RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 17-18, 2007

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]

September 17-18, 2007

The Recombinant DNA Advisory Committee (RAC) was convened for its 109th meeting at 8:00 a.m. on September 17, 2007, at the National Institutes of Health (NIH), Natcher Building (Building 45) Auditorium, Bethesda, Maryland; the afternoon of September 17 and the September 18 portion of the meeting were held on the NIH Campus, Building 31-C, Conference Room 6. Dr. Howard Federoff (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public from 8:00 a.m. until 5:45 p.m. on September 17 and from 8:15 a.m. until 10:15 a.m. on September 18. The following individuals were present for all or part of the September 2007 RAC meeting.

Committee Members

Steven M. Albelda, University of Pennsylvania *(via teleconference on Day 2)*
Jeffrey S. Bartlett, Columbus Children's Hospital
Stephen Dewhurst, University of Rochester Medical Center
Hildegund C.J. Ertl, The Wistar Institute *(via teleconference on Day 2)*
Howard J. Federoff, Georgetown University Medical Center
Jane Flint, Princeton University *(via teleconference)*
Ellen E. Grant, HealthNow New York Inc.
Jeffrey P. Kahn, University of Minnesota
Louis V. Kirchhoff, University of Iowa
Eric D. Kodish, The Cleveland Clinic Foundation
Robyn S. Shapiro, Medical College of Wisconsin
Nikunj V. Somia, University of Minnesota, Twin Cities
Scott E. Strome, University of Maryland Medical Center
Richard G. Vile, Mayo Clinic *(present on Day One only)*
David J. Weber, The University of North Carolina at Chapel Hill
Lee-Jen Wei, Harvard University *(via teleconference)*
John A. Zaia, City of Hope

Office of Biotechnology Activities (OBA)

Jacqueline Corrigan-Curay, Office of the Director (OD), NIH
Amy P. Patterson, OD, NIH

***Ad Hoc* Reviewers and Speakers**

Abdu Azad, University of Maryland
Baruch A. Brody, Baylor College of Medicine *(via teleconference)*
Wei-Mei Ching, Naval Medical Research Center
Mary K. Crow, Hospital for Special Surgery, Weill Medical College, Cornell University
Stephen J. Dumler, The Johns Hopkins Medical Institutions
Marina E. Eremeeva, Centers for Disease Control and Prevention (CDC) *(via teleconference)*
Karen Frank, University of Chicago Hospitals
Edward J. Fudman, Austin Rheumatology Research, P.A.
David W. Hackstadt, National Institute of Allergy and Infectious Diseases (NIAID), NIH *(via teleconference)*

[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.

John Hart, University of Chicago Hospitals
D. Kyle Hogarth, University of Chicago Hospitals
Carol A. Kauffman, University of Michigan/U.S. Department of Veterans Affairs *(via teleconference)*
Nancy M.P. King, Wake Forest University
Gideon Lack, King's College London *(via teleconference)*
Jay Lozier, Warren Grant Magnuson Clinical Center, NIH
Eric L. Matteson, Mayo Clinic
Philip J. Mease, University of Washington
Claudia A. Mickelson, Massachusetts Institute of Technology
Michael J. Miller, CDC *(via teleconference)*
Shyam S. Mohapatra, University of South Florida
Bernard Roizman, The University of Chicago *(via videoconference)*
Naomi Rosenberg, Tufts University *(via teleconference)*
Leonard B. Seeff, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH
Scott A. Sicherer, Mount Sinai School of Medicine *(via teleconference)*
Sonia Skarlatos, National Heart, Lung, and Blood Institute (NHLBI), NIH
David H. Walker, The University of Texas Medical Branch at Galveston *(via videoconference)*
Richard J. Whitley, The University of Alabama at Birmingham *(via videoconference)*
Christopher W. Woods, Duke University
Xiao Xiao, The University of North Carolina at Chapel Hill *(via teleconference)*

Nonvoting Agency Representatives

Paul Andreason, Office for Human Research Protections, U.S. Department of Health and Human Services (DHHS)
V. Ellen Maher, Food and Drug Administration (FDA), DHHS
Daniel M. Takefman, FDA, DHHS

NIH Staff Members

Elizabeth Adams, NIAID
Mary Allen, NIAID
Rosemarie E. Aurigemma, National Cancer Institute (NCI)
Ronald Barnett, OD
Christopher E. Beisel, NIAID
Valerie Bonham, Office of the General Counsel
Jan Casadei, NCI
Jay Chiorini, National Institute of Dental and Craniofacial Research
Robert Cofin, NHLBI
Connie Coldwell, OD
Stephen P. Creekmore, NCI
Linda Ding, NIAID
Matthew Fenton, NIAID
Linda Gargiulo, OD
Mary Groesch, OD
Charlotte Holden, OD
Bob Jambou, OD
Mary Joyce, NHLBI
Shahnaz Khan, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
Kathy L. Kopnisky, National Institute of Mental Health
Robert Kotin, NHLBI
Steve Krosnick, Center for Scientific Review
Catherine Laughlin, NIAID
Laurie Lewallen, OD
Lina Li, NHLBI
Catherine McKeon, NIDDK

Maureen Montgomery, OD
Anna Nicholson, NIAMS
Stuart Nightingale, OD
Glen H. Nuckolls, NIAMS
Marina O'Reilly, OD
Roland Owens, NIDDK
Michael N. Pensiero, NIAID
Marshall Plaut, NIAID
Julian Poyser, NIAID
Vijaysmitha Rayadurg, National Biosafety and Biocontainment Training Program
Maryann Redford, National Eye Institute (NEI)
Gene Rosenthal, OD
Rita Sarkar, NHLBI
Dick Sawyer, NIAID
Tom Shih, OD
Santa Tumminia, NEI
Frosso Voulgaropoulou, NIAID
Anthony Welch, NEI
Bruce Whitney, OD
M. Virginia Wills, NIAID
Hao Zhang, NIAID

Others

There were 209 attendees at this two-day RAC meeting.

Attachments

Attachment I contains lists of RAC members, *ad hoc* reviewers and speakers, participants, 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 this document.

I. Day One Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff, RAC Chair, called the meeting to order at 8:00 a.m. on September 17, 2007. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) was published in the *Federal Register* on August 27, 2007 (72 FR 165). Issues discussed by the RAC at this meeting included discussion of a serious adverse event (SAE) on a human gene transfer trial using an adeno-associated viral (AAV) vector, public review and discussion of one protocol, a Gene Transfer Safety Assessment Board (GTSAB) (a subcommittee of the RAC) report, presentation and discussion of nanoparticle-mediated gene delivery, discussion of proposed experiments involving deliberate transfer of chloramphenicol resistance to *Rickettsia conorii* and *Rickettsia typhi* that would require a Major Action under Section III-A-1 of the *NIH Guidelines*, and presentation of information regarding the NHLBI's Gene Therapy Resource Program.

Dr. Corrigan-Curay reminded RAC members of the rules of conduct that apply to them as special Federal Government employees.

Dr. Federoff expressed the RAC's and the OBA's condolences to the family of the subject who had died and thanked them for providing access to the medical information necessary for the discussion. Dr. Federoff also thanked the experts and the physicians assembled for this discussion. The aim of the discussion was to gain a better understanding of the clinical course and determine whether the vector or transgene administered in the clinical trial played a role in theevent. While not all information would be available at the time of the meeting, the RAC would attempt to identify the gaps in the current understanding to be able to determine the potential role of gene transfer in this event. He also noted the

hope that the discussion would help identify knowledge generalizable to the use of AAV vectors, gene transfer and the design and conduct of future clinical trials.

II. Overview of AAV Vector-Based Clinical Protocols

Presenter: Jacqueline Corrigan-Curay, M.D., J.D., Acting Executive Secretary, NIH RAC

Dr. Corrigan-Curay noted that AAV-based protocols registered with OBA represent four percent of the protocols registered by the OBA (36 total). Diseases targeted in AAV-based protocols include cystic fibrosis, single gene disorders, cancer, Parkinson's disease, and Alzheimer's dementia. A number of these protocols have been completed, some are ongoing and others have not yet enrolled any participants. Approximately 86 percent of AAV-based protocols registered with OBA are Phase I trials. To date, more than 500 research participants have been dosed with recombinant AAV vectors; however, this figure is an underestimate because two large Phase III prostate cancer trials are actively enrolling and dosing participants but the exact number enrolled in these trials has not been reported to OBA.

Drawing on the Genetic Modification Clinical Research Information System database (GeMCRIS), 34 events in 13 AAV trials were judged initially to be SAEs. These events were determined by the principal investigator (PI) to be both unexpected and possibly related to gene transfer with an AAV-based vector. All of these events were reviewed by the RAC's Gene Transfer Safety Assessment Board (GTSAB), and no patterns that crossed the various AAV-based trials were found. Moreover, the types of SAEs observed in AAV vector trials did not seem to differ from those observed in other gene transfer trials.

A. FDA Comment

Dr. Takefman indicated that the FDA's review of the SAEs observed in AAV vector trials to date also had not identified any patterns across AAV trials and has not uncovered any specific type of SAE attributable to AAV vectors.

III. Overview of Rheumatoid Arthritis and the Role of TNF Inhibitors

Presenter: Mary K. Crow, M.D., Hospital for Special Surgery, Weill Medical College, Cornell University

Dr. Crow provided background information on rheumatoid arthritis (RA) and the role of TNFα-antagonists in the treatment of this chronic disease. Dr. Crow described RA as a systemic autoimmune disorder that primarily affects the joints, leading to pain, swelling and deformities that can lead to disability. RA is also associated with early mortality primarily due to associated cardiovascular disease and infections.

While there are many mediators of the inflammation and joint destruction characteristic of RA, TNF-alpha appears to be a primary mediator of pathogenesis. TNF-alpha is essential to the immune system's defense against microbes and, of interest in the current case an important action of TNF-alpha is to promote granuloma formation. Granulomas are aggregations of cells that help to wall off infection. Granuloma formation appears to be a particularly important mechanism for controlling infection with intracellular pathogens. When produced in excess, however, TNF-alpha can lead to chronic inflammation, and bone erosions in RA and is one of several mediators of septic shock in the setting of systemic infection.

The three available antagonists of TNF-α, adalimumab (Humira®), infliximib (Remicade) and etanercept (Enbrel), have been shown to be effective in inhibiting the destructive role of TNF-alpha in RA and contolling the clinical symptoms of the symptoms of the disease. Indeed, they have been characterized as revolutionary in the control of the disease. However, the decision to use these agents must be made with attention to the risks that are associated with their use including impaired defense against infectious agents. The infections that have been associated with anti-TNF therapy include reactivated latent and primary tuberculosis, fungal infections, including Histoplasma, Candida, and Aspergillus, and recurrent bacterial infections, including Streptococcus and Staphylococcus.

IV. TNF Antagonists in RA: The Clinician's Perspective

Presenter: Eric L. Matteson, M.D., M.P.H., Mayo Clinic

Dr. Matteson reviewed the clinical indications for instituting TNF-antagonist therapy in patients with RA. He noted that they are often used in combination with other disease modifying anti-rheumatic drugs but that he knew of no studies in which combinations of TNF-antagonists were used together. He noted that there are a number of known adverse events with TNF-antagonists including infection and that this risk is heightened by concurrent use of steroids. The baseline risk of infection in RA patients who are not on any TNF-antagonist therapy is two-fold that of the general population. The magnitude of possible increased risk of infection associated with the use of any TNF antagonist therapy is the matter of ongoing study. Estimates of this risk of increased infection with the use of these agents range from no increased risk in some databases (including clinical trials) to an increase in risk which may be about double that of the baseline risk of infection in patients with RA. Studies of patients taking infliximab or adalimumab suggest that the associated risk may be dose dependent. It is not known whether increased risk is related to genetic polymorphisms.

Dr. Matteson noted that the overall risk of opportunistic infections has not been adequately determined. With respect to tuberculosis, awareness of the increased risk and screening has decreased the occurrence of this complication in patients being given TNF antagonists. With respect to histoplasmosis, over 40 cases in patients on TNF antagonist therapy that have been reported to the FDA. Most of these infections have presented between 1 to 6 months after starting anti-TNF therapy. The initial symptoms may mimic the underlying inflammatory disease (for example, worsening of joint swelling) but patients may also present with an acute, fulminant course with fever, malaise, cough, dypsnea and interstitial pneumonitis. However, there is no consensus among rheumatologists with respect to screening for possible latent infections of *Histoplasma capsulatum,* the causative agent of histoplasmosis*,* in patients living in endemic areas.

V. Overview of OBA Protocol #0504-705: A Phase I Dose-Escalation Study of Repeat Intra-Articular Administration of tgAAC94, a Recombinant Adeno-Associated Vector Containing the TNFR:Fc Fusion Gene, in Inflammatory Arthritis Subjects with and without Concurrent TNF-α Antagonists

Principal Investigators: Philip J. Mease, M.D., University of Washington, and Edward J. Fudman, M.D., Austin Rheumatology Research, P.A.

A. Dr. Mease

Dr. Philip Mease provided an overview of the protocol. The investigational agent (tgAAC94)consists of an adeno-associated virus (AAV) that contains single stranded DNA encoding human TNFR:Fc complementary DNA. The DNA sequence is identical to that of the cDNA used for the production of etanercept (Enbrel™), a FDA approved TNF-antagonist drug. He explained that the ultimate goal was to develop an intra-articular theatment for patients with inflammatory arthritis who were on systemic therapy, including TNF antagonists, but who had incomplete responses. An incomplete response would be defined as persistent synovitis in critical joints in patients on systemic therapy. In addition, the local injection of the recombinant AAV might be used for patients with mono- or oligo-articular inflammatory arthritis who were not on systemic therapy. In these patients, providing local therapy might provide a safer alternative to systemic therapy.

Dr. Mease presented animal data that both demonstrated proof of concept and failed to show any toxic effects of the vector, tgAAC94. This information included the results of biodistribution studies showing that only minimal amounts of recombinant protein escaping into the systemic circulation. He also reviewed the

Phase I study that included 15 subjects, none of whom were on systemic TNF-antagonist therapy. In this study, no SAEs were determined to be related to the gene transfer.

