DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING
June 14, 1999

The Recombinant DNA Advisory Committee (RAC) was convened for its 74th meeting at 9:00 a.m. on June 14, 1999, at the National Institutes of Health (NIH), Building 31, Conference Room 10, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Claudia Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public on June 14 from 9:00 a.m. until 5:00 p.m. A committee roster is attached (Attachment I). The following individuals were present for all or part of the meeting:

Committee Members:

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

Ad Hoc Consultants:

Thaddeus P. Dryja, Harvard Medical School
Pedro R. Lowenstein, University of Manchester

Executive Secretary:

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

Nonvoting Agency Representatives/Liaison Representatives:

Melody Lin, Office of Protection from Research Risks
Andra Miller, Food and Drug Administration
Philip Noguchi, Food and Drug Administration

National Institutes of Health Staff:

Brenda Farmer, NIDR
Suzanne Goodwin, OD
Christine Ireland, OD
Becky Lawson, OD
Mike Miller, OD
Gene Rosenthal, OD
Steven Sabol, NHLBI
Thomas Shih, OD

Others:

Virginia Ackerman, Medeva Corporation
Andrea Anders, Capital Consulting Corporation
Robert W. Anderson, U.S. Food and Drug Administration
W. French Anderson, University of Southern California
Marie Laure Baës, Genethon
Saundra Bromberg, Capital Consulting Corporation
Audrey Chang, BioReliance
Yung-Nien Chang, VIRxSYS
Boro Dropulic, VIRxSYS
Diane O. Fleming, Safety Consultant
Scott Freeman, Schering-Plough Research Institute
Ken Garber, Writer/Journalist
Angus J. Grant, GenCell
Nancy L. Herring, Transgene, Inc.
Marc Horowitz, Baylor College of Medicine
Richard Hurwitz, Baylor College of Medicine
Dorothy Jessop, Public
Steven Kradjian, Vical, Inc.
Lavonne Lang, Parke Davis

William T. Lee, Cato Research
J. Tyler Martin, SyStemix, Inc.
Catherine Mathis, Transgene, S.A.
Valerie McDonnell, BioReliance
Lisa Needleman, Capital Consulting Corporation
Owen O'Conor, Memorial Sloan-Kettering Cancer Center
Anne Pilaro, U.S. Food and Drug Administration
Barry Polenz, Targeted Genetics Corporation
Isabelle Rivière, Memorial Sloan-Kettering Cancer Center
Dwaine Rieves, U.S. Food and Drug Administration
Brenda Ross, Johns Hopkins Medical Center Hospital
Donna R. Savage, Intelligent Fingers
Tomiko Shimada, Ambience Awareness International, Inc.
Barbara Singer, Capital Consulting Corporation
Lorna Speid, Valentis, Inc.
Rebecca Spieler, The Blue Sheet
Ruth S. Turner, Genzyme Corporation
Dominick Vacante, BioReliance
Rhea N. Williams, Schering-Plough Research Institute

Call to Order and Opening Remarks/Dr. Mickelson

Dr. Claudia A. Mickelson, RAC Chair, called the meeting to order at 9:00 a.m. on June 14, 1999. The notice of meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on May 28, 1999 (64 FR 29051). Issues discussed by the RAC at this meeting include: (1) a presentation on revised FDA policies regarding preclinical biodistribution studies, FDA guidance on the conduct of such studies, and related Informed Consent issues; (2) a discussion of future proposed actions to Appendix M of the *NIH Guidelines* regarding the appropriateness of conducting prenatal gene transfer research at the present time (based on the RAC's March 1999 consensus statement that such studies would be premature); (3) a discussion of the *NIH Guidelines* definitions of recombinant deoxyribonucleic acid (DNA) and human gene transfer, and their applicability to novel methodologies for modifying (either intentionally or inadvertently) the human genome; and (4) and public discussion and RAC review of a Phase I pediatric human gene transfer experiment involving direct injection of an adenoviral vector with the herpes simplex thymidine kinase (HSV-TK) transgene followed by intravenous (IV) administration of ganciclovir in patients with retinoblastoma.

Dr. Mickelson noted that many responses have been received for nominations to the Gene Transfer Vector Containment Working Group. This working group is charged with evaluating the *NIH Guidelines*, identifying currently unaddressed vector containment issues, and making recommendations to the RAC regarding appropriate containment practices and procedures that reflect the current state of science. The Working Group was encouraged to develop its recommendations in consideration of the current *NIH Guidelines* containment requirements and if possible, make recommendations regarding the format that might make this a more user-friendly document.

Dr. Mickelson noted that the American Society of Gene Therapy (ASGT) held its second annual meting in Washington, D.C., on June 9-13, 1999. During his keynote address to the ASGT, Dr. Harold Varmus, NIH Director, made particular mention of the important role that the RAC serves in maintaining pubic confidence in the field of gene therapy research. Dr. Varmus noted that RAC review of novel protocols prior to patient enrollment, its integration with Gene Therapy Policy Conferences, and clinical trial

follow-up are of particular importance to both the scientific community and the public. Dr. Varmus stated that the number of protocols requiring full RAC review has been significantly reduced in recent years. Since the *NIH Guidelines* were amended to eliminate the requirement for NIH approval of individual protocols, the RAC has recommended full review of less that 10% of the protocols submitted to the NIH Office of Recombinant DNA Activities (ORDA).

Minutes of the March 11-12, 1999, Meeting/Dr. Markert and Ms. King

Dr. Mickelson noted that the minutes of the March 11-12, 1999, meeting were reviewed by Dr. Markert and Ms. King prior to this meeting; both reviewers noted that the minutes were an accurate reflection of the previous RAC meeting and that minor editorial changes would be submitted to ORDA staff.

Committee Motion

The RAC approved a motion made by Dr. Greenblatt and seconded by Dr. McIvor to accept the minutes of the March 11-12, 1999, RAC meeting (with the incorporation of minor editorial changes submitted by the reviewers) by a vote of 9 in favor, 0 opposed, and no abstentions.

Data Management/Dr. Greenblatt

Dr. Greenblatt provided an overview of the human gene transfer clinical trials submitted to ORDA. To date, a total of 313 human gene transfer protocols have been registered with ORDA and are categorized as follows: (1) 277 clinical investigations involving gene "therapy" (primarily early phase studies designed to evaluate safety and toxicity of a gene therapy product that contains a potential therapeutic transgene); (2) 34 clinical investigations involving gene "marking" (cells transduced *ex vivo* with a non-therapeutic marker gene are readministered to research subjects to monitor cell distribution, duration of marker gene expression, etc.); and (3) 2 "non-therapeutic" clinical investigations involving healthy volunteers (intended to answer basic scientific questions about the nature of the immune response to specific classes of viral vectors).

The 277 gene "therapy" clinical investigations involved a broad spectrum of candidate diseases and disorders as follows: (1) 27 studies involving human immunodeficiency virus (HIV) infection, (2) 40 studies involving monogenic diseases (primarily cystic fibrosis), (3) 193 studies involving cancer, and (4) 17 studies involving other diseases and disorders, i.e., rheumatoid arthritis, coronary artery disease, peripheral artery disease, arterial restenosis, and cubital tunnel syndrome.

In accordance with the reporting requirements set forth in Appendix M of the *NIH Guidelines* the following documentation was submitted to NIH ORDA since the March 11-12 1999, RAC meeting: (1) 29 new clinical investigations (28 were determined to be exempt from full RAC review and one submission, #9904-304, that will be reviewed by the RAC during this meeting; (2) 27 amendments and updates (primarily minor modifications to clinical trial design, e.g., additional clinical sites, modifications to the patient eligibility criteria, and dosage changes); and (3) 8 safety reports (all involved complications that were not related to the gene transfer product.)

FDA Presentation and RAC Discussion of Revised FDA Policies and Draft Language Regarding Preclinical Vector Distribution Studies and Relevant Informed Consent Issues/Drs. Pilaro and Rieves

Background

This interagency discussion involving the FDA and NIH RAC is the fourth in a series of discussions regarding the issue of preclinical vector distribution studies. Previous FDA presentations and RAC discussions of this topic occurred on December 15, 1997; March 10, 1998; and March 12, 1999.

