

NIH Report

Assessment of Adenoviral Vector Safety and Toxicity: Report of the National Institutes of Health Recombinant DNA Advisory Committee

BACKGROUND

Given the nature of clinical research, it is not uncommon for gravely ill participants to succumb to their underlying disease in the course of a trial. However, in September, 1999, the gene transfer research community was alerted to the report of the death of a young man enrolled in a gene transfer trial at the University of Pennsylvania.¹ In this case, the research participant was not *in extremis* at the time of enrollment, nor did it seem likely that his death was due to his underlying condition, a partial deficiency of ornithine transcarbamylase (OTC). This raised critical questions as to whether the death might have been, in whole or in part, due to a toxic reaction to the adenovirus (Ad) vector employed to deliver a functional copy of the OTC gene to the participant, to conditions unique to the patient, and/or to the conduct of the trial. If the death was deemed directly attributable to administration of an experimental gene transfer vector, it would be the first such case in the history of gene transfer clinical research.

Federal agencies involved in the oversight of gene transfer clinical research took immediate actions as dictated by their respective missions and authority. In the case of the National Institutes of Health (NIH), oversight of human gene transfer research is embodied in the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* and the activities of the Recombinant DNA Advisory Committee (RAC; see Appendix A for roster). Both the *NIH Guidelines* and the RAC are administered by the NIH Office of Biotechnology Activities (OBA), which develops and implements NIH policies and procedures for the safe conduct of recombinant DNA activities, including human gene transfer clinical research.

The *NIH Guidelines* set forth policies and procedures designed to maximize the safety of basic recombinant DNA research and to provide guidance for optimal design of preclinical and clinical gene transfer research and standards for informed consent. Investigators conducting gene transfer re-

search, either funded by the NIH or carried out at an institution that receives NIH support for recombinant DNA research of any type, are required to comply with the *NIH Guidelines*. As advances in the science and safety of gene transfer research warrant, the RAC recommends changes in the *NIH Guidelines* to the NIH Director. The RAC's most visible role, however, is the public review of gene transfer clinical protocols. This public review involves an in-depth discussion of the preclinical safety data, the design of the protocol, the informed consent document, and any overarching scientific, safety, or ethical issues relevant to the specific protocol.

NIH RESPONSE TO A RESEARCH PARTICIPANT DEATH POSSIBLY ATTRIBUTED TO THE USE OF AN AD VECTOR

The possibility that the death of the research participant might be attributed to administration of the Ad vector prompted several actions on the part of the NIH. First, the OBA alerted all principal investigators and sponsors involved with gene transfer trials using Ad vectors that a research participant had died following administration of an Ad vector. At the time, Ad vectors had been employed in about 80 clinical trials since 1993, the year that Ad vectors were first used in gene transfer applications.² Because OBA determined that the case warranted an in-depth assessment of the risks of Ad-mediated gene transfer, OBA requested that investigators and sponsors involved in any Ad gene transfer clinical trial, regardless of the current status of the protocol, submit all relevant preclinical and clinical data regarding Ad vector safety, toxicity, and efficacy.

At the same time, OBA also established a RAC Working Group on Adenoviral Vector Safety and Toxicity (AdSAT) (see Appendix B for roster) to conduct a comprehensive review and analysis of the scientific, safety, and ethical issues associated

¹The trial—*A Phase I Study of Adenoviral Vector Mediated Gene Transfer to Liver in Adults with Partial Ornithine Transcarbamylase Deficiency*—was registered in 1995 with the Office of Biotechnology Activities (OBA) as protocol number 9512-139.

²These trials focused on several kinds of inherited diseases, including cystic fibrosis and OTC deficiency; diseases of the heart and circulatory system, including angina, coronary artery disease, and peripheral arterial disease; and also many kinds of cancer, including acute lymphocytic leukemia, bladder cancer, breast cancer, chronic lymphocytic leukemia, colon cancer, glioblastoma, malignant glioma, hepatocellular carcinoma, melanoma, neuroblastoma, non-small cell lung cancer, ovarian cancer, prostate cancer, renal cancer, retinoblastoma, and squamous cell carcinoma of the head and neck.

with Ad-based human gene transfer. The Working Group was asked to formulate findings and conclusions in regard to: (1) the OTC clinical trial specifically, including the postmortem analysis, the actual trial design, and the informed consent document and (2) vector safety and toxicity data associated with all Ad-derived clinical trials registered with NIH OBA. The findings of the Working Group were to be discussed by the full RAC and, if adopted, serve as a basis for the development of any additional NIH guidance on the use of virus-based vectors in human gene transfer research.