A total of 127 subjects have been enrolled in the current study and enrollment is complete. The majority of subjects enrolled have RA, but some subjects have psoriatic arthritis or ankylosing spondylitis. The primary target joint for the gene transfer is the knee, but injections have also done into metacarpophalangeal joints, ankles and elbows. Just over half of the subjects were concurrently on TNF-antagonists, most commonly etanercept. Roughly, one third of the subjects on TNF-α antagonists were also on methotrexate and prednisone, as was the subject who died.

The subject received two doses of tgAAC94 at the highest dose level, 1×10^{13} Dnase resistant particles (vector genome-containing particles). In total, 17 subjects have received two doses of tgAAC94 at the highest dose level. Data were presented showing the percentages of subjects experiencing adverse events after the first and second doses. In addition to a review of all adverse events, a review of all *serious* adverse events was presented. In addition to the death of the subject being discussed, only one other SAE was determined to be probably related to the gene transfer. This was a case of septic arthritis that occurred 15 weeks after dosing in a subject in the mid-dose cohort ($1x10^{12}$).

With respect to infections, those most commonly reported infections were upper respiratory infections, nasopharyngitis, sinusitis and urinary tract infections. Data presented did not show a clear pattern of occurrence of infection with increasing dose. Four cases of serious infection were reviewed in addition to that of the decedent. One was the case of septic arthritis mentioned above. In addition, one subject experienced an infected incision after surgical repair of a traumatic ankle fracture (the target joint was the wrist), another subject had a cellulitis of the leg (target joint wrist) and another subject experienced acute pyelonephritis. All three of these cases were determined to be unrelated to the gene transfer by the investigators.

Dr. Mease also reviewed abnormal results of liver function tests in some of the study subjects. All of the abnormal results were Grade 1 (< 2.5 x the upper limit of the normal value). Twenty-one of the 24 subjects with elevations in liver function tests were taking methotrexate, a drug known to be associated with abnormalities in these tests. Only five subjects had test results that were elevated more than 1.5 times the upper limit of normal, of which four subjects received the active agent. In three subjects the liver blood tests returned to normal spontaneously and in the other two subjects adjustment of the dose of methotrexate or adjustment of methotrexate and the dose of a cholesterol-lowering medication (from the statin class) resulted in resolution of the abnormality.

Data presented on the development of anti-AAV capsid neutralizing antibody titers showed that most subjects in the two higher dose cohorts had a substantial increase in their anti- AAV neutralizing antibody titers. The subject being discussed had anti-AAV antibody titers of 1:4 at baseline which had increased to1:128 when measured just prior to the second dose.

Vector biodistribution data were presented on subjects from the four dose cohorts used in the previous Phase I trial (OBA protocol 588) and in this Phase I/II study. The data demonstrated that as the dose increased, more subjects showed detectable levels of vector in the blood. At the highest dose, the dose the subject received, four of the eight subjects tested had detectable copies of vector DNA at four weeks and vector was still detectable at 8 weeks in three subjects.

Regarding the expression of the transgene, results of a radioimmunoassay that detects the level of functional TNF antagonist in the serum was presented. The limit of detection of this assay is 0.01 ug/ml. The assay was only be done on samples from subjects who were not on systemic TNF-antagonist systemic therapy because it can not distinguish between the transgene product and other TNF-antagonists. In eight subjects who received the highest dose ($1x10^{13}$) of vector, no TNF-antagonist was detected in the serum at four and twelve weeks. Further testing is ongoing on other samples.

Data were also presented on the expected systemic levels of TNF-antagonists for subjects on these agents. For adalimumab, which as noted was being given the decedent, the expected steady state drug concentration is 8-9 ug/ml. Levels detected in the subject's serum are presented below:

Date of Serum Collection	Timing	Result (ug/ml)
Feb 26, 2007	Prior to 1st injection	5.4
March 28, 2007	4 weeks after 1st injection	7.5
May 29, 2007	12 weeks after 1st injection	8.4
July 2, 2007	Prior to 2nd injection	8.6

Finally, Dr. Mease reiterated that the TNF-antagonists have a risk of serious infection including histoplasmosis. Specifically, based on data from controlled clinical trials and post-marketing surveillance, the incidence of clinically manifest histoplasmosis in patients on adalimumab is 4/4870 patients.

B. Dr. Fudman

Dr. Fudman focused on the recruitment of study subjects and the consent process. In particular he noted that study recruitment was tailored to the particular sites. Dr. Fudman noted that the informed consent is an ongoing process that does not end with the signing of the consent document but continues in the period leading up to dosing and beyond. Subjects are always permitted to withdraw their consent. In addition, subjects received a copy of the informed consent.

VI. Presentation of Case

Presenter: D. Kyle Hogarth, M.D., University of Chicago Hospitals

The case was presented by Dr. Hogarth, who was the subject's attending physician during her stay in the intensive care unit at University of Chicago Hospital. The subject was a 36-year-old female with a 15 year history of RA. While her disease initially involved her feet and knees, it subsequently involved the shoulders, elbows, wrists, knees and hands. She had been treated with disease modifying anti-rheumatic drugs (DMARDs) since the early 1990s, including TNF-antagonists since 2002 at which time she enrolled in an open label clinical trial for etanercept. In 2004, her treatment was changed to adalimumab in addition to methotrexate and low dose prednisone.

Her disease was characterized as well-controlled on her current medications with the exception of a persistently swollen and tender right knee for which she had received ten intra-articular steroid injections between 2000 and August of 2006. Her other medical history was only significant for recurrent herpes simplex virus (HSV) infections. Overall, she was active despite her disease and was a mother, married and worked full time.

On February 12, 2007, the subject enrolled in the study. She met all inclusion criteria and the results of her screening laboratory tests were normal. On February 26th, 2007, she received a first injection of the active study agent, total dose $5x10^{13}$ DNase resistant particles (DRP), into the right knee. Laboratory studies of samples taken before the injection, including a complete blood count, chemistries and liver function tests were normal. Synovial fluid drawn from her right knee showed no signs of infection.

In the weeks leading up to the second dose, the subject received treatment from outside medical providers. In late June, a private physician prescribed, by phone, a five day course of valacyclovir for a presumed recurrent HSV infection. On June 28th the subject received, by phone again, a seven day prescription for metronidazole for a gynecological infection. On June 29th and 30th the subject reported increased fatigue. On July 1st the subject reported unusual fatigue and low grade fevers. Nonetheless, she did go to work on July 2nd and after work went to the office of her rheumatologist to receive the second injection. A temperature of 99.6° F was recorded but her other vital signs were normal, as were the results of laboratory studies drawn that day. Synovial fluid drawn from her right knee that day did not show signs of inflammation and a culture for bacterial infection was negative. The subject was given her second injection of vector during that office visit.

During the evening of following her second injection (July 2nd), subject complained of nausea, and had vomiting, high fevers, and chills. Subject subsequently experienced diarrhea and abdominal pain, mainly in the epigastric area. Over the next several days the subject had episodes of fevers and she was seen by her primary care physician on July 5th, three days after receiving the second dose of the gene transfer product. Her physical examination and chest x-ray were both unremarkable at that time. She was prescribed an antibiotic, levofloxacin.

Two days after her visit to her primary care physician, the subject was seen in a local emergency room with complaints of nausea, vomiting , and headache. Her temperature there was 104°F. The evaluation there included a normal chest x-ray, normal chemistries and complete blood count and ultimately negative blood and urine cultures. She was given a diagnosis of viral syndrome, given an antiemetic, and was sent home.

A week after the second injection, the subject returned for the first time to the office of her rheumatologist, where she reported intermittent fevers, headaches and some vomiting. Her physical examination was significant for a tachycardia but her abdomen was not tender. She told her rheumatologist that she planned to see her primary care physician that day. At her primary care physician visit, she complained of "flu-like symptoms" nausea and difficulty sleeping. She reported having been seen in the emergency room. Laboratory studies were drawn by her primary pare physician that day and she was sent home. The results of those studies, which were reported at a later date for her physician showed an elevated white blood cell count of 29,000 and increased liver enzymes (aspartate aminotransferase (AST) 162, alanine aminotransferase (ALT) 125 and total bilirubin 1.2). In addition, a number of studies for etiologies of acute hepatitis, including cytomegalovirus, parvovirus, mononucleosis and Ehrlichia were negative.

On July 12th, the subject was admitted to a local hospital with abdominal pain, nausea, vomiting, diarrhea, fever and chills. Admission laboratory studies were significant forabnormal liver function tests, elevated white blood cell count and thrombocytopenia. Upon admission to the hospital the subject was started on antibiotics. Initial imaging indicated a possible cholecystitis. Viral studies done during the subject's hospitalization were significant for a positive PCR for HSV (types 1 & 2) from serum and a positive IgG antibody for HSV type 1.

During the first several days of the hospitalization, the subject continued to have fevers while on broad spectrum antibiotics and she developed worsening thrombocytopenia, liver function tests as well as coagulapathy. Five days after admission, she experienced an episode of hypotension, respiratory distress and a precipitous drop in her hemoglobin levels. She was intubated and transferred to the intensive care unit. Her blood pressure stabilized after admission to the intensive care unit, but she developed acute renal failure and had a sharp increase in one of her liver enzymes (AST). Imaging revealed what appeared to be a retroperitoneal hemorrhage. Arrangements were made for transfer to the University of Chicago Medical Center. At the time, her physicians thought that a liver transplant may be necessary because of worsening liver function and the coagulopathy.

After admission to the intensive care unit at the University of Chicago Hospital, she remained intubated, with hemodynamic instability and renal failure requiring continuous venous-venous hemofiltration. An enlarging retroperitoneal hematoma led to continued need for transfusions and caused an increase in abdominal pressure that altered respiratory mechanics. The large hematoma significantly displaced intra-abdominal organs. An evaluation for possible liver transplant was undertaken. A liver biopsy revealed that although there was liver injury, a transplant was not warranted because there was sufficient recoverable liver. Antibiotics were continued and multiple specialists were consulted.

Two days after being admitted to the University of Chicago Hospital, yeast was seen on a blood smear and antifungal treatment was started. Arterial embolization was attempted but was unsuccessful because a source of bleeding was not identified . Surgical evacuation of the clot was considered but due to her critical clinical condition was not thought to be possible. As a result of this large hematoma, the abdominal pressures continued to rise and the spleen and kidney demonstrated changes consistent with hypoperfusion. She became increasingly more difficult to ventilate, oxygenation requirements increased

and she developed what appeared to be acute respiratory distress syndrome. She became more hemodynamically unstable, requiring ionotropic medications.

As her clinical status continued to worsen, a decision was made to forgo resuscitation measures and the goal of care was changed to comfort care. Ventilatory and other support were withdrawn. She died on July 27, 2007, six days after being transferred to the University of Chicago Hospital.

VII. Presentation of Autopsy Data

Presenters: John Hart, M.D., University of Chicago Hospitals, and Karen Frank, M.D., Ph.D., University of Chicago Hospitals

A. Dr. Hart

Dr. Hart reviewed the autopsy data. Starting with the liver biopsy done the day after transfer to University of Chicago, he noted that there was a lack of significant necrosis. There were small areas of necrosis that were similar to those seen with adenovirus hepatitis;however, adenovirus was not detected in the subject's biopsy. Gomori methenamine silver (GMS) staining revealed histoplasmosis. Immunostains for herpes simplex virus were negative.

The liver on autopsy also showed no evidence of a viral infection but did show significant numbers of *Histoplasma capsulatum* spores in the random areas of necrosis. It was noted that there was an absence of granuloma formation in the liver around the histoplasmosis. Immunosuppression from the TNF-antagonists could lead to the absence of granuloma formation. However, he noted that granulomas are seen in some immunosuppressed patients, such as those with Acquired Immunodeficiency Syndrome. Dr. Hart also noted that there was no underlying liver disease.

Evidence of histoplasma infection was also found in the liver, lungs, bone marrow, spleen, lymph nodes, kidney and brain.

The retroperitoneal hemorrhage weighed 3.5 kilograms and caused significant displacement of the abdominal organs to the right and upward, including displacement of the diaphragm upward leading to compression of the lungs. It enveloped the left kidney leading to focal infarction of the kidney. No anatomic source for the bleeding could be identified on autopsy and there was no evidence of hemorrhage in the gastrointestinal tract, skin, lungs, bladder or other organs.

With respect to the subject's history of rheumatoid arthritis there was evidence of the surgical correction of the toe deformities but minimal swelling of the knees. The distal femoral condyles of both knees showed articular and subchondral changes consistent with RA but no evidence of synovitis. The bone marrow of the femoral condyles demonstrated *Histoplasma*.

Viral cultures were done on autopsy samples and the trachea, right and left knee and brain were positive for herpes simplex virus. There was no evidence of viral cytopathic effect in these or other tissues. Immunohistochemical stains for HSV performed on these tissues as well as the small bowel were negative. In addition, PCR by DNA extraction was done on samples of the brain and liver and were also negative for HSV.

B. Dr. Frank

Dr. Frank presented a summary of the microbiology and other findings. She noted that all blood and urine bacterial cultures were negative, as were tests for tuberculosis, HIV, Hepatitis B and C. The only positive cultures reported prior to the subject's death was a tracheal aspirate from the outside hospital that was positive for C*andida* and an urine culture that was done at University of Chicago that was positive for *Candida albicans*. As discussed by Dr. Hogarth, yeast was seen in peripheral blood smears starting three days after arrival to University of Chicago and a blood culture drawn prior to the subject's

death grew out *Histoplasma capsulatum* two days after her death. A post-mortem blood culture also grew out *Histoplasma capsulatum*.

Dr. Frank also noted that herpes simplex virus (HSV) was detected in the blood by PCR about a week prior to her death. The 300 copies/ml was on the low side of the range of results reported by that lab for HSV PCR (100 copies/ml to 450,000,000 copies/ml). A nasopharyngeal aspirate culture done during the hospitalization showed HSV Type I. As she explained, this can be seen in patients in the intensive care unit who have shedding of the virus. Only HSV Type 1 was seen in viral cultures at autopsy in samples from the brain, left and right knee and trachea.

