RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 6, 2001

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

CONTENTS

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
December 6, 2001

The Recombinant DNA Advisory Committee (RAC) was convened for its 84th meeting at 8:15 a.m. on December 6, 2001, at the Bethesda Marriott Hotel, Congressional Ballroom, 5151 Pooks Hill Road, Bethesda, MD 20814. Dr. Claudia A. Mickelson (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:15 a.m. until 4:45 p.m. on December 6. The following individuals were present for all or part of the meeting.

Committee Members

C. Estuardo Aguilar-Cordova, Harvard Gene Therapy Initiative
Dale G. Ando, Cell Genesys
Xandra O. Breakefield, Massachusetts General Hospital
Theodore C. Friedmann, University of California, San Diego
Jon W. Gordon, Mount Sinai School of Medicine
Jay J. Greenblatt, National Cancer Institute (NCI), National Institutes of Health (NIH)
Eric T. Juengst, Case Western Reserve University
Nancy M.P. King, University of North Carolina, Chapel Hill
Sue L. Levi-Pearl, Tourette's Syndrome Association
Ruth Macklin, Albert Einstein College of Medicine
M. Louise Markert, Duke University Medical Center
Claudia A. Mickelson, Massachusetts Institute of Technology

Executive Secretary

Amy P. Patterson, NIH

Ad Hoc Reviewers/Speakers
Myron S. Cohen, University of North Carolina, Chapel Hill
Terence R. Flotte, University of Florida
Elliott Grossbard, Avigen
Simon J. Hall, Mount Sinai Hospital
Katherine A. High, Children's Hospital of Philadelphia
Philip R. Johnson, Jr., Ohio State University
Mark A. Kay, Stanford University School of Medicine
Debra Leonard, University of Pennsylvania
William F. Raub, Department of Health and Human Services (DHHS)
Richard Jude Samulski, University of North Carolina, Chapel Hill
Sonia I. Skarlatos, National Heart, Lung, and Blood Institute
Lana R. Skirboll, Office of the Director (OD)

Nonvoting/Agency Representatives
Kristina Borror, DHHS
Philip Noguchi, U.S. Food and Drug Administration (FDA)
Stephanie L. Simek, FDA

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

NIH Staff Members
Bill Branson, NIH
Sarah Carr, OD
Laurie Harris, OD
Dennis Hickstein, NCI
Robert Jambou, OD
Robert Kotin, National Heart, Lung, and Blood Institute (NHLBI)
Bob Lanman, OD
Kathy Lesh, OD
Rebecca P. Link, NHLBI
Catherine McKeon, National Institute of Diabetes and Digestive and Kidney Diseases
Alec Liacouras, Center for Scientific Review (CSR)
Maureen Montgomery, OD
Marina O'Reilly, OD
Mi Sun Park, National Institute of Neurological Disorders and Stroke (NINDS)
Alexander Rakowsky, OD
Stephen M. Rose, National Institute of Allergy and Infectious Diseases
Gene Rosenthal, OD
Michael Sayre, CSR
Thomas Shih, OD
Allan Shipp, OD
Danilo A. Tagle, NINDS
H. Eser Tolunay, NHLBI

Others

Approximately 114 individuals attended this 1-day RAC meeting. A list of attendees appears in Attachment II.

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

Dr. Mickelson, RAC Chair, called the meeting to order at 8:15 a.m. on December 6, 2001. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* was published in the *Federal Register* on November 19, 2001 (66 FR 57971). Topics to be discussed by the RAC at this meeting included the following: recent accomplishments and future directions of the RAC; proposed response to the reports of neoplasms after vascular growth factor gene transfer; detection of adeno-associated virus (AAV) vector sequence in research participant semen; and the quarterly data management report.

Dr. Mickelson asked RAC members to note that information about the NIH Rules of Conduct and Conflict of Interest was provided to them in the premeeting materials.

A list of abbreviations and acronyms and their meanings appears in Appendix III.

II. Opening Remarks: Appreciation for Service to the Department of Health and Human Services (DHHS) and Presentation of RAC Member Certificates/William F. Raub, Ph.D., Deputy Assistant Secretary for Science Policy, Assistant Secretary for Planning and Evaluation, DHHS, and Lana R. Skirboll, Ph.D., Director, Office of Science Policy, NIH

Dr. Raub, representing the DHHS Office of the Secretary, noted the unique history of the RAC and saluted the hard work of RAC members in addressing myriad issues associated with gene transfer. Dr. Skirboll thanked the departing RAC members on behalf of the NIH.

The following outgoing RAC members received certificates: Drs. Aguilar-Cordova, Ando, Gordon, Greenblatt, Juengst, Macklin, Markert, and Mickelson. Congratulations for service to date were offered to

the four remaining RAC members: Dr. Breakefield, Dr. Friedmann, Ms. King, and Ms. Levi-Pearl. Dr. Friedmann will be the new RAC chair as of the March 2002 RAC meeting.

III. Spotlight on the RAC: Recent Accomplishments and Future Directions/Dr. Patterson

Dr. Patterson presented an overview of the current RAC's accomplishments over the past 5 years, highlighting major contributions. In 1997, fundamental changes were made to RAC procedures and function. In 1999, pivotal events led to reexaminations of the role and function of NIH and RAC oversight of human gene transfer research (GTR), the adequacy of overall Federal and local oversight, and the protection of human subjects.