Finally, to inform the public and provide important background information to the AdSAT Working Group, the NIH sponsored a public symposium on Ad vector safety and toxicity. This symposium brought together experts in the fields of adenovirus biology and pathophysiology, vector manufacture, hepatic physiology and coagulopathy, and cytokine and cell receptor biology.

The AdSAT Working Group met in December, 1999, in conjunction with the AdSAT Symposium and a RAC meeting, to review the details of the OTC trial as well as general vector safety concerns. The Working Group and the RAC heard a series of presentations from experts on Ad biology and pathophysiology, the ways Ad has been modified to serve as a vector in gene transfer research, and specific cases in which the vector had elicited toxic or other adverse responses (see Appendix C for presentations). In addition, the FDA presented its preliminary findings regarding the preclinical analyses and trial design as well as actions taken by the Agency in response to the death. This was an unusual action on the part of the FDA, one that required special approval of the FDA Commissioner, based on public health considerations, for departing from normal procedures. It reflected the Agency's commitment to a thorough and rapid assessment of adenoviral vector safety, toxicity, and efficacy and to a timely and appropriate response to this unexpected death.

The AdSAT Working Group developed draft findings and recommendations that were subsequently discussed, modified, and approved by the full RAC. The RAC's findings, conclusions, and recommendations are summarized below. Because the FDA has broad regulatory experience with all forms of product development, it may publish additional guidance and regulations on this topic.

FINDINGS AND CONCLUSIONS REGARDING THE UNIVERSITY OF PENNSYLVANIA OTC GENE TRANSFER TRIAL

The clinical trial conducted at the University of Pennsylvania targeted the correction of OTC, a recessive X-linked autosomal genetic defect that impedes the liver's metabolism of ammonia. This mutation is the most common of the inborn errors of the urea cycle and affects approximately 1 in 40,000 to 1 in 80,000 individuals (Matsuda *et al.*, 1991, *Am. J. Med. Genet.* 38:85–89). Individuals with OTC deficiency develop pronounced hyperammonemia and, in turn, excessive elevation of ammonium ion in the brain, which is associated with life-threatening encephalopathy, coma, and brain damage. Because the disease is X-linked, primarily males are affected. Females are usually asymptomatic carriers but, depending on the pattern of

X-chromosome inactivation and resulting mosaicism, they can also be affected and may require medical treatment. Hemizygous males with no residual OTC activity are born normal but become comatose within 24–72 hrs and usually die within 2 weeks. Males who are somatic cell mosaics for the OTC gene have a partial deficiency of OTC (Maddalena *et al.*, 1988), and they are susceptible to hyperammonemic crises throughout childhood and as adults. Dietary manipulation is only partially effective in managing OTC. Each episode of coma carries a known risk of mortality (5–10%) with a more significant risk of subsequent brain damage.

The approach used in the University of Pennsylvania clinical trial was to provide a functional OTC gene using an adenovirus-derived vector as the delivery vehicle. The E1 and E4 genes were deleted from the version of the Ad vector used in the trial. The Ad vector was administered to 18 study participants by direct infusion into the right hepatic artery. Delivery rates and volume of instillation were maintained constant for all research participants. Cohorts of three participants were assigned to six dosing regimens, with each cohort receiving a progressively higher dose of vector, adjusted for subject body weight. The first two participants in each cohort were proposed to be asymptomatic or symptomatic females, with male participants only eligible to be the third participant in each cohort. Doses ranged from 1.4×10^{11} vector particles (total) to 3.8×10^{13} particles. The 18-year-old male participant received the highest dose, following an asymptomatic female participant who received a slightly lower dose (3.6×10^{13} total vector particles).

Discussions of the Working Group centered primarily on the circumstances that may have predisposed the trial participant to a toxic reaction to the vector, such as the trial participant's health prior to vector infusion, the participant's cytokine profile, and origins of the pulmonary complication that led to the subject's death.

After reviewing clinical and postmortem findings as well as other related studies, members of the Working Group concluded, and the RAC concurred, that the research participant's death most likely resulted from a systemic, Ad vector-induced shock syndrome, due to a cytokine cascade that led to disseminated intravascular coagulation, acute respiratory distress, and multiorgan failure. Post-mortem bone marrow biopsy revealed red cell aplasia. The data suggested that the high dose of Ad vector, delivered by infusion directly to the liver, quickly saturated available receptors for the vector (coxsackievirus-adenovirus receptors) within that organ and then spilled into the circulatory and other organ systems, including the bone marrow, thus inducing the systemic immune response. Adenoviral capsid proteins elicit humoral immune responses resulting in the generation of anti-Ad vector antibodies. Although the Ad vector used in the OTC trial was incapable of replicating, the capsid proteins encoating the vector likely contributed to the participant's immune response. It was noted that dose-related toxicities were observed over a very narrow dose range, leading the Working Group to consider the advisability of arithmetic rather than logarithmic dose escalations in the dose range of toxicity.