During the December 15, 1997, RAC meeting, FDA representatives informed the RAC that preclinical studies from multiple Investigational New Drug (IND) applications indicated evidence of vector DNA in gonadal tissue following extragonadal administration; however, the specifics of these observations could not be publicly discussed without prior approval of relevant sponsors. The FDA noted that evidence of gonadal sequences consisted of positive polymerase chain reaction (PCR) signals (homologous to vector nucleic acid sequences) extracted from whole gonads subsequent to vector administration; and highlighted the fact that these observations involved multiple classes of vectors, formulations, and routes of administration. At that time, there was insufficient data to determine whether these sequences were intracellular or integrated. It was also noted that if the sequences were intracellular, there were no data indicating whether the sequences were in gametes or in somatic cells. In the absence of such data, the FDA raised a note of caution that administration of gene transfer vectors could potentially result in advertent germ-line integration, a circumstance that would pose unknown risk to subjects participating in gene transfer clinical trials. The FDA noted additional concern that sponsors were increasingly interested in gene transfer for less serious disease, earlier intervention before manifestation of disease, and gene transfer for augmentation or enhancement purposes. The RAC recommended that NIH ORDA send a letter to all principal investigators of clinical gene transfer trials and all Institutional Biosafety Committees, requesting submission of all available data (preclinical and clinical) related to persistence of vector sequences in gonadal tissue. NIH ORDA received approximately 80 responses to this request. During its March 10, 1998, meeting, the RAC discussed these responses. On the basis of the limited information available to the RAC at that time, the RAC emphasized the importance of initiating well-designed studies to adequately evaluate the implications of these findings and recommended that the NIH should fund these definitive experiments.

During its March 12, 1999, meeting, the RAC held a public scientific discussion of reproductive physiology, gonadal biodistribution results from industry and academia, and the potential risks of a positive signal and what it could mean to future generations. Experts were invited to present there data: (1) Dr. Haig H. Kazazian, University of Pennsylvania, Philadelphia, Pennsylvania, presented data on alteration of the human genome caused by retrotransposition; (2) Dr. Lonnie Russell, Southern Illinois University, Carbondale, Illinois, discussed the testis, barriers to the entrance of substances into the testis, and directed attempts to transfect germ cells; (3) Ms. Nancy King, a RAC member, discussed the ethical and social implications of inadvertent germ-line transmission. Based on these new data, the RAC concluded that the risk of foreign gene transfer to germ cells (and to future progeny) was perceived to be extremely low, and there was a general acceptance by those present that these risks were acceptable in the context of the early phase clinical trials involving somatic cell gene transfer. Further discussion at that meeting concluded that studies of biodistribution to gonadal tissue were not necessarily required prior to all phase I clinical trials. The RAC noted that a positive finding (gene transfer to germ cells confirmed) should not uniformly preclude a clinical trial from going forward; however, a negative finding (no *detectable* gene transfer to germ cells) should not be interpreted as zero risk to research subjects. The RAC emphasized that such findings (and their meaning and significance) should be adequately addressed in the Informed Consent document.

FDA representatives requested today's discussion for the purpose of presenting revised policies and draft language regarding preclinical vector distribution studies and relevant informed consent issues.

Preclinical Vector Distribution Studies/Dr.Pilaro, FDA

Dr. Pilaro discussed the FDA's revised policies regarding preclinical vector distribution studies, including guidance on preclinical study design, e.g., goals of vector biodistribution studies, situations in which biodistribution studies may or may not be postponed. She explained that one of the documents used by the FDA in assessing the appropriateness of preclinical studies (relative to an IND application), is the International Congress on Harmonization (ICH-M3). The ICH-M3 provides international standards for the design and conduct of studies (both preclinical and clinical) to assess safety and toxicity of pharmaceuticals. Although the ICH-M3 does not specifically address gene transfer vectors, many of the principles and approaches for evaluating the safety of pharmaceuticals are applicable.

According to the ICH-M3, the termbiodistribution is defined as "absorption, distribution, metabolism and excretion (ADME) of a drug or biologic." The document further specifies that exposure data in animals should be evaluated prior to human clinical trials and available for comparison to human data in clinical trials by the completion of a phase I study.ADME data can be derived from pharmacokinetic and toxicokinetic preclinical animal studies, and should be obtained prior to clinical trials so that the animal studies can be compared to the data obtained from Phase I clinical investigations. This comparison provides important information about whether the animal model adequately reflects theADME of a drug of biologic product in the human environment. The ICH-M3 defines biodistribution studies as "preclinical animal studies designed to determine distribution of vector to sites other than intended therapeutic site." The ICH-M3 definition of biodistribution studies is also the working definition used by the FDA.

The goals of vector biodistribution studies are two-fold: (1) to assess potential for dissemination of the vector to the germ-line and (2) to assess dissemination to non-target tissues, which provides information about the potential target organs for toxicity and helps with the design and conduct of toxicology studies. Both of these assessments can be achieved in the same preclinical studies. Dr.Pilaro noted that the FDA had several internal discussions following the March 12, 1999, RAC meeting. As a result, the FDA proposes to modify its policy regarding the timing of vectorbiodistribution assays.

Dr. Pilaro outlined potential scenarios under which vector biodistribution assays *may* be postponed (according to the revised FDA policy):

- A previously defined vector -- previous experience with a similar vector, route of administration, formulation, schedule, e.g., an adenovirus type 5 vector (significant data regarding known target organs for toxicity and vector dissemination patterns.)
- The transgene product is perceived to be innocuous if expressed ectopically, e.g., expression or over-expression of p53 wild type in normal tissue.
- The size of the vector, i.e., plasmid DNA, or the formulation of the vectorcapsid for a viral vector is not excessively different from vectors that have been previously discussed in public and evaluated extensively.

Dr. Pilaro outlined potential scenarios under which vector biodistribution assays *may not* be postponed (according to the FDA policy):

- A new vector class – minimal or no experience, e.g., adeno-associated viral vectors and lentiviral vectors.
- A change in formulation, e.g., a plasmid vector with a lipid carrier (changing from a cationic lipid to a nonionic lipid formulation could affect vector biodistribution).

- A change to intentional systemic route of administration with an established vector.
- Use of a transgene that could potentially induce toxicity if aberrantly expressed in a non-target organ, e.g., interleukin-2.

Dr. Pilaro outlined the following issues that should be considered in the design of preclinical biodistribution studies:

- Species – non-human primates are not needed in all cases. The FDA would require non-human primate studies only for instances when the transgene product is species-specific or the route of administration precludes the use of a smaller species.
- Gender – the gender of animals used in biodistribution studies should reflect that of the proposed patient population, e.g., use of male animals for hemophilia studies.
- Number – a minimum of 3 to 5 animals per gender per dose group per time point. If smaller species are used, e.g., rodents, a larger number of animals is preferable because it facilitates statistical validation. In those instances when biodistribution studies require the use of non-human primates which are a precious resource, the number of animals should be small.
- Dose Selection – when selecting doses, the following should be included: (1) a vehicle control group (serves as the control for biodistribution studies as well as the PCR readout); (2) a clinically relevant dose (not necessarily the highest clinical dose, but may be the maximum feasible dose for that particular species); (3) a low dose in order to determine a "no observable effect level" (some preclinical data suggest that vector biodistribution patterns may be related to the volume administered, i.e., large doses and large volumes may result in "leakage."
- Route of administration – animal biodistribution studies should reflect the clinical route of administration. It was previously thought that intravenous administration would be an appropriate "worst-case scenario" model; however, experience has shown that the kinetics of viral clearance following intravenous administration is very different from other routes of administration.
- Sacrifice time points – time points should be early (at the peak of vector transduction and expression), in order to provide important information about the kinetics of vector transduction and the persistence of that effect. A later time point should also be included and should be determined by the clinical design, e.g., if the vector will be administered to patients every two weeks, animals should be sacrificed at this time point. If a positive signal is identified in the germ-line or non-target tissue, or some degree of vector toxicity is observed, an even later time point should be included to determine the clearance of the vector signal from these sites, i.e., for a positive gonadal signal a 90-day time point is recommended so that a full cycle of spermatogenesis can be evaluated.
- Tissue panel for harvest – at a minimum, tissue harvested for vector biodistribution studies should include peripheral blood, gonads, and the site of vector injection. When evaluating potential toxicity, highly perfused organs such as brain, liver, lung, kidney, heart, and spleen should be harvested. Harvesting of additional tissue will be determined primarily by the route of administration and the transgene, e.g., contralateral lymph node tissue should be harvested for subcutaneous intramuscular injections to evaluate vector dissemination through the lymphatic system. It is extremely important to minimize contamination when harvesting tissues, i.e., clean instruments for each organ and each animal.
- Detection assay – the FDA recommends direct DNA PCR in order to detect vector sequence that is unique to the product. The FDA currently recommends that the sensitivity level of detection assays be less than 100 copies per microgram of DNA per tissue sampled. The methodology should be appropriate to adequately detect the sequence in the tissue samples from preclinical animal studies as well as clinical samples, i.e., semen and peripheral blood, which may be obtained during the Phase I study.