VIII. Discussion of Case by Expert Panel

Panelists: Carol A. Kauffman, M.D., University of Michigan/U.S. Department of Veterans Affairs *(via teleconference)*; Jay Lozier, M.D., Ph.D., Warren Grant Magnuson Clinical Center, NIH; Bernard Roizman, Sc.D., The University of Chicago *(via videoconference)*; Leonard B. Seeff, M.D., NIDDK, NIH; and Richard J. Whitley, M.D., The University of Alabama at Birmingham *(via videoconference)*

Dr. Kauffman discussed the role of histoplasmosis in the current case. Clearly there was evidence of disseminated histoplasmosis. The subject's risk factors for histoplasmosis include the use of TNF-antagonists since 2002, low dose prednisone and methotrexate. Histoplasmosis is the most common fungal infection associated with TNF-antagonists. It is unclear from the history provided whether this is a new infection or reactivation. The onset of the infection cannot be known, but clearly did not start right before admission. The subject was likely already ill with histoplasmosis when she received the second injection of the AAV vector.

She noted, however, that data presented on the detection of histoplasmosis antigen does not establish infection prior to dosing. The value on 7/02/07, the day of the second dose, was reported as positive. However, the value was reported at less than 0.6ng/ml, which was below the level of accurate sensitivity of the test. As a clinician, Dr. Kauffman did not feel this was a positive result and a diagnosis of histoplasmosis could not be based on that type of result. The clinical course, however, is strongly suggestive that she had an active histoplasmosis at the time of injection on July 2, 2007.

The course was fulminant sepsis with disseminated intravascular coagulation. The case reports for patients on TNF-antagonists include some severely ill patients who required intensive care unit care and some deaths. The subject appeared more severely ill than most reported cases, but certainly the clinical course was within the spectrum described for disseminated histoplasmosis in patients taking anti-TNF agents. Such a fulminant course has been described in patients with Acquired Immunodeficiency Syndrome (AIDS). Most AIDS patients with severe histoplasmosis do not have well-formed granulomas. This subject's histopathology is similar to what is described in AIDS patients who die of histoplasmosis.

Drs. Whitley and Roizman provided their assessment of the role of HSV in the clinical course. Dr. Whitley noted the 300 copies/ml of HSV was detected by PCR in her blood. This was an unexpected result since HSV is not latent in white blood cells as are cytomegalovirus (CMV) or other herpes viruses. Second, she had a positive nasopharyngeal culture for HSV Type 1. Given her history of HSV-1 infections demonstrated by the pre-existing antibody, this is likely a reactivation of latent infection.

The autopsy date is more difficult to interpret because multiple cultures from the brain, as well as both synovial spaces, were positive for HSV-1. While disseminated HSV-1 infections are seen in immunocompromised hosts, they are uncommon. There are approximately 29 cases in the literature of women with disseminated HSV-1 infection. The majority of these cases have been seen in pregnancy but a few are in immunocompromised individuals. However, in this case, there was no histopathologic evidence of herpes simplex infection in the brain tissue. Also inconsistent with the diagnosis of disseminated HSV, immunohistochemical tests for HSV 1 were negative in the knees, brain and other

tissues that were culture positive. Finally, PCR performed on extracted DNA from the brain and liver were also negative.

The order of tissues collected for autopsy should be taken into consideration. The tissues that were positive for HSV by culture were collected after the tonsillar tissue, an area that had been previously shown to be positive for HSV. Contamination by virus of the last tissues collected is very possible, despite precautions taken during a nonsterile autopsy.

Taken together it's difficult to explain the conflicting results and attribute a role for HSV-1 infection as a cause of death in this particular individual.

Dr. Seeff presented his assessment on the liver findings. As stated by Dr. Hogarth, the subject was originally transferred to University of Chicago for possible liver transplant but a biopsy revealed "recoverable liver." Dr. Seeff noted that the liver dysfunction was primarily evidenced by the transaminitis with a striking rise in the AST at around the same time as the hematoma and the elevated bilirubin. He postulated that the acute rise is AST may have been related to muscle injury in the context of this large bleed especially since the muscle enzymes (creatine phosphokinase) were also elevated. He noted that the rise in bilirubin and other liver dysfunction was likely multifactorial and secondary to possible sepsis, transfusions and renal failure. He noted that it is not uncommon to see some level of liver dysfunction in critically ill patients with multiple medical problems. He also discussed the possibility of drug induced liver injury. Drug induced liver injury is always in the differential diagnosis for any patient receiving multiple medications. He did not feel in this case it was likely the etiology of the liver problems. Finally, he concluded that the liver dysfunction was not a contributor to her death.

Dr. Lozier discussed the hematologic data and focused on a possible etiology for the large retroperitoneal bleed. He first noted that the size of the retroperitoneal hematoma was extremely large even in comparison to those in his patients with a coagulation factor deficiency such as hemophilia. The cause of this type of bleed was not entirely clear. He noted that the lab tests were consistent with disseminated intravascular coagulation (DIC), a condition that is associated with histoplasmosis and can cause bleeding. He also noted her low platelets, which are characteristic of DIC; however, the levels were not as low as would be expected to cause this degree of hemorrhage. He concluded that DIC and thrombocytopenia were unlikely to explain this degree of bleeding.

He discussed one abnormal blood test result that was obtained around the time of the discovery of the retroperitoneal hemorrhage, a prolonged Russel viper venom time. This abnormal test could indicate an inhibitor to one of the coagulation factors. Such an inhibitor could help to explain the degree of hemorrhage. There are case reports of patients with acquired antibodies to prothrombin who experienced large flank hematomas. He suggested that further testing be done to look specifically at factor II (prothrombin), factor V and factor X levels.

Dr. Strome brought up the possibility of a mycotic aneurysm being the source of the bleed rather than a coagulation defect. Dr. Hart noted that due to the size of this hematoma, there was no possible way to identify a small vessel mycotic aneurysm. Despite careful analysis, no aneurysm was found; however, mycotic aneurysm could not be ruled out as a possible cause of the hemorrhage.

IX. RAC Discussion: Assessment of the Possible Role of Gene Transfer

Moderator: Dr. Federoff

The RAC members first addressed whether there was any evidence of contamination of the product by an infectious agent. Targeted Genetics provided product lot release data that demonstrated it passed sterility testing and adenovirus and herpes simplex virus were not detected. A *Histoplasma capsulatum* culture on the product was pending.

The second question addressed was whether the presence of a helper virus such as HSV could have led

to replication-competent AAV and an active liver infection. Initial PCR data from liver, lung and spleen demonstrated low levels in the liver and spleen of the vector but no detection of potentially replication competent AAV as demonstrated by detection of the wild-type AAV (wtAAV) rep gene. Dr. Bartlett noted that the detection of sample is very low and below the limit of quantitation in these assays and that the limited dissemination into extra-articular sites was predicted based on the preclinical data. Taken as a whole, he did not feel that the PCR data indicated ongoing replication of the vector genome in these tissues.

With respect to whether HSV could have acted as a helper virus, Dr. Bartlett noted that HSV has been demonstrated to be a helper virus for AAV in *in-vitro* assays but that *in-vivo* data are lacking. The main helper virus for AAV is adenovirus. Dr. Xiao and Dr. Roizman echoed that it was highly unlikely that HSV could have acted as a helper in the absence of evidence of wild type AAV. The lack of detection of wtAAV rep gene made it highly unlikely there was significant replication competent AAV. Moreover, for the HSV to act as a helper virus, it would have to occupy the same cell as the vector and/or replication competent AAV. As Dr. Federoff summarized, it was very unlikely that there was significant replication of the vector in the tissues.

The RAC members then considered whether there could have been expression of the transgene that led to unusually high systemic levels of TNF-antagonists causing over suppression of the subject's immune system. In animal studies, expression of the transgene had led to systemic detection of the TNF-antagonist. However, in previous subjects dosed at the highest dose level, who were not on TNF-antagonists, there was no detection of the transgene product in the serum using these same assays at four and twelve weeks. Targeted Genetic presented data performed by BioMonitor that indicated that serum levels of TNF-antagonists in this subject prior to dosing were not above the expected steady state. This assay does not distinguish between the two different TNF-antagonists: adalimumab, that was being taken systemically, and etanercept, the transgene product. Nonetheless, Dr. Bartlett explained that transgene expression from AAV vectors is typically not observed until one to two months after administration; therefore, it was unlikely that there was significant transgene product from the second dose.

The committee discussed whether there could have been an immune response to the AAV vector that contributed to the liver disease or overall clinical course. Immune response to AAV vectors was the discussion of a day long symposium at the RAC meeting June 19, 2007. In June, data were presented from a hemophilia trial in which subjects who received hepatic artery injection of an AAV vector experienced a transaminitis starting approximately four weeks after administration and simultaneously a decline in the expression of the transgene. The investigator in that trial presented data indicating there may have been a CD8+ T cell response against the AAV vector capsid that led to the destruction of transduced liver cells.

Dr. Ertl noted that although there were trivial amounts of AAV vector genome in the liver and in the spleen at autopsy, it does not exclude leakage of the vector outside of the knee prior to autopsy. The autopsy was done more than three weeks after administration. It is possible that that at an earlier stage, significantly more vector was present in these organs. Possibly, an immune reaction against the vector could have led to some slight liver damage but it would certainly not have led to the death of the subject, which, apparently, as far as she could tell, was in large part due to a very massive bleed. An immune reaction would not have contributed to this.

However, Dr. Bartlett noted that the subject had high titers of anti-AAV antibody at the time of dosing, 1:128. Based on studies of passive immunization strategies in mice, such a titer would be expected to severely limit dissemination of the vector. Dr. Ertl cautioned that this was certainly the case with adenovirus in which transgene expression is severely curtailed if you have these kinds of antibody titers but we do not know whether antibody bound AAV particles may not readily gain access to Fc positive cells and then enter a pathway that does allow access to the immune system. Dr. Bartlett agreed that this was not known, but stated that in experiments done in his lab, pre-existing immunity does not seem to increase innate inflammatory responses in the liver of AAV-treated animals.

It was also noted that if a T-cell response occurred against the vector, then inflammation would be expected in the knee where most of the vector was present. However, at autopsy not only was there a lack of synovitis but also the left and right knee appeared identical from a pathological perspective.

X. RAC Discussion: Informed Consent and Subject Selection

Discussion Leader: Dr. Kodish

Dr. Kodish framed the discussion by stating that the key ethical concepts that drive the modern understanding of research ethics are risk and benefit. Risk versus benefit must be balanced. With regard to risk, both probability and magnitude are important to consider. Unlike clinical ethics, where benefit and risk are weighed for an individual, research ethics considers potential benefits to society and to other individuals in addition to benefits to individual research participants. The central ethical question for this protocol is when is gene transfer an appropriate therapy for non-life-threatening conditions? Such a consideration takes into account quality of life issues and the failure of conventional therapy.

Ms. Shapiro discussed two inherent challenges regarding the therapeutic misconception in clinical trials: (1) how an informed consent document can clearly explain the theory behind the intervention without creating the misconception that the theory has been proven and (2) how the overall goal of a trial can be described in a way that does not imply that the goals of the current phase of the study (i.e., safety in early phase studies) are the same as the long-term goals (i.e., efficacy). Research studies suggest that participants systematically misinterpret the risk-benefit ratios of participating in research because, in part, they do not understand the underlying scientific methodology. Because most people have been socialized to believe that doctors always provide personal care, it may be difficult to persuade prospective participants that the clinical trial encounter is different, especially if the researcher is also the treating physician. In addition, research often involves people who are acutely ill and in distress. Such patients may tend to trust their well-being to any medical-related authority figure, which can undercut efforts to dispel the therapeutic misconception. In this case, a further complication to the therapeutic misconception was that the trial was designed such that re-administration of the active agent was timed, in part, on clinical symptoms in the target joint, as opposed to at a pre-scheduled interval. This timing could lead a participant to conclude that these injections were therapeutic. Ms. Shapiro suggested three methods of reducing the therapeutic misconception: (1) emphasize the research nature of the intervention and appropriately qualify statements or claims about anticipated outcomes and potential benefits in the "benefits" and "purpose" sections; (2) include in the "benefit" section a statement such as, "We do not expect you to receive any direct medical benefit from participation in this study"; and (3) consider using a neutral discloser who is distinct from anyone on the prospective participant's treatment team.

Dr. Kahn discussed the ethical issues related to recruitment of potential participants, including ensuring that individuals are adequately informed in their decisions to participate and that they are participating voluntarily. Any discussion of physician vs. investigator roles should include clear disclosure of the potential conflict of those roles. Waiting periods and/or a consent monitoring process are additional measures that can be taken to insure the consent process is objective and is not influenced by any pre-existing relationships between a subject and the investigator. He noted that the *NIH Guidance on Informed Consent for Gene Transfer Research*, (a guidance that is available to investigators, sponsors, institutional review boards [IRBs], potential participants, and the public) specifically discusses the conflict and offers sample language for disclosing the competing roles of investigators and physicians and the use of waiting periods.

The RAC members discussed briefly the decision to enroll subjects with non-life threatening illness in gene transfer and the best way to avoid therapeutic misconception. Dr. Brody noted that during the public discussion of the related phase I protocol, the RAC had considered the ethics of enrolling participants without immediately life threatening conditions. Ms. King noted, however, that the therapeutic misconception can be amplified for potential participants for whom conventional therapy has failed. In her view, consideration should be given to reimbursing subjects to reinforce the message that research is to benefit future patients and not current research participants. It was also noted that investigators as well

as participants can suffer from therapeutic misconception and that if the informed consent document and process reflect the investigator's bias, this could affect the potential subject's belief.

XI. Summary of Meeting/Dr. Federoff

Dr. Federoff summarized this complex case,and the possible future studies of AAV vector distribution, and the roles of HSV and coagulation factors.

A. RAC Recommendations

Dr. Federoff summarized the RAC's recommendations:

- The RAC proposes that its Gene Transfer Safety Assessment Board (GTSAB) continue to work with the University of Chicago Hospitals, Targeted Genetics Corporation, and invited experts in consultation with the FDA to identify all available blood and tissue samples from Subject 1209.

- The GTSAB should establish the priority of tests on these samples that would help clarify the role of the gene transfer product and the role of immune response against the vector in the death of Subject 1209.

- The GTSAB should offer advice as to how and where such testing would be accomplished.

Dr. Federoff reminded everyone that the advice of the GTSAB is not the same as the advice of the full RAC, and he stated that the GTSAB will deliver its final report at the December 2007 RAC meeting.

B. Committee Motion 1

Although not officially moved and seconded, the RAC accepted the above recommendations by a vote of 15 in favor, 0 opposed, 0 abstentions, and 0 recusals.