Relevant preclinical studies using the Ad vector were also considered. Prior to the initiation of the human trial, several versions of the OTC Ad vector were tested in a number of an-

imal models, including mice, baboons, and rhesus monkeys. In mouse studies, earlier versions of the Ad vector used in the OTC trial were shown to induce a self-limiting form of hepatitis that takes several days to develop and resolves within 1 month. Toxicity studies in rodents and in non-human primates using other Ad vectors also suggested the potential for this vector system to induce liver toxicity or to stimulate potentially harmful immune system responses, particularly when the vector is administered at high doses (Lozier *et al.* 1999). Such toxicity was reportedly diminished in the modified version of the Ad vector used in the OTC trial.

FINDINGS AND RECOMMENDATIONS REGARDING THE USE OF AD-BASED VECTOR SYSTEMS IN HUMAN GENE TRANSFER CLINICAL RESEARCH

After reviewing the preclinical and clinical data from the OTC trial and other Ad-based clinical trials and considering the presentations and discussions from the December 1999 AdSAT Symposium and RAC meeting, the AdSAT Working Group concluded that human gene transfer experiments using Ad-based vectors should continue—with caution. The RAC endorsed this conclusion.

The Working Group also developed a number of draft recommendations for strengthening the design, evaluation, and conduct of Ad-based gene transfer research. These conclusions and recommendations were presented and discussed at two subsequent RAC meetings and were endorsed by the RAC with some modifications. The RAC's final recommendations are summarized and discussed below.

Recommendation: Standards should be developed to improve the comparability and value of experimental data collected during clinical trials. These standards would apply to the determination of vector potency (particle number, titer, dose); vector strength (transgene expression, transduction efficiency and specificity); vector quality (identity, purity, integrity, homogeneity); and vector or treatment-related toxicity (standard reporting criteria).

The lack of appropriate standards for measuring the quantity of vector administered to study participants was of particular concern, because the post-mortem data from the OTC trial suggested that the fatality was possibly the consequence of a threshold effect involving a dose–response “elbow” above which toxic responses increase abruptly. Inaccurate and inconsistent methods for determining titer and vector particle number could mean that the dose administered actually exceeded the intended dose. The lack of uniformly recognized standards for measuring vector concentration or potency from one gene transfer research group to another also complicates meaningful comparisons of the raw data across clinical trials. Vector titer standards, model Ad vector particle measurement assays, and suitable reagents

and reference standards need to be available for use among all laboratories involved in these efforts. Standardized assays should also be developed for use with vectors that are based on viruses other than adenovirus and with nonviral vectors. The standard reagents and assays that have been developed for use with retroviral vectors might serve as a model.³

The quality and integrity of all vector preparations need to be routinely evaluated, especially if they are intended for administration in high doses to human research participants. For example, during the OTC clinical trial, there appeared to be considerable variability among different lots of Ad vector. In some cases, the dose of vector administered required use of an entire lot of Ad vector. Therefore, multiple lots of vector were employed throughout the trial, and different participants received vector from different lots. Stringent quality control on each lot would be essential to ensure product purity, potency, and uniformity throughout such a trial. Other points for consideration in product quality and safety include the development of robust methods and formulations for the storing and shipping of vectors, because changes in physical conditions such as temperature and pH can affect product stability over time.

Determination of vector potency and strength should include analysis of transgene expression measured as levels of messenger RNA or protein. In addition, gene transfer researchers should routinely report transduction levels in quantitative terms (*e.g.*, as the proportion of target cells expressing the transgene and quantity of specific transgene product produced) rather than as a qualitatively described clinical effect. Preclinical results obtained through animal modeling may be poor predictors of results in humans, because many factors critical to the interpretation of the data cannot be accounted for by simple proportional scaling between animals and humans. Examples of factors that affect the application of preclinical results to human studies include differences in body size, vector pathogenicity, preexisting immunity, sensitivity to the vector, and biological parameters (such as organ blood flow, body temperature, tissue receptor densities, and cellular regulatory components) across species.

The attention drawn to this issue by the RAC prompted action in other quarters. An October, 2000, conference on adenoviral vector testing, organized by the Williamsburg Bioprocessing Foundation, led to the formation of a working group to develop a standard that would allow for the comparison of toxicities observed in preclinical and clinical studies. This group, which is led by the FDA and includes academic, industry, and government representation, is currently overseeing the selection of entities to manufacture, characterize, and distribute a wild-type Ad standard.