Dr. Pilaro explained that there may be certain circumstances when "generic" platform studies may provide useful information about biodistribution, e.g., for an entire vector class. A platform study would involve a large number ofanimals and would be specifically designed to address all biodistribution issues, e.g., all intended routes of administration for the clinical investigation, non-target organ tissue, systemic administration, gonads, etc. Conducting a larger platform biodistribution and toxicity study would allow for the comparison of signals by various routes and provide a basis for calculating a hazard ratio. Platform studies for a specific class of vectors would also provide supporting data for other vectors within that class, regardless of the transgene. She stated that sponsors who decide not to conduct preclinical vector biodistribution studies prior to the Phase I trial will be required to submit these data as the product evaluation moves through Phase II and Phase III development. She read a portion of a sample letter that would be sent to sponsors by the FDA when vector biodistribution studies are not conducted prior to the Phase I clinical investigation. The letter reads:

"The present submission does not contain data that demonstrate the extent to which this vector is able to disseminate out of the injected site and distribute to gonadal tissues. These data are necessary to determine the risk of inadvertent gene transfer to the germ cells, which may result in genetic changes in subsequent progeny.

"In the course of development of your product, you will be required to obtain these data and provide them to the Agency for review and comment. Data may be obtained either from biodistribution studies in animals, analysis of clinical samples, or from a combination of preclinical and clinical sample analyses. Clinical data should be derived from peripheral blood cells and semen samples during the treatment and follow-up periods for the clinical trial, and from gonadal tissues (primarily ova) obtained at autopsy from consenting patients. We will require that these data be provided in a timely fashion, so that the results may be used to guide further development and optimization of your product as a therapeutic agent.

"Please update the Agency on the status of these studies at the time of each annual report."

Vector Biodistribution - Informed Consent Document Considerations/Dr. Rieves, FDA

Dr. Rieves presented an overview on FDA's recent discussions regarding gonadal biodistribution of vector sequences and the inherent Informed Consent issues raised by such findings. The FDA has recently changed its policy concerning biodistribution studies. In the past several studies were placed on clinical hold (prior to the initiation of phase I studies) until vector biodistribution studies had been conducted. Presently, the FDA is allowing some phase I studies to proceed before such studies have been completed. When reviewing an IND application, one FDA consideration is the extent to which the preclinical data can adequately assess the degree of risk to research subjects. At the same time, the FDA is sensitive to the need for allowing some trials to proceed. The FDA's position is that some phase I trials may proceed with some level of potential or unknown risk with the proviso that potential research subject are fully informed and understand the nature of such risks.

The FDA requests that sponsors submit the proposed Informed Consent document with the IND application. Although FDA cannot require sponsors to submit their Informed Consent documents (it is not within its statutory authority to do so), the FDA does request submission of these documents. In general, sponsors submit these documents to the FDA. In turn, the FDA reviews Informed Consent documents and makes recommendations as appropriate. Dr. Rieves noted that Institutional Review Boards (IRBs) are also responsible for reviewing and approving Informed Consent documents. He read the following sample

language developed by the FDA that might be used as a template (and modified as appropriate to facilitate patient understanding) for Informed Consent documents. The sample language reads:

"Risks associated with treatment in this study include the possibility of permanent genetic alterations in some of your sperm (men) or eggs (women). Some of these changes could lead to miscarriage or abnormalities in your children. Other changes may have no apparent effects but could still be passed on to future generations. These changes could be neutral or may eventually cause abnormalities. The likelihood of such outcomes is currently unknown."

He emphasized that the FDA requires sponsors to conduct their studies under IRB review. The FDA recognizes that some clinical gene transfer investigations may have a reasonably foreseeable risk of germ line alterations and recommends that potential research subjects be fully informed of such potential risk.

IRBs expect that information given to subjects will be written in understandable language and that reasonably foreseeable risks will be enumerated. The sponsor is requested to ensure adequate informed consent (acknowledged to be a major challenge), and the consent form should be submitted along with the protocol for approval. Dr. Rieves read sample consent form wording.

RAC Discussion

Dr. McIvor brought up the topic of reasonably foreseeable risks and asked whether 1 in 10^4 is considered a "reasonably foreseeable risk" in terms of a potential insertional mutation that may or may not have subsequent phenotypic consequences.

Dr. Noguchi stated that the risk is sufficiently low (based on current data and information) to allow some studies to go forward, realizing that in the event of an untoward experience, there are mechanisms that the FDA would use to halt the study. At this time, the FDA's position is that the scientific community feels comfortable in moving forward; however, it is likely that new scientific findings or untoward consequences could result in changes to this position. Relevant research results and Informed Consent issues must be revisited on an ongoing basis.

Dr. Mickelson emphasized the importance of quantifying risk as much as possible and emphasized that patients should be very involved in the Informed Consent process. Dr. Ando agreed that the Informed Consent language proposed by the FDA should include some mention of the severity level of the risk. Dr. Rieves explained that the FDA language is largely a template from which to expand the discussion, i.e., severity of risk. In response to Dr. McIvor's concern about how to inform and educate the patient and how to convey the relative risk in comparison with natural mutagenesis, Dr. Rieves characterized it as "a major challenge." Dr. Noguchi stated that Informed Consent documents should explain that there is a potential risk, even if the magnitude of such risk is unknown. Regardless of the difference between the risk of a natural insertion versus that of a vector, gene transfer is a deliberate intervention with potential risk and potential research subjects deserved to be informed about the possibility of such risk.

Dr. Aguilar-Cordova asked Dr. Pilaro if biodistribution studies could be conducted using an appropriate animal species as a surrogate for determining toxicity for instances when species-specific proteins or cytokines may not adequately reflect the human environment. Dr. Pilaro stated that such an approach would be acceptable, if the appropriate transgene were available and the vector formulation were identical.

In such instances, the FDA would weigh heavily the *in vitro* data and previous experience with similar

vectors and similar products. If non-human primates are used, the FDA expects that both the vector biodistribution and toxicity studies will be conducted simultaneously, thereby making optimal use of this precious resource.

Dr. Mickelson inquired about the time line for submitting vector biodistribution studies for clinical investigations that proceed absent such data prior to initiation of phase I studies. Dr. Pilaro answered that the ICH–M3 document defines timely as "by the end of phase I." The FDA believes that these data should be provided before beginning a large patient population study, but not necessarily before the pivotal trial. By the time researchers have treated a large number of patients, the FDA would want to see the biodistribution data.

Dr. Mickelson asked Dr. Pilaro to further explain the concept of "generic" platform studies. Dr. Pilaro explained that these would be extremely large studies for a single vector class. She noted that the FDA held a planning meeting with the NIH Office of Rare Disorders to discuss platform studies for AAV. It is foreseeable that platform studies would involve multiple research investigators, each addressing specific aspects. These data could then be shared and made accessible to the scientific community and thus used as a reference for individual studies. The goal of such a platform study would be to facilitate the rapid accumulation of data, while at the same time, reducing duplication of effort and conserving resources.

Based on the recent finding of gonadal biodistribution of vector sequences, Dr. Mickelson asked whether the FDA planned to notify patients retroactively who have previously participated in gene transfer clinical investigations about the potential risk. Dr. Noguchi mentioned that the FDA previously required such an action for a xenotransplantation clinical trial. When it was discovered that an endogenous retrovirus from the donor animal was capable of infecting human cells, the FDA placed a clinical hold on these studies, and sponsors were required to "re-consent" all participants based on this new finding. Dr. Macklin argued that notification of patients about the potential risk is ethically desirable, but the process of "re-consenting" is reasonable only when new data come out in the course of the trial, and people are still undergoing the intervention.

