RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 16, 2004

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

CONTENTS

[Note: The latest Human Gene Transfer Protocol List can be found at the Office of Biotechnology Activities' Web site at <www4.od.nih.gov/oba/rac/protocol.pdf>.]

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]

December 16, 2004

The Recombinant DNA Advisory Committee (RAC) was convened for its 98th meeting at 8:30 a.m. on December 16, 2004, at the Bethesda Marriott Hotel, 5151 Pooks Hill Road, Bethesda, MD. Dr. Diane Wara (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 3:30 p.m. on December 16. The following individuals were present for all or part of the meeting.

Committee Members
Steven M. Albelda, University of Pennsylvania Medical Center
W. Emmett Barkley, Howard Hughes Medical Institute
Martha C. Bohn, Northwestern University
Neal A. DeLuca, University of Pittsburgh
David L. DeMets, University of Wisconsin Medical School
Stephen Dewhurst, University of Rochester Medical Center
Thomas D. Gelehrter, University of Michigan Medical School
Helen Heslop, Baylor College of Medicine
Philip R. Johnson, Jr., Columbus Children's Hospital
Terry Kwan, TK Associates
Bernard Lo, University of California, San Francisco
Nicholas Muzyczka, University of Florida
Glen R. Nemerow, The Scripps Research Institute
Madison Powers, Georgetown University
Naomi Rosenberg, Tufts University
Robert D. Simari, Mayo Clinic and Foundation
Diane W. Wara, University of California, San Francisco
David J. Weber, University of North Carolina, Chapel Hill

RAC Executive Secretary

Stephen M. Rose, Office of the Director, National Institutes of Health (NIH)

***Ad Hoc* Reviewers/Speakers**

Jeffrey S. Chamberlain, University of Washington
Kathryn V. Holmes, University of Colorado Health Sciences Center, Fitzsimons *(via teleconference)*
Crystal L. MacKall, National Cancer Institute (NCI), NIH
Jonathan W. Simons, Emory University *(written response)*

Nonvoting/Agency Representatives

Kristina C. Borror, 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

Betsy Earp, OD
Kelly Fennington, OD
Linda Gargiulo, OD
Kathryn L. Harris, OD
Robert Jambou, OD
Laurie Lewallen, OD
Maureen Montgomery, OD
Marina O'Reilly, OD
Eugene Rosenthal, OD
Thomas Shih, OD
Gisele White, OD

Others

There were 61 attendees at this 1-day RAC meeting. Attachment I lists RAC members, *ad hoc* reviewers/speakers, nonvoting/agency liaison representatives, and Office of Biotechnology Activities (OBA) staff members. Attachment II lists public attendees.

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

Dr. Wara, RAC Chair, called the meeting to order at 8:30 a.m. on December 16, 2004. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on November 18, 2004 (69 FR 67597). Issues discussed by the RAC at this meeting included public review and discussion of two protocols, a data management report, follow-up on the September 2004 safety symposium, and a presentation on the immune response to lymphopenia.

Dr. Rose reminded RAC members of the rules of conduct that apply to them as Special Government Employees.

II. Minutes of the September 23, 2004, RAC Meeting/Drs. Bohn and Dewhurst

Dr. Bohn stated that the minutes of the September 2004 RAC meeting had been well prepared. No suggestions were made for changes to the minutes.

Committee Motion 1

Dr. Bohn moved that the RAC approve the September 23, 2004, RAC meeting minutes. Dr. Muzyczka and Ms. Kwan seconded the motion, which was unanimously approved.

III. Discussion of Human Gene Transfer Protocol #0410-679: Phase I Clinical Trial of rAAV2.5-CMV-Minidystrophin Gene Vector in Duchenne Muscular Dystrophy

Principal Investigator:	Jerry R. Mendell, M.D., Columbus Children's Research Institute
Other Presenters:	R. Jude Samulski, Ph.D., University of North Carolina, Chapel Hill, and Xiao Xiao, Ph.D., University of Pittsburgh
Sponsor:	ASKLEPIOS BioPharmaceutical, Inc.
RAC Reviewers:	Drs. Gelehrter, Lo, and Nemerow
Ad hoc Reviewer:	Jeffrey S. Chamberlain, Ph.D., University of Washington

[Note: Drs. DeLuca, Johnson, and Weber recused themselves because of conflicts of interest.]

A. Protocol Summary

Duchenne muscular dystrophy (DMD) is the most common, severe form of muscular dystrophy. It is inherited as an X-linked recessive disorder. Incidence is estimated at 1 in 3,500 live male births. Four clinical stages are recognized in the progression of DMD: (1) clinical onset at ages 3 to 5 years, recognized by mild impaired motor function; (2) progressive loss of function at ages 6 to 10 years; (3) ambulatory loss at ages 10 to 12 years, requiring use of a wheelchair; and (4) various life-threatening potential events thereafter. Currently, there is no treatment that can reverse DMD. Prednisone, a steroid with potentially serious side effects, can partially benefit the patient with DMD; however, it has limitations for long-term use. Most other pharmacologic approaches for the treatment of DMD have been disappointing.