C. RAC Summary Statement

Dr. Federoff offered the following statement as a summary of the comments received from many RAC members and other experts related to this protocol:

- The RAC held an in-depth review and public discussion in September 2003 of the first phase I protocol (OBA Protocol 0307-588) using this same vector and transgene for rheumatoid arthritis (RA). The follow-up protocol (OBA Protocol 0504-705), in which this adverse event occurred, was not selected for public review. The RAC recognizes that they have the discretion to select protocols for public review based on a number of factors including (1) a new vector/new gene delivery system; (2) a new clinical application; (3) a unique application of gene transfer; and/or (4) other issues considered to require further public discussion. In reviewing this case, the RAC heard concern from its rheumatology consultants about the decision to use two different TNF antagonists in a single patient and the decision to include patients with RA, psoriatic arthritis and ankylosing spondylitis in a single trial. Therefore, the RAC noted that in selecting protocols for public review, the apparent safety (i.e. few or no adverse events) of the vector in a previously reviewed phase I or II study may not necessarily be indicative of a similar outcome in a subsequent trial. The safety of the vector as delivered must be considered with attention to the subject population chosen for a particular study.

- The importance of collecting adequate blood and tissue samples for any research protocol cannot be overstated; especially when the intervention targets such complex physiologic systems as the immune system or metabolic pathways. Redundancy in sample collection is important since it may be difficult in advance to anticipate all of the tests that may be needed. This becomes particularly evident when one needs to determine the causality for an adverse event.

- The RAC noted that both the sponsor and the investigator attempted to gather samples early in the initial hospitalization; however, since tests can only be ordered by the treating physicians, these efforts were not successful. Therefore, not only does the list of samples to be collected (e.g. whole blood, peripheral blood mononuclear cells, sera, tissue) need to be thought out in advance, but also the logistics of collecting and storing these samples must be determined. One method to accomplish this may be to develop a medical card that subjects could carry that could request that blood samples be collected at times of hospitalization and provide a mechanism for reimbursement, collection and preservation of the samples. This would not only help in the retrospective understanding of an event but might also be critical in other cases in helping to make a diagnosis.

- The RAC also noted that, not surprisingly, there was some initial confusion about whether the adeno-associated virus vector was derived from an adenovirus and therefore whether the abnormal liver tests were related to an adenoviral infection. While this initial misunderstanding did not lead to any changes in overall management, certainly having the most accurate information is critical to treatment of patients. It is incumbent upon the gene transfer community to educate their subjects, their families and outside providers as to the nature of the viral vectors often used in gene transfer as well as the specific transgenes. One mechanism to make this information accessible may again be the provision of medical cards with easily understood information, including contact information for investigators and sponsors. This card might include a URL for a web-based information source that would allow 24 hour access to critical information.

- The *NIH Guidelines* require that a discussion of autopsy occur as part of the informed consent process. Since the subject's family may not always be involved in that discussion or prepared to make that decision in the face of the death of a loved one, consideration should be given to developing written instructions outlining the subject's wishes that could be provided to their family in advance.

- In addition, the logistics of autopsy must be thought out in terms of the types and amount of tissues to be collected, how instructions for the autopsy will be communicated if performed at an institution that is not a trial site, and whether there could be a mechanism in place for transfer from an outside institution to the designated institution that understands the protocol for the study.

- A comprehensive plan for collection of blood and/or autopsy samples should be included in protocol development.

- Retrospectively, with all of the information we have before us, the non-specific, relatively benign sounding symptoms expressed by the subject the day of dosing take on more significance. The question is whether there are generalizable principles that can be discerned about timing of dosing, especially in safety trials where the potential side effects are not well characterized. In the case at hand, the protocol provided clear contraindications to receiving a second dose including pregnancy, a history of previous reaction to the injection or an adverse event that was probably related to the investigational agent. However, there may be more subtle considerations that will require some clinical judgments as to whether to delay a scheduled intervention for several days or longer. Even the practice of drawing labs the day of dosing but not necessarily having those labs back prior to the dose administration may need to be reconsidered. To optimize these clinical judgments, these issues require considerable forethought and clear articulation to investigators and in the protocol document.

- While gene transfer may have a relatively safe record compared to other therapeutic modalities, the perception may be that it is still a high risk therapy; therefore, the risk : benefit calculus that underlies offering gene transfer to subjects with chronic but non life-threatening conditions should clearly be articulated. Failure of conventional therapy should at a minimum be a consideration although it may not be determinative. Moreover, a subject's decision to either defer conventional

treatment or seek investigational treatment in addition to conventional treatment must also be considered provided it is an informed decision.

- It is not an exceptional situation for an investigator to also be the subjects' physician and indeed some subjects may seek out physicians because they are investigators and thus may be able to enroll the patient in a clinical trial. What is critical in this situation is that the potential conflict be acknowledged and discussed during the informed consent process. This includes a discussion of the different roles that the same individual, e.g., physician and researcher, will undertake. In certain circumstances, additional mechanisms may be advisable to ensure the decision is an informed one.

- As a corollary, some potential subjects, as well as investigators, may believe gene transfer has the potential to do what other conventional therapies cannot. To overcome the therapeutic misconception that may be part of human nature, the consent process must clearly articulate to subjects the goals of early safety trials and the unknown risks. This may go beyond just the consent document and include offering potential subjects information from outside sources that will help them make a more informed decision. Nonetheless, the consent document and process remains the critical tool to educate the subject. It should be developed in consultation with outside resources and evaluated periodically to ensure that it clearly communicates the nature of the trial and its risks and benefits.

XII. Public Comment

Mr. Robert Mohr, the husband of the subject, described his wife's life and asked the provocative question, "Would my wife still be alive today if she had not participated in this study?" He exhorted everyone related to this clinical trial to figure out what happened and not to let it happen to anyone else.

Other comments and questions were offered by Arthur W. Nienhuis, M.D., president of the American Society of Gene Therapy and faculty member at St. Jude Children's Research Hospital; L. Joseph Wheat, M.D., president and director of MiraVista Diagnostics; and several members of the press.

XIII. Discussion of Human Gene Transfer Protocol #0707-868: A Phase I Safety Study of Heat/Phenol-Killed, *E. coli*-Encapsulated, Recombinant Modified Peanut Proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) in Normal Volunteers Followed by Subjects Allergic to Peanuts

Principal Investigators:	Robert A. Wood, M.D., Johns Hopkins University, and Scott A. Sicherer, M.D., Mount Sinai School of Medicine *(via teleconference)*
Sponsor:	Allertein Therapeutics, LLC
RAC Reviewers:	Drs. Albelda, Kodish, and Vile
Ad hoc Reviewer:	Gideon Lack, M.D., King's College London *(via teleconference)*

Dr. Strome recused himself from discussion of this protocol due to a conflict of interest.

A. Protocol Summary

Allertein Therapeutics, LLC, is developing EMP-123 to treat peanut allergy. EMP-123 consists of heat/phenol-killed, *E.* coli-encapsulated, recombinant modified peanut proteins, Ara h 1 , Ara h 2 and Ara h 3 suspended in a thick solution for rectal administration. The coding regions of Ara h 1 , Ara h 2, and Ara h 3 peanut protein genes were modified to encode single amino acid substitutions designed to interfere with known sequential IgE-binding epitopes. These alterations are hypothesized to reduce the ability of these proteins to produce allergic responses following administration during immunotherapy by reducing the responses of mast cells. The product is designed to act as a vaccine, or immunotherapy, in which peanut-allergic patients are succesively vaccinated with increasing doses of EMP-123 to reduce or

eliminate sensitivity to peanut allergens and to prevent life-threatening or fatal reactions in peanut-allergic patients who are inadvertently exposed to peanut proteins.

Peanut allergy is estimated to occur in 0.5 percent to 1 percent of the U.S. population, and there is evidence that the prevalence of peanut allergy in the United States is rising. Currently there is no approved drug available to treat peanut allergy. Accidental ingestions are common, with up to 50 percent of peanut-allergic patients having an allergic reaction during a 2-year period. Allergic reactions to peanut can be severe and even fatal, and peanut and/or tree nut allergies account for the majority of cases of fatal food-induced anaphylaxis. Only about 20 percent of children outgrow peanut allergy, and thus for the majority of affected people peanut allergy is life-long.

The primary objectives of this study are to 1) determine in a small cohort of normal volunteers whether weekly rectal administration of escalating doses of EMP-123 is associated with any symptoms, and 2) determine in a small cohort of subjects with peanut allergy whether weekly rectal administration of escalating doses of EMP-123 is associated with more than mild allergic symptoms. The secondary objectives of this study are to 1) determine the rate of serious adverse events and adverse events reported, and 2) determine the rate of desensitization, as determined by oral food challenge (OFC) in peanut allergic subjects on EMP-123. The tertiary objectives of this study are to conduct and evaluate several indicators of immune function in the peanut allergic subjects.

The sponsor is currently conducting two animal studies of EMP-123 with the goal of providing support for the proposed clinical trial. A study to measure desensitization to peanut allergens in a mouse model of peanut anaphylaxis also serves as a safety study of the anaphylactic potential of EMP-123 in peanut-sensitized mice. In this mouse model, mice treated with EMP-123 showed no symptoms of anaphylaxis during the EMP-123 treatment period, indicating that EMP-123 by itself lacks anaphylactic potential. In addition, rectal administration of EMP-123 once a week for 3 consecutive weeks in peanut-sensitized mice resulted in desensitization of some but not all of the mice to orally administered peanuts. A 16-week repeated dose toxicity study of rectally administered EMP-123 in dogs has resulted in no adverse findings attributable to EMP-123 during the 16 weeks of dosing and the 2-week followup.

B. Written Reviews by RAC Members

Seven RAC members voted for in-depth review and public discussion of the protocol. Key issues included the construct, indication, and route of administration; significant potential safety concerns; and the involvement of healthy volunteers.

Three RAC members and an *ad hoc* reviewer provided written reviews of this proposed trial.,

Regarding preclinical issues, Dr. Albelda asked the investigators to discuss their data demonstrating that the point mutations introduced into the peanut allergens prevent IgE binding, to review the preclinical models used to test efficacy and toxicity, and to present updated results from the toxicology models. He requested that the investigators justify their use of normal participants in the first phase of this study. He requested that the investigators discuss how they know the procedure will not inadvertently sensitize the normal participants to become allergic to peanut antigens and how they can be certain they will not trigger an allergic reaction in the allergic participants or further sensitize them to the peanut allergens. Dr. Albelda further asked why the investigators chose not to conduct an open food challenge test in participants before they enter this study, why they propose using phenol-killed bacteria that could induce severe irritation when administered rectally, and what data indicate that the proposed two hours of observation postadministration is sufficient. Noting that the informed consent documents were generally clear, Dr. Albelda made several suggestions for improving the form to be signed by the normal participants, including including the specific exclusion of persons infected with the human immunodeficiency virus (HIV) and discussing in more detail the possibility of becoming sensitized to peanut allergens as a result of this study. For the peanut-allergic participant form, he suggested explaining “peanut IgE levels” in simpler language, including estimates about the potential side effects of the oral food challenge test, and stating whether there is a risk that this experiment could make

participants' allergies worse. In addition, any potential research participants who are also patients of the principal investigators in this study should be consented by an independent party.

Dr. Kodish limited his review to the two informed consent documents. One is for potential participants in the healthy volunteer group and the second is for potential participants who are peanut-allergic. Overall, he noted that if the investigational agent could induce new-onset peanut allergy, that possibility should be included as a specified risk in the healthy participant consent form, and the risk of exacerbation of peanut sensitivity should be included in the form for the peanut-allergic participants. In the healthy participant form, Dr. Kodish suggested changes to clarify the goal of the study, clarify the existence of the two phases of the study, and assist potential participants in distinguishing between the total length of the study and their time commitment to it. In the peanut-allergic participant form, Dr. Kodish asked for greater clarification of the different instructions to participants who pass the open food challenge test compared with those who fail it.

Dr. Vile requested a detailed presentation of the immunological basis of the concepts involved in the underlying rationale of this trial, including how the escalating dose of an allergen/immunogen can induce immunological tolerance, which models are available to test these concepts in the current setting, and how the mouse and dog allergy models relate to the human allergy. He asked the investigators to provide data to address the possibility that the normal participants might become sensitive to the peanut allergens and wondered whether any experimental models could address this concern. Dr. Vile also asked about the possibility that the vaccine would exacerbate the reactivity to the allergen proteins in peanut-allergic participants and how this question could be addressed in experimental models. He stated that all of the issues surrounding new or increased peanut sensitivity should be addressed more specifically in the two informed consent documents. Dr. Vile also requested that the investigators discuss what is known about the rectal administration of phenol in humans.

Dr. Lack focused his review on safety issues in normal and peanut-allergic participants, and clinical end points. For the normal participants, he asked whether any evidence exists that sensitization to the vaccine and allergic symptoms could develop in animals. Noting the possibility that exposing normal participants to low levels of peanut allergens might stimulate specific IgE responses and thus make them allergic, Dr. Lack suggested that the normal participants be clinically tolerant to peanut, be nonatopic, and have negative specific IgE and negative skin prick test to peanut. He suggested that the investigators demonstrate specific IgE binding prior to administering the vaccine, using known concentrations of recombinant modified Ara h 1, Ara h 2, and Ara h 3, and comparing them with wild-type Ara h 1, Ara h 2, and Ara h 3 in known concentrations. Dr. Lack stated that the safety profile of this trial would be enhanced considerably if the allergic participants could be shown by skin prick test prior to dosing to have relatively less reactivity to the engineered allergens compared with wild-type allergens. Regarding clinical end points, he suggested that the investigators conduct a baseline oral food challenge test in each participant prior to initiation of dosing and also that the end point skin prick test titration (for normal and peanut-allergic participants) be conducted not only to whole peanut antigen but also to wild-type Ara h 1, Ara h 2, and Ara h 3. Dr. Lack asked whether rectal administration of phenol is known to have any irritant or proinflammatory effects and stated that the investigators should provide an algorithm for managing allergic reactions and anaphylaxis in study participants.

C. RAC Discussion

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

- Dr. Kodish asked about the possibility of bringing in a third party to witness the consent process, which would be similar to using a research subject advocate (RSA).

- Several RAC members raised the question of whether the PIs' former patients should be excluded from participating in this study.

- Dr. Weber offered three suggestions for the informed consent document: The investigators should change "doctor" to "investigator," define the method of birth control requested, and include

a warning about not driving after taking antihistamines, which might be necessary should a reaction occur.

