg, or eightyfold more than the maximum clinical dose. Deaths were seen in the high-dose monkeys, by both the IV and intraportal vein infusion routes.

Additional studies included IHA administration in two baboons. Toxicity seen in the liver—a transient elevation in transaminases—resolved by day 28. The severity of these findings was similar to portal vein infusion in rhesus monkeys in similar doses. After the RAC made the recommendation to use IV administration, the FDA requested biodistribution studies conducted intravenously. In the C3H mouse, there was widespread distribution of the vector to nontarget organs including gonads, a dose-related increase in the amount of vector signal present in the ovaries that persisted for 90 days. As a result, the IND submission was placed on clinical hold because, at that time, the RAC's policy was that vector signal in gonadal tissue was an "absolute stop."

Dr. Pilaro also summarized proposed changes in the vector to a newer construct, several studies of evidence of gene transfer to gonads and to progeny, and adenoviral distribution in baboons after IHA infusion using the new third-generation construct. The new construct was found to be less inflammatory to the liver in the mice after IV administration and in the baboon after IHA administration. Similar toxicity profiles were seen in the baboons and the mice, regardless of route of administration, and there was no evidence of distribution to the gonads, even at doses in the baboon that were half the highest human clinical dose (the maximal achievable dose these researchers could give the animals using this route of injection).

After Mr. Jesse Gelsinger's death, the FDA asked the investigators to provide data that showed any additional toxicity information regarding the infusion of these vectors. The investigators provided data from rhesus monkey studies in which some deaths occurred at the highest doses (1×10^{13} particles/kg). All three animals showed evidence of DIC, but the animal that survived showed evidence that DIC was resolving. The investigators also examined the same lot of vector that was given to Mr. Jesse Gelsinger. At the same dose that was given to Mr. Jesse Gelsinger, all animals survived to terminal sacrifice, and only minor toxicities to the liver were observed—minor increases in liver enzymes and adequate or slightly increased platelet counts. The histology information is still pending.

From the data presented yesterday, from the information presented this morning by Dr. Wilson and colleagues, and from review of the file on this clinical trial and its aftermath, Dr. Pilaro stated that the FDA has learned that there is a threshold level for adenovirus toxicity to the liver. Twofold to fivefold differences exist between the maximally tolerated dose in the rhesus monkey and the mouse and death of the animals. The effects are seen regardless of the vector backbone (first, second, or third generation), regardless of the route of administration (IV, IHA, or portal vein infusion), and regardless of the transgenes being studied (marker gene or OTC). Dr. Pilaro explained that the IHA route was deemed by the FDA to be safer than others for vector injection, since there was no dissemination of signal to the gonadal tissue (which went along with the current policy of the RAC at the time this file was submitted) and since only minimal toxicities were observed even at close to the highest human dose when given to baboons by the IHA route.

Clinical Perspectives/Dr. Thomas L. Eggerman, CBER, FDA

Dr. Eggerman reviewed the clinical aspects of the OTC trial from the FDA's perspective, emphasizing important safety aspects in the design of the trial and including those aspects that were changed after FDA review. He commented on the Informed Consent document and indicated changes recommended by the FDA. He also reviewed the conduct of the trial, which in part reflects an ongoing FDA analysis of what has occurred during the past 3 months, and indicated protocol changes that were made in response to the events in this trial.

In review of this protocol, the FDA, the RAC, and the sponsor were concerned about the safety of administering the adenoviral vector to hemizygotic males and symptomatic heterozygotic females with OTC who had adequate control with drug and/or diet therapies and to heterozygotic women who were sufficiently healthy not to require any treatment. At the pre-IND stage, the FDA raised the question of whether testing this adenoviral vector might be more appropriate in significantly affected male infants who otherwise might die. The sponsor indicated it would be difficult to differentiate between death related to the natural course of disease and potential adverse events related to vector administration, wanting to study first a relatively healthy population that carried the genetic defect to ascertain an appropriate dose and to characterize the safety profile.

The clinical trial design was an open-label, dose-escalation study. As a safety measure, the starting dose was 2×10^9 particles/kg (a 500-fold reduction below the no-effect dose in nonhuman primates), and the dose was escalated in half-log increments sequentially in each of the five cohorts. Exclusion criteria were pregnancy or nursing, history of liver disease or cardiovascular disease, or high level of neutralizing adenoviral vector antibody. If two patients developed grade 2 NCI Common Toxicity Criteria toxicities or if a single patient developed a grade 3 or higher toxicity, the study would be put on clinical hold pending an explication acceptable to the University of Pennsylvania Institutional Review Board (IRB) and to the FDA. If evidence of significant metabolic correction was exhibited in the absence of toxicity, the study would be considered completed even if the maximal dose had not been reached.

After review of the IND and discussions with the sponsor, additional safety considerations were added, including that a hemizygotic male would be the third patient in a cohort and (by verbal agreement) that a phone call would be made to the FDA before proceeding with the next cohort. A major intention of this safety study was to establish good communication between the FDA and the sponsor; toward this end and until September 1999, this IND included 22 formal amendments and more than 50 additional communications by fax, phone, or letter. The Informed Consent form indicated that some primates had died after receiving high doses of adenoviral vector; after reviewing the consent form, the FDA recommended several changes, including the addition of statements about potential germ-line effects of gene therapy and all risks related to liver biopsy.

Dr. Eggerman reviewed the history and toxicity results of the cohorts in this trial. Cohort 4 (dose level, 6×10^9 particles/kg) included one patient with transient liver enzyme elevations rated at grade 3, which triggered the stopping rule. After receiving approval from the FDA to enroll a third patient, that patient also developed transient liver enzyme elevations rated at grade 3. Although the protocol dictated that the stopping rule had been met, the sponsor did not contact the FDA to review these results before treating a fourth patient in the cohort. The same results occurred for the fourth patient, and the sponsor submitted summary data for the third and fourth patients approximately 2 months after their treatments. However, this result was not discussed with the FDA at the end of cohort 4 (prior to beginning cohort 5), an aberration from the sponsor's actions after cohorts 1, 2, 3, and 5, which featured discussions with the FDA prior to progressing to the next cohort.

The first patient in cohort 6 (dose level, 6×10^{11} particles/kg) showed grade 3 toxicities of transient decreased phosphate and fever. The second patient enrolled in cohort 6 was Mr. Jesse Gelsinger. The agreement with the sponsor was that males would not be treated as one of the first two patients in each cohort; unlike cohort 5, the sponsor did not contact the FDA to obtain permission to treat a male as the second patient in this cohort. When the FDA was informed of serious toxicities observed following Mr. Jesse Gelsinger's vector therapy, the study was placed on clinical hold.

Dr. Patricia Keegan, CBER, FDA

Dr. Keegan reviewed the actions taken by the FDA after being contacted by the sponsor about Mr. Jesse Gelsinger's death. The FDA initiated a search of the database to identify all protocols involving adenovirus gene therapy. The protocols were assessed for those with therapeutic (rather than vaccine) intent, for systemic routes of administration, and for direct injection into the liver. Six INDs were identified that used the IV or intra-arterial route of administration; six additional INDs were identified that contained protocols administering a virus by percutaneous direct injection into the liver.

The FDA then contacted each IND sponsor by telephone in late September and early October 1999. Sponsors were informed of the preliminary information about the death of the patient at the University of Pennsylvania and were asked about toxicities in ongoing trials to determine whether similar events had been observed in current trials; no similar toxicities were reported. Sponsors also were asked to identify the current dose level under study.

After obtaining information from the sponsors, the FDA advised all sponsors to reevaluate the adequacy of eligibility criteria; some protocols were modified to include this request for more aggressive monitoring, particularly for DIC. The FDA also instructed the sponsors to revise Informed Consent documents and to reconsent patients on study with this new information. Protocols were modified by agreement to remain at or below a dose of 2×10^{12} vector particles as a total dose for injection. One protocol was proceeding at a dose that exceeded this level; it was placed on hold pending review of clinical data from that study and additional information from the University of Pennsylvania.

Dr. Jay P. Siegel, CBER, FDA

Dr. Siegel clarified the terms “serious,” “severe,” and “significant” adverse events, which represent different concepts in the regulatory construct.

A *serious* adverse event is based largely on the clinical implications of the event. Although all adverse events are reported to the FDA, seriousness is part of the requirement for expedited reporting. *Severe* is a term used in the National Cancer Institute and World Health Organization toxicity grading criteria and is commonly used in clinical trials to describe a grade 3 toxicity. Severity is not a trigger for expedited reporting; however, most Phase I clinical trial protocols contain provisions based on whether a grade 3 toxicity occurs. A toxicity may be severe but not serious. For example, a marked abnormality of liver function may qualify as grade 3 (severe), but if it does not cause or prolong hospitalization, it may not meet the criteria for serious. In the OTC protocol under discussion, *significant* adverse events were defined to mean events that were grades 3 or 4 (severe or life-threatening).

Dr. Siegel commented about the relationship between FDA review and RAC review. The FDA appreciates the expert scientific analysis and discussion of the RAC, as well as the role the RAC plays in facilitating public discussion. In addition to *ex officio* participation in the RAC, several FDA scientists routinely attend RAC meetings, and the FDA always considers carefully the opinions and advice of RAC members.

The FDA scientists who review gene therapy research bring a unique perspective based on their broad background in overseeing drug development and their specific involvement in reviewing the growing numbers of gene therapy applications and amendments. Any such review occurs at a specific point in time and is based on the information available at that time; frequent modifications in response to new information are to be expected.

Dr. Siegel stated that FDA authorization of this OTC deficiency trial was conditioned on agreements regarding specific interactions of the sponsor with the FDA when certain events occurred. Commonly, after FDA review and authorization, an investigator and sponsor will carry a Phase I trial to completion guided by their judgment and by protocol-specific rules for matters such as dose escalation and stopping, and investigators contact the FDA periodically for annual reporting, expedited adverse event reporting, and approval of protocol modifications. The type of intensive interaction that took place around this OTC deficiency trial is uncommonly requested by the FDA.

RAC Questions on Human Gene Transfer Protocol #9512-139

Dr. Mickelson asked RAC members for their questions. Dr. Mickelson summarized the RAC's questions as follows: (1) What might the researchers have done differently? (2) What can be learned? (3) What recommendations can be made for other researchers and the RAC? and (4) What kinds of experiments should be considered?