The primary objective of the study is the assessment of the safety of intramuscular administration of recombinant adeno-associated virus-2.5 (rAAV2.5)-minidystrophin gene vector using a cytomegalovirus promoter in dystrophin deficient DMD subjects. The secondary objective is to determine the dose of rAAV2.5-CMV-minidystrophin vector required to achieve a detectable level of dystrophin in muscle. The vector has been shown to initiate the production of an attenuated functional dystrophin in laboratory animals and reverse the dystrophic phenotypes in the mdx mouse, an animal model for DMD. Intramuscular injection of the vector restores muscle histology to normal and increases muscle strength although not to the level of wild-type mice.

The proposed human clinical trial is a phase I, double-blind randomized protocol. Two cohorts of subjects with DMD null mutations will undergo gene transfer in a standard three-six-dose escalation scheme to establish maximum tolerated dose (MTD). Subjects will receive three injections of vector directly into the muscle on one side of the body and the same number of injections of a placebo, saline or empty capsid vector, in the same muscle on the opposite side of the body. The placebo treatment will serve as a control, making certain that the observations following gene transfer are correctly interpreted. Six weeks after the shots, the injected muscles on both sides of the body will undergo biopsy to determine whether dystrophin protein is present on the side of the gene injections. Safety end points to be assessed include inflammatory reaction to the vector assessed by muscle biopsy; changes in hematology, serum chemistry, urinalysis, and immunologic response to AAV and minidystrophin; and reported history and observations of symptoms.

B. Written Reviews by RAC Members and *Ad Hoc* Reviewer

Five RAC members recommended in-depth review and public discussion of the protocol. Key issues included that rAAV2.5 is a novel vector, the minidystrophin transgene has not been used in humans, and that the protocol proposes the enrollment of research participants with DMD as young as 10 years old. RAC reviewers Drs. Gelehrter, Lo, and Nemerow and *ad hoc* reviewer Dr. Chamberlain submitted written reviews, to which the investigators responded in writing and during this meeting.

Dr. Gelehrter noted that the proposal was well presented with a useful review of the extensive preclinical data using the mdx mouse. He asked if it would be possible to inject the vector in the same muscle group for all six participants. He inquired about the choice of the cytomegalovirus (CMV) promoter rather than the muscle creatinine kinase (MCK) promoter, and the status of the biodistribution, toxicity, and steroid studies in the mouse model. He recommended that possible germ-line incorporation be studied in ejaculate samples three months after vector administration. Regarding the informed consent document, he recommended that it not be written in first person and clearly state that no benefit to subjects is expected.

Dr. Lo asked the investigators about the rationale for enrolling subsequent participants before the biopsy results are analyzed from the previous participant. He wondered whether participation in this study would make the participants ineligible for future gene transfer studies that use the same vector and stated that such information should be included in the informed consent document. Dr. Lo asked that the distinction between informed consent and assent from children be clarified in the informed consent document and that more detail be provided regarding how the assent process will be modified according to the age of the participant so that it is developmentally appropriate. He suggested that the investigators ask the

participants specifically about their use of anabolic steroids, marijuana, and other drugs not prescribed by a physician and possibly add use of such drugs to the list of exclusion criteria.

Dr. Nemerow noted the AAV2.5 vector differs by five amino acids from the AAV2 vector capsid, and asked whether the new residues are derived from a different AAV serotype, and alter receptor specificity or affinity. He asked whether the onset of transgene expression is similar to that of AAV2 vectors, and whether the investigators had analyzed AAV2.5 vector biodistribution in animal models to determine whether it differs substantially from that of AAV2. He requested that characterization of the placebo injection be clarified and made consistent. He asked whether the 3-week interval between participant dosings is adequate to determine safety, given that the minidystrophin expression in muscle is expected to occur approximately 6 weeks after gene transfer. He requested discussion about whether potential participants with neutralizing antibody to AAV2 should be excluded from this study. He asked whether the investigators had considered using a muscle-specific promoter to drive transgene expression rather than the constitutively expressed CMV promoter. To assess transgene expression, he recommended that the investigators consider using reverse-transcriptase polymerase chain reaction instead of immunostaining and Western blotting, since these latter two are likely to yield only semiquantitative information and may lack sufficient sensitivity. He asked whether the participants will be offered the opportunity to receive additional vector injections—and, if so, under what conditions—if some benefit is perceived in the bicep muscle receiving the rAAV2.5-minidystrophin.

Dr. Chamberlain expressed concern about the potential for the rAAV2.5 vector and/or dystrophin to elicit a cellular immune response. Because several strong, muscle-specific promoter/enhancer cassettes have been shown to be highly active in rAAV vectors and to not induce cellular immune responses, he suggested that the investigators use a muscle-specific regulatory cassette for this study. Dr. Chamberlain also recommended studying potential cellular immune responses against AAV2.5 capsid in the canine model of DMD, adding that there appears to be no need for biodistribution or toxicologic studies in dogs. He noted that results from a canine study would enable more careful planning for potential immune responses that might be encountered in humans.

C. RAC Discussion

During the meeting, the following additional questions and issues were raised:

- Dr. Albelda asked why the AAV2.5 vector is being proposed for use rather than an AAV1 vector, since AAV1 seems to be more efficient in muscle.

- Dr. Simari asked about the volume of muscle that would be obtained at biopsy. Dr. Mendell explained the procedure that should result in obtaining any transduced tissue.