Recommendation: A centralized database should be developed for collecting and organizing gene transfer vector safety and toxicity data.

If information on vector toxicity is to be converted into generally accessible and useable knowledge, data collection practices

³In October 2000, the FDA issued supplemental guidance on testing for replication competent retrovirus in retrovirus-based gene transfer products and during follow-up of patients in clinical trials using retroviral vectors.

among oversight bodies need to be more uniform. For example, with regard to adverse event data collection, both NIH OBA and FDA provide a suggested format outlining essential data elements. OBA's suggested format, while closely modeled after FDA's MedWatch adverse event report form, is tailored specifically to clinical gene transfer research. OBA accepts reports of adverse events using either the NIH OBA format or the FDA MedWatch report form. However, neither NIH nor FDA requires specific formats for data reporting. Wherever possible, establishment of standardized reporting formats across agencies could lead to improved compliance, more complete, informative reports, and thus enhanced quality of data.

The NIH and FDA are working together to create a database—the Genetic Modification Clinical Research Information System (GeMCRIS)—to enhance the science and safety of gene transfer clinical trials and public awareness of clinical gene transfer research. GeMCRIS will facilitate the sharing of information, increase public awareness of this arena of research, optimize patient safety, and enhance public confidence in the oversight of clinical gene transfer research. GeMCRIS is designed to serve a diverse group of users including principal investigators, other researchers, sponsors, the NIH, FDA, patient communities, the general public, policymakers, and the media.

A precursor database has been accessible on the Web since December, 2000. It includes basic information about all protocols registered with OBA, such as registration number, title, and investigator, the disease under study, vector, gene, route of administration, phase of study, and clinical trial sites. Technical and lay language summaries of the gene transfer protocols are also posted. A link is provided to the data management reports, which contain summaries of adverse events and other relevant preclinical and clinical safety information and are provided to the RAC at each quarterly meeting. The database, summaries, and data management reports can be accessed at <http://www4.od.nih.gov/oba/rac/clinicaltrial.htm>.

The first version of GeMCRIS is scheduled to be posted on the OBA Website in 2002. The Phase I database will be able to support queries by disease category, disease, phase of study, protocol number, and principal investigator. The Phase II version will contain more information organized to allow in-depth queries and permit more complex analyses to be performed. The final database will organize all available information in a highly systematic way to facilitate cross-trial analyses, searches for specific variables, analyses of aggregate data and the identification of emerging trends in the safety and efficacy of human gene transfer research.

Recommendation: The RAC will heighten attention to the issue of collection of biological data necessary to support safe product development in initial clinical trials. All vector systems should be evaluated using traditional drug development approaches, to include assessments of biodistribution; pharmacokinetics; target receptor distribution and concentration (preclinical models and human research subjects); routes and rates of administration; and characterization of therapeutic and toxic thresholds (dose escalation and response profile).

The information submitted to OBA in response to its request for preclinical and clinical AdSAT data, and presented at the

December, 1999, AdSAT symposium, generally provided little or no information about the critical element of vector distribution in research participants. Moreover, although many reports described a variety of routes for administering vectors—including intrahepatic, intratumoral arterial, venous, dermal, and aerosol—they did not provide information as to how the route may affect the eventual distribution of each of the vectors. Quantitative information about biodistribution profiles is needed for assessing toxicity profiles. Increased knowledge in this area would benefit the field of human gene transfer research.

Efforts are also needed to improve the collection and analysis of vector distribution data at the preclinical stage. Critically, researchers need to examine more thoroughly the validity of animal model systems used for determining pharmacodynamic and toxicity profiles of vectors. For example, the distribution of the coxsackievirus–adenovirus receptor may differ significantly between mouse and human, suggesting that the mouse liver is not necessarily the best system for modeling the biodistribution pattern or toxicity profile of Ad vectors in the human liver. However, the limitations of particular animal models must be weighed against their usefulness as genetic models to examine important features of the proposed experimental therapy in the context of the underlying disease. Other important considerations are the potential for an animal-derived transgene product to trigger a human immune response that can affect any number of study parameters, and the extent to which an animal-derived transgene product will be able to function adequately in a human environment.

Traditional approaches to pharmacokinetic analyses may not always be sufficient for all aspects of gene transfer research. For example, traditional pharmacokinetic analytic methods for measuring serum levels of an active substance might apply if a transgene-derived substance is secreted into the bloodstream, as in the case of clotting factors. However, in the majority of cases, such as cell cycle regulatory proteins used in experimental cancer therapies, the transgene-derived active substance is not secreted but remains intracellular. For most gene transfer research applications, pharmacokinetic profiles are determined by measurement of transgene expression (or transgene-derived product activity) and persistence in target and nontarget tissues.