New developments in GTR have also occurred in the last five years and the RAC has charted new territory in the review of protocols. Among the developments have been new vector systems, clinical indications, clinical strategies, human subject protection, policy and guidance for potential applications of GTR, biosafety provisions of the *NIH Guidelines*, and safety information. The RAC has provided significant advice for development of a GT safety assessment board and national database. During this time, there have been four GT safety symposia and four policy conferences.

This RAC's legacy is a review process that enhances the analysis and synthesis of scientific and safety information; provides information and feedback useful to investigators, sponsors, and oversight bodies; enhances the ethical conduct of GTR; and builds the public trust and confidence.

IV. Perspectives From the RAC Chair/Dr. Mickelson

Dr. Mickelson thanked all members for their service on the RAC. She expressed her hope that in the future, the RAC will expand its concerns to include helping the GTR field develop scientific and ethical excellence while continuing to be a forum for public input. Continuing to work with the FDA and other DHHS offices will strengthen the coordinated framework. She also supported efforts to enhance public outreach.

V. Minutes of the September 6-7, 2001, Meeting/Dr. Aguilar-Cordova and Ms. Levi-Pearl

The two RAC reviewers noted that the minutes of the September 2001 RAC meeting were accurate in content, and that minor typographical errors had been corrected.

A. Committee Motion 1

As moved by Dr. Gordon and seconded by Dr. Markert, the RAC unanimously accepted the September 6-7, 2001 minutes by a vote of 11 in favor, 0 opposed, and 0 abstentions.

VI. Proposed Response to Reported Appearance of Neoplasms After Vascular Growth Factor Gene Transfer/Dr. Gordon

This initiative was first discussed at the September 2001 RAC meeting and Dr. Gordon briefly reviewed that discussion. OBA has received some safety reports of tumors arising in gene transfer research participants who received vascular or fibroblast growth factors for cardiovascular or peripheral vascular disease. While a causal relationship between the tumor growth and the gene transfer product has not been established, Dr. Gordon proposed in Sept. that potential gene transfer recipients be screened for any underlying neoplasms or malignancies prior to study enrollment. This is based on the theoretical possibility that growth factors could enhance the vascularization of tumors and foment tumor growth. As part of the RAC's role in identifying trends and knowledge gaps in clinical gene transfer research, Dr. Gordon offered to gather additional information and report back to the RAC at the December 2001 meeting.

On the basis of his consulting with the NCI and OBA staff, Dr. Gordon proposed that the following four recommendations be considered by the RAC:

OBA should:

1.) Send a letter to all investigators involved in vascular growth factor gene transfer
2.) Work with investigators to augment data collection on research participants receiving growth factor gene transfer
3.) Work with recognized authorities on cancer risk (e.g., American Cancer Society and the National Cancer Institute) to identify screening protocols for neoplastic disease in these research participants
4.) Advise the NIH Director of these actions.

Dr. Gordon then presented draft language for the proposed letter for discussion.

A. **RAC Discussion**

Dr. Aguilar-Cordova questioned whether there was sufficient information known from these trials to compare the prevalence of neoplasms in the research participants with the expected prevalence in a similar non-enrolled control group. Dr. Gordon responded that at this point in time we do not know whether there is a cause-effect relationship or increase in prevalence, but that this should prompt us to gather more data. Ms. King pointed out that some of the studies using VEGF do have control groups, so this may provide some useful comparative information.

Acknowledging that the current level of knowledge about these tumors is insufficient to draw any definite conclusions, Dr. Macklin suggested that at some point the consent forms for these trials include some pertinent information. This should include a statement of risk and the current information available.

Dr. Skarlatos noted that the Data Safety Monitoring Board at the NHLBI currently recommends that investigators conducting NHLBI-supported research of this nature include in the informed consent document wording that indicates that studies have suggested a possibility of tumor development.

Dr. Noguchi noted that the FDA requires that cancer be a contraindication for enrollment in these studies. As a parallel, he also noted that licensed colony stimulating growth factors have been used in the clinical setting for many years, and he is not aware of any data suggesting an increased incidence of lymphoma/leukemia in those receiving post-transplant GCSF. But, he added that perhaps it would be worthwhile to consult the hematopoietic transplant community for information on patients who received these factors to determine whether malignancies have been looked for following treatment.

Dr. Ando noted that there is a complex variety of growth factors: hematopoietic, vascular, mesenchymal, etc. If a letter were to be sent, the principal investigators would likely be interested in more details, such as the histologic types of the tumors and exactly which growth factors were involved in the cases. Dr. Patterson responded that OBA has compiled a lot of the data that Dr. Ando mentioned, but there are major gaps in the data such as whether there was presence of vector sequence or transgene expression detectable in the tumor. She also noted that even if we had that data, the distinction between causality and association would still be difficult to determine without proper controls.

Dr. Markert raised the question of who would pay for these screening tests, particularly relevant for individuals who do not have third-party insurance coverage. She wondered whether this would decrease the enrollment of potential participants who lack the resources to pay for the screening tests. She also questioned how ongoing studies might be affected if future studies have these financial considerations built into their proposed budgets.