D. Investigator Response

Dr. Mendell and his colleagues responded to RAC questions and concerns with the following information:

- In response to concerns about whether AAV can be readministered, Dr. Mendell noted In the proposed experiment, neutralizing antibody against AAV2.5 vector will be assessed and a profile of persistence will be followed in the subjects. The relationship between neutralizing antibody and gene expression will also be studied. The consent form will use appropriate language to indicate that there is a possibility that readministration of the vector may not be possible.

- The timing of enrollment will be adjusted to follow the safety data gathered on each subject at the six week time point, which will comprise clinical and laboratory findings including an initial examination of the muscle biopsy. The entire safety profile will be presented to the Data Safety Monitoring Board before proceeding with each subsequent enrollment.

- Use of anabolic steroids or marijuana would be added to the exclusion criteria.

- Regarding whether the study could be conducted in all participants in the bicep muscles, the investigators stated their preference for using only one muscle group but also explained that, due to a number of factors that influence muscle degeneration, not all the participants may present the same usable muscle target.

- The controls would differ between the two cohort groups. The first group would be injected with saline in the contralateral muscle while the second cohort would receive a control consisting of empty capsids.

- Regarding the choice of promoter, CMV was selected because of previous clinical use in AAV vectors and to lay the groundwork for subsequent studies involving delivery to cardiac and diaphragm muscles.

- The Investigators agreed with Dr. Gelehrter's suggestions regarding examination of semen at three months post vector administration. Two negative semen sample analyses will be required before a research participant no longer is required to use barrier contraception.

- The biodistribution and toxicology studies in the mouse model while exposed to steroids are currently ongoing but will be completed prior to the initiation of this clinical trial.

- Dr. Samulski explained that the AAV2.5 vector differs by five residues from the standard AAV2 vector capsid. The vector was chosen because of the accumulated safety data; AAV serotype 2 derived vectors have been used in a trials for hemophilia and ∀-antitrypsin in muscle. However, with the AAV2.5 vector, a 40X lower dose could be used efficiently in muscle. Heparin sulfate is the primary receptor. Onset of transcription is more rapid than with AAV2 vectors. The immune profile of the vector is more similar to AAV1.

E. Public Comment

Dr. Borror suggested a few revisions to the informed consent document. She suggested changing the wording in the assent and permission forms to be consistent with wording in the benefit section stating "there will not be any benefit." In the assent form, Dr. Borror noted a statement that "the study doctor needs you to volunteer"; she expressed concern that such a statement might be perceived as coercive.

F. RAC Recommendations

Dr. Wara summarized the following RAC recommendations:

- The presence of a cellular immune response may limit the expression of the mini-dystrophin transgene. As such, the investigators should consider using ELISPOT or a comparable assay to assess cellular immune response to the mini-dystrophin transgene. If an immune response to the mini-dystrophin is found, further assays should determine which component of the dystrophin protein is immunogenic.
- The proposed highest vector dosage in humans (3×10^{13} genome copies per the targeted muscle) should be reexamined to be sure that it is comparable to the dosage in the preclinical mouse model (2.5×10^{11} genome copies per the injected tibialis anterior muscle).
- The protocol should be considered in light of emerging data from the biodistribution and toxicity studies that are being carried out in a preclinical study involving a mouse model of exposure to steroids. Participant enrollment should not begin until the data have been assessed in the context of this protocol.
- The protocol's dose escalation is modeled on oncology studies involving subjects whose conditions are more acute and life expectancies are more limited. Since the condition under study is a chronic illness, the plan to base dose escalation solely on the frequency of serious adverse events should be reconsidered.

- For future studies, which may involve systemic administration, it will be important to determine the biodistribution of the AAV2.5 vector in the mouse model.
- The constitutive CMV promoter used in the vector for control of transgene expression may not be appropriate in future trials using systemic delivery. A more restrictive or tissue-specific promoter for systemic delivery may need to be employed in order to prevent unintended transgene expression in non-target tissues.
- By using the term "treatment," the informed consent document may mislead subjects about the potential benefits of participation. The term should be replaced with "experimental intervention" or "study agent." For further information, please refer to the *NIH Guidance on Informed Consent for Gene Transfer Research* http://www4.od.nih.gov/oba/rac/ic/.
- In the assent form, the statement "The study doctor needs you to volunteer…" could be coercive and should be revised.

G. Committee Motion 2

It was moved by Dr. Gelehrter and seconded by Dr. Bohn that the recommendations summarized orally by Dr. Wara be included in the letter to the investigators and the sponsor as expressing the comments and concerns of the RAC. The vote was 14 in favor, 0 opposed, 0 abstentions, and 3 recusals (Drs. Johnson, DeLuca, and Weber).