Recommendation: Experiments involving vector controls, whenever appropriate and meaningful, should be included in the preclinical and clinical gene transfer procedures.

Without appropriate controls in experimental gene transfer procedures, it can be very difficult to determine the actual efficacy and safety of a particular vector-based experimental treatment. In the absence of properly designed controls, it may be impossible to determine whether an observed toxicity is due to underlying disease or to the use of a specific vector. This situation can lead to erroneous estimations of the safety and efficacy of a vector; overestimation can place research participants at risk and underestimation can mean that a potentially life-saving product does not get developed. If proper controls were used more widely, and if gene transfer experiments in general were more uniformly designed, it might also become possible to combine vector safety-related observations from many protocols for the purpose of conducting trend analysis.

Certain types of clinical trials are inherently more amenable to the use of null or control vectors than are others. For instance, trials involving direct intradermal or subcutaneous injection of vector products into or near well-defined lesions lend themselves to this approach because some of the lesions can be treated with null vectors to serve as controls. In other studies, however, such controls might not be necessary because the efficacy of the candidate gene transfer can be directly assayed. Therefore, it is important to assess independently the risks associated with the administration of the active substance (the transgene product), the clinical intervention (*e.g.*, administration requiring general anesthesia versus a simple injection), and the vector itself (the control or null vector).

Recommendation: All research participants enrolled in gene transfer clinical trials should be monitored for several types of acute toxicities before and after vector administration. For better surveillance across all such studies, monitoring should routinely include a research participant's immune status (both humoral and cellular), cytokine profile, and predisposing or underlying conditions that might elevate an individual's sensitivity to a particular vector (research participant genotype, secondary and concurrent infections).

Because immune status can vary considerably from one individual to another, and may affect a research participant's reaction to a specific experimental treatment, researchers conducting gene transfer clinical trials should systematically monitor the immune system status of all research participants. In designing the immunological work-up, the investigator should consider the research participant's underlying disease and the anticipated host reaction to the vector and/or the transgene product. This monitoring should include measurements conducted both before and after vector administration. Establishment of a standardized approach to gauge immune competence prior to administration of a gene transfer product would be very useful in assessing the pharmacodynamic effects of gene transfer products on the immune system.

There are at least three considerations important to designing the assessment of immune system function in gene transfer research participants. First, does the participant's immune system react against the vector and does vector administration affect the participant's response to the vector? For instance, with adenoviral vectors, the titer of preexisting antibodies should be assessed. It is recommended that, at least in a subset of patients, T cell responses to the vector be assessed pre- and post vector administration. A second consideration involves trials with cancer vaccines. The research participant's ability to mount an immune response against a standard antigen should be assessed. Then if the participant does not mount an immune response to the gene transfer product, it will be possible to determine if the

failure is secondary to a problem inherent in the participant's immune system or to the design of the gene transfer product. A third consideration involves protocols in which all T cells are destroyed as by chemotherapy. In this regard, it is important either to replace the T cells with cryopreserved autologous T cells or HLA-matched donor T cells, or to show that the research participant's thymus can function to regenerate T cells from bone marrow stem cells. The specific tests to be done will be protocol-specific, but these immune issues should be carefully considered.

In addition, pre- and post-treatment values drawn from larger numbers of trial participants could be pooled and analyzed in various ways to provide broad data sets from which general immunomodulatory trends might be identified. For immunogenic vectors such as adenovirus, presence or absence of an immune response pre- or post-administration is likely to affect both the efficacy and tolerability of the vector. In addition to the immunogenicity associated with the viral particle, any immunologic response against the gene insert product should be thoroughly characterized. Immune responses against the active substance of the gene transfer vector may have a profound clinical impact on the potency of the experimental product and may interfere with subsequent or alternate methods of treatment. Of note, the Immune Tolerance Network may provide a model and/or resources for addressing some of these types of issues.⁴

Steps should also be taken to minimize the complications that could be caused by secondary infections. Research participants in gene transfer protocols should be screened for infections and, under most circumstances, excluded from participation until any such infection has resolved. This set of precautions can be modeled on those that are now standard practice for solid organ transplant procedures. Prescreening for concurrent viral and bacterial pathogens should be based on specific concerns related to the viral vector used in the experimental procedure. Such concerns may include the potential for *in vivo* recombination in the research participant between the vector and a concurrent viral infection (*e.g.*, generation of a replication-competent retroviral vector), and the potential for direct or indirect interactions between the vector and pathogens that may increase the severity of either the underlying infection or the reaction to any vector toxicity. The Committee realizes that it may not be possible to eliminate infection in patients with primary and acquired immunodeficiency; however, these issues should be considered in the protocol.