Drs. Aguilar-Cordova and Breakefield urged careful attention to the wording of the proposed letter and that the purpose be clearly stated.

Dr. Macklin felt that the proposed screening could do no harm, but possibly could prevent some harm which would justify sending such a letter to investigators. Dr. Greenblatt agreed and noted that it would be in keeping with the RAC's role to be proactive in providing information to the public.

Ms. Levi-Pearl reiterated Dr. Macklin's previous point that once such a letter was sent to investigators, some sort of wording regarding the possible risk of tumor development should be included in the informed consent documents. Dr. Gordon concurred.

At Dr. Mickelson's request, Dr. Skarlatos agreed to provide OBA and the RAC with the wording suggested in informed consent documents used in NHLBI-funded growth factor studies.

Dr. Mickelson summarized the key discussion points noting that it should be understood that OBA is gathering more data; the RAC would vote on the intent of the letter with the understanding that a draft letter would be presented to all members for comments before being sent to investigators; and that the NIH Director be informed of the letter.

There were no comments offered from the public.

Committee Motion 1

Dr. Gordon then moved that the letter be drafted, the committee see the final form before it goes out, and the NIH Director be informed of the letter. Dr. Aguilar-Cordova requested that the committee be provided with the data available from OBA. Dr. Mickelson added this to the motion and Dr. Greenblatt seconded the motion. The vote was eleven in favor, with 0 abstentions.

With respect to points 2 and 3 of Dr. Gordon's presentation, Dr. Patterson added that OBA should work with colleagues at the NHLBI, the NCI, the FDA, and the RAC to determine which additional data elements should be collected. Also, she made the point that the intention of this initiative is not that the RAC be a body that would promulgate screening criteria, rather that the RAC would serve as a catalyst to spur the experts in the field of cancer screening to look at this particular question and perhaps have them come to some form of consensus on what may or may not be appropriate in these patient populations with somewhat diverse growth factors. Dr. Mickelson called for, but there were no comments from the public. Dr. Aguilar-Cordova suggested that a statistician help in the analysis of the data.

Committee Motion 2

Dr. Gordon then moved that the committee adopt points 2 and 3 of his proposal. Dr. Markert seconded the motion. The vote was 11 in favor, 0 opposed, and 0 abstentions.

VII. Data Management Report/Dr. Greenblatt

Dr. Greenblatt reported that a total of 500 GTR protocols had been submitted to the OBA since June 1988; 17 new protocols were submitted to the OBA in the August 1 to November 1, 2001 reporting period. No protocols were chosen for public review. Of the 500 protocols, 40 were for gene marking, 2 were for nontherapeutic protocols in normal volunteers, and 458 were aimed at development of a therapeutic response. Of these:

- 314 were for cancer.
- 54 were for monogenic diseases (cystic fibrosis and hemophilia were the most numerous).
- 38 were for infectious diseases (predominantly for human immunodeficiency virus [HIV]).
- 52 were for other diseases (coronary artery disease and peripheral artery disease were the most numerous).

A. Amendments and Updates and Adverse Events

In the past reporting period, 85 amendments and responses to Appendix M were submitted to the OBA. These amendments included minor changes such as the addition of new investigators and new study sites. None warranted detailed discussion.

Analysis of serious adverse event (SAE) reporting for this period indicated that, of the 173 reports submitted to the OBA, 125 were initial reports and 48 were follow-up reports. One report was deemed serious, possibly associated, and unexpected, and no discussion of this or the other SAEs was found to be warranted.

Of note, after the close of the quarterly reporting period, there was the submission of a SAE that generated a teleconference between OBA and the study sponsor. This event involved a research participant enrolled in protocol 412. Nine days following completion of administration of the study agent, the research participant was admitted to the hospital after the participant's family was unable to arouse the participant. The research participant was noted to have elevated liver enzymes and altered mental status. Narcan was administrated and the participant had a withdrawal response with improved mental status. Within 24 hours, the participant was more alert and decreased enzyme values were seen in follow-up lab tests. Although all the details are not yet available, the participant was on multiple pain medications, which may have accounted for this event. This event will be discussed in more detail at a future RAC meeting.

B. Thanks From RAC Members

Dr. Mickelson thanked Dr. Greenblatt for his service in presenting the data management report over the past several years.

VIII. Detection of Adeno-Associated Virus Vector Sequence in Research Participant Semen: Report and Analysis

RAC Working Group: Dr. Aguilar-Cordova, Dr. Breakefield, Dr. Friedmann, Dr. Gordon, Ms. King, Dr. Markert, and Dr. Mickelson

Ad Hoc Consultants: Myron S. Cohen, M.D., University of North Carolina, Chapel Hill; Terence R. Flotte, M.D., University of Florida; Simon Hall, M.D., Mount Sinai Hospital; Philip R. Johnson, Jr., M.D., Ohio State University; and Richard Jude Samulski, Ph.D. (via conference call), University of North Carolina, Chapel Hill

Dr. Mickelson introduced the next agenda item which was a review of the detection of DNA sequences from adeno-associated viral (AAV) vector found in the semen of the first research participant of protocol #0001-371: "A Phase I Safety Study in Patients With Severe Hemophilia B (Factor IX Deficiency) Using Adeno-Associated Viral (AAV) Vector To Deliver the Gene for Human Factor IX Into the Liver." The data from this study were presented and the potential significance of this data for the risk of germ-line gene transfer was discussed.