- Dr. Ertl asked whether the proposed two-hour waiting period following rectal dosing is adequate, in particular because of the potential for a more delayed reaction due to the allergen being encapsulated in bacteria.

Dr. Ertl requested a description of the clinical manifestations of rectal peanut allergy.

D. Investigator Response

1. Written Responses to RAC Reviews

The purpose of the proposed clinical trial is to determine whether EMP-123 delivered via the rectum is safe and in followup studies whether it can induce tolerance in peanut-allergic research participants. The delivery of the modified peanut proteins encapsulated within an intact cellular delivery system (*E. coli*) is anticipated to reduce the potential for allergic reactions following administration of the proteins by "hiding" the proteins from mast cells; that is, the antigens are not available to trigger mast cell degranulation while encapsulated.

The rectal route was chosen for delivery of EMP-123 following early discussions with the FDA. The investigators explained that earlier studies in a murine model of peanut anaphylaxis demonstrated a marked decrease or elimination of allergic symptoms when an EMP-123 prototype was delivered subcutaneously, intranasally, or intrarectally—but not orally. Although the rectal route was not initially considered for human trials, in the murine model it was the most efficacious route of delivery.

A previously characterized murine model of peanut anaphylaxis was used to test the efficacy of EMP-123 as well as the potential of EMP-123 to induce anaphylaxis in peanut-sensitized mice. The model uses female C3H/HeJ mice that are sensitized by repeated intragastric administrations of peanut and cholera toxin as an adjuvant and then challenged orally with peanut. This model mimics human peanut allergy both physiologically and immunologically and therefore is a useful tool for developing immunotherapeutic approaches for treating peanut allergy. However, it remains unclear whether this murine model is an appropriate model for studying whether EMP-123 will sensitize naive mice since the sensitization regimen used in this model requires an adjuvant and is species specific.

Regarding anaphylactic symptoms in the mice during treatment, the investigators explained that sensitized mice treated with vector control, vehicle control, or EMP-123 during the desensitization period showed no signs of anaphylaxis during the 1-hour observation period following each desensitization treatment. The mice were also observed for clinical signs the following day, and no symptoms were seen from the instillation of EMP-123 in the rectal vault of peanut-sensitized mice. Rectal irritation was not observed in any of the mice that received rectal desensitization treatments. Additional results of the murine trials of rectal administration of EMP-123 once a week for 3 consecutive weeks in peanut-sensitized mice included an increase in mean body temperature relative to vehicle control, a decrease in mean plasma histamine levels relative to vehicle control, a decrease in the mean serum peanut-specific IgE levels, and a decrease in the mean spleen cell production of interleukin (IL)-4, IL-5, IL-10, and IL-13 relative to vehicle control.

Results of the beagle studies indicated that no toxicity was produced in male or female dogs rectally administered low-dose (approximately equivalent to the anticipated maximal clinical dose) or high-dose EMP-123 (approximately 10 times the anticipated maximal clinical dose) once a week for 16 weeks. According to the investigators, these results provide reassurance that EMP-123 did not cause sensitization to any of the modified peanut proteins.

Regarding the possibility of inadvertently sensitizing normal participants to become allergic to peanut allergens, the investigators stated that individuals who are not allergic consume peanuts regularly in their diet without adverse allergic symptoms. These individuals generally do not make peanut-specific IgE but

instead make peanut-specific IgG as a part of the normal immune response to any ingested food protein. If individuals routinely ingest peanuts in their diets, the administration of the small amounts of peanut proteins in EMP-123 is not likely to alter the immune response. Testing of the nonallergic participants for sensitization to peanuts will be added to the protocol, at the request of RAC reviewers. In multiple correspondences between the sponsor and the FDA regarding EMP-123, the FDA specifically recommended that EMP-123 be tested in nonallergic participants before being tested in participants with a history of peanut allergy.

The risk that a peanut-allergic participant might become more sensitive or reactive to peanut, rather than becoming tolerant, cannot be ruled out. However, based on the results in the murine model of peanut allergy, which showed desensitization to peanuts (and thus no additional sensitization of mice to peanuts as a result of EMP-123 dosing), the sponsor believes it is unlikely that peanut-allergic participants who receive EMP-123 will become more sensitive to peanuts.

Although the sponsor plans to revise the clinical protocol to eliminate all oral food challenges, immunologic studies and end point titration skin tests will be conducted before and after EMP-123 dosing in both normal and peanut-allergic participants to evaluate any immunologic effects of EMP-123. The end point titration skin prick test will use extract from whole peanuts; doing so will address the main concern of whether EMP-123 sensitizes normal volunteers or further sensitizes peanut-allergic participants to subsequent exposure to peanuts.

The use of epinephrine to treat a reaction during dosing with EMP-123 will result in the discontinuation of an individual from continued active dosing but will not stop the overall study so that, as the investigators explained, the use of epinephrine is not inhibited by the potential to stop the study. The protocol requires that the study be stopped if more than one participant requires more than one injection of epinephrine during the dosing of EMP-123.

Dr. Wood explained that stool guaiac testing is the most sensitive test available for early colitis. The investigators intend to figure out how best to deal with the chance of false positive stool guaiacs due to the irritation caused by rectal administration.

2. Responses to RAC Discussion Questions

Regarding the issue of witnessing the informed consent process, both Drs. Wood and Sicherer stated that an RSA would be available to assist in this protocol.

Dr. Wood argued that a 2-hour waiting period following dosing represents more than adequate safety, since most reactions occur within minutes and, even if delayed, would be extremely unlikely to exceed 90 to 120 minutes. Study participants will be provided with medications for self-administration, as they would be for their peanut allergy anyway, to be used if a delayed reaction occurs. The mouse model never revealed a single symptom with the EMP-123; the only symptoms occurred with the peanut challenge.

Dr. Wood agreed to exclude anyone with an asthmatic condition from the normal participant group (but not the peanut allergy group) to rule out the possibility that an asthma attack could be confused with an adverse reaction.

Regarding the possibility of this product sensitizing non-peanut-allergic participants, Dr. Wood stated that it would be inconceivable that someone who is eating peanut regularly at doses thousands of times larger than what will be given in this trial could be immunologically changed by way of this product. The protocol has been modified to exclude anyone with any peanut-specific IgE, since that individual might have some risk of an allergic reaction even though she or he is clinically tolerant to peanuts.

Dr. Wood explained that the clinical manifestations of rectal peanut allergy are not known. However, he offered examples of latex allergy in barium studies that are conducted using latex instruments. Those manifestations included localized itching and swelling, systemic manifestations of urticaria and pruritus,

and quick absorption of the latex allergen, resulting in distant manifestations similar to what would occur if the allergen had been ingested. Dr. Wood noted that anaphylactic shock would be a possibility.

E. Public Comment

Dr. Shyam S. Mohapatra, University of South Florida, asked the investigators whether they had looked at T-cell responses and whether the investigators believed that the five-participant control group was adequate.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

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

Clinical/Trial Design Issues

- Understanding the safety of this intervention is critical. As such, normal controls with a history of asthma, even a mild form, should not participate in the study because the similarities in asthma symptoms and allergic symptoms would make safety data difficult to interpret.

- A better measure of peanut tolerance in the controls is needed. In addition to documented consumption of peanuts in a concentrated form, a serum peanut-specific IgE test and a skin prick test should be administered prior to enrollment. Only controls with negative test results should be enrolled.

- There were questions about whether a stool guaiac test to monitor local rectal reactions to the EMP-123 is adequate and whether the 2-hour waiting period after administration is long enough to monitor serious delayed allergic reactions to EMP-123. Although the rationale for the use of the stool guaiac test and the 2-hour waiting period were determined to be sound, administering a questionnaire would provide an additional measure of safety and enable more data to be gathered. The questionnaire should include questions about any local rectal reactions and symptoms of inflammatory bowel disease as well as any signs or symptoms of an allergic response to the vaccine between study visits. In addition, it may be advisable for any participant who develops signs or symptoms of rectal inflammation or has a positive guaiac test to undergo further evaluation of the rectum (e.g., sigmoidoscopy).

- The inclusion criteria should mandate use of an "effective method of birth control." The acceptable methods of contraception that would be considered effective by the investigators should be defined.

Ethical/Social/Legal Issues

- Although the study is limited to adults, it is possible that some participants may have been patients, as children, of the investigators. In these cases, such participants could misperceive the investigator's role. This potential for role conflict should be addressed during the consent process and in the informed consent document. It may also be advisable for participants who are former patients of the investigators to delay signing the informed consent document for 24 hours after presentation of the protocol. Further information on role conflicts is available in the *NIH Guidance on Informed Consent for Gene Transfer Research*, available at http://www4.od.nih.gov/oba/rac/ic/index.html.

- Consideration should be given to involving an observer in the consent process who is not associated with the study. The observer's role would be to document the validity of the consent, including that the participant understood the study's risks and lack of direct benefit, and gave consent voluntarily without duress. Any concerns about the consent process should be reported to the IRB by the observer.

- Since participants who experience allergic reactions will be given antihistamines, the informed consent document and process should make clear that antihistamines have sedating effects, and therefore, participants will need a designated driver if they receive antihistamines.

G. Committee Motion 2

Dr. Federoff summarized the RAC recommendations, which will be included in the letter to the investigators and the sponsor expressing the comments and concerns of the RAC. No formal motion was made or seconded regarding these summarized recommendations. The vote was 14 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XIV. Consideration of a Proposed Major Action: Under Section III-A-1 of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*: Deliberate Transfer of Chloramphenicol Resistance to *Rickettsia conorii* (*R. conorii*) and *Rickettsia typhi* (*R. typhi*)

Presenters: Abdu Azad, Ph.D., University of Maryland, and David H. Walker, M.D., The University of Texas Medical Branch at Galveston *(via videoconference)*

A. Introduction

Dr. Corrigan-Curay explained that under the *NIH Guidelines* (Section III-A), a Major Action is required for experiments involving the transfer of a drug resistance trait to a microorganism if the transfer could compromise the treatment of disease. Prior to proceeding, such experiments require publication in the *Federal Register*, a review by the RAC, RAC recommendations to the NIH Director, NIH Director approval, and then institutional biosafety committee approval. For this proposal, notice was published July 24, 2007, in the *Federal Register* (72 FR 40320), and materials submitted by the PIs were made available on the OBA Web site shortly thereafter. Three public comments were received. The proposal was reviewed by the RAC Biosafety Working Group and by outside experts.

The Major Action under discussion was the transfer of chloramphenicol resistance to *R. typhi* and *R. conorii*. This research was proposed by Dr. David Walker and Dr. Abdu Azad.

Dr. Corrigan-Curay introduced the expert consultants for this Major Action: Dr. Wei-Mei Ching, Naval Medical Research Center; Dr. Stephen J. Dumler, The Johns Hopkins Medical Institutions; Dr. Marina E. Eremeeva, CDC *(via teleconference)*; Dr. David W. Hackstadt, NIAID *(via teleconference)*; and Dr. Christopher W. Woods, Duke University. *Ad hoc* members for the purpose of this discussion were Dr. Claudia Mickelson, Massachusetts Institute of Technology (former RAC Chair), and Dr. Naomi Rosenberg, Tufts University School of Medicine (former RAC member, *via teleconference*). Dr. Michael J. Miller was present *(via teleconference)* as the CDC liaison.

B. Dr. Walker

Dr. Walker presented a summary of *Rickettsia* biology and rickettsial diseases, the proposed approaches to this project, and a discussion of the benefits and risks of the proposed project, and. He provided information about the safety and security of the facilities and the personnel training at the two locations where the proposed project will be done,. He explained that all work involving the handling of live rickettsial organisms would be performed in biosafety level 3 (BSL-3) facilities, which are select agent secure. At both locations current policies dictate that all laboratory personnel must be cleared by the U.S. Department of Justice prior to being granted access to the BSL-3 facilities, that trainees are required to pass the biological laboratory institutional examinations to obtain access to the BSL-3 facilities, and each person must be trained by a mentor in the BSL-3 laboratory for a minimum of 2 weeks.

Rickettsia are obligate intracellular, gram negative bacteria that reside in the cytosol of host cells. They are vector-borne pathogens transmitted by fleas, lice, mites or ticks. There are more than 20 species of

Rickettsia which range in pathogenecity from nonpathogenic to causing fatal disease. Regarding treatment of *Rickettsia* infection, doxycycline is the drug of choice for treating presumptive or confirmed rickettsial diseases in adults (including pregnant women) and children. Chloramphenicol was formerly the second line drug used to treat diseases primarily in children and pregnant women for whom tetracyclines were at one time contraindicated. Clarithromycin can be considered an effective alternative to tetracyclines for children infected with *R. conorii* (Mediterranean spotted fever). The most effective antibiotics against *Rickettsia in vitro* are doxycycline, fluoroquinolones, rifampin, thiamphenicol, and telithromycin.

Dr. Walker explained that several antibiotics had been tested unsuccessfully for use in selecting Rickettsia transformants. Rickettsial transformants' resistance to chloramphenicol will be more easily selected than those resistant to other antibiotics. Knockout strains of *R. typhi* will be used to generate information on the virulence factors of typhus group *Rickettsia* possibly useful for vaccine development and as targets for drug development. He also discussed random inactivation of rickettsial genes with the Himar1 transposon, site-specific gene inactivation by homologous recombination, and the rationale for knocking out the methyltransferase gene. The knockout mutant would not be more virulent than the parental strain because knockout strains are generally less fit for growth, and the investigators will attempt to knock out virulence factors and select for less virulent strains.

Dr. Walker explained that *R. typhi* and *R. conorii* will be used in the proposed project rather than *R. prowazekii* because the latter is a select agent. He also indicated that the mouse models that best mimic human vasculitis involve *R. typhi* and *R. conorii*. Knowledge obtained from this project may be used in the future to knock out genes in the Madrid E strain of *R. prowazekii*. Although many researchers are attempting to find non-antibiotic selection markers for Rickettsia, to date none have worked. Dr. Walker believes that chloramphenicol will be an effective marker with which to study virulence genes. The data from the proposed studies on the experimentally determined virulence factors may be useful for the development of a vaccine against *Rickettsia*.

