RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 15, 2006

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]

March 15, 2006

The Recombinant DNA Advisory Committee (RAC) was convened for its 103rd meeting at 9:00 a.m. on March 15, 2006, at the National Institutes of Health (NIH), Building 31C, Conference Room 10, Bethesda, MD. Dr. Diane Wara (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. until 2:30 p.m. on March 15. The following individuals were present for all or part of the meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania Medical Center
Stephen Dewhurst, University of Rochester Medical Center
Howard J. Federoff, University of Rochester
Helen Heslop, Baylor College of Medicine
Terry Kwan, TK Associates
Glen R. Nemerow, The Scripps Research Institute
Steven Piantadosi, Sidney Kimmel Cancer Center
Naomi Rosenberg, Tufts University
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities
Richard G. Vile, Mayo Clinic College of Medicine
Diane W. Wara, University of California, San Francisco
David J. Weber, The University of North Carolina at Chapel Hill

Office of Biotechnology Activities (OBA) Director/Acting RAC Executive Secretary

Amy P. Patterson, Office of the Director (OD), NIH

***Ad Hoc* Reviewer**

LouAnn C. Burnett, Vanderbilt University

Nonvoting Agency Representatives

Kristina C. Borror, Office for Human Research Protections, U.S. Department of Health and Human Services (DHHS)
Stephanie L. Simek, U.S. Food and Drug Administration (FDA), DHHS

NIH Staff Members

Jeffrey Dixon, OD
Kelly Fennington, OD
Linda Gargiulo, OD
Mary Groesch, OD
Katherine Harris, OD
Robert Jambou, OD
Mary Joyce, National Heart, Lung, and Blood Institute (NHLBI), NIH

[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.

Laurie Lewallen, OD
Maureen Montgomery, OD
Marina O'Reilly, OD
Eugene Rosenthal, OD
Rita Sarkar, NHLBI
Michelle Saylor, OD
Thomas Shih, OD

Others

There were 40 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, *ad hoc* reviewers and speakers, and nonvoting agency and liaison representatives. Attachment II contains a list of public attendees. Attachment III is a list of abbreviations and acronyms used in these Minutes.

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

Dr. Wara, RAC Chair, called the meeting to order at 9:00 a.m. on March 15, 2006. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on February 23, 2006 (71 FR 9361). Issues discussed by the RAC at this meeting included public review and discussion of one protocol, a gene transfer safety assessment board report, and a presentation and discussion regarding biosafety considerations for research with lentiviral vectors.

Dr. Wara noted that recently RAC discussions have frequently focused on issues related to trial design which are pertinent not only to the gene transfer field but to all clinical research. To assist the NIH research community with such issues, the clinical trial design working group is being formed under the aegis of the Clinical Research Policy Analysis and Coordination Program (in the OD, NIH), which is also headed by Dr. Patterson. The working group will include RAC members as well as investigators from other clinical fields, bioethicists, and experts in clinical trial design. The working group will address the challenges of designing studies with appropriate scientific, statistical, and ethical rigor.

Dr. Patterson reminded all RAC members of the rules of conduct that apply to them as special Federal Government employees.

II. Minutes of the December 13-14, 2005, RAC Meeting/Drs. Federoff and Vile

Dr. Federoff noted that the December 2005 RAC minutes appeared to be accurate, and he recommended acceptance.

Committee Motion 1

It was moved by Dr. Heslop and seconded by Dr. Federoff that the RAC approve the December 13-14, 2005, RAC meeting minutes. The vote was 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

III. Risk Assessment for Research with Lentiviral Vectors

Presenter:	Dr. Somia
RAC Reviewers:	Drs. Dewhurst and Rosenberg
Ad hoc Reviewer:	LouAnn C. Burnett, M.S., CBSP, Vanderbilt University

A. The Issue

The OBA has been receiving inquiries regarding the determination of containment for research involving lentiviral vectors. The *NIH Guidelines* don't address containment for research with lentiviral vectors explicitly. As with other viral vectors, OBA has interpreted the *NIH Guidelines* to allow vector research to be performed under the same containment that might be applied to the infectious parent virus (i.e., for HIV-1, Biosafety level 2 (BL2) facility using additional practices and containment equipment recommended for BL3). However, a comprehensive risk assessment and containment determination for specific experiments with specific vectors should be based on a number of considerations including the nature of the recombinant agent and how it is to be manipulated. It would be helpful to IBCs and investigators to have guidance regarding the conduct of risk assessments and determination of containment levels for lentiviral vector research. OBA organized a working group of RAC and *ad hoc* consultants with virology and biosafety expertise to develop general criteria to be considered when conducting a risk assessment of lentiviral vector research.