A. Liver-Directed Gene Transfer of rAAV for Hemophilia B: Summary of Clinical Protocol and Data (Including Summary of Semen Vector Analysis) and Statement of the Problem/Mark A. Kay, M.D., Ph.D., Stanford University

Dr. Kay provided background information on the clinical protocol, AAV vectors, and the data. The study design is a Phase I open labeled dose escalation safety trial with hepatic artery administration of an AAV-hFIX vector. The objectives of the trial were to determine safety, characterize the immune response to the transgene, determine the dose capable of producing therapeutic levels of FIX in the blood, and to determine whether germ line transmission of vector occurs following hepatic administration. The first research participant, a 63 year old male with severe hemophilia B, received 2 x 10^{11} vector genomes/kg on Aug. 13, 2001. Triplicate semen samples were analyzed for vector sequence by a PCR assay and tested positive from week one to seven. Samples were negative on week eight, but one of three tested positive on week 10. All samples from weeks 12 and 14 were negative. The sample from week 3 was fractionated and vector sequence was detected in the cell pellet and seminal fluid, but not in motile sperm. Enrollment in the trial was closed following placement of an FDA hold on the trial, pending three consecutive negative monthly semen samples.

AAV vectors contain a single stranded DNA genome. Following vector administration, there is an increase in gene expression that occurs three to six weeks post-administration concomitant with the conversion of the single strand DNA into double stranded DNA forms. The vector DNA can be in monomeric or concatomeric forms that can remain episomal or become integrated in a low proportion of cells. In mouse studies, in which AAV vectors are administered intravascularly, almost all hepatocytes are transduced 24 hours post administration but only approximately five percent remain stably transduced.

1. RAC Discussion

Dr. Gordon suggested that since AAV vectors could reach the semen or primitive spermatagonia, further studies should be done to determine whether sperm or spermatogonia could actually be transduced. While these studies are being performed, however, the study should be able to continue. Steps can be taken to prevent germline transmission though sperm-banking.

To clarify the risk-benefit ratio, Dr. Samulski asked how much Factor IX had been detected in this research participant. Dr. Kay replied that on the basis of the dog data, he expects only the middle or last cohorts of the clinical trial to show some benefit (i.e., circulating Factor IX). In response, Dr. Samulski suggested that investigators concentrate on gathering most of the safety data on the sperm risk from the

other two members of this first cohort, even if doing so slows down the trial. By answering most of the safety questions early, the trial can proceed more quickly within the cohorts that are expected to show some benefit.

Dr. Patterson asked the total sperm count in this research participant and what percent of the sperm were motile. Dr. Kay responded that the sample was not fresh but did contain a few motile sperm.

Dr. Friedmann asked about the recent studies suggesting a correlation between the presence of AAV and male infertility. Dr. Hall found the significance of AAV infection difficult to determine since the patients are usually co-infected with helper viruses such as CMV. Even for mumps orchitis, it is unclear whether infertility is caused by viral infection of the sperm or inflammation causing atrophy of the testicles.

B. Brief Explanation of Assay Used for Semen Vector Analysis/Debra Leonard, M.D., Ph.D., University of Pennsylvania

Dr. Leonard described the assays used to analyze the research participant's semen samples. DNA was extracted from 0.5 or 1 ml of semen. The PCR was performed with primers specific to the human α-1 antitrypsin promoter and FIX sequence in the vector. As an inhibition control for the PCR reaction, a second vector was constructed with a 100 bp deletion (to distinguish the products of the PCR reaction), and spiked into some of the samples tested. The semen sample PCR reactions were run in triplicate along with the controls. The sensitivity of the assay was 10 copies of vector.

To confirm that the bands amplified by the PCR reactions were vector, two other assays were performed. The band was sequenced and found to match the expected sequence. Southern blot analysis was also positive. In samples from subsequent weeks, the number of positive samples detected among the triplicates and the intensity of the signal decreased.

In addition, the semen sample from week 3 was fractionated. This sample contained a borderline normal total number of sperm but only 5% were motile, much lower than the average 50% motile sperm. This could be due to the age of the research participant or the delayed processing of the sample. Four fractions were tested: total semen, seminal fluid, motile sperm and nonmotile cells that include nonmotile and immature sperm, and white blood cells. The motile sperm were negative while vector was detected in the three other fractions.

1. RAC Discussion

In response to Dr. Cohen's question, Dr. Leonard clarified that only the week 3 sample was fractionated and it was not possible to fractionate any of the other samples. With future research participants, all samples would be fractionated to ensure a complete set of data. Dr. Cohen also asked which other types of samples had been tested for presence of vector. Dr. Leonard responded that investigators had also tested serum, saliva, urine, and stool; white blood cells were not tested consistently in the past but will be tested consistently for future research participants. Over the seven week time frame, vector continued to be recovered only from semen. She suggested that while urine and blood turn over regularly, the only "washout" of sperm occurred when the investigators were collecting the semen specimens because this research participant was not sexually active.