The measurement of cytokine release as a result of vector administration would help to elucidate the interdependence of specific cytokine responses, vector tolerance, and potential toxicity outcomes. Data obtained to date are not sufficiently comprehensive to establish useful correlations that might influence clinical decision-making. By accumulating a larger body of knowledge, it is conceivable that cytokine profiles may in future be used as a basis for predicting dose ranges, scheduling, and modification.

⁴The Immune Tolerance Network is a collaborative effort funded by the NIH and the Juvenile Diabetes Foundation International. The Network solicits, develops, and assesses clinical strategies and biological assays for the purposes of inducing, maintaining, and monitoring tolerance in humans in transplantation and a number of diseases. For example, the Network studies determine safety, toxicity, and efficacy of promising tolerogenic strategies; investigate the basic mechanisms of immune tolerance as an integral part of clinical trials; and develop and/or refine and validate immune and surrogate marker assays to monitor the induction, maintenance, and loss of tolerance.

Recommendation: Research participants in gene transfer clinical trials should be encouraged to agree to post-mortem examinations.

The serious adverse event reports and clinical data submitted to NIH/OBA were notably lacking in autopsy data. Although problem is likely part of a national trend in which fewer individuals and families agree to post-mortem analysis. The inability and/or failure to collect such information in the context of clinical gene transfer research makes it more difficult to detect potential trends in vector toxicity and to confirm or reject hypotheses about vector safety. Investigators should work with trial participants and their families to explain the importance of these data. While Appendix M of the *NIH Guidelines* speaks to this issue, and the NIH OBA has emphasized the importance of post-mortem analysis, further efforts are needed to ensure that a discussion of the scientific need for, and a request for permission to perform a post-mortem examination is carried out within the consent process.

Recommendation: Informed consent documents need to be improved to clarify risks and potential benefits faced by those who participate in gene transfer clinical trials.

Research participants must provide informed consent to participate in research. The informed consent document is an integral part of the informed decision-making process. The document alone, however, may not be sufficient, and other sources of information may be used to achieve the goal of fully informing the trial participant. The language used in these documents often reflects the local norms and preferences of the Institutional Review Board. Efforts to standardize the information content of the consent document as well as the process would greatly benefit study participants, their families, and the field of human gene transfer research.

Human subjects regulations and the *NIH Guidelines* specify essential elements to include in the informed consent document. In the case of the *NIH Guidelines*, this includes, for example, discussion of animal study outcomes and prior human experience, including a brief, focused description of potential benefits and risks, including the most frequent and serious adverse events relevant to the proposed study, and discussion of any potential for germ line transmission and alteration.

The use of neutral parties, whose responsibility is to ensure that the interests and well-being of the participant are protected, may be appropriate in the consent process. Well-trained advocates or independent counselors may be helpful in ensuring that the participant receives an unbiased accounting of the risks and potential benefits of the study. However, establishing, implementing, and managing such a system may pose many challenges, not the least of which are resources and oversight.

The nature of the relationship between the advocate/counselor and the investigator/institution might be considered for future RAC discussions. The issue of participant remuneration for participation in a clinical trial might also be addressed at some point.

Recommendation: Gene transfer clinical data should be reviewed and analyzed on a regular basis in a public forum.

Periodic review of aggregate gene transfer clinical trial data will help identify trends indicating potential areas of promise or concern. Discussion of these data in a public forum will promote awareness of these trends among research participants, their families, research investigators, industry sponsors, and the general public. The NIH, FDA, and professional organizations, such as the American Society of Gene Therapy, are encouraged to convene symposia similar to that co-sponsored by the NIH and FDA in December, 1999, in response to the death of the research participant in the OTC trial.

In fact, as part of ongoing efforts to ensure participant protection in gene transfer trials, the Department of Health and Human Services established Gene Transfer Safety Symposia to strengthen further safeguards in place for individuals enrolled in human gene transfer studies. The safety symposia are public forums for the review by scientific experts of emerging issues in the medical, scientific, ethical, and safety aspects of clinical gene transfer research. By fostering discussion and information exchange, the symposia will help: (1) enhance understanding of the safety and toxicity of gene transfer; (2) identify critical gaps in current knowledge; (3) maximize patient safety; (4) enhance informed consent processes; and (5) optimize the development of gene transfer clinical trials.