Discussion on Future Proposed Actions to the *NIH Guidelines* Based on the Gene Therapy Policy Conference on Prenatal Gene Transfer/Drs. McIvor, Markert, Macklin, and Ms. King

Background

On July 30, 1998, Drs. W. French Anderson, University of Southern California; and Esmail Zanjani, Veterans Hospital, Reno, NV, submitted two preliminary protocols for prenatal gene transfer to the RAC to provide a context for discussion and to initiate a national dialogue on the substantive public policy issues raised by prenatal gene transfer research. At the RAC meeting on December 15-16, 1997, Dr. Anderson first suggested that a Gene Therapy Policy Conference (GTPC) be held on the topic of *in utero* gene transfer research, and the RAC subsequently made such a recommendation at the March 10, 1998, meeting. Following the RAC meeting of September 24-25, 1998, Committee members and *ad hoc* experts were assigned to one or more of the three working groups based on their individual areas of expertise: (1) Preclinical Research Issues; (2) Clinical Research Issues; and (3) Ethical, Legal, and Societal Issues. The working groups were asked to develop preliminary responses to questions that related specifically to the primary focus of their assigned group. On January 7-8, 1999, the RAC sponsored a GTPC entitled "Prenatal Gene Transfer: Scientific, Medical, and Ethical Issues." RAC working group and *ad hoc* participants were brought together to deliberate further on the scientific, safety, ethical, legal, and societal implications of prenatal gene transfer research. Following this GTPC, working group participants were

asked to further refine their responses to the assigned questions, incorporate any additional questions that were raised as a result of the conference presentations, identify unresolved issues, and propose next steps in the deliberative process. The working group chairs presented their findings and recommendations at the March 11-12, 1999, RAC meeting as part of the RAC's continuing service as a public forum for discussion of the science, safety, ethics, and development of Federal policy for recombinant DNA research.

During this meeting the RAC was asked to think about how the findings and recommendations would be eventually incorporated into the *NIH Guidelines*. Drs. Macklin and Mickelson suggested that RAC members reread the executive summary and send ORDA comments on these issues. Dr. Markert noted that some of the policy issues identified by the working group are not necessarily limited to *in utero* research. In terms of revising the *NIH Guidelines*, Dr. Markert recommended that revisions to Appendix M should not be limited to language about prenatal gene transfer. Appendix M should be revisited in its entirety to reflect new scientific methodologies, consistent research design issues, informed consent issues, etc. Dr. McIvor agreed that issues such as efficacy, safety, and gene expression are not limited to *in utero* gene transfer. Dr. Mickelson suggested that any statements adopted by the RAC should be accompanied by a footnote stating that policy issues would be revisited by the RAC in light of new scientific findings and include an acknowledgment that there is a diversity of opinions on prenatal gene transfer research.

The RAC was asked to review a draft executive summary of the RAC's findings and recommendations on prenatal gene transfer research based on the working group reports. Discussion centered on how to approach issues where there was not a consensus among the working groups. Dr. Markert noted that when developing a summary report such as this, it is sometimes difficult to accurately reflect the full range of opinions on diverse issues. The RAC discussed whether or not the working group reports should reflect differences of opinion. Ms. Knorr stated that one important role of the RAC is public education, and for that reason, it would be appropriate to reflect diverse opinions. Dr. Noguchi concurred that it is important to elucidate the points of controversy. Dr. Macklin noted that the RAC as a whole, should not be divided on some issues. The consensus of the RAC was that there would be insufficient time during this meeting to finalize the working group reports and executive summary. Ms. Levi-Pearl suggested that once the executive summary and working group reports are finalized, the information should be summarized in non-technical language that is easily understandable by the general public. Ms. King reminded the RAC that the proposed changes to subpart B of 45CFR 46, *Protection of Human Subjects*, are still pending. Once a final rule is published, these changes may impact the language of the working group reports relating to pregnant women and fetuses as subjects.

Proposed Definition of Recombinant DNA for Human Subjects Research/Dr. Mickelson

Dr. Mickelson explained that the definition of recombinant DNA, which defines the scope of the *NIH Guidelines,* was developed in 1976. That definition, which is still in use at the present time, reads:

"Section I-B. *Definition of Recombinant DNA Molecules*

"...recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. ..."

When Appendix M, was added to the *NIH Guidelines* in 1990 to address recombinant DNA research

involving human subjects, human gene transfer research was further defined as:

"Section I-A-1-a. Experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects (human gene transfer)... ."

Dr. Mickelson noted that both of these two definitions may need to be revisited by the RAC in light of emerging technologies and methodologies that have the potential for modifying the human genome. As background for this discussion, she noted that the FDA working definition of "human gene therapy" is substantially different from those in the *NIH Guidelines*. FDA's working definition is taken from its October 14, 1993, *Federal Register* publication entitled: *Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products*. The FDA working definition reads as follows:

"Human gene therapy is defined as a medical intervention based on the administration of genetic material in order to modify or manipulate the expression of a gene product or to alter the biological properties of living cells. Cells may be modified *ex vivo* for subsequent administration or altered *in vivo* by gene therapy products given directly to the subject, including but not limited to autologous bone marrow stem cells modified with a viral vector, intramuscular injection of a plasmid DNA vector, use of antisense oligonucleotides to block gene transcription, ribozyme technology, and use of sequence-specific oligonucleotides to correct a genetic mutation."

RAC Discussion

Dr. Noguchi stated that the phrase "to alter the biological properties of living cells" establishes a useful distinction between unaltered human tissues, e.g., those used for transplantation, and cells and tissues in which the biological characteristics have been changed. This definition does not include human fertilization because the FDA considers it to have normal biological properties. In terms of the FDA's jurisdiction over human cloning, the present technology for cloning animals, if applied to human beings, would constitute a dramatic alteration of the biological properties of living cells from a somatic as well as a genetic basis. Dr. Macklin noted that the *NIH Guidelines* definition of recombinant DNA describes "molecules" whereas the FDA working definition describes a "process."

Dr. Wolff stated that if the RAC develops a new definition for the use of antisense in humans this should be excluded from NIH oversight because it is not used for permanent genetic modification, but as a transient means of expressing a drug, e.g., to suppress a viral infection. In contrast, a new definition should encompass sequence-specific oligonucleotides used to correct a genetic mutation.

Dr. Markert noted that many human gene transfer protocols currently covered by the *NIH Guidelines* do not involve vectors that integrate into the genome. Many of the cancer and HIV protocols involve transient expression; however, there could be safety or other issues related to these studies that should be discussed in public and be subject to long-term follow-up.

Several RAC members stated that revisions should not be a matter of dropping or changing a definition, but rather adding other definitions to those that already exist. Dr. McIvor suggested that adding new definitions to cover the consideration of human gene therapy protocols could be better accomplished in appendix M, as also suggested by Ms. King. Dr. Wolff stated that, at a minimum, language should be included about small oligonucleotides that permanently modify genetic material.

Dr. Mickelson stated her interest in the fact that naturally occurring viral mutants can be used therapeutically without RAC review. Dr. Noguchi reminded the RAC that most of the viral vaccines

currently on the market or being used experimentally are mutated viruses, and that there are other similar natural flora as well. Those kinds of products are reviewed extensively nationally and internationally; for example, a Secretary's Committee for xenotransplantation will cover transgenic animal organs (but not transgenic human tissues and cells).

Committee Motion

A motion was made by Dr. Mickelson and seconded by Dr. Aguilar-Cordova to form a working group to develop a revised definition of recombinant DNA. The motion was approved by a vote of 13 in favor, 0 against, and no abstentions. The working group will be composed of Drs. Aguilar-Cordova, Ando, McIvor, Mickelson, Wolff, and Juengst.

Human Gene Transfer Protocol #9904-304, "Pediatric Phase I Study of AdV/HSV-TK Followed by Ganciclovir for Retinoblastoma"

Investigator: Dr. Richard Hurwitz, Baylor College of Medicine, Houston, Texas

Reviewers: Drs. Breakefield and Chow and Ms. Levi-Pearl

Ad Hocs: Dr. Thaddeus P. Dryja, Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts
Dr. Pedro R. Lowenstein, University of Manchester, Manchester, United Kingdom

Background

Retinoblastoma is the most common primary malignant tumor of children; usually occurring in children under three years of age. While some children are diagnosed with tumors that are small enough to be eradicated with cryotherapy or laser photocoagulation, thus salvaging the eye, the majority of children diagnosed with non-metastatic retinoblastoma have tumors that are so large that enucleation (removal of the eye) is required. Although enucleation results in blindness in one eye and may have cosmetic implications as the remaining area of tissue and bone undergoes normal growth and development, enucleation has almost a 100% cure rate. Although the cure rate is extremely high, this procedure results in blindness (in one eye) and possible slight facial deformity. Long-term follow-up studies of patients with unilateral retinoblastoma show that patients undergoing enucleation go on to lead productive lives, i.e., similar rates of relationships, employment, and ability to perform other important function such as driving.