H. Additional Public Comment

The following individuals provided comments after the RAC had considered and voted on its recommendations:

- Margaret Wahl, representing the Muscular Dystrophy Association (MDA), showed the film "Ivan's Story," the story of Ivan Garcia of Miami, Florida, a 13 year old with DMD. Usually, there is a slow loss of function in DMD, with a loss of the ability to walk between the ages of 9 and 13 years, progressing to death often in the late teens or early 20s from respiratory or cardiac failure or both. The "treatments" for DMD, despite many years of research and about $100 million spent by the MDA in trying to find a cure for this disease, are primarily corticosteroids, which have significant side effects, and spinal surgery to straighten spinal curvature, which is risky and painful. The cutting of various tendons which sometimes prolongs walking with braces, assisted ventilation, treatment for cardiac failure, power wheelchairs and computers help people function but do not cure the disease. There is extensive knowledge of the disease and gene function. Prenatal genetic testing is available for DMD, but more than one-third of DMD cases are new mutations with no family history who would not have been tested. Ms. Wahl reiterated that the DMD community represented by the MDA supports this protocol.

- Peter Renzi summarized his experience with DMD. He is currently a college student. He stated that being a student was one of his few choices because of limitations imposed by his disease. He reiterated his support for gene transfer as a means to treating and curing DMD. In response to several RAC members' questions, Mr. Renzi averred that he understood clearly that taking part in an early-phase clinical trial would mean he likely would not benefit personally from the experimental agent being tested. He also expressed his understanding of the clear difference between current treatment for DMD and experimental attempts to development new treatments.

- Marie Pichaske, Mr. Renzi's mother, explained that she has been waiting for 18 years, since her son's diagnosis and shortly thereafter the discovery of dystrophin, for this kind of research to begin. She stated support for DMD research with minimal risk such as this protocol to move forward.
- Patricia Furlong, President of Parent Project Muscular Dystrophy, whose two sons died from DMD at ages 15 and 17, stated that her sons would gladly have been part of research in the hope that the results would mean that someone with DMD could regain or preserve their independence. She shared her understanding of the importance of this clinical trial as a first step,

noting that she welcomes safety and caution but also wants to encourage moving forward in finding a cure for DMD.

IV. Data Management Report/Drs. Albelda, Heslop, Simari, and Wara

Dr. Simari reported that 10 protocols were submitted since the RAC meeting of September 2004. Three were selected for in-depth review and public discussion. Review of one of these protocols was postponed at the request of the investigator. Of the seven trials not selected for review, five were for cancer and two were for peripheral artery disease. Five employed plasmid vectors, one employed an adenoviral vector, and one employed a retroviral vector.

Between August 7, 2004, and November 3, 2004, 111 serious adverse events (AEs) were reported to OBA. Of these, 36 were A events, 17 of which were classified as A1, which is defined as serious, possibly associated with the gene transfer, and unexpected. The term "unexpected" encompasses the specificity, frequency, and severity of the event; any of which can cause the event to be classified as unexpected. Dr. Simari updated the RAC on three of the trials in which A1 events occurred:

- #0304-581, "A Phase I Study of Intravesical Recombinant Fowlpox GM-CSF and/or Recombinant Fowlpox-TRICOM in Patients with Bladder Carcinoma Scheduled for Cystectomy." Three subjects were found to have elevations in aspartate transaminase (AST) and alanine transaminase (ALT) within days or weeks following vector delivery. These subjects were followed closely and, at last report, all of the elevations resolved or were resolving. All of these elevations were deemed unexpected and possibly related to the study agent.

- #0308-600, "A Phase II Randomized Double-Blind Controlled Study to Evaluate the Safety and Efficacy of PROSTVAC®-VF/TRICOM™ in Combination with GM-CSF in Patients with Androgen Independent Adenocarcinoma of the Prostate." One subject in the active arm experienced a serious AE after being dosed on September 14, 2004. On October 9, the subject developed thrombotic thrombocytopenic purpura (TTP) and had a myocardial infarction. Despite the complicated course that followed, the subject recovered.

- #0312-619, "Administration of a Replication Deficient AAV Gene Transfer Vector Expressing the Human CLN2 cDNA to the Brain of Children with Late Infantile Neuronal Ceroid Lipofuscinosis" (known as Batten disease). An 8-year-old subject received the study agent on October 5, 2004, and was discharged from the hospital in stable condition. On October 13, 2004, the subject developed recurrent seizures that evolved into status epilepticus. At the conclusion of a complicated course of hospital treatment, the subject was discharged via air ambulance to a hospital in England, her home. She subsequently died in supportive hospice care. Study investigators referred to the development of status epilepticus as a serious related but expected complication. It was classified as expected because the principal investigator (PI) considered the risk of seizures a potential complication. However, there were questions as to whether the event meets the criteria for the specificity and severity of an expected event. Dr. Patterson stated that the PI placed the study on voluntary hold. She said the PI will be invited to the RAC meeting in March 2005 to review the experience and describe any changes made to the protocol and informed consent documents.

Dr. Wara reported that 127 protocol amendments and 10 responses to Appendix M had been filed between August 7 and November 3, 2004. Of the 127 amendments, 47 were for PI or site changes and 39 involved annual updates and/or safety reports. Dr. Wara then described Protocol #9904-304, "A Pediatric Phase I Study of AdV/RSV-TK Followed by Ganciclovir for Retinoblastoma," which had been discussed briefly by the RAC. The results of a preclinical study were reported in the group's 2003 Investigational New Drug (IND) report. A xenograft model of retinoblastoma was used to test the efficacy of adenoviral vector expressing thymidine TK plus ganciclovir versus adenoviral vectors expressing the full length or truncated retinoblastoma gene. The TK vector efficacy was superior to the retinoblastoma vectors in the animal model. Changes were made to the clinical protocol to incorporate the RAC and FDA recommendations that the first three participants have bilateral disease (one eye lost, with failed

treatment in the second eye). The pretreatment assessment was amended to include the levels of antibody to adenovirus in serum, urine, and nasal swabs within 1 week of enrollment. In addition, the investigators modified the informed consent document to read, "and could result in the death of your child if the tumor spreads outside the eye."