As to whether it might be possible that the genetically modified *R. typhi* strain could persist over time and infect a flea being used to deliver the challenge strain, Dr. Walker noted that the BSL-3 and animal BSL-3 (ABSL-3) facilities at the two proposed locations are flea free, and fleas will not be used in any aspect of the proposed research. He responded to concerns about whether a resistance trait could be transferred from *R. typhi* or *R. conorii* to other *Rickettsia* by stating that there is no evidence that *R. typhi* or *R. conorii* can be transformed naturally or that they can be transformed experimentally without electroporation.

Because *Rickettsia* cannot be transmitted from person to person, the only humans at risk of becoming infected as a result of this proposed research are the laboratory workers.

C. Dr. Azad

Dr. Azad explained that *R. typhi* occurs in the United States sporadically, mostly in Hawaii, Texas and a few other Southern States. The goal of the proposed research is to knock out *R. typhi* virulence genes as well as genes encoding effectors of the type IV secretion system, all with the purpose of developing attenuated mutant strains that might be useful as live vaccines. To create such strains, the researchers will first knock out the known rickettsial virulence genes as well as each conserved type IV effector in *R. typhi*. To achieve this objective, the researchers need a robust selection marker, and they anticipate that the chloramphenicol acetyltransferase (CAT) resistance cassette will serve this purpose.

Dr. Azad reviewed the difficulties in developing Rickettsial genetic systems due to the lack of robust selectable markers, the rationale for use of the CAT marker, and previous studies using that marker. He also discussed the rationale for gene knockout, the proposed strategy of genetic manipulation in *R. typhi*, genome-wide Himar1 transposon mutagenesis of *R. typhi*, schematic presentation of plasmid construction for transformation of a Himar1-based transposon into *Rickettsia*, and the additional identification step of genetic barcoding (third base substitution and modification of restriction enzyme sites) of *Rickettsia*.

He concluded by saying that the risk of infection of laboratory personnel working with these bacteria in the a BSL-3 facility is very low, and the danger to the public is essentially nil.

D. Outside Experts

Dr. Woods briefly reviewed the literature and resources for physicians relating to the current recommendations for treating rickettsial infection in the. Doxycycline is considered first-line therapy for all rickettsial infections regardless of the patient's age. Chloramphenicol remains the drug of choice for most rickettsial infections in pregnant women and those who have a true tetracycline allergy. Chloramphenicol is listed as the leading alternative therapy for most rickettsial infections. Despite the latter recommendation, the use in the United States of chloramphenicol for rickettsial infections is infrequent and is unlikely to increase.

Dr. Kirchhoff read into the record comments received from Dr. Didier Raoult, Marseilles School of Medicine. Dr. Raoult's remarks concluded by stating that it is dangerous to transform typhus group *Rickettsia* (i.e., *R. typhi*) with a chloramphenicol-resistance gene unless this work is performed in a BSL-4 laboratory. He suggested amoxicillin as an alternative marker; its minimal inhibitory concentration is 128 micrograms per milliliter, and at this concentration, it inhibits rickettsial growth.

Dr. Kirchhoff also read into the record comments received from Dr. David Heymann, Assistant Director-General for Communicable Disease and Representative for the Director General for Polio Eradication of the World Health Organization (WHO). His comments were submitted on behalf of the WHO in response to a query from the OBA. *R. typhi* and *R. conorii* are found widely in the developing world. Only doxycycline and chloramphenicol are recognized treatment options, with doxycycline being the preferred treatment for both and chloramphenicol considered to be second-line treatment. No data are available on the frequency of use of chloramphenicol in the developing world for treatment of rickettsial diseases. Regarding worldwide availability of chloramphenicol for the treatment of these diseases, both oral and intravenous chloramphenicol are readily available, although shortages of the intravenous form occur.

E. RAC Discussion

Dr. Kirchhoff noted that all three comments received in response to the *Federal Register* notice supported allowing the proposed experiments to proceed.

Dr. Kirchhoff summarized the steps taken by the RAC Biosafety Working Group to address issues surrounding this proposed research. Questions and concerns from RAC members included the possible use of other antibiotic markers, evaluation of the specific goals of the proposed project ,the risk to laboratory workers, the risk of escape from a BSL-3 facility versus a BSL-4 lab, and whether *R. conorii* and *R. typhi* should be considered separately. In comparing BSL-3 to BSL-4, Dr. Weber indicated that BSL-4 labs are designed to protect people and do not include any additional protections to prevent escape from the lab.

Dr. Kirchhoff brought up that one of the points the RAC had struggled with was whether the investigators had established that there were no alternative markers. In particular, Dr. Raoult had suggested the possible use of ampicillin resistance for this purpose. Dr. Walker responded that *Rickettsia* had beta-lactamase genes and that his lab had tried to use ampicillin as a marker. Dr. Azad commented that they had also tried rifampin without success. Dr. Mickelson responded that she was not sure the same problems with spontaneous resistant mutants would not occur with chloramphenicol. She wondered if it appeared to be a better marker only because it had not been used yet and therefore there was no resistance.

Dr. Mickelson noted that at her institution it is policy not to allow use of antibiotic markers that are second line treatments. The concern is not human to human transmission but protection of the laboratory workers, who may have an unknown sensitivity to the first line treatment.

For *R. typhi*, Dr. Walker responded that ciprofloxacin is equivalent to the response of chloramphenicol; thus, ciprofloxacin could be used as a second-line treatment and chloramphenicol as a third-line treatment. For *R. conorii*, erythromycin is equivalent to chloramphenicol, so erythromycin could be used as a second-line treatment and chloramphenicol as a third-line treatment.

Dr. Dumler explained that concepts such as the use of chloramphenicol have become entrenched over the years, and it has been difficult to dislodge those recommendations because of a relative lack of clinical trials. However, in recent years it has become clear that doctors are not inclined to use chloramphenicol for a variety of reasons: It is less available in the United States than it is in other countries, and it is not favored for many reasons, including that epidemiologic data indicate that it is an inferior drug compared with doxycycline and other antibiotics. In his opinion, based on his experience in the U.S., chloramphenicol is no longer an appropriate drug to be used for rickettsial infections, except in rare circumstances.

Dr. Kirchhoff asked whether the data available on alternative antibiotics for *R. typhi* vs. *R. conorri* were sufficiently different to justify separate consideration of the proposed experiments involving these two species. Dr. Dumler noted that Dr. Raoult had referred to case reports of treatment failure with the other drugs. Dr. Dumler explained that in-vitro testing of *R. typhi* strains has indicated that they are uniformly sensitive to chloramphenicol and ciprofloxacin, and variably so to macrolide antibiotics. Clinical data for alternative drugs for treating *R. typhi* were available from case reports but not clinical trials. Dr. Mickelson asked whether this relative lack of clinical data on alternative drugs for treating *R. typhi* versus *R. conorii* should factor into the RAC's considerations. Given the difficulty in performing controlled clinical studies, Dr. Federoff asked whether the RAC accepted the currently available, albeit rather meager empirical data that there are active antibiotics against these bacterial species other than chloramphenicol. If the committee accepted the premise that chloramphenicol need not be considered the only second line antibiotic, then the committee could proceed to discussing the proposed studies.

Dr. Federoff then asked both Dr. Dumler and Dr. Walker whether the proposals should be considered together or separately. He accepted their recommendation to consider them together as one proposal.

Dr. Kirchhoff discussed the concern of the Biosafety Working Group regarding the possibility of containment failures, such as that which apparently occurred in the U.K. (foot and mouth disease virus release from a laboratory), . Dr. Walker stated that Rickettsia has never spread out of a laboratory, and he noted the long record of success in public safety of protecting the community by use of BSL-3 facilities. The conclusion from the discussion was that, based on experience, there is a very small, but nonetheless finite, possibility of a containment failure, with no documented evidence of such an event occurring to date with Rickettsia.

Dr. Kirchhoff explained that the Biosafety Working Group had discussed biosecurity concerns related to the possible use of chloramphenicol-resistant *R. conorii* and *R. typhi* as bioweapons but concluded that these concerns were not determinative.

Dr. Somia noted that the Biosafety Working Group had also discussed the issue of sharing of rickettsial strains produced by the proposed project. He asked what procedures would be in place to ensure that recipient laboratories requesting modified strains also had equivalent levels of biosafety and security. Dr. Walker and Azad explained that strains would be sent only to investigators having BSL-3 facilities as well as specific approval for work with rickettsial agents.

Dr. Dewhurst asked about the training of personnel and medical surveillance at the two institutions. Dr. Walker explained that before working in the BSL-3 facility, staff must pass several examinations. Dr. Azad explained that laboratory workers are required to carry a card with information about the agents, disease, and emergency contacts.

Dr. Azad stated that no animal research is involved in the proposed project

F. RAC Consensus

Dr. Federoff summarized the RAC consensus on this proposed research as follows:

These studies and discussion of them did not consider aerosol challenge.

Stipulations:

- The discussion and development of recommendations are based on the opinions of infectious disease experts in *Rickettsia* that for *R. typhi* after doxycycline, antibiotics other than chloramphenicol are suitable and preferable as second-line treatments .

- With regard to the containment of experiments in the laboratories of Drs. Walker and Azad, all research should use BSL-3 practices. Access should be restricted to well-trained personnel, and a standard initial and ongoing training program should ensure that these personnel are properly trained.

- Backup power for the BSL-3 facility should be used to ensure that security remains in place, even in the event of a power failure.

- Genetic barcoding should be employed, as described by Dr. Azad.

Health surveillance program:

- Baseline rickettsial titers should be determined for all laboratory workers, and baseline blood samples should be taken from all laboratory workers and then stored.

- Individuals with allergy to doxycycline should be excluded from working on the proposed project.

- A medical card to be carried by all laboratory workers should identify the organism to which the laboratory worker may be exposed, list the key personnel responsible for diagnosis and treatment, and list the relevant CDC telephone number and a 24-hour contact number for the PI.

- A detailed standard operating procedure in case of laboratory exposure should be developed, including key personnel who are charged with diagnosing and treating exposed workers, and the steps an exposed laboratory worker should take when the key personnel are not onsite.

The authority to create chloramphenicol-resistant *Rickettsia* is limited to Drs. Walker and Azad. If chloramphenicol-resistant *Rickettsia* are transferred to the laboratory of another investigator, all work with them must be carried out at the BSL-3 level with the stipulations outlined above and must utilize the security measures that are currently being used in the laboratories of Drs. Walker and Azad.

G. Committee Motion 3

Dr. Federoff asked the RAC to vote on the above consensus, although he noted that the wording might undergo minor changes before it is finalized. The vote was 12 in favor, 0 opposed, 0 abstentions, and 0 recusals.

XV. Day One Adjournment/Dr. Federoff

Dr. Federoff adjourned Day One of the September 2007 RAC meeting at 5:45 p.m. on September 17, 2007.

XVI. Day Two Call to Order and Opening Remarks/Dr. Federoff

Dr. Federoff opened Day Two of the September 2007 RAC meeting at 8:15 a.m. on September 18, 2007.

XVII. Minutes of the June 19-21, 2007, RAC Meeting/Drs. Ertl, Kahn, Somia, and Wei

Having reviewed the AAV portion of the minutes, Drs. Ertl and Somia declared that portion to be a concise summary of what occurred, with only minor changes suggested by Dr. Ertl. Dr. Kahn noted a few minor corrections but otherwise thought the minutes reflected the conduct of the RAC's discussion.

A. Committee Motion 4

It was moved by Dr. Kahn and seconded by Dr. Somia that the RAC approve the June 19-21, 2007, RAC meeting minutes. The vote was 12 in favor, 0 opposed, 1 abstention, and 0 recusals; three of the in-favor votes were cast by teleconference (Drs. Albelda, Ertl, and Vile).

XVIII. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Albelda, Federoff, and Strome

A. Submissions and Amendments

Dr. Federoff reported that of the 13 protocol submissions received by the OBA in the past 3 months, 11 were not selected for public review at this RAC meeting. Of the 11 protocols not selected, 10 are for cancer, and one is for HIV. Five protocols employ an adenovirus vector, three employ a plasmid vector, two employ a fowlpox/vaccinia virus vector, and one employs *Saccharomyces cerevisiae*. During the reporting period, 120 amendments were received by OBA, including 18 protocol design modifications, 37 PI and/or site changes, 42 annual reports, seven responses to *Appendix M-I-C-1* of the *NIH Guidelines* (three of the seven were protocols that had been reviewed publicly by the RAC), and 16 other amendments.

Three protocol amendments were discussed briefly:

- 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. The amendment concerned the timing for reinstating enzyme replacement using pegylated ADA in order to improve the engraftment of the gene-modified engrafted cells to take hold as well as to mitigate the risk of infection in the participants.

- Protocol #0401-625 (an *Appendix M-I-C-1* response): A Phase I Study of a Tropism-Modified Conditionally Replicative Adenoviral Vector (Ad5-Delta-24-RGD) for Intraperitoneal Delivery in Ovarian and Extraovarian Cancer Patients. This protocol was discussed at the March 2004 RAC meeting, and as was requested, biodistribution and toxicology data sets were submitted and are available for review.

- Protocol #0612-821: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study to Evaluate the Efficacy and Safety of Ad5FGF-4 in Female Patients with Stable Angina Pectoris Who Are Not Candidates for Revascularization. This trial was discussed at the March 2007 RAC meeting. The amendment stated that robust biodistribution studies would be conducted prior to application for licensure and that revisions had been made to the informed consent document to include the necessity of a placebo group and define the risk of cardiac catheterization.

B. Adverse Events

Dr. Strome discussed the AEs that were reported to OBA during this reporting period (Dr. Federoff recused himself from this discussion due to a conflict of interest). A total of 101 AEs were reported from 24 trials, with the majority unrelated to the gene transfer products; 15 new reports submitted were considered by the PIs to be possibly related to the gene transfer products; for three AEs, follow-up reports changed the attribution of the AE from possibly related to unrelated. In total, the GTSAB reviewed 33 AEs, including 17 initial reports and 16 followup reports that were submitted by the investigators and sponsors from 12 trials, including 3 initial reports resulting from the death of the subject participating in Protocol #0504-705, discussed earlier at this RAC meeting.