The objective of the study is to estimate the maximum tolerated dose and dose-limiting toxicities of an adenoviral vector , (AdV/HSV-TK) delivering the herpes simplex thymidine kinase (HSV-TK) transgene injected directly into retinoblastoma followed by the intravenous administration of ganciclovir every 12 hours for 7 days. Retinoblastoma is the most common primary malignant tumor of children and usually occurs in children younger than 3 years of age. Current standard treatment for nonmetastatic retinoblastoma is enucleation, which although results in a high rate of survival also results in blindness and severe cosmetic facial deformity. Recent attention has been turned to finding alternative therapies that not only will result in a high cure rate but also will allow salvage of the affected eye. Occasionally, a child presents with a small tumor that can be eradicated with cryotherapy or laser photocoagulation while still preserving the eye and useful vision. Unfortunately, most children present with tumors that are too large for these types of therapies. In an attempt to shrink a larger tumor to a size that can be managed by these local therapies, clinical investigators have begun trials using systemic chemotherapy instead of enucleation. Although preliminary studies have shown promise, chemotherapy has significant side effects, including an increased rate of secondary malignancies. Because patients with retinoblastoma have a significant second malignancy potential as a natural course of their disease, especially osteogenic sarcoma, an alternative therapy without systemic toxicity is desirable.

Approximately 12 patients will be enrolled in the study; at least 3 patients will be treated at each dose level (maximum of 4 injections at 2-week intervals per patient). The proposed dose levels (viral particles) for the 4-dose cohorts are: 1 x 10^9, 1 x 10^{10}, 1 x 10^{11}, and 1 x 10^{12}. Intraocular injection of the virus will use a stereoscopic surgical microscope to visualize the tumor during the surgical procedure. Ganciclovir treatment will begin 24 hours post-virus injection at a dose of 5 mg/kg IV over 1 hour every 12 hours for 7 days (14 doses).

Preclinical animal data in support of this clinical investigation suggest that intraocular injection of AdV/RSV-TK and subsequent ganciclovir treatment may effectively reduce the size of the retinoblastoma tumor.

The RAC recommended full public discussion of this protocol based on the following issues: (1) the proposed study represents a new route of vector administration, i.e., intraocular injection; (2) a new candidate disease, i.e., retinoblastoma; (3) the study involves a pediatric population, i.e., 12 to 18 months average age; and (4) potential safety issues related to the gene transfer procedure.

RAC and *Ad Hoc* Reviews

Dr. Aguilar-Cordova recused himself from the discussion of this protocol due to the fact that he is a co-investigator on this clinical protocol.

Dr. Breakefield

Dr. Breakefield explained that this proposal involves intraocular injection of a first-generation adenovirus vector carrying the HSV-TK gene, and subsequent systemic administration of ganciclovir. The hypothesis (based on preclinical results) is that the proposed intervention may result in a significant reduction in tumor volume such that local therapies can be administered and enucleation avoided. She noted that the during its preliminary evaluation of this proposal, RAC members had varying opinions about the necessity of full public review. While some RAC members were of the opinion that this proposal does not raise any novel issues deserving of public review and deliberation, others expressed concern that the study raises several safety issues related to increased risk to the patient. She stated that the animal model that was used for the supporting preclinical studies may not be an appropriate model for assessing some of these concerns.

The proposed vector is a first-generation adenovirus vector. She inquired whether the investigators had considered using a newer generation of adenovirus vector because these later generation vectors lack replication-competent virus. She explained that first-generation Ad.TK vectors have significant amounts of replication-competent virus due to recombination with the 293 packaging cells. This is less of a problem when used in the peripheral setting, e.g., mesothelioma and ovarian cancer, since the immune system can adequately clear any replication-competent virus from the body. However, these first-generation adenovirus vectors can cause significant toxicity in the brain, even though replication-competent virus can eventually be cleared over time. She expressed concern that injection of a first generation adenovirus vector into the eye might results in similar untoward effects as those observed in the brain although there is likely to be a significant barrier to the immune system. She noted studies conducted by Dr. Jean Bennet at the University of Pennsylvania in which intraocular adenovirus injection (studies involving retinitis pigmentosa) caused significant eye inflammation.

One significant concern is that of potential adverse effects resulting from an immune response to the adenovirus vector. If a significant immune response to the vector were to occur, patients could be at

increased risk of damaging the second, healthy eye. Second, the data that Ad.TK causes chronic autoimmune inflammatory disease in the brain are weak at best. The work was all done in Lewis rats which are prone to autoimmune diseases. The investigators have looked at Ad.TK in the brains of Wistar rats, Fisher rats, cotton rats and monkeys and never seen any autoimmune disease. They have never seen anything like this in their patients and one is still alive after 2 years. Dr. Breakefield stated that it reminded her of the data that Savio Woo published about the toxicity of Ad.TK in baboon brains (2 died). She stated that she could not reproduce that data either and it was not seen in the clinical trial or in anyone else's preclinical studies. The investigators have stated that the vector stock has been tested for recombinant virus, and that the level is less than 1 in 10^8 viral particles; however, the proposed starting dose is 10^9 viral particles. Dr. Breakefield expressed concern that any recombinant-competent virus in the context of eye, which is immune privileged, might cause significant inflammatory or toxic responses that could damage the vasculature of the eye. If the vasculature were to be damaged, tumor cells could potentially escape into the bloodstream.

Toxicity studies for this vector in brain tumors in mice indicate that there is some risk of hemorrhage and some inflammatory responses at doses of vector up to 10^{10} viral particles. She noted that the doses used in these preclinical experiments are three orders of magnitude lower than that proposed for the clinical investigation.

Another concern noted by Dr. Breakefield was that the injection procedure could result in the inadvertent release of tumor cells into the vasculature and subsequent development of malignant disease. She explained that the needle will be repositioned within the tumor once it has been injected. Previous clinical results have shown that use of these vectors in the brain can result in a hemorrhagic response and disruption of the blood-brain barrier. The vector itself, as known from brain tumor studies, can cause hemorrhagic responses that would open the blood-brain barrier. Another related concern is that endothelial cells within the tumor might be killed upon subsequent treatment with ganciclovir (having taken up the virus upon dividing); which might also result in disruption of the vasculature.

She explained that in her preliminary review, she noted that the Informed Consent document did not provide an adequate explanation about the potential risks, i.e., developing metastatic disease that may be incurable (should tumor cells inadvertently escape into the vasculature), and possible adverse consequences if an immune response to the adenovirus vector were observed. She acknowledged that the investigator has since informed her that the Informed Consent document is undergoing revision.

Dr. Chow

Dr. Chow concurred with the overview of the protocol and the concerns raised by Dr. Breakefield. She reiterated the fact that the disease (tumor) in the proposed study group is virtually 100 percent curable by enucleation. She stated that the long-term objective of the proposed intervention is to prevent the need for enucleation and possibly save some level of vision; however, the procedure raises the possibility of developing metastasis. She explained that the level of risk cannot be quantified in this case.

Dr. Chow referred to the section of the protocol that describes the possible toxicities that could be expected, e.g., retinal detachment, lens toxicity, and optical toxicity. She questioned whether such toxicities were easily repaired so that vision would be preserved.

Dr. Chow noted the Informed Consent document explains that the study costs related to the adenovirus vector, antibody to the virus, ganciclovir, and some cell culture tests will be covered by the institution; however, all other costs associated with the study are expected to be covered by the patient's insurance

provider. She expressed concern that insurance providers may not cover many of the costs associated with this experimental procedure.

Dr. Lowenstein

Dr. Lowenstein noted that the primary objective of the proposed study is to improve quality of life, as opposed to saving life. In that sense, the protocol raises unique issues about intervention for cosmetic purposes.

Dr. Lowenstein brought up several issues of concern, the first of which related to possible acute and long-term cytotoxicity and inflammatory potential of the virus. In his opinion, the preclinical studies submitted in support of this study do not adequately assess these potential risks. He noted that the preclinical animal data showed that the test animals all died from the tumor; such a model does not allow for adequate evaluation of long-term toxicity and immune response to the vector. He expressed concern that the vector could cause acute inflammation based on recent data (submitted for publication). These data indicate that there is a very rapid release of cytokines up to 90 minutes following injection of an adenovirus vector into the brain. The acute cytokine release can determine subsequent responses to the vector and could be examined in the absence of the chronic model. Widespread diffusion of the adenovirus has been seen; there is no evidence of adenovirus starting an autoimmune response in the brain. (Dr. Lowenstein offered to share the data from his study, and the RAC invited him to do so later in the meeting.)

He noted an inconsistency in the proposed doses in the clinical protocol. In one section of the protocol, the investigators state that 1×10^8 infectious units will be used as the lowest dose; in another section 1×10^9 is proposed. This discrepancy brings into question how many possible replication-competent viruses could be in the adenovirus stock.