Dr. Gordon asked whether Mr. Jesse Gelsinger’s adverse reactions might have been due to infusion line sepsis and whether his medications were discontinued during the gene therapy trial. Dr. Raper responded

that a full sepsis workup, including blood and urine cultures, indicated no evidence for sepsis, although the lungs showed some evidence of staphylococcus infection at postmortem. Mr. Jesse Gelsinger's medications were continued throughout his participation in the trial but were switched from oral to IV forms.

Dr. Markert wondered whether the rhesus monkeys (in the study with the same vector lot used for Mr. Jesse Gelsinger) were preimmune to adenovirus and whether introduction of adenoviral proteins into the liver could worsen the OTC status of patients. Dr. Wilson replied that the University of Pennsylvania screens its colonies for animals that have no preexisting neutralizing antibodies, a process that may not reflect the human population. In preclinical studies in the spf mouse, when hepatitis was produced, their metabolic measures improved nonspecifically, but the researchers were not confident that that result would be duplicated in humans. Dr. Batshaw added that the majority of patients were asymptomatic, and no differences in OTC status were observed.

In response to Dr. Horwitz's question about whether any ongoing active parvovirus infection was observed in Mr. Jesse Gelsinger, Dr. Wilson responded that a plan is currently being developed to review this possibility using polymerase chain reaction.

Dr. Gooding asked whether trends were seen in cytokines—in addition to the IL-6 and IL-10 data presented—and what is known about the aggregation of virus in the material infused into patients. Dr. Wilson reported that another cytokine of interest is TNF-α, which has been surprisingly low in all the patients, probably due to its relatively short half-life. Dr. Joseph Hughes, University of Pennsylvania Health System, who runs the vector manufacturing facility used by the University of Pennsylvania, responded that examination of other adenoviruses made and stored in the same way as Mr. Jesse Gelsinger's lot indicates no evidence of aggregation.

Dr. Warren asked whether administration of adenovirus could have resulted in a greater load to the liver due to release of glutamine and alanine from extremities; he also wondered whether dialysis could have aggravated the situation, since it can activate clotting cascades and cause platelet aggregation. Dr. Batshaw replied that at least 2 hours after the acute infusion, the ammonia level had dropped compared with the preceding day, and there was no evidence of glutamine accumulation. Dr. Wilson added that, in addition to hemodialysis, one difference between Mr. Jesse Gelsinger and the other patients was intubation and then ventilation. In response to Dr. Wilson's question to all researchers present, no one else had experience with patients who received high-dose vector, had a reaction, and then were intubated. Dr. Warren followed up by noting that the issue of pulmonary sequelae deserves more focus, because the series of events that took place were prompted by the ARDS that Mr. Jesse Gelsinger suffered. The critical question, as stated by Dr. Raper, is whether infusion initiated some sort of priming event that led to some secondary event (hemodialysis, intubation, or some other aspect of subsequent therapy).

Dr. Markert wondered whether Mr. Jesse Gelsinger's need for intubation in December 1998, in response to respiratory failure, indicated a different response—different pulmonary reactivity—from most patients with OTC deficiency. Dr. Raper responded that Mr. Jesse Gelsinger's response to that intubation appeared not to be different than expected.

Dr. Friedmann asked about the implications of this adverse event for other *in vivo* deliveries of adenovirus vectors. Dr. Wilson answered that the role of the large "bump" in IL-6 and IL-10 is not known, and it would be valuable for the field to evaluate serum cytokines following the infusion of a vector. In response to Dr. Friedmann's followup query as to whether anything different might have occurred if the injections had been done intraparenchymally in the liver, Dr. Wilson stated that less innate immunity is activated when vector is given directly to an organ or a tissue.

Dr. McIvor asked whether data were available from patients other than Mr. Jesse Gelsinger on hepatic circulation vs. peripheral circulation in the presence of vector; data from studies with monkeys indicated that there was release into the peripheral circulation. Dr. Wilson responded that these experiments were still in progress, although some evidence has begun to show up in the lower doses of vector for proportionately less virus in the blood. Biodistribution of the vector in Mr. Jesse Gelsinger was surprising; although the vector was administered into the hepatic artery, it was not as hepato specific as the researchers had hoped.

Dr. Chow asked whether the researchers could confirm that the virus had entered into any of the patients' hepatocytes, to which Dr. Wilson responded that the virus can definitely be observed in the hepatocytes.

Dr. Gordon asked whether spf mice differ from normal mice in sensitivity to adenoviral infusion, in terms of toxicity. Dr. Wilson responded that, in the limited studies conducted with the spf mouse, no significant differences in the profile for toxicity appeared.

Dr. Beaudet stated his belief that reaction to adenovirus is relatively flat but becomes a steep curve at some point and that IL-6 is one of the markers of that steep curve, meaning that perhaps it is not necessary to infer anything special about Mr. Jesse Gelsinger's case. Dr. Wilson echoed his concern about the steepness of that response curve and that, with natural patient-to-patient variation, dose escalation may significantly increase adverse effects.

Dr. Beaudet reported that his experience in administering adenoviral vectors to newborn calves in Australia resulted in immediate, severe anaphylactic reactions, which some of the animals did not survive; none of the researchers present indicated any knowledge of the same reaction having occurred in humans.

Dr. Breakefield wondered whether high ammonia levels could be a risk factor that would predispose a patient to having an adverse reaction to adenovirus. Dr. Batshaw responded that one study with the spf mouse indicated no further elevation in ammonia level after administering adenovirus on top of a susceptible organism. Dr. Wilson indicated that, through discussions with the FDA, University of Pennsylvania researchers are considering repeating the vector toxicity studies in spf mice that have been challenged with protein.

Dr. Beaudet queried whether normal volunteers given the same dose in the same way as Mr. Jesse Gelsinger would have the same result. Dr. Warren responded that the pulmonary toxicity observed in Mr. Jesse Gelsinger's case has not been seen in the dosing of 25 patients, using an E1/E3-deleted p53 expressing virus to 2.5×10^{13} particles/kg.

Dr. Friedmann wondered whether research restricted to newborns marked with OTC deficiency could constitute a meaningful study. Dr. Batshaw replied that the one advantage in using the ^{15}N study, in which ^{15}N ammonia conversion to ^{15}N urea is measured, is that that conversion is not affected by dialysis and therefore could be used as a mechanism for measuring any changes in urea synthetic capacity following adenoviral gene transfer. Researchers at the University of Pennsylvania had planned to propose a Phase II study on neonates and other individuals in acute hyperammonemia, if this Phase I study had shown a safe and potentially effective dose.

Dr. Wilson concluded the question-and-answer session by offering one specific consideration for the RAC and the FDA in terms of trial design—decreasing the usual half-log increments of vector administration. The relationship between adenovirus dose and toxicity appears to be "elbow-shaped," a phenomenon that might warrant smaller dose increments for Phase I trials. As dose is escalated, dose-limiting toxicities begin to appear slowly, and then an extreme reaction appears.

Public Comments

Patricia Simon, OTC Patient

Ms. Simon favors the continuation of gene therapy trials. She participated in the University of Pennsylvania's OTC gene transfer trial as patient 001 (who dropped out) and again as patient 015. She is an asymptomatic carrier of OTC deficiency who lived with treating OTC deficiency for the 14½ years her son was alive. Babies are not the only individuals who might benefit from successful gene therapy; children and teens may also benefit. Ms. Simon described what it is like to live with this disease on a daily basis by caring for a child with OTC deficiency. No data are available on the long-term effects of the medication that controls (not cures) this disease; the medication tastes bad at best and often causes vomiting. When the disease goes out of control, the child must be hospitalized. Watching a child's diet is not an easy task; the diet itself is incompatible with life because most of the foods associated with childhood are denied children with OTC deficiency. These children are always willing to subject

themselves to additional tests, because the hope is always there that something will help or make a difference. Ms. Simon implored the RAC to let these gene therapy trials continue because it is the only hope that parents and these children have.

(Dr. Mickelson reiterated that the RAC is not considering halting gene therapy but rather is trying to optimize the quality of clinical trials so they are worthy of the time, effort, pain, and altruism that patients invest.)

Cynthia Le Mons, National Urea Cycle Disorders Foundation (NUCDF)

Ms. Le Mons listed the names of several children who have died from OTC deficiency and showed their pictures. OTC deficiency is an orphan disease. It took the death of Mr. Jesse Gelsinger to bring awareness to the plight of sufferers of OTC deficiency. Although the media report that this disease is controllable by drugs and diet, it is not that simple. Every parent and physician knows that this is a daily struggle and that, no matter how well controlled a child is one day, the reality of the disease looms: It may kill that child the next day. The window for treatment is short; death may occur within hours. It is devastating to watch your child suffer on a daily basis, not only from the disease but also from the medications used to control it. Researchers and bioethicists collaborated with NUCDF families at the inception of this gene therapy trial. This study was a safety study; the families knew the risks involved. Mr. Jesse Gelsinger died trying to advance this research. Ms. Le Mons concluded by reading the names of children currently living with OTC deficiency.

Mindy Rosen, NUCDF

Ms. Rosen has a 17-year-old son with OTC deficiency disease. She discussed her son's diet, which is limited to the same breakfast, lunch, and dinner, supplemented with a vile-tasting formula and drugs. Any deviation from this regimen will make him sick. Even with diet and drug therapies, half of the individuals diagnosed with this disorder die. Gene therapy is the only hope for sufferers. Her son is merely one infection away from an episode that could end his life. The message from her son, who is a patient at the NIH Clinical Center, is that he wants gene therapy because all he wants is to live and be normal.

Professor Klaus Cichutek, Paul-Ehrlich-Institut, Germany

Dr. Cichutek reported on an official meeting that was convened in Germany as a result of Mr. Jesse Gelsinger's death. All ongoing trials in Germany using adenoviral vectors were reported on; no serious adverse event reports were related to vector use. All adenoviral vector use was in cancer patients involving various routes of administration, with maximal particle doses of about 1×10^{13}. Dr. Cichutek recommended closer collaboration of all ethics committees, advisory boards, and authorities because many of the gene therapy trials conducted in the United States are also being done in Europe. To ensure patient safety, collaboration must occur. The Commission on Somatic Gene Therapy and the Paul-Ehrlich-Institut will continue to support gene therapy.

X. Recommendations of the RAC Working Group on Adenovirus Safety and Toxicity

Co-chaired by Dr. Verma, the Working Group on Adenovirus Safety and Toxicity was charged with reviewing the submitted adenovirus safety and toxicity data. These data were submitted to the Office of Biotechnology Activities (OBA) (formerly the Office of Recombinant DNA Activities) in response to its request for relevant safety and toxicity data for all registered trials that involved the use of adenovirus; most requests resulted in submissions. The Working Group met after Day One of this RAC meeting and arrived at preliminary findings and conclusions. The report will be finalized by e-mail correspondence.