The AEs experienced by one participant in Protocol #0307-591, "An Open-Label Safety Study of Escalating Doses of SGT-53 for Systemic Injection in Patients with Advanced Solid Tumor Malignancies" were discussed. Dr. Esther Chang (Georgetown University) and Dr. Leanne S. Sleer (Synergene Biotechnology Group) were present to answer RAC questions about the protocol. The primary goal of the study is to evaluate the safety of escalating doses of SGT-53, a plasmid encoding the p53 tumor suppressor and anti-transferrin receptor single chain antibody fragment to target the complex to tumor cells, administered twice weekly for five weeks. The secondary goals is to evaluate the pharmacokinetics of SGT-53 and the therapeutic effect relative to tumor size and progression. The study participant with colonic adenocarcinoma metastatic to the liver who experienced the AEs had a medical history of poorly controlled hypertension, type 2 diabetes, gout, and glaucoma. The signs and symptoms associated with the AEs included fever, hypertension, bilateral streaking perihilar opacities and peribronchial cuffing revealed on chest X-ray, 3-mm nodules in his left upper lung and small bilateral effusions revealed on computerized axial tomography scan, anemia, thrombocytopenia, and elevated D-dimer, lactic acid dehydrogenase, fibrinogen, and haptoglobin.

Dr. Strome asked Drs. Chang and Sleer to explain the binding specificity of their single-chain fragment in humans and whether any other studies or further characterizations were anticipated. Dr. Chang stated that their data safety monitoring board (DSMB) had recommended that the dose be reduced by one-half, and that the investigators were implementing this change. In addition, they will increase the early management of participants who experience chilling, will predose with acetaminophen, and will admit each participant for 48 hours of observation prior to the first infusion. Both the protocol and the informed consent document are in the process of being revised to reflect all these changes.

C. RAC Recommendations

Dr. Strome requested that the RAC express its appreciation to the investigators in Protocol #0307-591 for complying with the DSMB recommendation to reduce the dose. He also asked that the RAC vote on the following three recommendations:

- The investigators should develop a specific protocol for monitoring hematologic parameters in study participants and the results of the monitoring should be reported to the RAC.

- The investigators should consider readjusting the inclusion/exclusion criteria based on this experience with poorly controlled hypertension.

- The informed consent document should be modified to include the risks of these AEs.

D. Committee Motion 5

It was moved by Dr. Weber that the RAC approve the above recommendations. The vote was 9 in favor, 0 opposed, 0 abstentions, and 1 recusal.

XIX. National Heart, Lung, and Blood Institute Gene Therapy Resource Program

Presenter: Sonia Skarlatos, Ph.D., NHLBI, NIH

Dr. Skarlatos provided information about the NHLBI's Gene Therapy Resource Program (GTRP). The goals of the GTRP are to provide resources and regulatory assistance for NHLBI-funded investigators and to support gene transfer clinical trials. The GTRP infrastructure consists of the Preclinical Vector Core laboratory, which produces large- and small-scale viral and nonviral vectors for studies in basic research directed toward clinical applications. The Clinical-Grade AAV Vector and Lentiviral Vector Cores produce scalable clinical-grade AAV and lentiviral vectors for use in heart, lung, and blood clinical studies. The Pharmacology Toxicology Core performs toxicology testing and biodistribution studies for vectors in large- and small-animal models as a prerequisite for use in clinical studies. The Clinical Coordinating Center manages and coordinates all GTRP activities in conjunction with the NHLBI, including the resource application process, the Scientific Review Board, and the Steering Committee; develops and maintains the GTRP database and Web site; provides regulatory assistance to NHLBI-supported investigators; and administers clinical trial funds.

Three entities or boards provide oversight for the GTRP. The Scientific Review Board, a virtual board that conducts the initial peer review of applications, is composed of scientific experts in heart, lung, and blood diseases, gene transfer, vectors, biostatistics, ethics, and clinical trial design. To date, about 50 experts have agreed to serve on the Scientific Review Board. The Steering Committee will ensure compliance with GTRP procedures and will conduct secondary reviews of applications; face-to-face meetings will take place quarterly, and teleconferences will be arranged on an as-needed basis to supplement the quarterly meetings. The NHLBI Gene Transfer Working Group will govern the program as a whole, and prioritize funding for approved applications based on programmatic relevance and budget. It is made up of NHLBI staff members from all divisions, meets weekly, and plans to conduct periodic site visits of the core facilities and possibly to host scientific meetings.

Starting in 2008, applications will be accepted for preclinical vectors and regulatory assistance at any time during the year. February 15 and September 15 will be the deadlines for pharmacology/toxicology studies, clinical-grade vectors, and clinical trial support. The GTRP will attempt to process applications quickly, striving for an 8-week review period and providing a response to each investigator within 2 weeks of Steering Committee review.

A. RAC Discussion

Dr. Strome asked whether the FDA considers nonhuman primate (NHP) studies as essential preclinical studies in gene transfer. Dr. Takefman responded that the FDA typically does not recommend nonhuman primate studies but does recommend that researchers use a more relevant animal species in the preclinical trials conducted after small-animal (usually murine) studies have been completed. Dr. Skarlatos noted that the Pharmacology/Toxicology Core would be able to perform studies with many types of animals including rodents, sheep, pigs and NHP.

In response to Dr. Federoff's statement that some of the academically based contract manufacturing groups are concerned about liability of their product in clinical trials, Dr. Skarlatos explained that the GTRP will have an office that is responsible for reviewing potential liabilities and assisting in agreements between investigators and core facilities.

XX. Nanoparticle-Mediated Gene Delivery

Presenter: Shyam S. Mohapatra, Ph.D., University of South Florida

Dr. Mohapatra reviewed the potential role of nanotechnology in gene transfer and described how natural polymeric nanoparticles, specifically, chitosan nanoparticles, play a role in gene transfer. Chitosan is a modified carbohydrate polymer derived from the chiton components of the shells of crustaceans. It has been sold as a dietary supplement for many years. As a gene delivery system, chitosan has many advantages, including that it is natural, biocompatible, biodegradable in the human body by natural

enzymes, nonimmunogenic, immunostimulatory, nontoxic, nonhemolytic, and cost effective. Chitosan can be modified into many different forms, including powder, paste, pill, and fiber.

Chitosan is positively charged and thus binds negatively charged DNA electrostatically. Dr. Mohapatra discussed several *in vitro* studies of gene transfer efficacy and *in vivo* targeted delivery in preclinical experiments. He concluded that chitosan-based nanoparticles hold great promise in gene delivery for chronic diseases because they offer targeted delivery and thus can be administered at lower doses, are noninvasive, have a long shelf life, and are cost effective. Preclinical studies suggest that they may be useful for a variety of human diseases.

A. RAC Discussion

RAC members offered several comments about the safety of nanoparticles and about protecting laboratory workers, particularly since these are small enough to pass through high-efficiency particulate air filters, which have a maximal efficiency of around 200 nanometers.

XXI. Closing Remarks and Adjournment/Dr. Federoff

Dr. Federoff thanked the OBA staff members for all their work in preparation for the discussion of Protocol #0504-705, which was discussed on the morning of September 17.

Dr. Federoff thanked the participants and adjourned the meeting at 10:15 a.m. on September 18, 2007.

[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.]

_____
Jacqueline Corrigan-Curay, J.D., M.D.
Acting RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following 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 into the Minutes after that meeting.

Date: _____

_____
Howard J. Federoff, M.D., Ph.D.
Chair
Recombinant DNA Advisory Committee

Attachment I
Recombinant DNA Advisory Committee Roster

Chair

FEDEROFF, Howard J., M.D., Ph.D.
Executive Vice President and Executive Dean
Georgetown University Medical Center
Building D, Room 120
4000 Reservoir Road, NW
Washington, DC 20007

Members

ALBELDA, Steven M., M.D.
Professor of Medicine
Pulmonary, Allergy, and Critical Care Division
Department of Medicine
School of Medicine
University of Pennsylvania
Abramson Research Center, Room 1016B
3615 Civic Center Boulevard
Philadelphia, PA 19104

BARTLETT, Jeffrey S., Ph.D.
Associate Professor
Gene Therapy Center
Columbus Children's Research Institute
Columbus Children's Hospital
Room WA3010
700 Children's Drive
Columbus, OH 43205

DEWHURST, Stephen, Ph.D.
Professor
Department of Microbiology and Immunology
University of Rochester Medical Center
Box 672
601 Elmwood Avenue
Rochester, NY 14642

ERTL, Hildegund C.J., M.D.
Director
Vaccine Center
The Wistar Institute
School of Medicine
University of Pennsylvania
3601 Spruce Street
Philadelphia, PA 19104

FAN, Hung Y., Ph.D.
Director
Cancer Research Institute
University of California, Irvine
Sprague Hall, Room 102
Mail Code 3900
Irvine, CA 92697

FLINT, Jane, Ph.D.
Professor
Department of Molecular Biology
Princeton University
Lewis Thomas Laboratory, Room 234
Princeton, NJ 08544

GRANT, Ellen E., Ph.D., LCSW-R
Vice President, Community Affairs
HealthNow New York Inc.
257 West Genesee Street
Buffalo, NY 14202-2657

KAHN, Jeffrey P., Ph.D., M.P.H.
Maas Family Chair in Bioethics
Director
Center for Bioethics
University of Minnesota
Boynton Health Service Building, Room N504
410 Church Street, SE
Minneapolis, MN 55455-0346

KIRCHHOFF, Louis V., M.D., M.P.H.
Professor
Departments of Internal Medicine (Division of Infectious Diseases) and Epidemiology
University of Iowa
Bowen Science Building
Room 4-403
51 Newton Road
Iowa City, IA 52242

KODISH, Eric D., M.D.
F.J. O'Neill Professor and Chair
Department of Bioethics
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195

SHAH, Prediman K., M.D.
Director
Division of Cardiology
Atherosclerosis Research Center
Cedars-Sinai Medical Center
Suite 5531
8700 Beverly Boulevard
Los Angeles, CA 90048

SHAPIRO, Robyn S., J.D.
Professor and Director
Center for the Study of Bioethics
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226-3548

SOMIA, Nikunj V., Ph.D.
Assistant Professor
Department of Genetics, Cell Biology and Development
Molecular Genetics Institute
University of Minnesota, Twin Cities
Jackson Hall, Room 6-160
321 Church Street, SE
Minneapolis, MN 55455

STROME, Scott E., M.D.
Professor and Chairman
Department of Otorhinolaryngology-Head and Neck Surgery
School of Medicine
University of Maryland
Suite 500
16 South Eutaw Street
Baltimore, MD 21201

VILE, Richard G., Ph.D.
Professor of Immunology
Consultant in Molecular Medicine
Department of Molecular Medicine
College of Medicine
Mayo Clinic
Guggenheim Building, 18th Floor
200 First Street, SW
Rochester, MN 55905

WEBER, David J., M.D., M.P.H.
Professor of Medicine, Pediatrics and Epidemiology
Division of Infectious Diseases
Schools of Medicine and Public Health
The University of North Carolina at Chapel Hill
Bioinformatics Building, Room 2163
Campus Box 7030
Chapel Hill, NC 27599-7030

WEI, Lee-Jen, Ph.D.
Professor
Department of Biostatistics
School of Public Health
Harvard University
677 Huntington Avenue
Boston, MA 02115

WILLIAMS, David A., M.D.
Professor of Pediatrics
Director
Division of Experimental Hematology
Children's Hospital Medical Center
University of Cincinnati
Mailing Location 7013
3333 Burnet Avenue
Cincinnati, OH 45229

ZAIA, John A., M.D.
Chairman
Division of Virology
Beckman Research Institute
City of Hope
1500 Duarte Road
Duarte, CA 91010-3000

OBA Director

PATTERSON, Amy P., M.D.
Director
Office of Biotechnology Activities
Director
Recombinant DNA Program
Recombinant DNA Advisory Committee
Office of Science Policy
Office of the Director
National Institutes of Health
U.S. Department of Health and Human Services
Suite 750, MSC 7985
6705 Rockledge Drive
Bethesda, MD 20892-7985

Acting Executive Secretary

CORRIGAN-CURAY, Jacqueline, M.D., J.D.
Acting Executive Secretary
Recombinant DNA Advisory Committee
Medical Officer
Office of Biotechnology Activities
Office of Science Policy
Office of the Director
National Institutes of Health
U.S. Department of Health and Human Services
Suite 750, MSC 7985
6705 Rockledge Drive
Bethesda, MD 20892-7985

Participants

AZAD, Abdu, Ph.D.
Professor of Microbiology and Immunology
Department of Microbiology and Immunology
School of Medicine
University of Maryland
Health Sciences II Building, Room 433
20 South Penn Street
Baltimore, MD 21201

BRODY, Baruch A., Ph.D. *(via teleconference)*
Leon Jaworski Professor of Biomedical Ethics
Director
Center for Medical Ethics and Health Policy
Baylor College of Medicine
Andrew Mellon Professor of Humanities
Department of Philosophy
Rice University
1 Baylor Plaza
Houston, TX 77030-3498

CHING, Wei-Mei, Ph.D.
Senior Scientist
Viral and Rickettsial Diseases Department
Naval Medical Research Center
Building 503, Room 3N85
503 Robert Grant Avenue
Silver Spring, MD 20910-7500

CROW, Mary K., M.D.
Professor of Medicine
Benjamin M. Rosen Chair and Director of Rheumatology Research
Hospital for Special Surgery
Weill Medical College
Cornell University
535 East 70th Street
New York, NY 10021

DUMLER, J. Stephen, M.D.
Professor
Division of Medical Microbiology
Department of Pathology
The Johns Hopkins Medical Institutions
Ross Building, Room 624
720 Rutland Avenue
Baltimore, MD 21205

EREMEEVA, Marina E., M.D., Ph.D.
(via teleconference)
Senior Service Fellow
Rickettsial Zoonoses Branch
Division of Viral and Rickettsial Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Coordinating Center for Infectious Diseases
Centers for Disease Control and Prevention
U.S. Department of Health and Human Services
CLFT
Atlanta, GA 30329-4018

FRANK, Karen, M.D., Ph.D.
Assistant Professor of Pathology
Department of Pathology
University of Chicago Hospitals
MC 0001
5841 South Maryland Avenue
Chicago, IL 60637

FUDMAN, Edward J., M.D.
Austin Rheumatology Research, PA
Suite 702
1301 West 38th Street
Austin, TX 78705

HACKSTADT, David W., Ph.D.
(via teleconference)
Chair
Biosafety Committee
Head
Host-Parasite Interactions Section
Laboratory of Intracellular Parasites
National Institute of Allergy and Infectious Diseases
National Institutes of Health
U.S. Department of Health and Human Services
Room 6212
903 South Fourth Street
Hamilton, MT 59840