In response to Dr. Patterson's question about whether any studies are being conducted to localize the PCR signal to a particular cell type in the pellet, Dr. Leonard stated that such studies are being considered for future research participants. Since this research participant is currently negative, such tests can not be currently performed.

Dr. Breakefield pointed out that based on one analysis of a sample that was not prepared in an optimal manner, it was not yet valid to say that sperm were negative for AAV vector sequence.

Dr. Mickelson asked how the determination about whether integration had occurred would be made. Dr. Leonard responded that this question is currently under discussion among the investigators.

In response to Dr. Cohen's question, Dr. Leonard stated that the cell pellets had been saved but had been frozen so that they are not preserved in a manner that would allow morphologic analyses to be conducted.

C. Overview of Spermatogenesis/Dr. Hall

Spermatogenesis occurs during a cycle of 64-74 days but depending on frequency of sexual activity, that sperm may be retained for up to another 90 days. On average, over 100 million spermatozoa are produced per day. Spermatogenesis occurs in the seminiferous tubules. The type A and B spermatogonia are found outside the blood-testis barrier while meiosis and spermatid development occurs within the barrier. Therefore, transduction of spermatogonia is more likely than transduction of mature sperm. About 75% of semen is made in the seminal vesicle with the remaining 25% produced in the prostate gland.

Microbial infections of the epididymis and/or testicles may originate in the urethra and proceed through the duct system in a retrograde manner. Another mode of infection is hematogenous as occurs in mumps orchitis. It is unclear how HIV enters semen. HIV RNA has been detected in sperm, but also in semen of men who had undergone vasectomies.

Preclinical studies have shown spread through the genitourinary tract of adenovirus injected into the prostate of a mouse, but it was not detected in sperm. However, rodent models differ from humans in that the testicles can move freely from the scrotum to the peritoneal cavity. Adenovirus injected into the epididymis was detected in the interstitium, but did not affect spermatogenesis. Retroviral vectors can be used to generate transgenic mice by the transduction of testicle tissue, but in these experiments the natural barriers are bypassed.

More studies are needed to examine the efficiency of the blood-testis barrier and to identify which of the cell types involved with spermatogeneis can be transduced by vectors.

1. RAC Discussion

Dr. Friedmann asked about transduction of Leydig cells and the epithelial cells lining the epididymus. Dr. Hall responded that Leydig cells are transducible by adenovirus as are the epithilial cells following epididymus injection but not following intravenous or testicle injection. He explained also that a PCR-based assay of semen would detect vector DNA from cells sloughed off from anywhere along the tract: the testes, epididymis, vas deferens, seminal vesicles, ejaculatory duct, prostate lining, and/or urethra.

Dr. Friedmann asked whether studies had been done of germ-line transduction in female animals. Dr. Gordon had conducted ovarian injections in mice of adenovirus but was unable to penetrate the ovarian follicle. No positive embryos were observed following *in vivo* exposure of oocytes to adenovirus. Low levels of transduction were detected following *in vitro* exposure of zona free oocytes, but this was attributed to the difficulty in completely washing off vector.

Dr. Cohen asked whether prostatic fluid that was contaminated on day 1 of a gene transfer study might still contain contaminated material 3 months later. Dr. Hall responded that such a finding is possible and would vary widely, in part due to the frequency of sexual activity of the research participant.

Dr. Aguilar-Cordova asked about the pH of fluid in semen. Dr. Hall answered that the pH of the sperm stored in the vas ampulla is basic, and the prostatic secretions are acidic. When mixed together, the pH normally is between 7 and 7.5.

D. Preclinical Studies of AAV-hFIX Vector Safety and Biodistribution/Katherine A. High, M.D., Children's Hospital of Philadelphia

Dr. High presented results from several animal studies. Extensive studies were done in mice injected intramuscularly with an AAV vector. The mice were sacrificed at 31 or 91 days post-injection, and the gonads were assayed for vector presence. As dose increased, more animals tested positive for vector in

the gonads. The intensity of signal and number of positive animals decreased over time post-administration. Semen samples from hemophiliac dogs tested negative for vector sequence up to 16 months post intramuscular injection.

Rabbits were intramuscularly injected with AAV vector, and semen samples were collected out to 90 days when the animals were sacrificed to analyze gonadal DNA. No vector DNA was detected in semen samples but was detected in the gonads. Fluorescence in situ hybridization analysis of testicular tissue harvested seven days post-injection showed vector localized to the basement membrane and vessel walls. Double label experiments with antibodies to AAV capsid and heparin sulfate, a receptor for AAV, showed co-localization along the basement membranes and vessel walls. Attempts to directly transduce murine spermatogonia cells failed.

Studies of intra-hepatic artery delivery in rats showed positive vector signal in gonadal DNA preparations in a dose dependent manner. Similar studies were performed on normal dogs and no vector was detected in semen samples. Further studies of vector clearance and localization are being done with rabbits injected intravenously with different doses of vector. Studies have also begun on fractionated rabbit semen samples.