Dr. Verma summarized the 3 hours of Working Group discussion, stressing that these are talking points that have yet to be answered and that no decisions have been made yet.

1. Standards of vector quantification are needed. The narrow window between early toxicity and severe toxicity underscores the need for effective and accurate standardization. Fundamental questions that have been answered for other vector systems are not in place for adenoviral vectors, including the

issue of how many particles equal one infectious unit, who does the assay, and how titre is measured. Other questions include the following: How is quantification carried out? How is the expression of a gene monitored? Should there be a standard measure of particle number, infectious unit number, or both? Should there be a standard measure, or should a centralized facility exist where all samples and data are sent to be compared to the standard?

(Dr. Noguchi, FDA, agreed with this assessment and with the FDA's primary responsibility for establishing standards, but he also stated a need for help in figuring out the standards. He called for active participation by the NIH, industry, and academic institutions.)

2. What are the endpoints for measuring vector biodistributions, e.g., vector DNA, RNA, or encoded proteins? How can the amount of messenger RNA per cell be quantified so it is possible to discern how much protein is being synthesized? What proportion of cells within a target tissue are transduced?

3. What clinical endpoints should be established for subjects before and after vector administration? For example, would patient immune status, secondary infection, and cytokine profile be useful information for clinical trials?

4. What is the biodistribution, using different routes, in the profile of animal models and patients? Is there a reasonable expectation that, when a vector is infused, it will arrive at the desired cell type?

5. What happens to a vector when it is inserted into a particular cavity or tissue?

VI. Would it be possible for the field, the FDA, and the RAC to discuss and evaluate the ongoing clinical trials of other vectors to discern the relevant toxicities, rather than waiting for adverse events to occur?

7. Now that such high-titre viruses are being used, it becomes even more important to evaluate the quality and integrity of the vector. Does the vector contain what it is intended to contain? Has it undergone any deletion, mutation, or other major changes? Are there lot-to-lot variations, especially at the higher doses, when each patient receives his or her own dose?

8. An organized database is needed for figuring out the mechanisms and toxicities for each vector. How can the raw data be converted into knowledge? How can data be gathered, and then how can knowledge be gained from the collected data?

9. Well-planned controls are critical at all stages of research, although ethical issues surround the use of controls (administering a adenovirus vector without the transgene) in Phase I safety trials.

10. Participants in gene transfer trials should be informed that lack of autopsy data represents a loss of crucial information. How can patients be encouraged to allow autopsies, and how can IRBs assist investigators in obtaining such permission?

Comments from investigators in the audience included an agreement that advances can be made in the short run regarding the issue of standards. Standardization should refer to all vectors, not just adenovirus.

Dr. Beth Hutchins, Canji, Inc. (a subsidiary of Schering-Plough), reported on one result of the Viral Vector and Vaccine Bioprocessing conference held in November 1999 in Williamsburg, VA. Several adenovirus researchers at that conference discussed the need for a 1-day symposium to discuss characterization of adenovirus, including development of a reference standard, physical characterization, purity assays, and biological activity issues. Dr. Hutchins asked Dr. Noguchi for a contact at the FDA who could assist in planning such a symposium, and Dr. Noguchi volunteered. Dr. Verma volunteered Dr. Mulligan from the Working Group to participate in this planning committee.

Dr. Beaudet expressed the concern that patients often are so enthusiastic about gene therapy that they may be willing to take risks they do not understand. He recommended that patient advocates may need to be added to the consent process.

Dr. Verma suggested that additional input for the Working Group on Adenovirus Safety and Toxicity be e-mailed to OBA at <ci4e@nih.gov>. Dr. Mickelson stated that a draft of these points, based on today's discussions, will be sent by e-mail to RAC members for comment. A final report will be prepared and brought before the RAC for discussion at its next meeting in March 2000.

Public Comments

Dr. Dinko Valerio, IntroGene, The Netherlands

Dr. Valerio shared recent data related to the role of preexisting neutralizing activity in the side effects of adenovirus gene therapy. In some gene therapy trials, the effect of the gene transfer may not be present due to neutralizing activities. Studies have shown that preexisting immunity completely mitigated the toxicity as well as the gene transfer, suggesting increasing dosage. However, assuming patient variability and a steep toxicity curve at some point, high dosages can be dangerous in situations in which the window between effectiveness and toxicity becomes narrow. Possible solutions include generating vectors from adenovirus serotypes with a low prevalence of neutralizing activity, targeting to administer as low a viral dose as possible, and eliminating patient variability through prescreening and prevaccination.

Mr. Paul Gelsinger, father of Mr. Jesse Gelsinger

Mr. Paul Gelsinger stated that all the people, including Jesse, who participated in the OTC deficiency trial entered the trial with the same pure intent in mind—to help others by participating in finding a cure for this disease. He referred to the statements written on coins—"In God We Trust" and "Liberty." Jesse understood his freedom to choose and knew he made the right choice. Everyone participating in the trial demonstrated "E Pluribus Unum"—one for all and all for one. Mr. Gelsinger explained how Jesse set aside his personal life to participate in this trial, including taking an unpaid leave of absence from his job. Mr. Paul Gelsinger implored researchers and regulators to keep working together.

Dr. Mickelson expressed the RAC's deep sympathy for the loss suffered by Mr. Gelsinger and his family.

XI. Data Management/Dr. Greenblatt

Dr. Greenblatt reported that, as of the current reporting period, 357 gene transfer protocols have been registered with the OBA: 35 gene-marking protocols, 320 therapy protocols, and 2 nontherapeutic protocols. Further breakdown of the therapy protocols showed 30 protocols for infectious diseases (all in human immunodeficiency disease), 43 for monogenic diseases, 29 for other diseases, and 218 for cancer.

Since the last reporting period, 17 new protocols had been submitted to the OBA, three of which were recommended for RAC review; two of these were scheduled for discussion during Day Three of this RAC meeting.

There were 30 amendments and updates submitted to the OBA in the last reporting period. Most amendments were relatively minor, such as additional investigators, sites, or patient testing; minor modifications to eligibility or exclusion criteria; and minor changes to provide protocol clarification. A few protocols increased the dose of vector, and one protocol decreased the vector dose. One amendment modified the Informed Consent form for the hemophilia A protocol in response to suggestions by RAC members at the September 1999 meeting; the minimum age for this protocol was also reduced from 25 to 18 as suggested by RAC members.

The OBA received 17 safety and adverse event reports since the last reporting period. Two deaths were reported to OBA within 24 hours of treatment, both of which included an autopsy. Protocol #9806-260 is a Phase I study of Allovectin-7 (a plasmid DNA/cationic liposome expressing human leukocyte antigen-B7 and beta2 microglobulin cDNA) by direct gene transfer with concurrent low-dose, subcutaneous IL-2 protein therapy as an immunotherapeutic regimen in malignant melanoma. The patient had widely metastatic malignant melanoma and prior treatment with multiple cycles of chemotherapy and radiation therapy. Allovectin-7 was administered to the left anterior lymph node, and 2 weeks later the same lymph node was injected again. Within 1 minute after the second injection, the patient went into cardiac arrest

and died. Autopsy revealed that death was attributable to the injection procedure—a puncture to the pericardial sac, revealing the pulmonic trunk—and not directly to the vector.

The second report of death occurred in Protocol #9902-294, a multicenter, open-label, dose-escalation study of intramyocardial vascular endothelial growth factor 2 gene therapy in refractory patients with stable exertional angina who were not candidates for revascularization procedures. The patient had severe coronary artery disease with previous bypass surgery, and he died from cardiac arrest the morning after receiving the four experimental injections of vascular endothelial growth factor-2 DNA into the anterior wall and septum of the heart. Autopsy showed no evidence of inflammatory infiltrate, interstitial edema, or DIC. Preliminary findings did not indicate a certain basis for death, but the sponsor concluded that the procedure was not implicated as a cause of death.

XII. Minutes of the September 2-3, 1999, RAC Meeting

Copies of the minutes were provided. The minutes were reviewed previously and were approved by a subcommittee composed of Dr. Greenblatt and Dr. Juengst, who indicated that the minutes needed a few minor changes but otherwise represented an accurate reflection of the technical, scientific, and procedural discussions that took place during the September 2-3, 1999, RAC meeting. Dr. Greenblatt, speaking also for Dr. Juengst, recommended that the RAC approve the September 1999 minutes, with minor modifications.

Committee Motion 1

The RAC approved a motion made by Dr. Friedmann and seconded by Dr. Markert to accept the minutes (with the incorporation of minor modifications) of the September 2-3, 1999, RAC meeting by a vote of 10 in favor, 0 opposed, and 0 abstentions.

XIII. Day Two Closing/Dr. Mickelson

The proposed action Working Group on Adverse Event Reporting was scheduled to meet at the close of this public meeting in Building 10, Room 102C118.

Dr. Mickelson adjourned the second day of the December 1999 RAC meeting at 3:15 p.m. on December 9, 1999.

XIV. Day Three Opening Remarks/Dr. Mickelson

Dr. Mickelson opened the third day of the December 1999 RAC meeting at 8:30 a.m. on Friday, December 10, 1999, in Rooms E1-E2 of the Natcher Building at the NIH. She provided background information on adverse event reporting. In July and August 1999, the OBA received several adverse event (SAE) reports that were marked “confidential.” During the September 2-3, 1999, meeting, the RAC developed the following consensus statement with regard to serious adverse event reporting to OBA and the RAC: “Adverse event reports shall not be designated as confidential, either in whole or in part. Adverse event reports are essential to decision-making by IBCs, IRBs, and potential subjects of gene transfer research in humans. The public disclosure of adverse events [in human gene transfer research] is also essential to public understanding and evaluation of gene transfer in humans. Adverse event reports must be made available for public discussion [by the RAC] without the inclusion of proprietary or trade secret information.” At that point, the Working Group on Adverse Event Reporting was established (chaired by Dr. Ruth Macklin) to come up with issues raised by the proposed action, engage the RAC and the FDA in discussion, and reach conclusions after public comment.

Dr. Patterson summarized the proposal, which was recently published for public comment in the *Federal Register* on November 22, 1999 (64 FR 63827). It would add three provisions to the current *NIH Guidelines*:

1. SAEs would be defined, as would the timeframe in which they are to be reported.

2. SAE reports could not contain any trade secret or confidential commercial information, because all information submitted in accordance with the *NIH Guidelines* is considered public.