V. Followup on Safety Symposium: Safety Considerations in Recombinant DNA Research with Pathogenic Viruses—Development of a Web-Based Resource/Dr. DeLuca; Kathryn V. Holmes, Ph.D., University of Colorado Health Sciences Center, Fitzsimons *(via teleconference)*; and Marina O'Reilly, Ph.D., OBA

Dr. O'Reilly reviewed the objectives of the safety symposium held September 21-22, 2004, in Bethesda, Maryland and presented the Web pages being developed as resources for institutional biosafety committees (IBCs). These Web pages include materials developed for and during the safety symposium, the webcast of the presentations, frequently asked questions (FAQs), and other relevant resources.

The goals of the safety symposium were to review novel recombinant research with pathogenic viruses, such as 1918 influenza virus, highly pathogenic avian influenza viruses, and SARS corona virus, enhance awareness of biosafety issues, review current guidance, discuss associated risk assessment issues, and draft a Points To Consider document to assist IBCs in reviewing this research.

The Points To Consider document drafted at the safety symposium has been converted into a Web-based resource. Dr. O'Reilly showed the draft Web pages that include background for the meeting, the full webcast, the agenda, the participant list, and linked PDF documents for slides and individual presentations. The resources page includes general biosafety guidance, an introduction to risk assessment, current biosafety guidance for wild-type influenza and SARS coronaviruses, examples of risk assessment templates for pathogenic virus research, examples of risk assessments, occupational medical services for biomedical research, and references. FAQs were created to guide users through the resources using links embedded in the answer sections.

A. RAC Discussion

Dr. Rosenberg noted that at the safety symposium, the RAC discussed the need for guidance on the type of training and education that should be provided for investigators establishing new higher containment labs. Dr. DeLuca stressed the need for timely guidance in the area of research with emerging viruses. Dr. Holmes suggested the possibility of developing a means through which researchers could share new information and guidance on work with emerging viruses or novel recombinant viruses. Dr. Wara noted that one outcome of the safety symposium should be a public statement that these needs were identified. The statement should also encourage methods to address the need for information sharing.

Dr. Patterson asked the RAC whether it would be appropriate for OBA to move forward in partnership with the American Biological Safety Association, American Society for Microbiology (ASM), and others to formulate a training program and more specific algorithms for risk assessment. Dr. Barkley cited the successful collaboration between the RAC and the ASM to provide training resources in the early days of recombinant DNA research. Drs. Barkley and Rosenberg volunteered to participate in a working group to help conceptualize the next steps in dealing with the training issue and defining the role of the OBA and the RAC in coordinating the various groups that might be interested in this effort.

VI. The Immune Response to Lymphopenia/Crystal MacKall, M.D., NCI, NIH

Dr. MacKall summarized research focusing on how the human body replaces T cells following depletion by disease or chemotherapy. Researchers are attempting to exploit this process therapeutically in the context of cancer therapy. Innate immunity, which involves cells that do not manifest immunologic memory or specificity, can be reconstituted by cell populations derived from hematopoietic pluripotent progenitor cells. Dr. MacKall gave the examples of immune recovery post-bone marrow transplants. In pediatric patients receiving intensive chemotherapy, CD4 T cell levels can recover rapidly in three to six

months. In young patients, the thymus contributes naïve cells (CD45 RA and CD45 RO) resulting a diverse T cell receptor repertoire and normalized T cell counts. However, in adult patients over 40 years old, thymic rebound does not occur. Immune reconstitution occurs more slowly and prolonged depletion is often associated with opportunistic infections. Immune recovery occurs by homeostatic peripheral T cell expansion involving activated cells. The activated cells undergo expansion, but recovery can be unstable because these cells also have high rates of programmed cell death.

Dr. MacKall provided an overview of research conducted with lymphopenic mice exposed to antigen which skewed the T cell repertoire. Other studies identified interleukin-7 (IL-7), a member of the gamma C cytokine family involved in early T cell development in humans, as largely responsible for homeostatic proliferation. In humans who were subject to sustained T cell depletion, IL-7 inversely correlates with CD4; as the count of one goes up, the other goes down. Dr. MacKall said researchers believe this does not reflect increased production of IL-7, but rather decreased utilization.

Regarding lymphopenia and autoimmunity, she cited a number of mouse models of autoimmunity that involve lymphopenia, immune repopulation syndromes observed in HIV patients who experienced a dramatic recovery due to antiretroviral therapy, and autoimmune iritis detected in some lymphopenic cancer patients administered large numbers of antitumor cells. However, autoimmune responses did not occur in all cases and may involve other factors such as inflammation.