Of additional concern is the possibility that endothelial cells will be transduced when the vector is injected into the tumor. Since endothelial cells will continue to divide, there is the possibility that subsequent ganciclovir treatment could kills these cells as well as tumor cells. Similar brain tumor studies have resulted in hemorrhages due to this effect. With regard to the brain tumor studies, the possibility of hemorrhage was a risk that these patients were willing to accept because they had a Stage 4 cancer that would most likely result in death within a relatively short-time period. In contrast, the proposed retinoblastoma patients can be cured of their disease by enucleation. In one case (brain tumor studies) the objective of the study was to save life whereas the objective of the proposed study (retinoblastoma), is saving the eye.

Dr. Dryja

Dr. Dryja explained that he is an ophthalmologist and well known expert on retinoblastoma. He explained that there are two categories of retinoblastoma: bilateral and unilateral. Bilateral retinoblastoma is considered to have a hereditary component which puts the population at risk not only for developing tumors in both eyes, but for developing other forms of cancer later in life. In general, unilateral retinoblastoma is not considered to have a hereditary component, thus this population is not at significant risk of developing other neoplasms. The average age of patients diagnosed with unilateral retinoblastoma is between 12 and 18 months, with the range being between birth and 5 years of age. Compared to many underdeveloped countries that do not have adequate health care (poor survival rate for this disease), mos U.S. citizens have access to quality health care. As a result, most unilateral retinoblastoma patients are diagnosed early enough to receive timely treatment (enucleation), which has almost a 100% survival rate and vision in the non-diseased eye remains unaffected. If timely diagnosis and treatment are not received,

the tumor usually spreads outside of the eye. If tumor spreads outside of the eye, chemotherapy is not usually very effective. In such instances retinoblastoma has a very high mortality rate.

Dr. Dryja stated his opinion that the proposed benefit that these children would receive is primarily cosmetic. He explained that long-term studies have been conducted to evaluate the social and emotional effects of children who have undergone enucleation as a treatment for unilateral retinoblastoma. These studies have found that these individuals have the same rates of marriage, employment, and income levels as those who grow up with both eyes intact. Therefore, the loss of an eye early in life does not appear to be a major psychological or economic handicap.

Dr. Dryja acknowledged that the ultimate goal of the proposed study is a praiseworthy one; however, the proposed patient population (unilateral retinoblastoma that can be cured by enucleation) is probably not a desirable test group. He explained that there remains a small subset of patients with unilateral retinoblastoma who were diagnosed late, and patients with the bilateral form of disease and who will lose both eyes. These patients would likely gain more benefit because their tumors have either metastasized or they will be totally blind. The reason ophthalmologists view retinoblastoma as having a high cure rate is that the eye is not violated. Once the eye has been violated-once the tumor gets out of the eye-the cure rate drops dramatically. The procedure proposed for this trial, placing needles into the eye, is one of the recognized ways of spreading this tumor. For example, the tumor has been spread inadvertently by ophthalmologists who misdiagnose a case, perform a surgical procedure on the eye, and inadvertently spread the tumor through the needle tracts or by way of other therapeutic maneuvers.

Dr. Dryja explained that he had consulted with two other ophthalmologists who are recognized as experts on retinoblastoma, Drs. David Walton and Bob Petersen, about the proposed study. He read Dr. Walton's comments to the RAC:

"I feel the protocol should not be approved because of the intrinsic risk of spread of the retinoblastoma tumor outside the eye following compromise of the integrity of the eye to make the injection."

Dr. Dryja then read the comments submitted by Dr. Petersen:

"I agree with you that the protocol should be rejected. I will go through the protocol point by point. The author states that enucleation results in blindness and severe cosmetic facial deformity, neither of which is true. When enucleation is done for large unilateral retinoblastoma, the cure rate approaches 100 percent in these patients. The author does not mention external beam radiation therapy or plaque therapy which are the appropriate treatments with large tumors in an eye where useful vision can be saved. Chemoreduction therapy is, indeed, associated with side effects but probably a lot less severe than the danger of spreading the tumor through the needle tract. The risk associated with putting a needle in an eye is currently unquantifiable. This is a trade-off-the increased risk versus the benefit for the patients who participate in this study."

RAC Discussion

Dr. McIvor expressed concern about whether it is appropriate for the RAC to review this study since many of the concerns that have been noted are not specific to gene transfer. DrBreakefield stated that in her opinion, RAC review of this study is appropriate because many of the potential risks related to the adenoviral vector, i.e., immune response to the adenovirus vector and hemorrhagic potential related to the procedure. She believed this protocol was appropriate for RAC review because the risk of releasing the tumor cells into the vasculature is increased specifically through the use of the vector, which has some known inflammatory hemorrhagic potential, and by the combination with ganciclovir therapy. The issues

related to vector safety in this model are ones that fall under the RAC domain.

Dr. Macklin suggested revisiting the issue of what constitutes "novelty" that then triggers a RAC review of a protocol. Dr. Mickelson concluded that the issue of what would trigger a vote for a RAC review of a protocol should be revisited, and she suggested bringing up this issue at another RAC meeting for further discussion.

Dr. Hurwitz began by discussing the issue of dosing of the virus. He advised RAC members to check the units carefully because sometimes they refer to infectious units and at other times to viral particles. He explained that this first-generation adenovirus vector was chosen because it has been used in several other clinical studies involving the central nervous system. Researchers at Baylor have extensive toxicity data on this vector. The toxic dose is approximately 1×10^{11} infectious units, which corresponds to the highest clinical dose that is proposed. He stated that there is also extensive animal data related to intraocular injection of this vector. He explained that the eye is a relatively immune privileged site, and that inflammation would not be expected in the eye as with other routes of administration.

Dr. Hurwitz explained that retinoblastoma is a disease exclusively of human children; thus there is no available animal model for studying this disease. Although various transgenic animals have been developed in an attempt to mimic retinoblastoma; no appropriate model has been developed that would adequately reflect the disease condition as proposed for the clinical study.

In response to Dr. Chow's question about costs associated with the study, Dr. Hurwitz stated that Baylor would be responsible for covering any costs not covered by insurance. He said that the Informed Consent document will be altered to address and clarify this issue.

Dr. Hurwitz provided additional information as to why he believes that the injection procedure can be performed without risk to the patient and disruption of thevasuclature. Retinoblastoma can grow either from the retina out, or from the retina toward the back of the eye. He explained that Carol and Jerry Shields at the Wills Eye Center have published data from more than 150 patients, demonstrating that tumor cells are not observed in the needle track with the procedure that is being proposed for this study. An older procedure, which involved the insertion of a relatively large needle through the conjectiva and the pars plana (which are heavily vascularized), and filling the vitreous; resulted in tumor spread. This new procedure involves the insertion of a very small needle into the periphery of the cornea in an avascular region-through the iris, which is relatively avascular. Care is taken to avoid the ciliary body and the pars plana, which are vascular, and the lens so as to avoid creating a cataract. The advantages of this technique are the ability to see the pathway and avoid insertion into the vascular tracts.

Dr. Hurwitz explained that retinoblastoma are relatively, although not completely, avascular. There is vascularity to the tumor, but relative to other tumors, this one is avascular. The route of metastasis of retinoblastoma is through the choroid, which is highly vascular, and the optic nerve. Even when cryotherapy and laser therapy are used in conjunction with chemotherapy in the vitreous of the eye (which disrupts the blood-retina integrity), metastasis are not observed.

Dr. Hurwitz provided additional explanation as to why patients with unilateral disease were chosen for the proposed study. He stated that there is currently significant medical interest in trying to salvage the eyes of these children. Since these tumors can now be cured by enucleation, pediatric oncologists are now trying to develop treatments that would not only be curative, but would have decreased side effects and would improve the patient's quality of life. He explained that it is very traumatic for parents to be informed that their child's eye must be removed. Although cosmetic benefit might be a side effect of this protocol, this study involves only patients who have a chance of having some level of vision preserved if the eye

were salvaged.

Dr. Hurwitz offered an analogous patient population in which this approach has been taken - patients with osteogenic sarcoma. A majority of the children and adults with osteogenic sarcoma can be saved if their leg is amputated, but now there are techniques to save the leg. There was a theoretical risk related to saving the leg when the studies were begun, but data indicate that the same percentage of patients are viable after limb salvage with the increased quality of life of having two legs rather than one. Trying to preserve important functional body parts is, therefore, not a new concept.