3. To ensure patient confidentiality, SAE reports should not contain individually identifiable patient information.

The intent in proposing these changes is to ensure that all pertinent information regarding the safe and ethical conduct of gene transfer trials is transmitted in a timely fashion to the RAC for review and analysis and, as necessary, for public discussion.

Dr. Skirboll, NIH Office of Science Policy (OSP), shared information about the NIH Director's recent announcement of the formation of a working group of the Advisory Committee to the Director, NIH, to consider recommendations to the Director regarding the role of NIH oversight of clinical gene transfer research and the function of the RAC. Four broad questions will be considered:

1. Is the current NIH framework for the oversight of public discussion of clinical gene transfer research appropriate, especially with regard to the respective roles of the RAC and the *NIH Guidelines*?

2. Are current NIH mechanisms adequate for coordination of oversight of clinical gene transfer research with the FDA, the NIH Office for Protection from Research Risks (OPRR), and IRBs and Institutional Biosafety Committees (IBCs)? Should that coordination be strengthened in new ways?

3. Are there additional NIH measures needed to minimize risk associated with clinical gene transfer research? Should more public meetings be held?

4. What should be the NIH role with regard to reporting, analysis and public discussion of adverse events?

The membership of this working group has not been finalized, but it will represent a variety of patient groups, constituencies, and the public. This working group is expected to consult with other experts. Information about this working group will be available on the OBA Web site as soon as members and meeting times are set.

XV. Current Issues in Adverse Event Reporting and Proposed Action
Working Group: Drs. Macklin (chair), Ando, King, McIvor, Mickelson, and Verma

FDA Presentation on Adverse Event Reporting/Dr. Karen Weiss, CBER

Dr. Weiss discussed adverse event reporting within the FDA—reporting requirements, definitions, and FDA procedures after a report is filed. All adverse events associated with the use of a biological product must be reported to the FDA, whether or not the event is considered product related. Reporting is divided into two categories: (1) expedited reports and (2) annual reports and informational amendments. Expedited reports are transmitted in written form via the U.S. Postal Service or other delivery service or verbally via telephone or facsimile (fax). Postmarketing reporting of adverse experiences is set forth in *US Code of Federal Regulations* (21 CFR 6000.80).

Reporting requirements for INDs of biologics are set forth in *US Code of Federal Regulations* (21 CFR 312.32) and a *Federal Register* publication of October 7, 1997 (62 FR 5237). Written reports must be provided for (1) any adverse event associated with the study drug that is both serious and unexpected and (2) any findings from tests in laboratory animals that suggest a significant risk for human subjects. The sponsor must notify the FDA and all participating investigators as soon as possible but no later than 15 calendar days after receipt of the information. For safety reports provided by telephone or fax, the sponsor must notify the FDA about any unexpected fatal or life-threatening experience associated with use of the drug as soon as possible but no later than 7 calendar days after the event occurs.

Dr. Weiss shared several FDA definitions regarding adverse events. *Associated experience* is defined as a reasonable possibility that the experience may have been caused by the drug. *Serious adverse drug*

experience is defined as any adverse event that results in death; is life threatening; requires inpatient hospitalization; results in prolonged, persistent, or significant disability or incapacity; or results in a congenital anomaly or birth defect. *Life-threatening adverse drug experience*, which requires a 7-day telephone/fax report if it is unexpected, is defined as any adverse drug experience that places a patient or clinical trial subject in immediate risk of death from reaction to the drug. Unexpectedness is another criterion that must be met before sponsors' reports rise to the level of expedited; an *unexpected adverse drug experience* is an adverse drug experience that has not been observed previously and therefore is not consistent with the investigator's brochure or the risk information described in a general investigational plan.

When FDA reviewers receive safety reports, they first gather information. A full review includes current and prior human data and nonclinical data—for the reported IND, for related products, and for the same product in other INDs. FDA reviewers then consider possible regulatory actions, including determining whether sponsors who hold the INDs for related products should be notified and whether protocol modifications are necessary (possibly, changes in the dose, route, and/or schedule). Other possible regulatory actions include requests that the sponsor change the Informed Consent document to reflect new toxicity information or reconsent current participants, update the investigator's brochure, consider the need for new animal studies, and place the IND on clinical hold or partial hold.

Patient Privacy and Confidentiality/Dr. Skirboll, NIH Office of Science Policy

Dr. Skirboll stated that the U.S. Department of Health and Human Services' (DHHS) proposed regulation on patient privacy and confidentiality, for which the comment period was open through January 3, 2000, covers individually identifiable information that is or has ever been electronically transmitted. The 1996 Health Insurance Portability and Accountability Act required Congress to pass a health privacy legislation bill by August 1999, or by February 2000, the DHHS Secretary would have to finalize regulations containing confidentiality standards related to the transmission of electronic data records. In the proposed rule, the information protected is that which is individually identifiable and is or has ever been electronically transmitted. The NIH Office of Science Policy recently completed a full analysis of the impact of these proposed regulations for the conduct of research; relevant questions and answers will be posted on the NIH Web site at <www.nih.gov/news/>.

Researchers must disclose individually identifiable information to patients on request unless one of five specific conditions is met:

1. Information identifies another individual and could cause harm to that individual.

2. Disclosure is likely to reveal the source of the information provided under a promise of confidentiality.

3. Inspection could reasonably be likely to endanger the life or physical safety of the patient.

4. The trial is in progress, and denial of access has been approved by an IRB or a privacy board and agreed to by the participant when consenting to participate in the trial.

5. Disclosure is forbidden by a legal proceeding.

In summary, researchers can deny a patient access to information about a trial during the course of the trial but only if that option is stated in the Informed Consent form and the IRB has agreed to it.

Dr. Skirboll explained that information that is coded and therefore not individually identifiable is not covered by the DHHS proposed regulation. Once a record has been deidentified, information from that record can be made available to a public source. Because the proposed regulation would not reach to secondary disclosure, the issue of deidentifying a record becomes critical. Deidentification must pass a two-part test: (1) All specified identification must be removed, and (2) there can be no reason to believe that the remaining information could be used to identify the individual. It is the second condition that may cause some difficulty, especially, for example, for an individual with a rare disease who lives in a small town. Specific patient identifiers defined in the proposed regulation include name, address, photographic images

and fingerprints, names of relatives or employers, birth date, telephone and fax numbers, e-mail address, Social Security number, medical record number, health plan beneficiary number, vehicle number, and other unique identification number, characteristic or code.

A long list of allowable disclosures is included in the proposed rule. Without patient consent, identifying patient information could be made available to other health care providers, payment processors, law enforcement agencies, and oversight bodies. Because the RAC is an oversight body, consent may not be needed. However, the RAC is also a forum for public discussion; DHHS has not yet dealt with this aspect.

Dr. Skirboll asked whether it is possible to deidentify information related to gene therapy patients. If not, the Informed Consent document should include information about the possibility of patient information disclosure to the RAC in the case of a serious adverse event. In so doing, patients would be aware before they enter a clinical trial that the RAC's public discussion may include the kind of discussion that has occurred at this RAC meeting regarding Mr. Jesse Gelsinger.

RAC Questions to Drs. Weiss and Skirboll

Dr. Friedmann asked Dr. Weiss why a grade 3 thrombocytopenia would not be considered life threatening. Dr. Weiss responded that certain types of thrombocytopenia are merely laboratory findings, especially those that are of short duration and limited platelet count deviation, from which patients recover and then proceed to the next cycle of chemotherapy. Reactions that are of great severity or intensity, like hepatic enzyme elevations or certain types of cytopenias, will not necessarily rise to the level of "serious," which is defined explicitly.

Dr. Macklin asked two questions: (1) whether the FDA communications are entirely with sponsors as opposed to investigators and (2) what the FDA does when it learns of compliance failure on the part of the sponsor, including any followup or sanctions imposed on sponsors who fail to communicate in a timely manner. Dr. Weiss clarified that the FDA is only able to communicate with the sponsor or the sponsor's authorized representative. However, in the case of important events, sponsors often gather the investigators and other critical people to help them address that event, and the FDA will meet with this assembled group or will participate in a conference call with the group. Dr. Weiss stated that failure to report is a serious problem. When reporting failures come to light as a result of bioresearch monitoring, various sanctions can be taken against the investigator, the most serious of which is that the investigator is forbidden from participating in clinical trials (termed "debarred"). In response to a request for definition from Dr. Macklin, Dr. Weiss defined "sponsor" as the entity that holds the IND and that is responsible for administering the IND, which can be an individual academician, an institution, or a university. Dr. Noguchi further clarified Dr. Macklin's concern that, in a hypothetical situation in which the investigator reports a serious event to the IRB but the sponsor does not report it to the FDA, the investigator would receive sanctions. He stated that the FDA takes sanctions against the person who commits the indiscretion or violation. When deviations and possible violations are discovered, a thorough investigation is conducted, and the response and sanction are graded to the severity, the consequences, who did it, and how widespread the violation was.

Dr. Macklin asked Dr. Skirboll about characterizing information that has been deidentified as being "anonymous," indicating concern that encrypted identification could, in principle, be relinked. Dr. Skirboll responded that she agreed it was not appropriate to call deidentified information "anonymous" and suggested the word "anonymized" instead. Dr. Macklin reiterated her concern that Informed Consent forms be clear about information being anonymous (meaning that all identifiers are removed and discarded) vs. anonymized (meaning that information is coded and could be linked back to the individual).

Dr. Juengst noted that one feature of the current RAC rules about reporting adverse events is that they will be discussed publicly at an open RAC meeting. He wondered about the constraints or restraints on the FDA's ability to disclose to the public the adverse outcome reports received. Dr. Noguchi responded that several situations allow FDA disclosure: if the event has been acknowledged publicly, if it was submitted to the RAC without a confidentiality label, if it was announced in a press release, or if it is referenced in material submitted to the Securities and Exchange Commission. In addition, Dr. Noguchi cited a section of the *NIH Guidelines* under which summary information that is relevant to a particular field can be discussed

in public. If an event does not fall under any of these categories, it receives intense scrutiny from the FDA Commissioner before it can be disclosed. Dr. Weiss added that disclosing information to inform other sponsors using related products about adverse events, in gene therapy and other fields, has never been questioned by the sponsor.