In summary, Dr. MacKall noted that inducing lymphopenia to augment an immune response may have both positive and negative effects. The advantage would be increased proliferation to high affinity, low affinity or self antigens, such as tumor antigens. However, in adults, CD4 lymphopenia that is induced will be prolonged and will likely result in opportunistic infections. She also noted that the T cell repertoire diversity will also be diminished. Dr. MacKall said the field would like to develop a more targeted approach that avoids broad immunosuppression and retains the T cell receptor repertoire diversity that's needed for robust anti-tumor response.

A. RAC Discussion

Dr. Heslop asked for comment on the fact that autoimmunity is rare after autologous transplantation that induces lymphopenia. Dr. MacKall responded that, if rapid homeostatic proliferation to low affinity and self-antigens is occurring, then regulatory factors must be coming into play to prevent autoimmunity. However, the data on this issue are preliminary and are not yet completely understood.

Dr. Dewhurst asked whether any studies using the mouse model address the long-term consequences of loss of repertoire. For example, if an animal's tumor is rejected as the result of vaccination during a lymphopenic stage, would that animal be predisposed to opportunistic infections regardless of the CD4 count much later? Dr. MacKall responded that this is difficult to determine because methods of measuring repertoire diversity are currently not well developed and that no researchers have investigated the intermediate loss of diversity in mice. Some researchers have tried to investigate whether, in HIV infections, the nadir of the CD4 count predicts subsequent opportunistic infections, but the activity of the thymus makes it difficult to determine the answer.

VII. Discussion of Human Gene Transfer Protocol #0410-675: Development of Effective Immunotherapy for Prostate Cancer Patients: Phase I/II Study of Human GM-CSF Gene-Transduced Irradiated Prostate Allogeneic Cancer Cell Vaccines (Allogeneic Prostate GVAX™) in Advanced Prostate Cancer Patients: Patients Made Lymphopenic by Chemotherapy and Infused with Autologous Peripheral Blood Mononuclear Cells

Principal Investigators:	Walter Urba, M.D., Ph.D., and Bernard Fox, Ph.D. *(via teleconference)*, Earle A. Chiles Research Institute
Sponsor:	Cell Genesys, Inc.

RAC Reviewers: Drs. Heslop, Muzyczka, and Powers
Ad hoc Reviewer: Jonathan W. Simons, M.D., Emory University *(written response)*

A. Protocol Summary

The phase I protocol explores a novel treatment strategy for advanced cases of hormone refractory prostate cancer (HRPC), based on the recent discovery that naïve T cells proliferate rapidly and can become "tumor killers" when they are transferred into lymphopenic hosts (hosts that have a decreased number of T cells). It is hypothesized that lymphopenia creates a space in which naïve cells can grow. The study will use a low dose of chemotherapy to induce lympho-depletion, followed by a series of vaccinations with irradiated Allogeneic Prostate GVAX™, a vaccine that has been used in previous clinical studies. The vaccine is composed of two prostate cancer cell lines that have been transduced with an adenoviral-associated virus (AAV) vector expressing granulocyte macrophage colony-stimulating factor (GM-CSF). At the time of vaccination, the subjects will be infused with autologous peripheral blood cells collected prior to treatment in the hope that infusion of normal lymphocytes will help with recovery time and reduce the likelihood of infection. The major goal of the study is to determine whether vaccination during lymphopenia will result in greater amounts of cancer-fighting T cells.

The aim of the study is to determine whether vaccination of human subjects during lymphopenia will skew naïve T cells toward a specific antigen, resulting in a dramatic expansion of therapeutic, tumor-specific T cells. Subjects will be randomized into three groups. All participants will be given the vaccine, but one group will receive a low dose of chemotherapy prior to being vaccinated, and a third group will receive a larger dose of chemotherapy prior to being vaccinated. Vaccinations will take place every two weeks for a 6-month period. At the time of vaccination, the participants will also be infused with autologous peripheral blood cells that were collected prior to treatment. Infusion of these normal lymphocytes may help with recovery time and maintain a significant component of the pretreatment repertoire of cells. The investigators will test the types and numbers of tumor-killing cells in the blood of subjects before, during, and after the 6-month series of vaccinations. The participants will also be followed for signs that their tumors are shrinking. The investigators hope to be able to draw a correlation between the number of tumor-killing cells in the blood of vaccinated participants and the ability to induce regression of prostate cancer.

B. Reviews by RAC Members and *Ad Hoc* Reviewer

Nine RAC members recommended in-depth review and public discussion of the protocol. RAC reviewers Drs. Heslop, Muzyczka, and Powers and *ad hoc* reviewer Dr. Simon submitted written reviews, to which the investigators responded in writing and during this meeting. Key issues included the risk of infection due to chemotherapy-induced lymphopenia, and the possibility of autoimmunity and autoimmune disease. Reviewers also recommended significant changes to the informed consent document.

Dr. Heslop commented on previous studies with the GVAX™ prostate cancer vaccines and the considerable preclinical data that supports the proposed study. However, she expressed concerns about the lymphodepletion approach. She said that in studies at NCI using a similar strategy, reconstitution of the T cell response was biased toward the infused melanoma-specific cells so that some patients were deficient in virus-specific responses. She asked if the investigators had considered saving an aliquot of peripheral blood mononuclear cells as a backup in case this were to occur. She also asked if the researchers were planning to monitor immune recovery to common viruses, such as CMV and EBV. Dr. Heslop asked the investigators to discuss the risks of generating an autoimmune response in subjects with prostate cancer. She requested that additional information about the risks of delayed immune recovery and of autoimmune disease be added to the informed consent document.