Vector shedding experiments were performed in rhesus macaques by Philipe Moullier's group at INSERM. Various body fluids tested positive for vector at time points up to 48 hours but were negative by five days post-injection. Vector was detected for up to nine months in peripheral blood mononuclear cells, which may be present in semen.

Dr. High concluded that while animal studies are continuing, some answers might be gained only from clinical studies. Barrier contraception and sperm banking can decrease risk to participants, partners, and potential offspring, so a plan for moving forward is needed.

1. RAC Discussion

Noting that spermatogonia can be transduced by retroviruses in a method for generating transgenic mice, Dr. Friedmann asked whether spermatogonia have AAV receptors. Dr. Kay responded that transduction efficiency differences between retroviral and AAV vectors had been observed in other cell types such hematopoetic cells. Also with AAV, unlike retroviruses, vertical transmission of virus has not been observed.

Regarding the possibility of immune rejection of transduced cells, Dr. Breakefield asked about the effect of exogenous transgene expression (e.g., lac Z or neomycin resistence). Dr. High responded that the studies were designed to include control promoterless null vectors that did not express any proteins. While the AAV inverted terminal repeats can act as promoters, they do not promote detectable expression in most cell types.

Dr. Flotte asked whether the FISH assay could be adapted to identify the types of cells in the pellet. Dr. High replied that they could try, but there were two points to consider. One was that the semen samples must be collected at the Philadelphia or Palo Alto sites to be fractionated quickly for valid results. Also, frequent sample collection requires cooperation of the research participant. For these reasons, it may be easier to address these questions in rabbits rather than burdening research participants.

Dr. Aguilar-Cordova asked about the function of white blood cells in semen, and Dr. Gordon asked the route by which white cells reach the semen. Dr. Hall explained that neither question can be answered definitively. White cells, especially CD4 T cells, are known to cycle in and out, and their function is to reduce the risk of antisperm immune responses. Entrance of the white cells is likely to be downstream from the seminiferous tubule.

Dr. Cohen questioned how much research effort should be expended on rabbit, dog, and cat studies given that the relevance of nonhuman animal studies to humans is unclear. He noted that HIV researchers have found relevance only between humans and other primates.

Dr. Cohen emphasized the inadvisability of relying on condoms as a failsafe barrier, in part because of human behavior and in part because of condom failure rates. These factors need to be considered in the determination of the risk/benefit ratio.

E. Position Statement and Clinical Trial Perspective/Elliot Grossbard, M.D., Avigen

Dr. Grossbard noted that the detection of vector sequence in research participant semen may be likely to occur in other participants as the vector dose is increased and as assay sensitivities increase. Under the terms stipulated by the FDA, the next participant may not be enrolled until three months after the previous participant's semen converts to negative. If vector clearance takes longer as the dose is increased, enrollment of 10-15 participants in the phase I trial may take five years or longer.

The vector sequence detected in semen does not present a significant clinical risk to the participant and the small theoretical risk to partners or potential offspring that can be reduced by the use of barrier contraception and sperm storage prior to gene transfer. To evaluate the significance of the positive signal, participants can be followed in the early investigational phase to obtain data to define the risk/benefit ratio for gene transfer for hemophilia B. In order to complete the trial in a timely manner, Dr. Grossbard proposed the following:

- Monitor participants for vector in semen until there are three consecutive negative samples at least one month apart. A positive result should not be the basis for a clinical hold.
- Participants should be informed of the data to date.
- Participants should be encouraged to store sperm and practice barrier contraception until vector sequence has not been detected over a three month period.
- Working with the FDA, develop a reporting method to ensure appropriate public disclosure and an investigative plan to determine the significance of the observation while allowing the clinical trial to proceed in a timely and responsible manner.

1. RAC Discussion

Dr. Flotte noted that the potential for horizontal sexual transmission is of only minor concern because this agent is not a pathogen. He encouraged additional data-gathering regarding the vector DNA that is shed in the semen to determine whether it is in sperm or infectious.

F. FDA Procedures Regarding Potential Germ-Line Transmission and Impact on Gene Therapy Clinical Trials/Stephanie L. Simek, Ph.D., Center for Biologics Evaluation and Research, FDA

The FDA has developed an action plan to be followed in the event of detection of vector sequence in semen samples. Following the report of a positive PCR signal in the participant's semen, the protocol is put on clinical hold. The research participant is notified, reconsented, and required to use barrier contraception until the three month negative interval is reported. The sponsor is required to retest semen samples until samples are negative three times over a three month interval. If the samples remain positive after three months, follow-up testing including semen fractionation are requested.

Regarding AAV vectors, the FDA has concerns about the duration of expression from episomal forms, whether animal models are predictive of human findings, and the potential for higher doses of vector to result in persistence of vector sequence through one phase of spermatogenesis. These concerns have raised the following issues:

- What is the significance of the consistent presence of vector sequence in participant semen samples?
- Should the studies be allowed to proceed or should there be assessment of risk/benefit for each clinical application or patient population?
- What are the implications of a gene transfer product that may require life-long barrier contraception?
- Does the potential benefit of the gene transfer product outweigh the potential risk of

developing a transgenic human?