Since the December, 1999, symposium on AdSAT, the NIH and FDA have sponsored additional gene transfer safety symposia. For example, a March, 2000, symposium focused on the use of internally deleted, helper-dependent adenoviral vectors—a new generation of Ad vectors proposed to be safer because they do not express viral proteins. However, there were also new safety issues specific to the production and clinical application of these vectors that needed to be addressed. In December, 2000, NIH held a symposium on safety considerations in cardiovascular gene transfer to address questions about the vectors, transgenes, patient selection, and follow-up in these studies. In October, 2000, the FDA held a symposium on long-term patient follow-up. Safety considerations in the use of adeno-associated vectors in gene transfer clinical trials were the topic of a symposium sponsored by NIH in March, 2001. The NIH, in consultation with FDA, organized this symposium to provide a forum to discuss, in the broader context of the other adeno-associated vector clinical and preclinical data, the preclinical data from one animal study that suggested a possible association between adeno-associated vector gene transfer and tumorigenesis. Future safety symposia may review additional gene transfer vector systems, applications, or other features critical to human gene transfer clinical trials.

Another initiative of particular relevance to this recommendation is an ongoing NIH effort to establish a standing working group of the RAC, to be known as the NIH Gene Transfer Safety Assessment Board. The Board is to play a key role in the analysis of safety information in gene transfer research studies. The Board would review of safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials and identifying significant trends or single events. Significant findings and aggregated trend data would be reported to the RAC in public session. This process has the potential to enhance review of new protocols; improve the development, design, and conduct of human gene transfer trials; promote public understanding and awareness of the safety

of human gene transfer research studies; and inform the decision-making of potential trial participants.

CONCLUSION

This report describes some Federal responses—those of the NIH and the NIH RAC—to the untimely death of a research participant in an adenoviral gene transfer vector. The report sets forth a number of suggestions by the RAC for strengthening the design and conduct of Ad-based human gene transfer clinical research and which, if implemented, should increase the safety of the research participants in these trials. These include, for example, ways to improve multiple aspects of gene transfer vector production and administration; the use of a vector standard and proper controls to increase the value and comparability of data across studies and trials; and the importance of and need for information regarding vector biodistribution, pharmacokinetics, target receptor distribution, effects of method of administration, and characterization of therapeutic and toxic thresholds. At the clinical level, we make recommendations aimed at improving clinical monitoring and the process of informed decision making by the research participant. At a broader level, we encourage that the safety, toxicity, and efficacy data accumulated from trials be regularly reviewed, analyzed, and publicly discussed, to identify areas of potential promise or concern and to promote awareness of these findings among the various sectors of the public. For our part, as we review gene transfer protocols, we are emphasizing the issues outlined in this report and identifying areas for improvement.

This report also highlights some of the positive outcomes and progress that has been made towards implementing these recommendations, including the development of an Ad vector standard; establishment of National Safety Symposia that have focused on vector systems; the development of a national database to organize systematically the data from gene transfer clinical trials, make the data accessible to the scientific community and the public, and facilitate cross-trial analyses to identify

emerging trends in the gene transfer field; and the proposed establishment of a Gene Transfer Safety Assessment Board to analyze safety information from gene transfer research. Another initiative of relevance to enhancing the safety of gene transfer protocols also warrants mention. In October, 2000, NIH, with the advice of the RAC, made important modifications to the *NIH Guidelines* to ensure that oversight bodies at the local level have the benefit of RAC deliberations on protocols prior to participant enrollment. The changes also instituted new feedback mechanisms to ensure that the NIH OBA and RAC are apprised in a timely fashion of the manner in which investigators respond to protocol changes recommended by the RAC.

We encourage continued implementation of the recommendations and activities described within this report. They will be beneficial to Ad vector clinical research, to the gene transfer field, and, most importantly, to the research participants in human gene transfer clinical studies.

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Appendix A
Roster of the NIH Recombinant DNA Advisory Committee

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Appendix B
Roster of the AdSAT Working Group of the NIH RAC

Co-Chairs

Claudia A. Mickelson, Ph.D.

Biosafety Officer
 Environmental Medical Service
 Massachusetts Institute of Technology
 (RAC member)

Inder Verma, Ph.D.

American Cancer Society Professor of Molecular Biology
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Linda Gooding, Ph.D.

Dept. of Microbiology & Immunology
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Marshall S. Horwitz, M.D.

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 University of Minnesota
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Richard C. Mulligan, Ph.D.

Professor, Dept. of Genetics and
 Howard Hughes Medical Institute
 Harvard Medical School

Glen Nemerow, Ph.D.

Associate Professor
 Department of Immunology
 Scripps Research Clinic

Robert Warren, M.D.

Chief, Surgical Oncology Program
 Department of Surgery
 University of California, San Francisco

FDA Representatives

(Center for Biologics Evaluation and Research)

Steve Bauer, Ph.D.

Patricia Keegan, M.D.

Philip D. Noguchi, M.D.

Anne Pilaro, Ph.D.

Jay P. Siegel, M.D.