Dr. Ando asked how metastasis would be diagnosed and under what conditions a clinical decision would be made to enucleate the eye. Dr. Hurwitz responded that, if at any point there is either stable disease or progressive disease, the patient would be treated by enucleation. Disease would be diagnosed both by examination under anesthesia (done weekly) and by magnetic resonance imaging and 3-D ultrasound (every 2 weeks). Patient examinations will be conducted daily. In terms of metastasis, examinations would focus on optic nerve involvement and extension to the choroid. As a precautionary note, the protocol will include a "stop rule." Specifically, if two cases of metastatic disease occur, the study will be stopped.

Dr. Hurwitz explained that his institution does not treat unilateral retinoblastoma patients with chemotherapy because of the known potential risk of second malignancy, but many institutions that see retinoblastoma patients are using chemotherapy for unilateral disease.

Dr. Dryja suggested that this initial Phase I study should involve patients who have had a second tumor developed and have exhausted all other means of treatment, having previously undergone enucleation of an eye due to tumor. This category of patients are at risk of becoming blind. Dr. Hurwitz responded that the idea was discussed, but dismissed because of ethical and scientific considerations. Patients with bilateral disease would benefit most obviously from this protocol; however, patients with bilateral retinoblastoma do not have metastatic disease; they have multifocal disease. Those are two different tumors; the tumor did not spread from one eye to the other eye. If both eyes of the bilateral retinoblastoma patients were enucleated, those patients would be cured of their disease just like a unilateral retinoblastoma patient would, assuming that the tumor had not spread to the choroid or the optic nerve.

From a scientific standpoint, there are two main issues: whether the tumor can metastasize and whether the tumor can cause an inflammatory response. First, patients who have been followed for months or years are at a significantly higher risk of developing metastasis simply because they have been examined and treated for years; the exact risk level is unknown. Regarding inflammation, these patients have already had chemotherapy and other therapies, all of which disrupt the blood-retina barrier and suppress the immune system. For both reasons, it will not be possible to address research questions "cleanly" if a bilateral patient population is used.

Dr. McIvor pointed out that the consequences of risk are more severe in unilateral patients because the load of disease is not as high and alternate therapies exist.

Dr. Hurwitz stated that the risk of an inflammatory response that would affect the child systemically is very low. If inflammation occurs locally, enucleation is a last resort. If the tumor is small enough to be treated with a laser or with cryotherapy or brachytherapy, one of those therapies would be used. Based on the way in which treatment decisions are made at this institution by its ophthalmologists, this study will enroll only patients whose only alternative is enucleation.

Dr. Markert asked Dr. Hurwitz for data to reassure the RAC that inflammation would not increase the

chance of metastatic spread. Dr. Hurwitz responded that there is no way of testing that specific issue in an animal model. The blood-retina barrier is not how this tumor spreads. The tumor spreads by local extension into the choroid and the optic nerve. Although many manipulations of this tumor have disrupted the vascularity and the blood-retina barrier, in 15 years there has never been a case of metastatic retinoblastoma reported by that route.

Dr. Ando queried Dr. Hurwitz about the introduction of phase I investigational agents in the metastatic setting of stage IV disease as salvage strategies after exhausting standard-of-care approaches. Dr. Hurwitz replied that patients with stage IV disease do not exist in this setting. He explained that he believes it is not ethical to use a therapy in a child when there is not even the remotest chance of there being a benefit.

Dr. Hurwitz explained that there has never been a report of spread of this disease through a needle tract or any other route in adults with brain tumors who have been treated with this vector. Patients with brain tumors have been treated with adenovirus, AdV-TK, with ganciclovir, and such a toxicity has not been reported. Retinoblastoma is classified as a brain tumor, a primitive neuroectodermal tumor.

In response to Ms. Levi-Pearl's question about the information and broadening of scientific knowledge that might emanate from this protocol, Dr. Hurwitz stated that this would be the first experience with a gene therapy approach in the eyes of humans. Toxicity data will be gathered from these individuals that will have ramifications for future trials. In addition, it will provide information about the toxicity of treating retinoblastoma patients as well as a dose of viral vector that can be used for further efficacy trials for retinoblastoma. Treatment for many other diseases of the eye will also benefit, especially those involving gene replacement therapies, and most significantly for patients with retinitis pigmentosa, the most common form of inherited blindness.

Dr. Ando expressed concern about adenoviral infection of the eye. Dr. Hurwitz responded that he has been actively searching for reports of intraocular inflammatory response and has found none, even though many researchers are setting up model systems to test adenoviral transduction. His experience includes putting adenoviral vector into more than 100 immunocompetent mice with the result being very minimal inflammatory responses. The inflammatory response seen in the eye from almost any antigen is markedly decreased, as is most dramatically shown in corneal transplants that can be done on any individual because the immune response in the eye is dramatically diminished.

Dr. Markert raised concerns about significant consent issues for this protocol. Of greatest concern is the statement about a 1 in 100 risk of a metastasis occurring and the child dying. Most families would do much to avoid enucleation. In the family's state of shock over this disease, they will need to have it explained clearly that there is a risk that the tumor could spread with this treatment. Dr. Hurwitz stated that several well-placed statements will take care of most of the RAC members' consent form concerns. For example, Dr. Hurwitz proposed the addition of the statements "and could result in the death of your child if the tumor spreads outside of the eye," and regarding the risk of injection, the statement, "if the tumor spreads outside of the eye, death from metastatic disease could result."

Ms. King believed that the potential for direct benefit is overstated. The consent form does not explain that this is a dose-escalation design, yet this is important information for the parents of these subjects because it helps to put into context how the dose is chosen. It also relates to safety and the timing of the child's entry into the study. The dose is not chosen to maximize potential benefit to the patient. Ms. King also was critical of the excess of treatment language in the consent form; treatment language in this context is potentially misleading to parents of subjects. The general benefits section seems to overstate the benefit given this stage of the study; therefore, Ms. King offered to provide in written form some suggestions for

clarification of the language.

Ms. Levi-Pearl stated her concern that subjects referred from specialists and other researchers may not have been given an adequate explanation of the nature of the research and may need to have conveyed to them clearly the risks as well as the fact that this may be a less than perfect "treatment."

Dr. Chow requested that Dr. Hurwitz address the issue of dose escalation and when dosing will stop. Dr. Hurwitz stated that toxicity exceptions to dosing increases were denoted because the problems enumerated were not related to the dose. Problems such as hemorrhages need to be addressed in other ways but do not obviate dose escalation. Retinal detachment, which occurs in treatment for retinoblastoma, will not increase the rate of the opportunity for metastasis.

Dr. Lowenstein posed the question about why laser therapy or cryotherapy is not an accepted mode of treatment to reduce tumor mass in larger tumors. Dr. Hurwitz, supported in his answer by Dr. Lowenstein, reported that much research has been done in this area that indicates definitively that the laser will not penetrate tumors of thickness beyond 1 millimeter and thatcryotherapy is not effective in tumors beyond 4 millimeters in thickness. Ophthalmology textbooks indicate exactly where the tumor must be and what size it must be in order to perform successfulcryotherapy or laser therapy.

Dr. Marc Horowitz, head of the Solid Tumor Program at the Pediatric Cancer Institute at Baylor College of Medicine, offered an overview of the standard treatments available. The management of retinoblastoma has gradually changed over the past few decades, with a trend away fromenucleation and external beam radiotherapy toward focal conservative treatments. A tremendous effort has been underway to preserve vision, and the ophthalmology community has indicated a willingness to take some risk to preserve it. Regarding the risk of metastasis, it is unknown whether this protocol will raise that risk. The proposed approach will not disrupt the blood-brain barrier any more than is already being done with cryotherapy or radiotherapy, both of which do cause local changes to the blood-brain barrier and have not increased the metastatic rate. The idea of taking some risk in order to save vision is not a novel concept and is generally accepted by the ophthalmology community. The general approach in the ophthalmology community-as witnessed in the multi-institution chemotherapy study in Toronto, Canada, and many of the major institutions in the United States - is a continuing attempt to further improve eye salvaging.

At the request of the RAC, Dr. Lowenstein presented data from his laboratory related to adenoviral vector injection of syngeneic glioma for which there is no other available treatment option. This model (Lewis rats) assimilates the human disease from which patients usually die within 6 to 12 months from the time of diagnosis. Similarly, Lewisrats will die within 20 to 30 days following tumor transplant. The tumor is implanted directly into the left side of the brain and monitored by nuclear magnetic resonance. The tumor is subsequently injected with an adenoviral vector similar to that being proposed for this clinical investigation (contains the thymidine kinase transgene). These animals receive ganciclovir at specified time points. He has found that rats that receive a control vector die within 20 to 30 days. Animals receiving the test vector and subsequent ganciclovir treatment survive for an extended period of time; however, all of the treated animals eventually die. He noted that most of the research in this area has reported data in terms of survival rather than studying the actual effect on the brain.