Dr. Ando queried Dr. Weiss about the definition of "unexpected" with respect to the investigator's brochure and previous toxicology studies. Dr. Weiss clarified that "expected" refers to an event that has happened (as opposed to one that is theoretically possible) and that is included in the investigator's brochure as having been observed in human subjects in clinical studies. In addition, an event that has happened before but has not yet been incorporated into the investigator's brochure must continue to be reported until it is added to the brochure. In response to questions from several RAC members, Dr. Weiss further clarified that serious events requiring medical intervention that are expected would not trigger a 15-day expedited report; that information would be collected by the FDA in an annual report or in an information update. The FDA has the authority to ask the sponsor to report about specific events more often than in an annual report, even though those events might be expected. Dr. Weiss noted that, in practice, it is not uncommon for the FDA to receive a 7-day phone call or a 15-day expedited report for an event that has already been seen and is included in the investigator's brochure; the tendency is for sponsors to err on the side of more reporting rather than less. Dr. Noguchi added that the FDA wants adverse event information not to be restricted to an individual IND; toward that end, information is disseminated by, for example, using the RAC, sending out letters, or issuing general warnings to investigators.

Dr. Verma resurrected Dr. Macklin's partially unanswered query to the FDA about sanctions, asking how other investigators or the public would know what measures the FDA takes against a sponsor and how that information is disseminated. Dr. Noguchi stated that, at the conclusion of an investigation, a list of deficiencies or violations is compiled. A warning letter is given to the sponsor, giving that person or organization the opportunity to respond to each of the violations via acknowledgment and/or indicating how they will ameliorate the problem. This letter is published routinely on the FDA Web site within a week of its issuance, and any individual can look through the (rather lengthy) list of warning letters included in this public record.

Dr. Verma then asked the FDA a related question, that is, how information is disseminated to other investigators when, for example, data from monkeys indicate toxicity. Dr. Noguchi answered that such information can be disseminated through the RAC, by letters, or by individually contacting the sponsors. He explained that the FDA tries to make such information as widely known as possible because of the importance for all investigators in a given field to know the related hazards.

In response to a query from Ms. Levi-Pearl about the reasons for constraints and the high degree of confidentiality under which the FDA, as a public office, operates, Dr. Noguchi stated that the FDA must protect patient identity, financial information, and trade secrets. In addition, a large body of information that is not well defined can be considered "commercial confidential," for example, a process used to create a high titre of a particular vector. The FDA realizes that some of these restrictions are no longer warranted, and work continues on a rule to permit more disclosure in gene therapy and xenotransplantation.

In response to a question from Dr. Aguilar-Cordova about results from animal studies, Dr. Weiss reiterated that significant findings from animal studies are to be submitted as 15-day written reports. Clinical investigators and sponsors have a high level of reporting requirement from the FDA, specifically because humans are being used in experimental conditions.

Dr. Markert was concerned that the FDA regulations about confidentiality, particularly regarding genetic testing, conflict with similar regulations from the OPRR. Often in research, patients' genes are being sequenced and, unless a laboratory is Clinical Laboratory Improvement Amendments (CLIA) certified, no information is allowed to be released to the patient regarding the results of genetic DNA sequencing. Dr. Skirboll explained that the DHHS has been discussing the issue of returning information to patients and that these kinds of conflicts will need to be resolved once the regulation is in place. It is not difficult for a laboratory to become CLIA certified; if patients want access to information, it is the laboratory's responsibility to obtain CLIA certification to provide that information.

RAC Discussion of Proposed Action/Dr. Macklin

Dr. Macklin explained that the Working Group on Adverse Event Reporting was charged with examining the proposed amendments to the *NIH Guidelines*, defining the changes, and deciding which of the changes might be controversial. As summarized by Dr. Macklin, the proposed Appendix M-VII-C states that investigators must report serious adverse events (SAEs) immediately in writing. It also clearly states that SAE reports must not contain privileged or confidential information, as they are not considered proprietary or confidential and the information is not a trade secret. The proposed new rule requires reporting of SAEs whether expected or not and whether related or unrelated to the experimental procedure under the definition described in Section I-E-7. If this proposal is accepted, it goes beyond FDA requirements; this expansion must be justified.

The proposed regulations require an acceptance of the definition of SAEs and acceptance of a larger universe of reports than the FDA requires, triggering concerns about what would be done with this new information and how the new reporting requirements would be implemented. The Working Group could not come up with a clear, comprehensive account of who would read, collate, and organize these reports. Some new mechanism would need to be put in place to deal with and address anticipated larger numbers of SAE reports. The Working Group proposed a close collaboration among the RAC, the NIH, and the FDA; these three entities would develop a mechanism whereby this new reporting system could be handled in a timely and efficient manner.

The Working Group reviewed a draft SAE reporting form, suggesting that the investigator report the SAE directly to the OBA/RAC (not through the sponsor, as under FDA rules) within 15 days of the event. Since SAE reports would come to the RAC, which is a public forum, the standard disclosure information for all Informed Consent documents would need to disclose the form in which reports are made to the RAC. The result of the new SAE reporting requirements might be a simplification of the process, because neither investigators nor sponsors would need to determine whether SAEs were related/unrelated or expected/not expected.

RAC Questions to the Working Group on Adverse Event Reporting

Dr. McIvor clarified, with Ms. Knorr's assent, that the proposal from the Working Group does not represent a change in the reporting requirements but, rather, represents a clarification of those expectations. Under the *NIH Guidelines*, Section M-VII-C, Adverse Event Reporting, reads "investigators who have received approval from the FDA to initiate a human gene transfer protocol must report any serious adverse event immediately to the local IRB, the IBC, OPRR (if applicable), the OBA, and the FDA, followed by submission of a written report filed with each group."

Dr. Aguilar-Cordova stated his concerns about the definition of "expected" and about the RAC's definitions being different from the FDA's definitions. An expected event would be defined as one that has already occurred in humans; any serious adverse event that has not occurred in humans would always be unexpected and would always trigger expedited reporting. Dr. Macklin clarified that the FDA definitions would remain the same as in FDA regulation, 21 CFR 312.32; what is proposed to differ is when and under what circumstances the SAE information should be reported. The Working Group suggests a uniform reporting process, in the form of a single submission that could be reported to the IRB, the IBC, the OPRR, the FDA, and the RAC; a draft form was provided to the RAC.

Dr. Greenblatt expressed his concern about the significant burden on investigators to report to the RAC using a different format and on a schedule different from that submitted to the FDA. If this burden is to be imposed on investigators and sponsors, the RAC should ensure that a sufficient infrastructure is in place to handle the incoming information.

Dr. Noguchi shared some statistical information with the RAC. In a typical week, the FDA receives 20 to 30 SAE reports for gene therapy, or approximately 1,000 to 1,500 reports each year. Dr. Weiss' group has the primary responsibility to review these reports; a medical doctor reviews the data, whether or not the investigator or sponsor deems that event related to the therapy. This medical reviewer often consults with the FDA's product reviewers. Therefore, even under the current regulations, a considerable amount of

time and work is spent on assessing the relatedness of SAEs and determining the appropriate action. In response to Dr. Verma's query about the number of FDA staff members assigned to review adverse event reports, Dr. Weiss replied that about 30 medical officers and a smaller group of pharmacology and toxicology experts are involved in reviewing SAE reports. Each medical officer is responsible for approximately 100 INDs, each of which currently generates approximately 1,000 amendments; the pharmacology/toxicology staff is responsible for even larger numbers.

Dr. Juengst agreed that an appropriate infrastructure must be in place before enacting a system that requires additional reporting. He explained that the requested reporting might not, however, rise to the same level of intense scrutiny currently afforded SAE reports at the FDA. One example of what might be done with these reports would be to generate an electronic database that would provide an annual review to the community. The RAC is interested in receiving more information than the FDA currently requests because gene therapy is a new and unproven area of research. Dr. Juengst predicted a mismatch between the SAE information the RAC is interested in and the level of reporting the FDA can handle; other examples exist of new areas of scientific interest that have been deemed publicly sensitive and important enough to require higher standards of reporting.

Dr. Aguilar-Cordova expressed his concern that the RAC would not have sufficient expertise to evaluate all the medical reports and reports on product procedures and purification methods that would be submitted to the RAC under the proposed reporting schedule.

Dr. McIvor reiterated his concern that, whatever form the proposal eventually takes, the information that is captured is adverse events associated with the gene therapy aspect of the protocol. After listening to the FDA's presentation, Dr. McIvor reported that he is more comfortable with the idea of the RAC not having to collect adverse event data that are both related and unrelated; the RAC should be able to accomplish its information goals by collecting only information on events that are "related" under the FDA definitions. Dr. Markert echoed Dr. McIvor's concerns, stating that she did not want the RAC to review expected complications and that the RAC should defer to the FDA's years of experience in defining what kinds of SAEs require immediate reporting.

Public Comments

In response to *Federal Register* of November 22, 1999 (64 FR 63827) on the proposed action regarding adverse event reporting, OBA received 16 written comments from representatives of organizations of gene therapy researchers, patient and public advocacy groups, and biotech industry, as well as from individuals concerned about human gene transfer research. Written comments were received from the following individuals:

Jeremy Rifkin, The Foundation on Economic Trends, Washington, D.C.
Debra Gessner, Collateral Therapeutics, San Diego, CA
Alan Whitaker, State Prison Corcoran, California
Paul I. Gelsinger, Tucson, Arizona, Father of Jesse Gelsinger who died from participating in the U Penn Ad-OTC trial
Martin Teitel, Ph.D., Executive Director and Board of Directors, Council for Responsible Genetics, Cambridge, MA
Abbey S. Meyers, President, National Organization for Rare Disorders, Inc., News Fairfield, CT
Jeffrey W. Carey and Henrik S. Rasmussen, M.D., Ph.D., GenVec, Inc., Gaithersburg, MD
Angus J. Grant, Ph.D., Rhone-Poulenc Rorer-Gencell, Collegeville, PA
LeRoy Walters, Ph.D., Georgetown University, Washington, D.C.
David Shoemaker, Ph.D., CATO/Vascular Genetics, Durham, NC
Savio L. C. Woo, Ph.D., President, The American Society of Gene Therapy, Thorofare, NJ
A Position Paper, Biotechnology Industry Organization (BIO), Washington, D.C.
Michael S. Perry, D.V.M., Ph.D., President and CEO
SyStemix, Inc. and Genetic Therapy, Inc., Novartis Companies, Palo Alto, CA
Nicholas J. Pelliccione, Ph.D., Schering Corporation, Kenilworth, NJ
Robert J. Beall, Ph.D., President and CEO, Cystic Fibrosis Foundation, Bethesda, MD
Robert Spiegel, M.D., Chief Medical Officer, Schering-Plough Research Institute, Kenilworth, NJ

Several individuals made oral presentation at the meeting as follows:

Dr. Savio L.C. Woo, Mount Sinai School of Medicine and the American Society of Gene Therapy (ASGT)

Dr. Woo stated that reporting requirements should be completely harmonized and that SAEs should be defined. The FDA and the RAC should jointly establish a harmonized set of requirements to satisfy legal and regulatory aspects of the FDA and to provide the public with useful information regarding gene therapy trials. The ASGT supports the amendments in principle but believes additional attention should be afforded to patient privacy. So that the public will not be misled about the safety or risk of gene therapy research, it is important to identify and segregate natural outcome from those SAEs that are related to gene therapy trials under clearly defined headings in the records maintained and updated regularly by the OBA. To fulfill the challenges inherent in administering pertinent data and to bring those data into the system and keep the system updated, the OBA will need appropriate additional resources so that the objectives of reporting SAEs in gene therapy trials can be met. Dr. Woo was impressed by the openness in reporting the scientific and clinical data at this RAC meeting. The ASGT would be pleased to work toward standardization of vectors.