HART, John, M.D.
Professor of Pathology
Department of Pathology
University of Chicago Hospitals
MC 6101
5841 South Maryland Avenue
Chicago, IL 60637

HOGARTH, D. Kyle, M.D.
Assistant Professor of Medicine
Department of Pulmonary and Critical Care Medicine
University of Chicago Hospitals
MC 6076
5841 South Maryland Avenue
Chicago, IL 60637

KAUFFMAN, Carol A., M.D. *(via teleconference)*
Professor
Department of Medicine
University of Michigan
Chief of Infectious Diseases
Ann Arbor VA Medical Center
U.S. Department of Veterans Affairs
2215 Fuller Road
Ann Arbor, MI, 48105

KING, Nancy M.P., J.D.
Professor
Department of Social Sciences and Health Policy
Director
Program in Bioethics, Health, and Society
School of Medicine
Wake Forest University
Medical Center Boulevard
Winston-Salem, NC 27517

LACK, Gideon, M.D. *(via teleconference)*
Professor of Paediatric Allergy
King's College London
Department of Medicine
St. Thomas Hospital
Fourth Floor, North Wing
Lambeth Palace Road
London SE1 7EH
United Kingdom

LOZIER, Jay, M.D., Ph.D.
Staff Physician
Department of Laboratory Medicine
Warren Grant Magnuson Clinical Center
National Institutes of Health
U.S. Department of Health and Human Services
Building 10, Room 2C306
MSC 1508
10 Center Drive
Bethesda, MD 20892-1508

MATTESON, Eric L., M.D., M.P.H.
Professor of Medicine
Chair
Division of Rheumatology
Department of Medicine
Mayo Clinic
200 First Street, SW
Rochester, MN 55905

MEASE, Philip J., M.D.
Chief, Rheumatology Clinical Research
Seattle Rheumatology Associates
Swedish Hospital Medical Center
Clinical Professor
School of Medicine
University of Washington
10th Floor
1101 Madison Street
Seattle, WA 98104

MICKELSON, Claudia A., Ph.D.
Deputy Director
Biosafety Program
Environment, Health, and Safety Office
Massachusetts Institute of Technology
Environmental Programs Office
Building N52, Suite 496
Cambridge, MA 02139

MILLER, Michael J., Ph.D. *(via teleconference)*
Associate Director for Science
Office of the Director
Division of Viral and Rickettsial Diseases
National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Coordinating Center for Infectious Diseases
Centers for Disease Control and Prevention
U.S. Department of Health and Human Services
CLFT
Atlanta, GA 30329-4018

MOHAPATRA, Shyam S., Ph.D.
Professor of Medicine
Director of Basic Sciences
Division of Allergy and Immunology
Department of Internal Medicine
University of South Florida
MDC-19, Room 2536
12901 Bruce B. Downs Boulevard
Tampa, FL 33612

ROIZMAN, Bernard, Sc.D. *(via videoconference)*
Joseph Regenstein Distinguished Service Professor
Departments of Molecular Genetics and Cell Biology and of Biochemistry and Molecular Biology
Cancer Research Center
The University of Chicago
Marjorie B. Kovler Viral Oncology Laboratories
Room 107
910 East 58th Street
Chicago, IL 60637

ROSENBERG, Naomi, Ph.D.
(via teleconference)
Professor
Department of Pathology
School of Medicine
Tufts University
Jaharis Building, Room 512
150 Harrison Avenue
Boston, MA 02111

SEEFF, Leonard B., M.D.
Senior Scientist for Hepatitis Research
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services
Claude E. Pepper Building, Room 9A27
MSC 2560
31 Center Drive
Bethesda, MD 20892-2560

SKARLATOS, Sonia, Ph.D.
Director
Vascular Biology Research Program
Division of Heart and Vascular Diseases
National Heart, Lung, and Blood Institute
National Institutes of Health
U.S. Department of Health and Human Services
Room 10186
MSC 7956
6701 Rockledge Drive
Bethesda, MD 20892-7956

WALKER, David H., M.D. *(via videoconference)*
Professor and Chair
Department of Pathology
The University of Texas Medical Branch at Galveston
301 University Boulevard
Galveston, TX 77555-3989

WHITLEY, Richard J., M.D.
(via videoconference)
Professor of Pediatrics, Microbiology, Medicine and Neurosurgery
Director
Division of Pediatric Infectious Diseases
The University of Alabama at Birmingham
Children's Harbor Building, Room 303
1600 Seventh Avenue, South
Birmingham, AL 35233

WOODS, Christoper W., M.D., M.P.H.
Chief of Infectious Diseases
Assistant Professor
Co-Director
Center for Global Health
Durham VA Medical Center
Division of Infectious Diseases
Department of Medicine
Duke University Medical Center
508 Fulton Street
Durham, NC 27705

XIAO, Xiao, Ph.D. *(via teleconference)*
Fred Eshelman Distinguished Professor of Gene Therapy
Division of Molecular Pharmaceutics
School of Pharmacy
The University of North Carolina at Chapel Hill
Campus Box 7360
Kerr Hall
Chapel Hill, NC 27599-7360

Nonvoting Agency Representatives

National Science Foundation
NSF Representative TBD

U.S. Department of Agriculture

JONES, Daniel D., Ph.D.
National Program Leader/Biotechnology
Cooperative State Research, Education, and Extension Service
U.S. Department of Agriculture
Waterfront Center, Room 3444
800 Ninth Street, SW
Washington, DC 20024

U.S. Department of Commerce

LEVIN, Barbara, Ph.D.
Project Leader
Biotechnology Division
National Institute of Standards and Technology
U.S. Department of Commerce
MSC 8311
100 Bureau Drive
Gaithersburg, MD 20899-8311

MCCAMMON, Sally L., Ph.D.
Science Advisor
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Unit 98
4700 River Road
Riverdale, MD 20737

U.S. Department of Energy

DRELL, Daniel W., Ph.D.
Biologist
Life Sciences Division
Office of Biological and Environmental Research
U.S. Department of Energy
SC-72
19901 Germantown Road
Germantown, MD 20874-1290

U.S. Department of Health and Human Services

Office for Human Research Protections

ANDREASON, Paul, M.D.
Compliance Oversight Coordinator
Division of Compliance Oversight
Office for Human Research Protections
U.S. Department of Health and Human Services
Tower Building, Suite 200
1101 Wootton Parkway
Rockville, MD 20852

BORROR, Kristina C., Ph.D.
Director
Division of Compliance Oversight
Office for Human Research Protections
U.S. Department of Health and Human Services
Tower Building, Suite 200
1101 Wootton Parkway
Rockville, MD 20852

Food and Drug Administration, Office of Cellular, Tissue, and Gene Therapies

MAHER, V. Ellen, M.D.
Team Leader
Division of Clinical Evaluation and Pharmacology/Toxicology
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
Food and Drug Administration
U.S. Department of Health and Human Services
1401 Rockville Pike
Rockville, MD 20852-1448

TAKEFMAN, Daniel M., Ph.D.
Chief
Gene Therapy Branch
Division of Cellular and Gene Therapies
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
Food and Drug Administration
U.S. Department of Health and Human Services
HFM-720
1401 Rockville Pike
Rockville, MD 20852-1448

U.S. Environmental Protection Agency

FREDERICK, Robert, Ph.D.
Program Manager
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Mail Code 8623D
401 M Street, SW
Washington, DC 20460

MILEWSKI, Elizabeth, Ph.D.
Senior Biotechnologist
Office of Prevention, Pesticides, and Toxic Substances
U.S. Environmental Protection Agency
East Tower, Room 625
MC 7201
401 M Street, SW
Washington, DC 20460

Liaison Representative

FAYL, Gilbert, Ph.D.
Secretary of External Affairs
European Academy of Sciences and Arts
Brussels, Belgium

Attachment II
Public Attendees

Anne Aberdeen, FDA, DHHS
Jim Ackland, Global BioSolutions
Glen E. Amundsen, Smith Amundsen, LLC
Pervin Anklesaria, Targeted Genetics Corporation
Ashok Batra, FDA, DHHS
Steve Bauer, FDA, DHHS
William Berlin, HCI/Allertein
Lilia Bi, FDA, DHHS
Diane E. Bovenkamp, Foundation Fighting Blindness
Haim Burstein, Targeted Genetics Corporation
Stacy D. Byars, Targeted Genetics Corporation
Catherine F. Cabot, Centocor, Inc.
Jeff Carey, Novavax, Inc.
Barrie J. Carter, Targeted Genetics Corporation
Jo Cato, Cato Research
Esther Chang, Georgetown University
Theresa Chen, FDA, DHHS
Shirley M. Clift, Cell Genesys, Inc.
Anna Derbij, Transgene
Chris Evans, Harvard Medical School
Joseph Fatandoni, MaxCyte, Inc.
Steve Ghiuizzani, University of Florida
Chris Goldrick, Edelman
Jaydee Hanson, International Center for Technology Assessment
Hiroto Hara, DNAVEC Corporation
Raymond D. Harris, SAIC-Frederick, Inc.
Changting Haudenschild, FDA, DHHS
Alison Heald, Targeted Genetics Corporation
Rebecca Hoffman, Abbott Laboratories
Atm S. Hoque, FDA, DHHS
Deborah Hursh, FDA, DHHS
Akihiro Iida, DNAVEC Corporation
Travis Che Jarrell, Summit Drug Development Services, LLC
Carl Johnson, Hereditary Disease Foundation
Sarah Kennett, FDA, DHHS
Deborah Kirschling, Allertein Therapeutics, LLC
William T. Lee, Cato Research
Susan Leibenhaut, FDA, DHHS
Aginz Lim, FDA, DHHS
Robert Lindblad, The EMMES Corporation
Stephen D. Litwin, The Biologics Consulting Group, Inc.
Diane Maloney, FDA, DHHS
Jennifer A. McDonnell, The Children's Hospital of Philadelphia
Maritza McIntyre, The Biologics Consulting Group, Inc.
Elizabeth McKenna, Foundation Fighting Blindness
Edmund V. Mickunas, Applied Genetic Technologies Corporation
Andra Miller, The Biologics Consulting Group
Hiroaki Mizukami, Jichi Medical University
John E. Mordock, Neurologix, Inc.
Keith Munson, Targeted Genetics Corporation
Lori Murray, Targeted Genetics Corporation
Arthur W. Nienhuis, St. Jude Children's Research Hospital

Patricia L. Novak, Cardium Therapeutics
Sarah Okada, FDA, DHHS
Jeffrey M. Ostrove, Ceregene, Inc.
Stewart Parker, Targeted Genetics Corporation
Richard Peluso, Targeted Genetics Corporation
John Perez, Abbott Laboratories
Kathleen Pirollo, Georgetown University
Ryan M. Porter, Harvard Medical School
Guang Qu, The Children's Hospital of Philadelphia
Brian Reid, WeissComm Partners
Paul Richards, FDA, DHHS
Stephen M. Rose, Foundation Fighting Blindness
Jeffrey J. Rudy, Celladon Corporation
Sheryl Ruppel, SAIC-Frederick, Inc.
Ruth M. Saltzstein, Targeted Genetics Corporation
Mercedes Serabian, FDA, DHHS
Jeff Siegel, FDA, DHHS
Stephanie Simek, FDA, DHHS
Leanne S. Sleer, Synergene Biotechnology Group
Aimee Smart, VIRxSYS Corporation
Jeff Smith, FDA, DHHS
Jennifer Spinella, Cardium Therapeutics
Don Stablein, The EMMES Corporation
Rachael Strong, FDA, DHHS
Michele Taylor, Cardium Therapeutics
A. Tesfaye, FDA, DHHS
Jie Tian, FDA
Gabor Veres, Applied Genetic Technologies Corporation
Amanda Wade, The Children's Hospital of Philadelphia
Samuel C. Wadsworth, Genzyme Corporation
Kim Wagner, Celladon Corporation
Jamie Weinstein, The EMMES Corporation
L. Joseph Wheat, MiraVista Diagnostics
Velma Wing, University of Pennsylvania
Celia Witten, FDA, DHHS
Robert Wood, Johns Hopkins University
Christopher W. Woods, Duke University
T. Fraser Wright, The Children's Hospital of Philadelphia
Ernest W. Yankee, Sanbio Research and Diagnostics
Yongjie Zhou, FDA, DHHS
Sheila Cohen Zimmet, Georgetown University Medical Center
Krisztina M. Zsebo, Celladon Corporation

Press
Andrew Bridge, Associated Press
Emily Brown, Bloomberg News
Jennifer Corbett Dooren, *Wall Street Journal*
Ángel Gonzalez, *The Seattle Times*
Calvin Jackson, Office of Communications, OD, NIH
Jocelyn Kaiser, *Science Magazine*
Brandon Keim, Wired
Joe Palka, National Public Radio
Meredith Wadman, *Nature*
Rick Weiss, *The Washington Post*
Kevin Wolf, *The Seattle Times*
Donna Young, AHC Media

Attachment III
Abbreviations and Acronyms

AAV	adeno-associated virus
AAV-2	adeno-associated virus serotype 2
Ad	adenoviral, adenovirus
AE	adverse event
BSL	biosafety level
CDC	U.S. Centers for Disease Control and Prevention
DHHS	U.S. Department of Health and Human Services
DIC	disseminated intravascular coagulation
DMARD	disease-modifying anti-rheumatic drug
DNA	deoxyribonucleic acid
DSMB	data and safety monitoring board
Fc	fragment crystallizable
FDA	Food and Drug Administration, DHHS
GTRP	Gene Therapy Resource Program, NHLBI
GTSAB	Gene Transfer Safety Assessment Board
GTWB	Gene Therapy Working Group, NHLBI
H. capsulatum	*Histoplasma capsulatum*
HIV	human immunodeficiency virus
HSV-1	herpes simplex virus type 1
IgE	immunoglobulin E
IL	interleukin
IRB	institutional review board
LFT	liver function test
NCI	National Cancer Institute, NIH
NEI	National Eye Institute, NIH
NHLBI	National Heart, Lung, and Blood Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
PI	principal investigator
RA	rheumatoid arthritis
RAC	Recombinant DNA Advisory Committee
RSA	research subject advocate
SAE	serious adverse event
TNF-α	tumor necrosis factor-alpha
TNFR:Fc	human TNF-α receptor-immunoglobulin G1 Fc fusion
WHO	World Health Organization