B. Background

Dr. Somia reviewed the lifecycle of the retrovirus. To create a vector, the viral genes are deleted; however, the vector retains regulatory elements such as the long terminal repeats (LTR), packaging sequence, and integration sequences. The functions of the viral genes, *gag, pol*, and *env*, are supplied by helper sequence in the packaging cells. In the early retroviral vectors derived from MLV, sequence overlap existed between vector and helper sequence permitting homologous recombination and the generation of replication competent retrovirus.

Lentiviruses are more complex and in addition to *gag, pol* and *env* encode multiple accessory proteins involved in viral pathogenesis. Vector systems derived from lentiviruses such as HIV-1 require one of the accessory proteins, Rev, involved in transport and stabilization of viral RNA. To decrease the likelihood of replication competent lentivirus (RCL) generation via homologous recombination, the viral helper functions are split among three or more plasmids in later generation vector systems. Another safety modification frequently used in lentiviral vectors is a deletion in the U3 region to create self-inactivating vectors in which the viral promoters are deleted in both LTRs. Currently, most lentiviral vectors are pseudotyped with VSV-G envelope rather than the HIV-1 envelope because VSV-G env transduces more cell types than HIV-1 env. Due to VSV-G toxicity, lentiviral vectors are generated by transient transfection systems or packaging cell lines with inducible systems.

C. RAC Reviewer Discussion

Dr. Somia

Dr. Somia noted that lentiviral vectors have become widely disseminated as they are available from academic laboratories and sold commercially in kits. Major safety considerations include

- Potential for replication competent lentivirus (RCL) generation during production of the vector
- Potential for productive infection in animals injected with a vector preparation contaminated with RCL
- Potential for viral vector mobilization
- Vector/virus shedding from animals
- Accidental injection of vector preparation into researchers
- The transgene in the vector

A number of assays have been adapted to test for the generation of RCL: 1) monitoring for the spread of virus in a target cell population by assaying for the presence of capsid protein (p24) in cell culture; 2) vector mobilization assays; and 3) PCR based approaches to detect *gag* and *pol* sequences in target cells. No RCL events have been detected by two groups with extensive experience with lentiviral vectors: Dr. Verma's laboratory and the National Gene Vector Laboratories.

Regarding animal research, species restrictions to productive HIV-1 replication provides an intrinsic safety component. Productive HIV-1 replication doesn't occur in most small animal. An exception to this would be experiments in which human cells infected with HIV-1 or lentiviral vectors were injected into immunodeficient mice. Vector shedding would be a greater concern if the vector expressed an oncogenic transgene. Risks would include contact with skin or mucous membranes or accidental injection.

Dr. Somia recommended that production of later generation lentiviral vectors could be performed under BL2 tissue culture practices. Because of the lack of productive infection in most small animals, animals could be housed in BLN-1 facilities. However, precautions should be taken to counter vector shedding during injections. Higher containment should be considered for animal experiments involving cells capable of supporting lentivirus replication.

Dr. Dewhurst

Dr. Dewhurst noted that containment may depend on a range of considerations including:

- Negative RCL testing
- The nature of the vector system (e.g., potential for generation of infectious virus from vector components, number of plasmids in the system, the genes deleted from the vector/packaging system, use of a self-inactivating vector)
- The nature of the gene insert (e.g., oncogenic potential)
- The vector titer
- The inherent biological containment of the animal host

Dr. Dewhurst expressed some concerns about IBCs requiring RCL assays. There is no reliable assurance of assay/data standardization between different labs (most of which have little or no experience with infectious HIV-1, its propagation, and p24 results). For many laboratories that do not work regularly with infectious HIV and lack the expertise to reliably grow the virus, a negative RCL assay is essentially meaningless. The use of wildtype HIV as a control would increase the risk in such a laboratory. In addition, there is the potential that a false-negative result would provide an unwarranted sense of security.

For animal research, vector administration and animal husbandry/housing can be considered separately. Vector delivery should be performed in BLN-2/BLN-2+ containment to minimize the risk of autoinoculation by the researcher. It may be permissible to reduce containment at some point after vector administration and cleansing of the inoculation site to BLN-1 for animals that have not been engrafted with human cells.