Ms. H. Stewart Parker, Biotechnology Industry Organization (BIO) and Targeted Genetics Corporation

Ms. Parker testified on behalf of BIO, which represents 850 companies, academic institutions, and State biotechnology centers engaged in biotechnology research on medicines, diagnostics, agriculture, pollution control, and industrial applications. The organizations represented by BIO are committed to providing the resources necessary to fully realize the promise of gene therapy for the treatment of serious medical conditions. BIO companies believe that it is vital that patients with these conditions have access to novel and innovative therapies and that gene therapy research and clinical trials be regulated in a thorough and efficient manner, in accordance with clear Federal statutes and guidelines. BIO is concerned that the adverse event reporting standards outlined for expedited reporting are inconsistent with currently recognized standards.

The industry's willingness to provide SAE data to the RAC is contingent on an agreement between the NIH and industry that would memorialize how the data will be used. As an alternative to the NIH proposal, BIO companies proposed the following structure for the future oversight of gene therapy: Sponsors agree to voluntarily provide to the NIH/OBA serious related and unexpected adverse event reports that are currently sent on an expedited basis to the FDA. Sponsors would also send to the RAC the safety data summarized in the IND annual progress report currently provided to the FDA. If, after an initial review, the RAC believes there may be potential safety concerns, BIO recommends a joint evaluation of the data between the RAC and the FDA, the results of which could form the basis for public discussion at a subsequent RAC meeting.

Ms. Parker stated that the industry looks forward to a collaboration with the OBA and the FDA and offered resources to help in that collaboration. A position paper on the issue of reporting of information from gene therapy clinical trials to the RAC is available on the BIO website at <www.bio.org>.

Dr. Robert J. Spiegel, Schering-Plough Research Institute

Dr. Spiegel suggested that the NIH and the RAC work more closely with sponsors. The RAC need not duplicate FDA functions, and the FDA's role should be formalized and recognized. There is a need to state formally when a signal occurs that warrants public discussion. He implored the RAC to weigh public interest in obtaining information against alarming the public unnecessarily. Schering-Plough is willing to help in formulating appropriate regulations.

Schering-Plough believes that the proposed alteration to the *NIH Guidelines* is deficient in that it does not adequately address the problems apparent in the current *NIH Guidelines*. The respective roles of the FDA, the NIH, and the RAC need to be identified in overseeing the safety of ongoing gene therapy trials. Input from the pharmaceutical and biotech industries needs to be reflected; these industries have been reporting

adverse events to the FDA and international regulatory authorities for more than 50 years. Schering-Plough's written comments made the following recommendations:

1. The NIH should codify its adverse event reporting requirements to match those of the FDA in scope, timing, and reporting format, possibly using the current MEDWATCH 3500 form.

2. All expedited or alert reports submitted to the FDA within 7 or 15 days should also be forwarded to the NIH but should not be released to the public until they have been reviewed adequately by the FDA and the RAC.

3. The FDA and the NIH should work together to develop a process to signal any need for broad public discussion of an issue relating to the safety of human gene therapy.

4. At each quarterly meeting or on an as-needed basis, the RAC should determine whether further discussion of adverse events is warranted.

5. The sponsor of the gene therapy research should be notified and given the opportunity to provide additional relevant information for discussion of safety issues with their products at a public RAC meeting. SAEs should be investigated before public release. Once an SAE is reported, it becomes part of that trial's process.

If DIC is of critical importance to the RAC in development of adenoviral vectors for gene therapy, it is appropriate to ask sponsors and investigators to come forward periodically and review their experience with DIC.

The credibility of any company and its product(s) is tied to compliance with internal procedures and with the FDA's procedures. Sponsors take seriously any deviations from that compliance.

Dr. Michael Coyne, National Hemophilia Foundation (NHF)

Dr. Coyne provided some background about bleeding problems in hemophilia. Because they are dependent on the purity of the blood supply, thousands of hemophilia sufferers have died of AIDS and hepatitis. Currently, factor protein must be administered intravenously, a procedure that is especially difficult for children and costly for all. Hemophilia can be cured by a single gene replacement, and minor changes in circulating factor can work significant positive changes. The NHF is raising money for researchers and laboratory grants and is bringing investigators together to collaborate.

Three Phase I clinical trials are currently under way. Participants in these trials want to be cured, but they also want to be pioneers and understand the risks necessary to pave the way for a cure.

The media report deaths in several trials as "gene therapy deaths," even those not related to the therapy but to the underlying disease, so Dr. Coyne strongly suggested that the FDA not report deaths unrelated to gene therapy. Quick reporting to the FDA should be mandated for those SAEs possibly, probably, or definitely related to the gene therapy procedure. Additional regulatory control would be burdensome.

Dr. Leroy Walters, Georgetown University (former RAC Chair)

In December 1999, 15 of 22 RAC members who reviewed the original OTC deficiency protocol sent a letter to Secretary Shalala and Dr. Varmus, expressing concern that changes in protocols were not being reported to the RAC; certain steps have been taken to deal with this problem. The second concern expressed in that letter was about SAEs being considered proprietary, which was not the intent years ago when the *NIH Guidelines* were first promulgated.

Not all research should come under the same kind of enhanced oversight as gene therapy. Innovative areas of biomedical research raise novel issues, and it is reasonable to proceed on different bases.

The goals of SAE reporting are to have real-time reporting of possibly important SAEs and an annual overview. Rationales for SAE reporting are to help protect human subjects who volunteer on behalf of humanity, to practice good medicine by helping clinicians avoid causing unnecessary harm, and to practice good science.

Dr. Walters offered a model that would include a data coordinating center function in the review of SAEs in gene therapy trials, enhanced cooperation between the FDA and the OBA/RAC, an online database to provide current information on all serious events, brief but complete data management reporting at each quarterly RAC meeting, and an annual "snapshot" of the gene therapy field in terms of safety and documented success. The Institute of Medicine's (IOM) 1995 committee report on supports this model.

Dr. Walters concluded by presenting three relevant recommendations from the 1995 IOM committee report, which was generated as a result of the death of five patients in a clinical trial of fialuridine (FIAU) for treatment of hepatitis B:

1. Some form of independent safety monitoring would be a valuable component of any clinical trial in which patients are treated for extended periods, but safety monitoring is especially important for all double-blind trials and any trial in which there is reason to anticipate that evidence of adverse reactions could be confused with evidence of disease progression or therapeutic response.

2. Ideally, data should be analyzed by the investigators or by an independent safety monitor in Phase I and Phase II trials and by data safety monitoring committees or data coordinating centers in multicenter Phase III studies on a continuing real-time basis.

3. Some form of cumulative adverse event reporting should be provided by the sponsor in a form that includes not only those events previously reported as serious, unexpected, and drug related but also any events judged to have met only the first (serious) or the first two (serious and unexpected) of those conditions, along with the sponsor's explanation of the event.

Dr. Angus Grant, Rhone-Poulenc Rorer-GenCell

Dr. Grant stated that the NIH and the FDA should work together to design a transparent system and a harmonized process for adverse event reporting. By so doing, unnecessary burdens would not have to be assumed by sponsors and investigators. The MEDWATCH form, currently used for postmarketing and IND reporting, is an appropriate mechanism for adverse event reporting.

Ms. Karen Rothenberg, University of Maryland School of Law

Ms. Rothenberg is a former member of the RAC. She stated that she is not convinced that the OBA and the FDA can work together and that the future of gene therapy must be based on public discussion and education.

Concerns about confidentiality are projecting the wrong message. It is not ethical to give clinical trial participants the presumption of complete privacy, and the Informed Consent document must be clear on the risk and benefit in terms of confidentiality.

The autopsy issue is critical to enhanced understanding of gene therapy. At the time people are asked to volunteer for clinical trials, researchers should make a strong point of requesting consent to autopsy, citing its critical role in information gathering.

Ms. Rothenberg suggested that serious consideration be given to taking the RAC out of the NIH. Along with research areas such as human cloning, embryonic stem cells, and use of animal organs in humans, gene therapy should be characterized as novel therapy and placed directly under the DHHS, similar to the move of the Office for Protection from Research Risks (OPRR) from NIH to DHHS. Uniformity for scientists and the public should be provided about who will ensure against future tragedies and who will ensure that the field learns from future adverse events.

Dr. Peter Cohen, physician and attorney

Public perception is one of failure in the system and on the part of researchers, which may or may not be true. Dr. Cohen reviewed portions of the OTC deficiency trial's Informed Consent form, which he believes to be flawed, noting that a lay person about to enter this trial might reasonably infer, from reading the Informed Consent form, that no evidence of liver disease occurred during the study. Attention by the RAC and the FDA should be focused on the Informed Consent process and document. In considering adopting new regulations, it is critical to ensure adherence to the regulations that are in place.

Additional Comments

Ms. Knorr stated that additional comments on the proposed action could be sent to the OBA at <oba@od.nih.gov> or by telephone at 301-496-9838.

Dr. McIvor filled in as acting Chair of the RAC.