Karen D. Weiss, M.D.

Appendix C
AdSAT Presentations and Discussions
December 8–9, 1999

Adenovirus Biology, Pathophysiology, and Adaptation to Gene Therapy

Adenovirus Molecular Biology and Disease

Marshall Horwitz, M.D. (AdSAT Working Group)
Division of Infectious Diseases, Albert Einstein College of Medicine

Adaptation of Adenovirus for Gene Transfer

Inder Verma, Ph.D. (AdSAT Working Group Co-chair)
Laboratory of Genetics, Salk Institute for Biological Studies

Examples of Adenovirus-induced Pathophysiology

Interplay Between Adenovirus and Proinflammatory Cytokines

Linda Gooding, Ph.D. (AdSAT Working Group)
Dept. of Microbiology & Immunology, Emory University

Receptors and Signaling Events in Adenovirus Cell Entry

Glen Nemerow, Ph.D. (AdSAT Working Group)
Department of Immunology, Scripps Research Clinic

Adenovirus-induced Hepatotoxicity

Robert Warren M.D. (AdSAT Working Group)
Department of Surgery, University of California, San Francisco

Disseminated Intravascular Coagulation

Margaret Rick, M.D. (AdSAT Working Group)
Hematology, Magnuson Clinical Center, NIH

Toxicity Experience with Adenoviral Vectors at BCM

Estuardo Aguilar-Cordova, Ph.D. (AdSAT Working Group and RAC)
Gene Therapy Laboratories, Baylor College of Medicine

Helper Dependent Adenoviral Vectors – Development, Performance and Safety

C. Thomas Caskey, M.D. (Invited Speaker)
Human Genetics & Vaccines Discovery, Merck & Company, Inc.

Safety and Toxicity Data from Clinical Trials Using Adenoviral Vectors

Summary of Phase I Studies with Adenoviral Vectors at U Penn

James M. Wilson, M.D., Ph.D. (Invited Speaker)
Institute for Human Gene Therapy, University of Pennsylvania

Safety of Local Delivery of Low- and Intermediate-Dose Adenovirus Gene Transfer Vectors to Individuals with a Spectrum of Comorbid Conditions

Ronald G. Crystal, M.D. (Invited Speaker)
Pulmonary and Critical Care Medicine, Weill Medical Center, Cornell University

Ad Vector Safety Assessment

David P. Meeker, M.D. (Invited Speaker)
Medical Affairs, Genzyme Corporation

Schering 58500 Safety Assessment

JoAnn Horowitz, M.D. (Invited Speaker)
Schering-Plough Research Corporation

Ad5CMV-p53 (RPR/INGN 201) – Global Safety Assessment

Lyndah K. Dreiling, M.D. (Invited Speaker)

Clinical Research, Aventis Pharmaceuticals (formerly Gencell/Rhone-Poulenc-Rorer)

Viral Cancer Therapy With Onyx-015

David H. Kirn, M.D. (Invited Speaker)

Clinical Research, Onyx Pharmaceuticals, Inc.

Adenovirus-Mediated Expression of Human Factor IX in Rhesus Macaques and Associated Dose-Limiting Toxicity

Richard A. Morgan, M.D. (Invited Speaker)

National Human Genome Research Institute, NIH

Discussion of Ornithine Transcarbamylase Deficiency**Clinical Aspects of Ornithine Transcarbamylase Deficiency**

Arthur L. Beaudet, M.D. (AdSAT Working Group)

Dept. of Molecular and Human Genetics, Baylor College of Medicine

Presentation of Serious Adverse Event on Human Gene Transfer Protocol #9512–139 entitled “A Phase I Study of Adenoviral Vector Mediated Gene Transfer to Liver in Adults with Partial Ornithine Transcarbamylase Deficiency (OTC)”

Mark Batshaw, M.D. (Invited Speaker)

Children’s National Medical Center, D.C.

Steven Raper, M.D. (Invited Speaker)

University of Pennsylvania

James Wilson, M.D. (Invited Speaker)

University of Pennsylvania

Presentations by Food and Drug Administration Center for Biologics Evaluation and Research**Introduction**

Kathryn Zoon, Ph.D. (Invited Speaker)

Toxicology Assessment

Anne Pilaro, Ph.D. (Invited Speaker)

Clinical Perspectives

Thomas Eggerman, M.D., Ph.D. (Invited Speaker)

FDA Actions

Patricia Keegan, M.D. (Invited Speaker)

Serious Adverse Events

Jay P. Siegel, M.D. (Invited Speaker)

Public Comment/Presentations**Interference of Pre-Existing Neutralizing Antibody**

Dinko Valerio, Ph.D.

Introgene, BV