Dr. Muzyczka asked whether the participants will have a significant risk of autoimmune disease following the proposed procedure, how the investigators plan to warn participants about this risk, and what the investigators and sponsors will do if autoimmune disease occurs. Dr. Muzyczka asked if subjects will be more susceptible to infectious diseases and/or reactivation of latent viral infections due to an altered

memory cell repertoire following chemotherapy with fludarabine and/or cytoxan. He requested any information available regarding the infectious disease history of individuals treated with these drugs. He pointed out that there are no murine AAV viruses, and asked the investigators to correct the protocol on this point.

Noting that the subject matter of the informed consent document is inherently complex, Dr. Powers stated that the investigators explained the procedure and the many known risks in reasonable detail and with clarity. He requested that any risks of an autoimmune response or delayed recovery of immune function, be described in the informed consent document. Dr. Powers suggested that the investigators clarify whether the subjects will be individuals who are not candidates for alternative therapies beyond supportive care. He also asked the investigators to disclose any financial relationships with the sponsor.

Dr. Simon's written comments were read into the record by Dr. Heslop. He noted that in the informed consent form, the term "chemotherapy" should be changed to "chemotherapy with agents like taxoterre." He said the risk of infection with the use of fludarabine and cytoxan and the fact that autoimmunity is a potential adverse event that could be life-threatening should be added to the form. Concerning protocol design, he noted that the small size of the study precludes the assessment of the influence of alternate vaccine boosting schedules on antitumor immunity that might be relevant after induction of lymphopenia and expansion of CD4 and CD8 positive cells. He suggested that subjects be screened for the use of the herbal remedy PC SPES which may affect prostate-specific antigen measurements and checked for deep vein thrombosis. Dr. Simon stated that induction of autoimmunity should be monitored as a possible toxicity and correlated with anti-prostate immunity. Since cross-priming of antigens occurs with GVAX™ in both animals and early human studies of poorly immunogenic tumors, evaluation of antibody responses from CD4 expansion is warranted on all three arms of the study. He noted that while the assays described are appropriate they are biased towards evaluating only T cell responses.

C. RAC Discussion

During the meeting, the following additional questions and issues were raised:

- Dr. Dewhurst requested more detail on the risks of tumor lysis syndrome (TLS) and the steps planned by the investigators to prevent it from occurring.

- Dr. DeMets requested clarification on whether the investigators intend to establish a maximum tolerated dose, as the protocol was inconsistent on this point. He noted that the investigators stated in the protocol that, if two-thirds of the participants experience a dose-limiting toxicity, the trial might be terminated. He asked how the investigators decided on two-thirds, as opposed to a smaller percentage of participants.

D. Investigator Response

Dr. Urba and colleagues responded with the following information:

- To address the concerns about the investigators' approach to induced lymphodepletion, Dr. Urba reviewed data from previous studies to clarify the rationale of the approach and the preclinical data on immunosuppression. He noted that the specific method used to induce lymphopenia doesn't seem to affect results. Overall, study results consistently indicate that increasing the degree of lymphopenia increases the amount of therapeutic T cells that respond to the vaccine.

- Dr. Urba also clarified that adequate lymphopenia could be achieved with improved safety by using lower doses of the immunosupression drugs than in previous studies. The investigators will use 25 percent of the cytoxan dose and 50 percent of the fludarabine dose used in other protocols cited. He stated that lower doses were chosen to reduce the risk of infectious complications in what will likely be an older patient population.

- Concerning the ability to maintain a T-cell repertoire during lymphopenia-driven proliferation, subjects will be reinfused with a repertoire of autologous peripheral blood lymphocytes harvested before chemotherapy. It is hoped that these infusions, administered at the same time as the vaccine, will help speed lymphocyte recovery and maintain a significant component of the pretreatment repertoire. Dr. Urba said the investigators will be monitoring subjects closely for any signs that they are developing infection.

- Regarding the potential generation of autoimmunity, the investigators believe that if their regimen is successful, some autoimmunity is not only likely, but will be a desirable outcome of the study. Dr. Urba said that enhanced immune responses clearly correlate with the ability to shrink tumors, but usually do not have any adverse consequences for the patient. In this protocol, there is additional protection because the antigens are prostate cancer antigens, and many of the subjects will have had their prostates removed or irradiated. However, Dr. Urba agreed that systemic autoimmune disease is a possibility. The investigators will be observing subjects carefully for any symptoms of disease and will treat with appropriate measures if disease presents. He stated that the most common treatment modality for autoimmune disease is steroids.

- To address Dr. Heslop's concern, the protocol was modified to monitor the recovery of subjects' immune responses to CMV and EBV using cytokine flow cytometry (CFC).

- In response to Dr. Simon, Dr. Urba stated that the trial will be limited to patients with metastatic prostate cancer who have failed hormonal therapy and have no curative options. The option of docetaxel-based chemotherapy, which may prolong survival for several months, is mentioned in the informed consent document and will be discussed with the subjects and their referring physicians.