XVI. Discussion of Human Gene Transfer Protocol #9906-322: *NGF Ex Vivo Gene Therapy for Alzheimer's Disease*

Principal Investigator: Dr. Mark Tuszynski, University of California, San Diego (UCSD)
Reviewers: Ms. King and Drs. Chow and Mickelson
Ad Hoc Consultant: Dr. Wilma Friedman, Columbia University

Background

The RAC recommended full public discussion of this protocol for several reasons: new disease category, use of autologous fibroblasts for the treatment of a noncancerous brain disorder, new disease and new gene, high level of public interest, first use of gene therapy to alter mental states (raising the prospect of using gene therapy for "enhancement" purposes in people without AD), requirement that the subjects forego other antidementia drugs for 1 year, ability of AD patients to consent, and the invasiveness of the procedure against a background of animal data that may be too inadequate. Dr. Wilma Friedman was asked to review the protocol as an *ad hoc* consultant.

Ms. King and Drs. Chow, Friedman, and Mickelson submitted written reviews to which the investigators responded in writing. Dr. Tuszynski provided oral responses to additional questions raised during the meeting.

Protocol Summary

Dr. Tuszynski provided an overview of the proposed trial. The researchers recognize that they are proposing an invasive procedure for a disorder that, although terminal, is terminal over a mean time period of 7 to 12 years. Because patients with AD have a compromised ability to provide informed consent later in the stage of the disease, the researchers have concluded that this trial should use patients in the earlier stages of AD.

This study is an open-label, Phase I clinical trial of *ex vivo* nerve growth factor (NGF) gene therapy for early AD. A total of eight patients will be enrolled in four treatment groups.

A group of cells in the brain, called basal forebrain cholinergic neurons, undergo regular and severe degeneration in AD. NGF is a nerve growth factor that has shown robust efficacy in mouse, rat, and large-primate models in preventing the degeneration of cholinergic neurons. When administered to aged rats or rats with lesions of the basal forebrain cholinergic system, NGF treatment reverses memory deficits and ameliorates morphological and biochemical features of neuronal degeneration. When NGF is delivered to the aged primate brain by *ex vivo* gene therapy, it reverses spontaneous, age-related atrophy and degeneration of cholinergic neurons in the brain. (Although animal models exist for cellular insults, spontaneous atrophy with aging, trauma, and excitotoxic injury to cells, no good animal model exists for AD because animals do not develop AD.) The robust effects of NGF on cholinergic neurons in various brain

models have been replicated by dozens of laboratories worldwide during the past decade, and NGF has clearly been identified as a molecule of substantial potential for the treatment of AD. However, a safe and effective method of delivering NGF to the brain has not been available until the recent development of gene therapy.

In animal studies to date, the researchers have grafted autologous NGF-secreting fibroblasts to 35 rhesus monkeys (230 total NGF-secreting grafts) and control grafts to 25 rhesus monkeys. In no cases have the researchers observed any of these cells forming tumors or inducing pain, and none of the monkeys has shown significant weight loss. The researchers have conducted a dose-escalation study that mirrors what they propose to do in humans. Safety results include no tumor formation, no cell migration, no NGF detectable outside the targeted area of the brain, and persistent biological efficacy for at least 1 year in primates and 18 months in rodents. Regarding adverse events, one animal that received the highest dose of injected fibroblasts failed to recover well from surgery and was euthanized 3 weeks postoperatively; autopsy findings were unremarkable, the cause of death was unclear, and the animal showed no evidence of traumatic injury to the brain and no vascular abnormality in the brain. In addition, one monkey had a problem with anesthesia, developed tracheal stenosis, and was euthanized; this adverse event was not a complication of surgery. Both animals that received the highest cell-grafting dose and injection rate of cells showed cell reflux from the injection site. To avoid this result in human trials, the researchers will reduce the injection rate tenfold from what was used in the monkeys and will use a lowered total dose of cells than that used in the smaller primate brain.

Patients' own skin cells (fibroblasts) will be genetically modified with a retroviral vector to produce and secrete human NGF *in vitro* and will then be grafted into the brain to prevent neuronal degeneration and to augment neuronal function. The "dose" of NGF delivered will be escalated by increasing the number of grafted cells. Neither the cell density nor the number of grafting sites will be varied; however, the first two study patients will receive only unilateral cell implants to ensure the safety of the grafting procedure. All subsequent patients will receive bilateral cell implants. The study will vary the gene therapy dose by varying the volume of implanted cells.

The study will be conducted over 1 year, although patients will be followed indefinitely at yearly intervals. In the first year, patients will be evaluated postoperatively at 2 weeks, 1 month, 3 months, 6 months, 9 months, and 1 year. Patients will receive a neuropsychological assessment postoperatively at 1, 3, 6, 9, and 12 months. The primary endpoint will be safety, and secondary outcome measures will include several established measures of cognitive function in AD. Patients with early stages of AD will be targeted because they will be most able to give informed consent to the surgery involved in this study and because this gene therapy aims to prevent cell degeneration and augment the function of remaining neurons. The second objective is most achievable in patients in earlier stages of the disease.

RAC Review and Discussion

Dr. Chow's review focused on the scientific basis for using this procedure—whether enough is known about AD to determine the cause of neuron loss. If the assumption is incorrect that amyloid deposit causes neuron death, then adding NGF may not solve the underlying problem. Because this procedure is invasive, Dr. Chow was concerned about the safety of the procedure, particularly in light of the monkeys that died; she disagreed with Dr. Tuszynski that the death of the monkey from tracheal stenosis was not related to the surgery. She was also concerned that information from a third monkey that was euthanized intraoperatively was not included in the appropriate summary table. Dr. Chow's final concern revolved around including in the Informed Consent form a clear indication that UCSD will take care of patients' medical care and bills.

Dr. Tuszynski responded that the real cause of neuronal death in AD is not known. If some of the postulated mechanisms that cause neuronal death do cause neuronal death in AD, there is a high probability that NGF could retard that process. One hypothetical mechanism that could prevent accelerated cell death induced by NGF is the presence of two receptors for NGF, a high- and a low-affinity receptor, i.e., Trk-A and p75; in cells that express both, the two receptors appear to interact and sustain cell function and cell survival under conditions of degeneration. In AD, researchers have not observed cells that express only p75 receptors, with the complete loss of Trk-A; therefore, susceptible to cell death

induced by NGF. In response to Dr. Chow's concern about payment for health care for patients who experience complications, the investigator stated that it is standard practice to bill insurance companies but that the researchers' intent is that no patient should pay for anything related to the procedure. Dr. Hoi Sang U, a co-investigator at Division of Neurosurgery at the Veterans Affairs hospital in San Diego, noted, in response to Dr. Chow's concern about tracheal stenosis, that the procedure will be performed under local anesthesia, with no intubation, so the risk of tracheal stenosis is not present.

Ms. King stated her satisfaction with the pre-RAC meeting exchanges between the reviewers and Dr. Tuszynski, noting that those exchanges have improved the Informed Consent form and have clarified many questions. She also expressed disappointment with the local IRB because it had approved an Informed Consent form that was not adequate. Ms. King's review focused on two areas of concern: invasiveness of the procedure and wording of the Informed Consent form. As a nonscientist, she found it difficult to understand the scientific rationale for using such an invasive procedure. Despite some of the changes that have been made, the information in the consent form still states the possibility of potential direct benefit for the subjects in this protocol; given the lack of analogies between AD and the kinds of brain injuries studied in rats and monkeys, statements about direct benefits may be premature. Ms. King preferred language in the consent form that would describe this study as only a safety study—that it is not intended to benefit the subject and that subjects understand they are participating in the study purely for the benefit of scientific knowledge. The investigator agreed to add more information about potential risk but continues to believe that some benefit to participants is possible.

Dr. Wilma Friedman's review focused on the effects of NGF in the brain. In the past 5 years, research has indicated that when NGF interacts with one of its receptors, the p75 receptor, cell death can result; NGF can support the survival of cells by interacting with another of its receptors, the Trk-A receptor. Given these two facts, it becomes critical to know which receptor is being expressed in the AD brain. Other research has shown that long-term exposure to NGF might upregulate expression over the p75 receptor, which could ultimately cause the death of cells. Dr. Wilma Friedman was also concerned about the axonal sprouting that the researchers have seen in the NGF-producing fibroblasts and whether this sprouting event would cause neurons to withdraw their terminals from their normal projection site. Dr. Wilma Friedman suggested that the researchers expose cholinergic neurons to beta amyloid, either in culture or by injecting fibrillar beta amyloid, to determine whether the cholinergic neurons die.

Dr. Mickelson was not present to speak about her review of this protocol. Dr. Ted Friedmann excused himself from discussion of this protocol because he is on the faculty at UCSD, where this protocol would be conducted.

Dr. McIvor raised the question of why the existing transgenic model is not considered a reasonable animal model for treatment and why it cannot be used to assess the potential for therapy for AD. Dr. Tuszynski responded that transgenic models only model one component of AD in a subset of patients that may have the familial, as opposed to the sporadic, form of the disease. None of the more thoroughly investigated transgenic models has proven to be satisfactory in replicating the spectrum of neuropathology and functional loss seen in AD. These models are useful in providing insight into the nature of normal biochemistry of particular pathways and the effect of a specific pathway in combining with other risk factors that lead to the more diverse spectrum of pathological events in AD.

Dr. Breakefield wondered whether, if fibroblasts are removed from the patients and are genetically modified to produce NGF, the fibroblasts will make more of the toxic beta amyloid. Dr. Tuszynski responded that, in those cells, the fibroblasts will make more of the toxic beta amyloid; he agreed to test this hypothesis in tissue culture. Dr. Breakefield suggested that the importance of autopsy data be stressed to participants. Dr. Tuszynski stated that the researchers intend to request consent for autopsy. Dr. Breakefield also requested a definition of "early onset" of AD, as defined by the researchers, in terms of patient function as well as amyloid plaque formation. Dr. Tuszynski answered that the Mini-Mental Status Examination (MMSE), among others listed in the protocol, will be used to evaluate efficacy. This index assesses mild, moderate, and severe stages of AD. In mild stages, patients should be able to give informed consent because intellectual function is still present. With 90 to 95 percent certainty, the MMSE can distinguish AD from normal aging. Regarding whether cells in the basal forebrain form plaques, Dr. Tuszynski stated that cells in this region of the brain do not have a heavy plaque load.

Dr. Markert echoed Dr. Wilma Friedman's earlier comments about potential worsening of AD symptoms. Although extremely unlikely, this result should be listed on the Informed Consent form as a possible risk. Dr. Tuszynski agreed and will alter the form accordingly. Dr. Markert also queried whether the frequency of enrolling patients would allow an opportunity to see adverse progression or detection of an effect. Dr. Tuszynski indicated that 3 months are allotted between patients to check for toxicity reactions.