Regarding non-HIV lentiviral vectors, Dr. Dewhurst explained that many of those are based on BL-1 agents, for example, FIV and EIAV. However, BL-1 containment may not be appropriate for vectors derived from these viruses. Typically, these vectors are pseudotyped with heterologous VSV-G env which expands host range and increases stability. Since the vectors are now capable of transducing human cells and are thus a risk for insertional mutagenesis, BL-2 containment may be required.

Dr. Rosenberg

Dr. Rosenberg stated that biosafety level determination is dependent on the nature of the genes and sequences that are retained by the parent vector—the more parental sequences that are left, the greater the chance that recombination may occur. Vectors should retain as little viral sequence as possible. For packaging, the viral genes should be separated on multiple plasmids. Because transfections with multiple plasmids are easily possible, a vector system using at least four plasmids should be standard. The vectors should be pseudotyped with a different viral env, such as VSV-G, and the use of the HIV-1 env avoided. The presence of transgenes that could cause disease (e.g., oncogenes) should be avoided. During vector production and tissue culture, care should be taken that the vector plasmids or cells not be contaminated with viral sequences or replication competent virus.

For animal research, the general use of sharps should be avoided. For animals that contain no human cells or lentivirus sequence, BLN-1 conditions would be reasonable to house animals after administration

and cleansing of the site is completed. In most experimental situations where vectors lacking viral genes and pseudotyped with VSV-G are being used in small volumes, it may not be necessary to perform rigorous testing for RCL. Given the wealth of literature indicating that RCL is highly uncommon, requiring RCL testing may not be advisable in a laboratory that has little or no expertise in screening for the presence of lentiviruses.

Ms. Burnett

Ms. Burnett approached this issue from how to assign a biosafety level under a worst-case scenario. In her opinion, the components of a BL-3 environment did not add to protection from or prevention of lentivirus exposure. She considered the following features useful to enhance BL2 containment:

- All manipulations of lentiviral vector in a biosafety cabinet or with appropriate eye, nose, and mouth or face protection
- Strict attention to sharps safety and positive confirmation of the use of safety devices
- Notification regarding theoretical mobilization of lentiviral vectors in persons infected with HIV-1 and offer of HIV-1 screening
- Use of BLN-2 for animal work

In general, she noted that at her institution, there is limited difference between an animal housed under BLN-1 conditions and one housed under BLN-2 conditions, except for the placement of a biohazard symbol on the cage that allows for notification of the animal care workers. Ms. Burnett stated that she would prefer that labs not perform RCL testing of lentiviral vectors unless they have the full expertise to do so. Given the availability of the Invitrogen lentiviral vector kits, which do not require much expertise, many labs using the vectors may lack the proper expertise.

G. RAC Discussion

Following the review and discussion by Drs. Burnett, Dewhurst, and Rosenberg, RAC members asked a variety of questions and further discussed the issues. Among the questions and concerns raised were the following:

- Dr. Wara asked whether RCL testing currently is recommended at the discretion of each IBC. Dr. Dewhurst confirmed that many institutions recommend or require RCL testing.

- Dr. Vile expressed concern that the RAC not appear to be recommending that RCL assays not be performed on the basis that many laboratories cannot perform these tests properly. He noted that this is distinct from a recommendation stating that there is no scientific reason to perform such tests at all. It is important to characterize vector stocks. RCL assays are relevant and provide necessary information to the field.

- Dr. Dewhurst agreed that the RCL testing is important for the broad community. He emphasized the point that it is possible to assign a biosafety level without requiring RCL testing by basing the biosafety level assignment on the nature of the vector system used and the experiment being conducted.

- Dr. Patterson asked whether the lack of detection of RCL across the field could be due to frequent failures to perform the assay properly. Dr. Somia responded that laboratories with the proper expertise had tested large amounts of vector without detecting RCL.

- Ms. Kwan suggested that investigators offer their IBCs information about the different classes of personnel that would be at risk, for example, to what kinds of risk the investigator, the technicians, and the animal handlers would be exposed to. It also would be important for community members of IBCs to know what kind of risks might exist for the surrounding community and, for clinical research, what kind of risk would exist for the research participant. For lentiviral vectors, she noted that it seemed that the most significant risks would exist for the

technical assistants and the animal care workers and not so much for the general community. Ms. Kwan also noted that the use of "regular language" would help community IBC members evaluate risk more accurately.