1. RAC Discussion

Dr. Gordon pointed out that the risk of germ line transmission in gene transfer will never be reduced to zero. Assuming this, the important questions are: What is the risk-benefit ratio? What kind of disease is being studied? What additional animal and human studies should be undertaken to increase the level of benefit and reduce the possibility of risk as much as possible? He suggested that the overall risk of germ-line transmission of vector in this case is low compared with the potential benefit of treating this disease.

Ms. King noted that the risk/benefit ratio can not be determined without considering not only the likelihood but also the consequences of a germ line transmission. Dr. Simek agreed that discussion of this issue is crucial and should encompass consequences not only for the research participant, but also for society. To address the question, Dr. Gordon described the consequences observed with retroviral integration: insertional disruption of a host gene or disregulation of a host gene by neighboring viral sequence. From experiences with the generation of transgenic mice, interruption of an active gene appears to occur 5% of the time, but since only one allele is affected, phenotypic change usually does not occur.

Ms. Levi-Pearl commented on the difficulty of stopping exciting scientific opportunities but also the need to keep in mind the clear prohibition against research that ventures into the area of germ-line transmission. She expressed hope for a compromise that would allow research to move forward yet still recognize that one sensationalized article in the media about germ-line transmission could do great harm to the entire gene transfer field.

Dr. Macklin suggested that in small early trials, the possibility of germ line transmission could be eliminated if only sterile subjects were included. However, if the investigational product were licensed for broad use, this would no longer be feasible. She asked whether sufficient data to address the germ line transmission issues can be generated in an early restricted study. Dr. High responded that data on human vector-shedding in semen could not be collected if only sterile individuals were allowed as research participants. In addition, of the approximately 3,300 individuals in the United States who have hemophilia B, most are younger than age 18 (due to deaths from HIV and hepatitis infections), so a large enough pool of sterile subjects does not exist.

Dr. Flotte asked whether the action plan could be modified to incorporate additional characterization of the positive seminal fluid pellet and study of motile sperm. If no data indicated that the sperm or sperm progenitors were being transduced, then keeping the trial on hold for 3 months past the first series of negative results may not be necessary. With fractionation data from an additional research participants and rabbit studies directly addressing vertical transmission, a data set might be generated that would support going forward with this research.

Dr. Markert suggested fractionation of the semen samples of all research participants in this study to determine if vector sequence is detected in motile sperm. She also suggested that white blood cell analyses be conducted. The female partner of the research participant should receive counseling to be advised about birth control pills or other standard methods to prevent pregnancy.

Dr. Cohen described the need to consider both vertical and horizontal transmission of vector. He stressed the need to develop a sensible surveillance method that allows the research to proceed while still being capable of detecting a rare event. He considered horizontal transmission to be the more important issue. The relative danger of the horizontal agent should be weighed. For the AAV vector, a high degree of confidence exists that this agent cannot replicate from one human to another and that it is nonpathogenic in humans.

Dr. Breakefield stated that it is likely that vector sequence will be detected in semen again; therefore, the issue is determining whether it is present in sperm. The current study has not clearly answered that question. Efforts should be made to improve the design of the fractionation experiment and develop appropriate standards for the PCR.

Dr. Noguchi noted that vectors are not viruses and that little is known about the biology of AAV vectors. There are significant differences between vector and viral transmission.

Dr. Aguilar-Cordova proposed that because of the existence of risk in the trial, it should be reinitiated at the dose level predicted to be potentially beneficial based on the dog studies. Dr. Mickelson disagreed because this is a phase I safety trial not intended for benefit. She suggested that the trial be optimized to generate data useful for other studies involving the same class of vector. The questions to be addressed are the timing between enrollment of participants and the development of appropriate assays. Ms. King also pointed out that in this trial, risk is not confined to the participant but extends to sexual partners and offspring.

Dr. Macklin stressed the distinction between the situation in this trial where there may be potential for inadvertent germ line transduction and the deliberate insertion of vector DNA into the germ line. However, the fact that the latter is taboo affects the risk/benefit analysis for this trial.

Dr. Friedmann asked the other RAC members to help define the specific data the committee would like the investigators to provide. Dr. Johnson would like to see more human data, particularly focusing on transduction of motile sperm. Dr. Friedmann asked about the difficulty and applicability of the rabbit studies. Dr. High responded that the rabbit studies should yield information about the kinetics of vector clearance. The fractionation technique will be improved to distinguish between motile sperm and other cell types in rabbit semen. If the rabbit motile sperm test positive, it will be necessary to determine whether the vector sequence was adherent to the outside of the sperm or intracellular in an integrated or episomal form.

Dr. Cohen wondered whether the rabbit studies would be distracting and efforts should instead focus on developing techniques for the study of human sperm, detection of integration events, and proper surveillance. Dr. Gordon responded that certain studies would be possible only in animals, but these could be done in parallel with the human studies.

Dr. Samulski suggested that the RAC form a working group to work with the FDA to improve the assays for studying the semen of the next research participants in this trial. He also suggested that the NIH consider ways to support research to answer questions about vector transduction of germ cells. Because the issue of germ line transmission will be revisited many times and in many contexts, the RAC should decide how these issues will be treated—either on a case-by-case basis or as a general policy.

On behalf of the RAC, Dr. Mickelson invited Dr. High and Dr. Kay to return to present additional data. Both investigators agreed to do so.