Dr. Ando wondered what kind of primate toxicology has been conducted, including the dose levels and how those dose levels compared with the anticipated human dose levels. Dr. Tuszynski responded that toxicity studies in primates are described extensively in the protocol. Doses have replicated and exceeded the dose-escalation study that will be done in humans. No evidence of toxicity has been seen in primates on a number of variables, including gene expression, weight, general animal activity, potential for cell migration, and potential for tumor formation. Dr. Tuszynski also noted that one monkey, in addition to those shown in the data, died, but that death occurred before the experiments began.

Dr. Breakefield requested clarification about whether this type of procedure in a human might carry with it a risk of some kind of hemorrhagic event. Dr. Hoi Sang U, the neurosurgeon for this protocol, responded that the number of perforations in the palatum made for treatment of Parkinson's disease is much larger than the number intended to be made in the basal forebrain nuclei for this AD trial. The needle trajectory in the proposed trial is similar to that used in the Parkinson's treatment. The risk of a hemorrhagic event in the proposed procedure is less than 1 or 2 percent.

XVII. Discussion of Human Gene Transfer Protocols #9910-342 and 343: *Phase I Trial To Evaluate the Safety of H5.020CMVPDGF-B for the Treatment of a Diabetic Insensate Foot Ulcer* and *Phase I Trial To Evaluate the Safety of H5.020CMVPDGF-B and Limb Compression Bandage for the Treatment of Venous Leg Ulcer (Trial A)*

Investigator: Dr. David J. Margolis, University of Pennsylvania Medical Center
Sponsor: Philip J. Cross, Institute for Human Gene Therapy, University of Pennsylvania
Reviewers: Drs. Ando, Macklin, and Markert

Background

The RAC recommended full public discussion of this protocol for several reasons: new diseases, new therapeutic application, new vector construct, new platelet-derived growth factor (PDGF) functional gene, and new patient populations.

Drs. Ando, Macklin, and Markert submitted written reviews to which the investigators responded in writing. Dr. Margolis provided oral responses to additional questions raised during the meeting.

Protocol Summary

Most patients with chronic wounds of the lower extremity fail to heal in a reasonable period of time. Despite considerable advance in elucidating the molecular basis of wound repair, attempts at developing new therapies have been disappointing. In the few studies where cytokine growth factors have been efficacious, their effect has been dramatically less than would have been predicted from animal studies. The long-term goal of this project is to evaluate a new approach for healing venous leg ulcer and insensate diabetic foot ulcer, which are two distinct types of chronic wounds usually treated with medical therapy.

Current methods of applying cytokines to chronic wounds are inadequate; human experience with topical application has been disappointing. The growth factor involved in this study is PDGF-B; PDGF-BB represents the dimeric, active form of this growth factor. This study will use adenovirus-Ad5 and the PDGF-B gene (called H5.020CMVPDGF-B) to ensure delivery of a cytokine growth factor to a nonhealing diabetic insensate foot ulcer. The growth factor PDGF-BB was selected because of its well-established importance in wound healing and because preclinical studies using animal wound-healing models have demonstrated the superiority of adenovirus-mediated delivery of PDGF-B and ultimate production of PDGF-BB compared with traditional topical application of PDGF-BB.

The aim of this study is to assess local and systemic toxicity in a dose-escalation Phase I trial and the feasibility of using the maximal tolerated dose associated with *in vivo* PDGF-B gene transduction via an intraulcer injection in patients with an insensate diabetic foot ulcer. The investigators will be able to evaluate the acute safety of this technique and its effect on two distinct chronic wounds—insensate diabetic foot ulcers and venous leg ulcers. The patients to be investigated in this study have diabetes mellitus and foot ulcer, lack protective sensation in their foot, and have adequate arterial blood flow. A total of 9 to 24 subjects will be enrolled in each of these two protocols, using four dose cohorts with a three-six design (i.e., 5×10^8, 5×10^9, 5×10^{10}, and 5×10^{11} particles per ulcer).

RAC Review and Discussion

Neither Dr. Ando nor Dr. Markert expressed any additional problems or questions that had not been addressed by the investigator.

Dr. Ando stated that this trial is an excellent study for gene therapy: It is a local study, it is extremely safe, the doses are well known, and gene transduction should be able to be correlated with efficacy parameters in the future. His only concerns related to immunogenicity and the inflammatory effects of the adenovirus, which the researchers have addressed as completely as possible in models and toxicology tests.

Dr. Macklin expressed some concern about the question of compensating participants; the investigators agreed to incorporate her suggestions and to simplify the Informed Consent form by using more ordinary language. The original plan was to give the subjects $100 for completing the study and no money to any of those who did not complete the study, unless some harm resulted from the study or the investigators or sponsor withdrew the participant. Dr. Macklin suggested that the payments be prorated; the investigators agreed to implement her suggestion.

Dr. Ted Friedmann asked why topical application of PDGF is so ineffective. Dr. Margolis responded that the answer is not known, but it is possible that touching the wound on a daily basis may be implicated or that the wound environment presents a hostile environment to the topical PDGF, which may deactivate the cytokine. Humans may respond differently to PDGF compared with animal models.

Dr. McIvor stated that a full-log escalation in doses is quite large and suggested a change to arithmetic increases. Dr. Margolis stated that the study will enroll one patient about every 6 weeks to check for reactions; the protocol's dose escalation may be modified if warranted.

Dr. Breakefield asked whether the relevant patients represent a more at-risk population (e.g., for reaction to virus or other concomitant illnesses). Dr. Margolis responded that the venous leg patients do not appear to be more at risk than other age-matched individuals. The diabetic insensate foot ulcer patients tend to have other problems, including retinopathy, nephropathy, and arterial disease. Although this study population does not have significant arterial disease, Dr. Margolis is not able to discern whether they will be more susceptible than others to the virus or to PDGF.

Dr. Ted Friedmans asked whether viremia studies have been conducted in animal systems. Dr. Margolis responded that biodistribution studies are currently under way.

XVIII. Other RAC Issues

Conclusions: Working Group on Adverse Event Reporting/Dr. Macklin

Dr. Macklin summarized the morning's proceedings regarding SAE reporting and then provided a summary of the Working Group's deliberations.

The first question dealt with by the Working Group was whether the proposed amendments to the *NIH Guidelines* go beyond the existing guidelines for reporting. The Working Group concluded that, although the proposed amendments appear to require more than is currently required, they merely spell out in detail the intent of the current *NIH Guidelines*. On the question of whether the current *NIH Guidelines* should be maintained or reduced, there was sharp disagreement. Some of the advocacy groups, the ASGT, and

public testimony appeared to endorse the explication of the existing regulations, yet others argued against them. No Working Group conclusion was reached.

The two sharpest points of disagreement were whether (1) there is a need to report allegedly unrelated as well as allegedly related SAEs and in what timeframe and (2) whether there is a need to report expected as well as unexpected SAEs. As denoted in FDA regulations, SAE definitions of expected and unexpected may not be appropriate or fully accurate for RAC discussions, so the RAC may need to redefine these terms.

Agreement in principle was reached by the Working Group on the following two issues:

1. SAE reporting procedures of FDA and the RAC should be better coordinated.

2. Harmonizing of reporting procedures is possible, but it may not be possible to achieve complete harmonization. Harmonization could be "up" (increasing reporting, thus imposing a significant burden on the FDA) or "down" (meaning less reporting than the RAC would want, thus possibly losing important information).

Items on which the Working Group still needs to focus include:

1. Timing of reports—expedited and annual

2. Establishing a sorting mechanism to set priorities for review and for reporting

3. Crafting the proposed guidelines to achieve the desired result—reports of adverse events associated with the gene transfer aspects of protocols and not from the underlying disease

4. Establishing a mechanism to review the data that are supposed to be provided

Dr. Macklin requested compilation of information on the degree to which compliance has been achieved with the current reporting requirements in the *NIH Guidelines*. Both the OBA and the FDA would need to review their records and devise an appropriate strategy for reporting that information. Dr. Noguchi pledged to work with OBA and to have the requested information available, although possibly not in time for the March 2000 RAC meeting.

Dr. McIvor pointed out two main problems with harmonizing reporting procedures between the RAC and the FDA: The FDA receives reports from the sponsor, whereas OBA receives reports from the investigator, the FDA receives material considered to be publicly nondisclosable, and the primary mission of the RAC is to provide public hearings on gene transfer protocols. Dr. Noguchi responded that the FDA's reporting system tries to provide the FDA with the information needed on SAEs but that the reporting form is probably not adequate because it does not provide the ability to obtain information in a standardized format. Industry representatives strongly believe that adverse events can be made public, but only after some deliberation process by the RAC and/or the FDA.

For the March 2000 RAC meeting, Dr. Markert requested that the RAC be given examples of 20 cases that would have fallen into the "unrelated and anticipated" group, which would be the kinds of cases the RAC would request to be reported within 15 days under the proposed reporting guidelines, so the RAC can decide whether it wants to see these kinds of cases. Ms. Knorr agreed to provide this information.

Ms. Knorr stated that, when the NIH requested reports on adenovirus adverse events in response to Mr. Jesse Gelsinger's death, a great deal of information was received that should have been submitted immediately. After the request, nearly 100 percent of the relevant information was captured; prior to the request, only a small percentage had been reported.

The Working Group on Adverse Event Reporting will work during the next 3 months and report its recommendations at the March 2000 RAC meeting.

(At this point, Dr. McIvor had to leave the RAC meeting due to transportation conflicts, so Dr. Macklin became acting chair for the remainder of this meeting.)

Miscellaneous

Ms. Levi-Pearl requested that the OBA keep the RAC informed about progress made with regard to the NIH Director's Advisory Committee working group on NIH oversight of clinical gene transfer research.

In response to Dr. Ando's question about when dual submission of gene transfer protocol applications to the RAC and the FDA will begin, Dr. Noguchi stated that the FDA prefers that protocols be sent first to the RAC for analysis as to whether public discussion will be needed. After the RAC makes that decision, the IND should be filed with the FDA.

XIX. Future Meeting Dates and Announcements/Dr. Macklin

The next RAC meeting will be held March 9-10, 2000, at the National Institutes of Health, Building 31C, Conference Room 10.

XX. Adjournment/Dr. Macklin

Dr. Macklin adjourned the meeting at 3:47 p.m. on December 10, 1999.

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

Debra W. Knorr
Executive Secretary

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

Date: December 10, 1999

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