H. Conclusion of RAC Discussion

Dr. Wara asked the members of the working group to work with the OBA to develop a brief guidance document that would be available on the OBA web site. The RAC will review this document at a subsequent meeting.

IV. Gene Transfer Safety Assessment Board Report/Drs. Albelda, Federoff, Heslop, and Wara

Dr. Heslop reported that 88 protocol amendments had been filed in the past 3 months, of which 11 were for change of site or of principal investigator (PI), 1 was for protocol design modifications, 42 were annual reports, 6 were responses to the *NIH Guidelines*, Appendix M(1)C(1), 5 were protocol status changes, and 23 were other amendments. Three protocols were summarized briefly, but none warranted public discussion:

- Protocol #9908-337, Transduction of CD34+ Cells from the Umbilical Cord Blood of Infants or the Bone Marrow of Children with Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency Disease (SCID). This study is on hold because of prolonged cytopenia in one patient after administration of Busulfan, which is given as conditioning prior to infusion of the genetically modified cells. The investigators plan to modify the study.

- Protocol #0301-567, A Multicenter, Randomized, Double-Blind, Dose Ranging Placebo-Controlled Study Evaluating Defined Doses of Percutaneously Delivered Via Boston Scientific Stiletto™ Endocardial Direct Injection Catheter System pVGI.1 (VEGF2) (Placebo, 20, 200, or 800 μg) in Patients with Class III or IV Angina. The company has placed this study on voluntary hold while it investigates three cases of pericardial effusion.

- Protocol #0502-699, A Pilot Study of Temozolomide and 0^6-Benzylguanine for Treatment of High-Grade Glioma, using Autologous Peripheral Blood Stem Cells Genetically Modified for Chemoprotection, was discussed at the June 2005 RAC meeting. This was a drug resistance study that was placed on hold because of proliferative disease in animal models. The investigators have completed their studies, and this study is now off hold.

Dr. Albelda reported that there had been 12 protocol submissions since December 2005, 1 of which was selected for public review at this RAC meeting. Of the 11 not selected for public review, 9 were for cancer, 1 was for peripheral arterial disease, and 1 was for macular degeneration. Five of the protocols employed plasmid vectors, two used adenoviral vectors, and one each employed a retroviral vector, a herpes viral vector, a pox viral vector, and RNA.

The OBA staff reviewed the 219 adverse events (AEs) reported, of which 24 were A events—16 reports and 8 followups. One protocol, for intractable angina, warranted public discussion—Protocol #0301-567, A Multicenter, Randomized, Double-Blind, Dose Ranging Placebo-Controlled Study Evaluating Defined Doses of Percutaneously Delivered Via Boston Scientific Stiletto™ Endocardial Direct Injection Catheter System pVGI.1 (VEGF2) (Placebo, 20, 200, or 800 μg) in Patients with Class III or IV Angina. This protocol, sponsored by Corautus Genetics, Inc., uses a delivery device produced by Boston Scientific Corporation. Of the 400 research participants proposed for the trial, 295 participants have enrolled to date.

The issue of concern regarding this fairly large trial was a pattern of serious AEs, including pericardial effusions, that appears to be associated with delivery of the plasmid to the heart. The increased number of AEs appears not to be related to the vector or to the transgene but to the delivery system. Dr. Albelda

noted that the AEs occurred soon after injection suggesting that trauma rather than inflammation contributed to the effects.

A. RAC Discussion Regarding Protocol #0301-567

Ms. Shapiro asked when publicly reviewing protocols, AEs and other safety and toxicity data, whether the RAC should focus solely on issues intrinsic to the gene transfer product or whether RAC review also should include in its scope the method of delivery, including mechanical devices. Dr. Patterson responded that the RAC should review the entire package because the product is both the delivery system and the gene transfer product. Other groups, such as the FDA's Center for Devices and Radiological Health, have expertise that they can lend to the RAC when needed. The RAC should concentrate on the gene transfer product, commenting on generalizable and teachable moments for the gene transfer field, and noting the safety issues for each protocol.

Dr. Simek explained that the Stiletto catheter is not an experimental device. It has been approved by the FDA Center for Devices and has been in use. Given that prior safety experience exists for the device, Dr. Piantadosi asked if the current AEs suggested a relationship between the gene transfer procedure and the device.