- Dr. Urba said that he has no financial relationship with Cell Genesys. Dr. Bernard Fox is a member of the Cell Genesys advisory board, but he will not be seeing patients. Dr. Hong-Ming Hu, a co-investigator on this trial, performed preclinical studies funded by Cell Genesys in 2003.

- To address the T cell bias in immune monitoring, the investigators will analyze patients at designated intervals for evidence of tumor-specific antibody production, using the techniques described by Dr. Simons.

- Dr. Urba indicated the areas in which the informed consent document has been changed. The tone has been modified to sound less optimistic, de-emphasize therapeutic outcomes, and emphasize safety considerations. It includes a statement that fludabarine is a known cause of death, as it can lead to tumor lysis syndrome (TLS). The risks of leukopenia and the potential risk of infection, including death, are now explained in several places in the informed consent document. A sentence has been added to inform subjects that that they may not use PC-SPES during the trial. The informed consent now explains the risk of autoimmunity relating to use of the vaccine. The phrase "like taxotere" was added to "chemotherapy drugs." All references to murine in the description of the virus used for gene transfer have been removed.

Dr. Urba also responded to RAC questions and concerns raised at this meeting by stating that:

- TLS is a clinical syndrome that occurs in individuals who, following treatment, appear to have a rapid response that causes millions of tumor cells to die at once. As a consequence, electrolyte or pH problems can develop or kidney failure can result. This syndrome has been observed in individuals with chronic lymphocytic leukemia who have large tumor burdens, hundreds of thousands of white cells per microliter in their peripheral blood, and lymph nodes, and who are extremely responsive to fludarabine. If an individual seems at high risk for TLS, they would be hospitalized, hydrated and urine would be alkalinized. Dr. Urba said the investigators did not

originally include mention of TLS in the study because it has not been seen with prostate cancer. However, TLS was added as a possible concern for the sake of completeness.

- The maximum tolerated dose of tumor cells has been determined elsewhere and will not be a goal of this trial.

- Regarding the statement that the trial might terminate if two-thirds of participants at a particular dose experienced dose-limiting toxicities, Dr. Urba clarified that the investigators intended to make the threshold two participants at any given dose.

E. Public Comment

Dr. Kristina Borror, FDA, stated that the use of words such as "treat" and "treatment" in the informed consent document could be misleading. She asked that the consent form be changed so that it does not imply clinical benefit. Dr. Urba indicated that he would remove any such terms if they remained in the latest version of document.

F. RAC Recommendations

Dr. Wara summarized the following observations and recommendations to be included in the OBA letter to the investigators and the sponsor:

- Given that the risks of infection and autoimmune disease are inherent in the strategy to induce lymphocytopenia prior to gene transfer, all subjects should be monitored closely for signs and symptoms of these complications.

- The investigators should clarify sections 5.3 and 11.5. of the protocol concerning the intent to identify the maximum tolerated dose.

- The determination of the percentage of research participants experiencing dose-limiting toxicity (currently 67 percent) should be re-assessed to ensure that this dose escalation endpoint produces an appropriate risk/benefit ratio.

- Use of the terms "treat" and "treatment" in the informed consent document could mislead subjects about the potential benefits of participation. These terms should be deleted.

- Information should be added to the informed consent documents concerning any financial or organizational relationships between the investigators and the sponsor (Cell Genesys).

G. Committee Motion 3

Dr. Heslop moved and Dr. Muzyczka seconded a motion that the above recommendations be included in the letter to the principal investigators and the sponsor as expressing the comments and concerns of the RAC. The RAC voted to endorse these recommendations with 17 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Glen Nemerow).

VIII. Closing Remarks and Adjournment/Dr. Wara

Dr. Wara thanked the participants and adjourned the meeting at 3:30 p.m. on December 16, 2004.

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

_____
Stephen M. Rose, Ph.D.
Executive Secretary

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

These minutes will be formally considered by the RAC at a subsequent meeting; any corrections or notations will be incorporated in the minutes after that meeting.

Date: _____ _____
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Attachment I
Recombinant DNA Advisory Committee Roster

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Attachment II
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Gerard J. McGarrity, Intronn Inc.
Maritza McIntyre, FDA
Jerry R. Mendell, Columbus Children's Research Institute
Sheila Mikhail, ASKLEPIOS BioPharmaceutical, Inc.
Andra E. Miller, The Biologics Consulting Group
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T. Shimada, Ambience Awareness International, Inc.
Walter Urba, Earle A. Chiles Research Institute
Margaret Wahl, Muscular Dystrophy Association, Inc.
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Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
AE	adverse event
ASM	American Society for Microbiology
CMV	cytomegalovirus
DMD	Duchenne muscular dystrophy
FAQs	frequently asked questions
FDA	U.S. Food and Drug Administration
HRPC	hormone refractory prostate cancer
IBC	institutional biosafety committee
IL	interleukin
MDA	Muscular Dystrophy Association
MTD	maximum tolerable dose
NCI	National Cancer Institute
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	NIH Office of Biotechnology Activities
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
rAAV2.5	recombinant AAV-2.5
SARS	severe acute respiratory syndrome
TLS	tumor lysis syndrome