He described a series of experiments in which the brains of treated and untreated animals were carefully examined. Using hematoxilin stain, they observed that the vector does not remain localized within the tumor; but it diffuses into the white matter. He explained that although intratumoral injection of the vector inhibited tumor growth, there is chronic activation and accumulation of inflammatory cells (macrophages) in the striatum three months following injection. No activation of macrophages was observed in the contralateral supportive white matter. This finding probably indicates the extent to which the vector

diffuses at the time of injection. These virus particles have the tendency to diffuse through planes of cleavage. Staining with vimentim shows that there is a subgroup of glial cells that are activated following injection; however, bilateral glial cells are also activated. He stated that in order to establish a dose-responsive curve, additional experiments are currently being conducted to evaluate the level of macrophage and glial cell activation at five and twelve month time periods.

Dr. Lowenstein presented additional data demonstrating the presence of cytotoxic T lymphocytes in the white matter and the striatum as evidence of positive staining for CD3 and CD8 positive lymphocytes. This appears to be a chronic, active inflammatory process because cytotoxic T lymphocytes are detectable three months after injection. He highlighted the observations that this inflammation results in separation of the myelin sheets (laterally) that is similar to demyelination, and no myelin separation was observed contralaterally. This separation of the myelin sheets is observed three months post vector injection. At this point it is unknown whether this observation is actually demyelination or a result of edema.

He explained that the thymidine kinase gene persists for quite a long period of time following vector injection. The transgene is expressed in the cortex, striatum, contralateral cortex, and contralateral striatum. The mechanism is not completely understood, but there are ongoing experiments to evaluate this finding further.

He explained that his laboratory has developed a PCR assay for detecting replication-competent virus sequences. At 90 days post vector injection they were still unable to detect the E1B sequence that would signify the presence of replication-competent virus. They have never observed any evidence of replication-competent virus. As a control, animals were injected with vector that was heavily contaminated with replication-competent adenovirus which was easily detected by PCR following treatment.

Dr. Chow inquired about the amount of vision retained by patients with large unilateral tumors. Dr. Hurwitz responded that the degree of vision is dependent on the size of the tumor. If the tumor is very large (fills the globe) and causes significant retinal detachment, those subjects would not be considered for this protocol. There are not data correlating the size of tumor with the degree of vision for this disease, so each patient has to be evaluated individually. Dr. Chow asked whether reduction of the tumor burden and retreating with another therapy would repair and rescue the retina or whether retina scarring would occur. Dr. Hurwitz responded that cryotherapy and laser therapy both cause scarring of the retina, even if successful. The proposed protocol will not provide the patient with 20/20 vision and the elimination of blind spots. Vision in the part of the retina that has not been affected might be preserved, but areas already affected by the tumor are probably beyond rescue. This conclusion is case-dependent.

Dr. McIvor inquired about the status of FDA review related to this study. Dr. Hurwitz answered that the FDA gave authorization to proceed with this study in February 1999. Dr. Noguchi responded that the issue of FDA authorization should not be a factor in the RAC's consideration of this protocol. He stated that the FDA appreciates the recommendations of the RAC; it should not be inferred that receipt of FDA authorization means that the FDA will never consider such recommendations.

A comment was made by one of the public members in attendance, Dr. Brenda Ross. She identified herself as a genetics fellow at Johns Hopkins Medical Center Hospital. She expressed concern that injection of an adenovirus vector into the anterior chamber of the eye, specifically the trabecular meshwork, could potentially place these patients at increased risk of developing glaucoma. Dr. Hurwitz reiterated that the virus will be administered directly into the vitreous chamber; not the anterior chamber. He explained that preclinical murine experiments demonstrated minimal gene expression in the trabecular meshwork following intratumoral injection. He emphasized that conducting such experiments is

technically difficult because a mouse eye is approximately 1/100th the size of a human eye.

Dr. Breakefield summarized the unresolved concerns involving this protocol:

- Additional experiments in an appropriate animal model would be desirable to evaluate the inflammatory response in the eye.
- Determining whether there is a recombinant virus in the eye could be addressed by culturing the virus stock in human retinoblastoma cells.
- It would be preferable to use a new generation adenovirus vector that is E1/E4 deleted but retained E3 that suppresses immune reactions to optimize safety; however, other RAC members did not feel that this should be a requirement.
- To minimize any toxic effects, it might be preferable to start with a lower dose than that proposed in the clinical protocol.
- Vascular spread of the tumor due to the repositioning of the needle in the tumor is a recognized risk; however, the degree of risk is difficult to assess. Introducing a longer waiting period between the initial doses (allowing for a longer period of evaluation between individual research subjects) would be prudent because it could take at least 30 days to detect whether the tumor has spread into the vasculature.
- The Informed Consent document should be clarified with regard to the potential risks that have been outlined.
- Some RAC members agree that the patient population should be individuals with bilateral retinoblastoma who have already had one eye enucleated and have a tumor in the second eye that requires enucleation, as opposed to subjects who have treatable disease in a single eye (by enucleation).

Dr. Breakefield acknowledged that the RAC has no authority to require that the investigators adopt these recommendations, but noted that it is important that the RAC's concerns be considered. She also emphasized the importance of reflecting these issues as part of the public record. The careful review and analysis provided by the RAC and *ad hoc* experts serve as a valuable resource to the relevant IRB and to other IRBs that may be asked to consider similar protocols in the future.

Dr. McIvor echoed support for Dr. Dryja's recommendation regarding patients with bilateral retinoblastoma. Although patients with the bilateral form have more advanced disease that is more difficult to treat, the bilateral patient population would seem to gain the most benefit from this Phase I protocol.

At the close of its discussion, the RAC made the following recommendations:

Committee Motions

A motion was made by Dr. Wolff and seconded by Dr. Breakefield to recommend that the investigators consider limiting the proposed study to subjects with bilateral retinoblastoma who have lost one eye and in whom the standard treatment has failed in the second eye. This patient population has a more favorable benefit-risk ratio than patients with unilateral retinoblastoma. The motion passed by a vote of 9 in favor, 0 opposed, and 4 abstentions.

A motion was made by Ms. King and seconded by Dr. McIvor to recommend that the Informed Consent document adequately disclose and discuss the risks of harm and potential for benefit, including the risk of the possible spread of retinoblastoma cells with subsequent malignant metastasis and possible death, as well as the possible compromise of vision to the normal eye due to inflammatory and immune responses to the vector. The term "treatment" should not be used to describe the investigational injections. Suggested language for the Benefits Section of the Informed Consent document reads as follows:

"The purpose of this study is to find out whether experimental injections of AdV/HSV-TK followed by ganciclovir are safe for children with retinoblastoma. This is the first time anyone will give these injections to children with this disease. We do not know whether these experimental injections can help the children who join this study. The most likely benefit, if there are any, would be that some children's tumors would shrink enough so that standard treatments could remove the tumors without having to take out the whole eye. It is possible that the experimental injections could have no effect on your child's disease. If your child's disease becomes worse, or if the side effects from the research become too great, or if there is any other reason to think that participation in this research is not in your child's best interests, we will stop your child's participation so that he or she can be treated with standard therapy."

The motion passed by a vote of 12 in favor, 0 opposed, and 1 abstention.

Dr. Markert closed the discussion of this protocol by acknowledging that the investigators have thought deeply and specifically about the best interests of their patients. Dr. Mickelson reiterated that this protocol was brought forward for RAC review as novel because of the number of committee members who believed it should be discussed in order to fulfill the RAC's role of public discussion and information.

Other RAC Issues/Dr. Mickelson

Dr. Mickelson reminded RAC members about future issues to be addressed by the RAC including the Gene Transfer Vector Containment Working Group and a definition of recombinant DNA as it relates to human gene transfer. Dr. Macklin pointed out the need to define "novelty" in deciding which protocols the RAC should review.

Future Meeting Dates, Announcements/Dr. Mickelson

The next RAC meeting will be held September 2-3, 1999, at NIH, Building 31C, Conference Room 10.

Adjournment/Dr. Mickelson

Dr. Mickelson adjourned the meeting at 4:12 p.m. on June 14, 1999.

[Note: RAC motions on proposed actions are considered recommendations to the NIH Director and are not considered final actions unless approved by the NIH Director and subsequently published in the *Federal Register*.]

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: June 14, 1999

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