Dr. Wara asked RAC members to consider whether the RAC should review studies which propose to enroll large number of participants who would be exposed to gene transfer. This Phase IIb study has a goal of enrollment of 400 and a current enrollment of 295; however, was not reviewed by the RAC.

Dr. Federoff made two procedural suggestions regarding protocols that use devices. First, if a history of device use exists when the RAC is considering a protocol for public review, having that information would be helpful in making that assessment. Second, when such a protocol is selected for public discussion, someone with technological expertise relevant to the device should be invited to the RAC meeting to inform RAC members, as part of the review process, about any potential safety issues.

V. Discussion of Human Gene Transfer Protocol #0512-752: *Phase I Trial to Assess Safety and Immunogenicity of Xenogeneic CD20 DNA Vaccination in Patients with B-Cell Lymphoma*

Principal Investigator:	M. Lia Palomba, M.D., Memorial Sloan-Kettering Cancer Center
Additional Presenters:	Miguel-Angel Perales, M.D., Memorial Sloan-Kettering Cancer Center; Jedd D. Wolchok, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center; and Andrew D. Zelenetz, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center
RAC Reviewers:	Dr. Heslop, Ms. Kwan, and Dr. Wara

Drs. Federoff and Vile recused themselves from discussion of this protocol because of conflicts of interest.

A. Protocol Summary

Lymphoma is the most common blood cancer and the third most common cancer of childhood. Since the early 1970s, the incidence of non-Hodgkin's lymphoma (NHL) has nearly doubled. Many people treated for NHL receive some form of chemotherapy, radiation therapy, biologic therapy, or a combination of therapies. New antibody treatments usually are used in combination with chemotherapy. The most widely used antibody is Rituximab, which attacks and destroys B lymphocytes (B cells) that carry on their surface a protein called CD20. Unfortunately, despite all the recent advances in therapy for lymphoma, between 20 percent and 70 percent of patients with this disease (depending on the aggressiveness of the particular type of lymphoma) eventually succumb. Participation in this study will be offered to individuals who have not had a good outcome after conventional therapy.

Tumor antigens that are co-expressed by tumor cells and their normal counterparts such as differentiation antigens are difficult targets for cancer immunotherapy, as they are not typically recognized by the immune system, which creates tolerance to such self-antigens to avoid autoimmunity. One way to educate the immune system to recognize self-antigens on tumor cells is to expose it to altered forms of a given antigen, such as another species homologue (xenogeneic) or an engineered, rationally or randomly mutated version of it. CD20 has been shown to be an excellent target self-antigen for passive immune therapy. The clinical efficacy of monoclonal antibodies against CD20 in patients with lymphoma is one of the major achievements in cancer therapy over the past decade. The investigators have adopted both approaches in several pre-clinical models, including melanoma, breast cancer, prostate cancer and lymphoma to show that immunization of mice with xenogeneic DNA can elicit humoral and cellular immune responses and protect them from otherwise lethal tumor challenges. The phase I clinical study proposes to test the hypothesis using xenogeneic DNA encoding a truncated mouse CD20 protein.

The primary objective of the study will be to assess the safety and toxicity of the delivery of a plasmid DNA carrying the mouse sequence of the extracellular domain of CD20 given intramuscularly using a needle-free infection device (Biojector® 2000, Bioject Medical Technologies Inc.), and to establish an optimal biological dose (OBD). Secondary objectives will include assessment of an anti-CD20 immune response and any evidence of anti-tumor response generated by administration of the vaccine.

The study will enroll up to 18 evaluable participants with relapse/refractory CD20+ lymphoma in a dose escalation scheme. The participants will receive a total of five vaccinations, administered every three weeks. Based on prior experience with ongoing DNA vaccine trials, the doses to be tested will be 0.5, 2 and 4 mg of purified DNA/injection. Participant's sera will be evaluated for cellular and humoral immune responses by ELISPOT assays and western-blot/immunoprecipitation assays. CT scans of the chest, abdomen, and pelvis will be prior to and at completion of five immunizations to assess for any anti-tumor activity.

B. Written Reviews by RAC Members

Three RAC members voted for in-depth review and public discussion of the protocol. Key issues included concern about the long-range impact of the vaccine on the immune system, particularly the viability of CD20+ cells, and the inability to "turn off" the gene transfer product proposed for testing in this study.