Dr. Mickelson summarized the RAC's recommendations. The committee concluded that there was a need to revisit the sequential enrollment of research participants and the duration of the hold following detection of vector sequence in participant semen. More data are needed to determine whether waiting for three consecutive negative samples over a three month period is appropriate.

The investigators should consider development of more sensitive and specific assays, in particular, consistent fractionation of semen samples. To facilitate the rapid processing of semen necessary for fractionation assays, a standard operating procedure should be developed to permit testing in local andrology labs. Further testing should determine whether a positive signal is due to the presence of virions or DNA localized outside or within sperm. Biodistribution studies should include other cell types such as lymphocytes. The animal model studies should be pursued including the rabbit studies and nonhuman primate studies. The investigators were invited to return and present their results to the RAC for further discussion.

There should be an ongoing evaluation of the informed consent document to incorporate any new information generated by the preclinical and clinical studies. This may require reconsenting currently enrolled research participants. Counseling may also be needed for partners of participants to explain the need for barrier contraception. In the event of germline transmission, monitoring and counseling for offspring will be necessary.

The NIH should consider issuing a request for applications (RFA) to develop technologies for improved detection of vector sequences in germ cells. The NIH has funded previous research on the topic, but now may be an appropriate time for additional research. OBA should also consider contacting other investigators to collect any relevant data on vector detection in semen from other trials using AAV or other vector systems.

Because the potential may exist in all *in vivo* gene transfer protocols, the issue of inadvertent germline transmission will arise more frequently in the future. This discussion should serve as a springboard for future discussions toward the development of a general policy.

G. Public Comment/Steven Faust, National Hemophilia Foundation (NHF)

Mr. Faust noted that since the advent of clotting factor concentrates, the greater threat to the lives of hemophiliacs comes not from the disease but from therapy. There are approximately 18 deaths/year related directly to hemophilia. Of the10,000 hemophiliacs who were infected with HIV or hepatitis C virus (HCV) via cont aminated blood pro ducts, on ly 2,200 survive. With recombinant protein therapy, currently hemophilia has an effective and relatively safe treatment. Hemophiliacs can choose between the existing treatment and participation in clinical trials of potential therapies su ch as gene transfer. The hemophilia community will support research trials if all appropriate preclinical research has been pursued prior to human research, and if the risks and benefits are made clearly known to participants.

The NHF reviewed the issue with this trial and drafted a recommendation that the FDA and RAC consider the risks to trial participants and following appropriate analysis allow the trial to proceed if such risks can be mitigated with appropriate safeguards and informed consent. NHF continues to advocate for safety while supporting gene transfer strategies that actively study more effective treatments and cures for patients with bleeding disorders.

IX. Adjournment/Dr. Mickelson

Dr. Patterson presented Dr. Mickelson with a memento in appreciation of her service on the RAC.

After thanking the *ad hoc* consultants and Office for Human Research Protections and FDA representatives, Dr. Mickelson adjourned the meeting at 4:45 p.m. on December 6, 2001.

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

…/s/…

Amy P. Patterson, M.D.
Executive Secretary

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

Date: …/s/…

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Attachment I
Committee Roster

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Attachment II
Attendees

Valder Arruda, Children's Hospital of Philadelphia
Mark L. Bagarazzi, Merck Research Laboratories
W. Emmett Barkley, Howard Hughes Medical Institute
Nell Boyce, *U.S. News and World Report*
Jeffrey W. Carey, GenVec
Janice Castillo, Avigen
Joy A. Cavagnaro, Access BIO
Amy Chew, Children's Hospital of Philadelphia
John Connelly, Genzyme
Linda Couto, Avigen
Joann C. Delenick
Neal DeLuca, University of Pittsburgh
Brooke Esteves, Wyeth Genetics Institute
Steven Faust, National Hemophilia Foundation
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James Foss, Stellar Systems
Thomas Gelehrter, University of Michigan
Patrick Gonin, Genethon
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Dorothy Jessop, citizen
Larry Johnson, University of North Carolina
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Kara Manning, Oregon Health and Science University
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Eliot Marshall, *Science*
Kevin McCleary, Stellar Systems
Alan McClelland, Avigen
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Andra E. Miller, The Biologics Consulting Group
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Natalie A. Ochs, F-D-C Reports
Thomas J. Paulson, Avigen
Shannon Penberthy, Marc Associates
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Sam Wadsworth, Genzyme
LeRoy B. Walters, Georgetown University
Craig D. West, A.G. Edwards & Sons
J. Fraser Wright, Avigen

Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
CSR	Center for Scientific Review
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
FDA	U.S. Food and Drug Administration
GTR	gene transfer research
HCV	hepatitis C virus
hFIX	human Factor IX
HIV	human immunodeficiency virus
IM	intramuscular
IV	intravenous
NCI	National Cancer Institute
NHF	National Hemophilia Foundation
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
NINDS	National Institute of Neurological Disorders and Stroke
OBA	Office of Biotechnology Activities (formerly ORDA, Office of Recombinant DNA Activities)
OD	Office of the Director
PCR	polymerase chain reaction
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RFA	Request for Applications
SAE	serious adverse event