Dr. Heslop noted that the major risk of the approach would be that if the vaccine was effective in generating a sustained immune response directed at CD20, it could potentially lead to long term B cell depletion, whereas the humanized monoclonal antibody Rituximab that reacts with CD20 antigen depletes the normal B cell compartment for only approximately six months. She requested that the investigators clarify how B-cell numbers and function will be monitored, including long-term monitoring, and what criteria will be used for instituting intravenous immune globulin (IVIG) replacement therapy. The informed consent document should include a discussion of the risks of significant viral reactivations or infection that have been reported in patients receiving Ritximab and a detailed explanation of the function of normal B cells in producing antibody and the use of IVIG has possible replacement therapy. She asked the investigators to clarify how the requirement for a normal B-cell count prior to vaccination might affect accrual to this protocol, since most potential participants will have been treated with Rituximab.

Ms. Kwan focused her review on the informed consent document. She noted that the revised informed consent document stated that "there is a risk of permanent and irreversible lack of B cells"; however, the document should clarify the consequences of immunosuppression including the possibility of death from uncontrolled infectious disease. The duration and type of monitoring should also be clarified especially to avoid any suggestion that performing follow-up blood tests could prevent the risk of permanent B cell depletion. The informed consent document should also include a request for autopsy.

Dr. Wara focused her concerns on the identification and management of potential risks, including B-cell depletion and immunodeficiency or autoimmunity, should the vaccine prove effective in establishing a long-term immune response to CD20. In regard to the preclinical studies, she requested discussion of potential long-term toxicity in the mouse model related to B-cell depletion, and the limited suggestion of

efficacy. She asked whether the intent of the protocol was to determine maximal tolerable dose (MTD) or the optimal biological dose (OBD) as stated in the scientific abstract. She also asked the rationales for both dose selection and the proposed time interval between dose escalations; the potential risk of autoimmunity; the percentage decrease in B-cell number that would trigger the use of IVIG to protect against infection; the length of followup time; and further discussion of the potential risks and consequences of this approach. The informed consent document understates the seriousness of various risks, including the remote, but real, risk of death, and should state the risk of not receiving Rituximab (because eligibility for this protocol excludes individuals who have received rituximab within the prior 90 days).

C. RAC Discussion

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

- Dr. Piantadosi expanded on Dr. Wara's concern regarding whether the protocol design was appropriate to attain the investigators' stated goals. No acute safety concerns appear to be present; therefore, it does not make sense to use a study design that includes the MTD as the primary research objective when the MTD is not the basic fundamental biological question. Dr. Piantadosi suggested correcting this situation by more clearly characterizing the purposes of the study and choosing a study design more appropriate for studying the safety and biological questions.

- Ms. Shapiro noted the link between study design and bioethics. It is of significant concern if research participants are exposed to risk in trials that are designed improperly so that the data obtained are flawed in terms of answering the research questions.

- Dr. Albelda expressed concern about the weakness of the preclinical data, particularly the small increase in survival that was observed in the mouse trial. He noted that, as a result, he would characterize this trial as one of low benefit and low risk.

- Ms. Shapiro requested that the investigators ensure that the informed consent document mirrors all changes made in the language of the protocol.

D. Investigator Response

Dr. Palomba agreed to further simplify the language in the informed consent document, per Ms. Kwan's request. The informed consent document was also modified to include a more extensive description of the possible risks of B cell depletion, the proposed monitoring, the potential use of IVIG as treatment, and a request for autopsy.

In response to Dr. Wara's query about why the investigators have retained MTD as the primary aim when they agree that reaching the MTD is unlikely, Dr. Palomba reiterated that the investigators' main interest is to look at the OBD for immunological response. Dr. Zelenetz further explained that, because of the difficulty in defining the OBD and the apparent safety of DNA vaccines in the melanoma model, the investigators believe that the most relevant way to do the dose escalation was to use the "maximal economic dose"—the amount of DNA vector that can be produced and given to research participants.

Efficacy in the mouse model may have appeared limited due to the use of the Burkett's-like lymphoma A20, which is an extremely aggressive lymphoma. Modifications to the plasmid and mode of administration are continued to be explored in the mouse model to determine if efficacy may be increased.

Selection of dose was based on immune responses achieved in previous melanoma, prostate cancer and malaria DNA vaccine trials. The dose escalation and monitoring schedules were based on the observation that B cell depletion occurs within a few days following administration of Rituximab. The use of IVIG will be determined by concomitant hypogammaglobulinemia and history of infections.

Regarding the risk of autoimmunity, CD20 antigen is not expressed on pro-lymphocytes or plasma cells; therefore, autoimmunity would be expected to affect only B cells. No autoimmunity to DNA has been detected in previous plasmid vaccine trials.

Regarding follow-up monitoring, the research participants' B cell counts and immunoglobulin levels will be evaluated pre-treatment, at week ten prior to the fourth injection, and at week 16, three weeks after the fifth injection. Participants will be evaluated with a physical examination and routine laboratory tests every four weeks for the first three months and every three months thereafter. Further testing for B cell depletion (by flow cytometry) will be performed at 3, 6, 12, and 24 month visits.

Dr. Zelenetz explained that participants who experience B cell depletion will be followed and treated as standard of care. Dr. Wara responded that long term follow-up could be part of the protocol, requested of participants, and provided through the institution. She also noted that when participants are followed off-study, the advantage of accumulating data centrally is lost which limits the ability to gather and analyze data across protocols with similar products. Dr. Wolchok explained that, in previous studies using DNA vaccines, Memorial Sloan-Kettering Cancer Center has committed to 15 years of follow-up for participants of DNA vaccine trials in accordance with FDA guidelines at the time. As such, he agreed it would be reasonable to follow all the participants on this proposed study for 15 years, despite the fact that the FDA recently decreased the required follow-up period to one year for plasmid DNA gene transfer.

E. Public Comment

Dr. Borror commented that the term "treatment" should be changed to "experimental intervention," "study agent," or a similar appropriate description.

F. Synopsis of RAC Discussion and RAC Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

- The primary objectives of the study will be difficult to achieve given the current study design. Changes in the study design or study objectives should be considered in order to better align the study design with the primary objectives. For example, since the concept of maximal tolerated dose may not be applicable to the evaluation of DNA vaccines, the determination of optimal biologic dose might be a more relevant and achievable goal.

- The duration of long term follow-up should be extended for research participants who have demonstrated depletion of B cells and/or autoimmunity.

- The informed consent document should be modified in the following ways:
    - the potential need for long term follow-up should be explained;
    - the reading level should be simplified to increase comprehension; and
    - potentially misleading terms and statements should be deleted. For example, terms such as "treatment," which can mislead prospective research participants about the potential study benefits, should be replaced with "experimental agent" or "study agent."

G. Committee Motion 2

It was moved by Dr. Heslop and seconded by Dr. Weber that the RAC 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 11 in favor, 0 opposed, 0 abstentions, and 2 recusals.

VI. Closing Remarks and Adjournment/Dr. Wara

Dr. Wara thanked the participants and adjourned the meeting at 2:30 p.m. on March 15, 2006.

[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.]

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Amy P. Patterson, M.D.
Acting RAC Executive Secretary/OBA Director

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: _____

_____
Diane W. Wara, M.D.
Chair

Attachment I
Recombinant DNA Advisory Committee Roster

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Public Attendees

Milton Axley, MedImmune Inc.
Richard Havlik, Arrow Health
M. Lia Palomba, Memorial Sloan-Kettering Cancer Center
Miguel-Angel Perales, Memorial Sloan-Kettering Cancer Center
Janet Peterson, University of Maryland, College Park
Daniel Takefman, FDA
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Susan Zwiers, MedImmune Inc.

Attachment III
Abbreviations and Acronyms

AE	adverse event
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BSL	biosafety level
DHHS	U.S. Department of Health and Human Services
DNA	deoxyribonucleic acid
EIAV	equine infectious anemia virus
FDA	U.S. Food and Drug Administration
FIV	feline immunodeficiency virus
HIV	human immunodeficiency virus
IBC	institutional biosafety committees
IVIG	intravenous immune globulin
MTD	maximal tolerable dose
NCRR	National Center for Research Resources, NIH
NHL	non-Hodgkin's lymphoma
NHLBI	National Heart, Lung, and Blood Institute, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	NIH Office of Biotechnology Activities
OBD	optimal biological dose
OD	NIH Office of the Director
PI	principal investigator
RAC	Recombinant DNA Advisory Committee
RCL	replication-competent lentivirus
VSV-G	vesicular stomatitis virus G protein