

Summary:
**Recombinant DNA Advisory Committee Discussion of Serious Adverse Event on
AAV Gene Transfer Trial, September 17, 2007**

On September 17, 2007 the Recombinant DNA Advisory Committee (RAC) undertook a review of the death of a participant on a gene transfer trial for patients with rheumatoid arthritis. Office of Biotechnology Activities (OBA) protocol 0504-705, entitled *A Phase I/II Study of Repeat Intra-Articular Administration of tgAAC94, a Recombinant Adeno-Associated Vector Containing the TNFR:Fc Fusion Gene, in Inflammatory Arthritis with and without Concurrent TNF- α Antagonist*, used a recombinant adeno-associated virus (AAV) serotype 2 vector containing a DNA fragment that encodes the extracellular region of tumor necrosis factor alpha receptor fused to the Fc fragment of human IgG1 (TNFR:Fc fusion gene). Expression of this fusion gene results in production of the TNFR:Fc protein, which has an amino acid sequence that is identical to etanercept. Such tumor necrosis factor (TNF) antagonists bind *TNF* alpha and are used in treating rheumatoid arthritis (RA). A 36 year old woman enrolled in this trial died of multiorgan failure just three weeks after receiving a second dose of the active agent by intra-articular injection.

The RAC received information from the principal investigator (PI) of the study, the clinicians and pathologists from the institution where the subject died and experts in rheumatoid arthritis (RA), infectious disease, hematology, hepatology, and gene transfer.

From the various sources, the RAC learned that after the death of the subject, clear evidence of disseminated histoplasmosis was discovered from blood cultures drawn just before and after her death. This subject was on a systemic TNF antagonist, adalimumab, for her RA at the time of enrollment in the trial. Experts presented data on the known risk of developing disseminated histoplasmosis in patients using these drugs. In addition, the RAC learned that during the course of her illness, she developed an acute and ongoing retroperitoneal bleed that resulted in a large hematoma (3.5 kilograms at autopsy). This bleeding led to the need for multiple transfusions of blood products. The expanding hematoma caused significant displacement of internal organs leading to splenic and renal ischemia and altered respiratory mechanics. The resulting increase in abdominal pressure likely led to hemodynamic instability. An anatomic site for the bleeding was not identified before the subject's death or at autopsy.

These pathology results appeared to negate a *primary* role for the injected recombinant vector; however, only initial data were available at the time of the meeting. Results presented at the meeting (received just two days prior) showed low levels of the vector in the subject's liver and spleen. These results are consistent with the findings of preclinical biodistribution studies, which showed some evidence of vector in tissues outside the injection site. There was no evidence of replication competent virus as demonstrated by the absence of AAV rep gene. Additional tissue and blood samples remain to be tested for presence of vector and replication competent AAV. Data on the level of TNFR:Fc protein produced by the transgene were not available at the time of the meeting. It was

not clear when such data would be available because a validated assay to distinguish between systemically administered TNF antagonist and the TNFR:Fc recombinant product has not yet been developed.

The RAC also heard presentations from bioethicists (both current and former RAC members) who reviewed some of the issues raised by this serious adverse event (SAE), including the issue of therapeutic misconception in early phase trials. Several members of the RAC underscored the importance of consulting outside resources, such as the [NIH Guidance on Informed Consent for Gene Transfer Research](#), when PIs and their staff are developing the informed consent document and processes.

The RAC discussion concluded with the adoption of a resolution to continue to work together with the FDA and the other parties involved in the analysis of the SAE, with the goal of determining if the gene transfer played any role. The results of these efforts will be presented at the December 2007 RAC meeting. In addition, Dr. Federoff shared a summary of observations on clinical design that were raised during the review of this adverse event. A more detailed summary of the proceedings follows.

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), OBA identified 34 serious adverse events (SAEs) in 13 AAV trials in which the principal investigator initially judged the event 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.

FDA Comment

Dr. Daniel Takefman, Chief, Gene Therapy Branch, Center for Biologics, Evaluation, and Research, Food and Drug Administration (FDA), indicated that their review of the SAEs observed in AAV vector trials to date also had not identified any patterns across AAV trials and had not uncovered any specific type of SAE attributable to AAV vectors.

Overview of Rheumatoid Arthritis and the Role of TNF Antagonist

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

Dr. Mary Crow, Director of Rheumatology Research and Associate Chief of Rheumatology at the Hospital for Special Surgery in New York provided background information on rheumatoid arthritis (RA) and the role of TNF 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 appears to be a primary mediator of pathogenesis. TNF is essential to the immune system's defense against microbes and, of interest in the current case an important action of TNF 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 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 FDA approved TNF antagonists, adalimumab (Humira®), infliximab (Remicade®) and etanercept (Enbrel™), have been shown to be effective in inhibiting the destructive role of TNF in RA and controlling the clinical 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 TNF antagonist therapy reactivated latent and primary tuberculosis, fungal infections, including histoplasmosis, candidiasis, and aspergillosis, and recurrent bacterial infections, including streptococcal and staphylococcal infections.

TNF Antagonists in RA: The Clinician's Perspective

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

The discussion of the role of TNF antagonists in the treatment of RA was continued by Dr. Eric Matteson, Professor of Medicine in the Division of Rheumatology at the 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 one to six months after starting TNF antagonist 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, dyspnea 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.

Overview of OBA Protocol #0504-705: A Phase I/II 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 antagonists

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

Dr. Mease

Dr. Philip Mease, the Principal Investigator on OBA Protocol 705 and the Chief of Rheumatology Research at Swedish Hospital Medical Center, provided an overview of the protocol. The investigational agent (tgAAC94) consists of an 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. He explained that the ultimate goal was to develop an intra-articular treatment 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 tgAAC94 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 escaped into the systemic circulation. He also reviewed the Phase I study¹ 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

¹ OBA Protocol #0307-588 titled *A Phase I Dose Escalation Study of Intra-Articular Administration of tgAAC94, a Recombinant Adeno-Associated Vector Containing the TNFR:Fc Fusion Gene, in Inflammatory Arthritis.*

knee, but injections have also been 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 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 (DRP) (vector genome-containing particles). Because the target joint was the knee, the total dose received was 5×10^{13} DRP. 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. Only one previous 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 (1×10^{12} DRP).

With respect to infections, the 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. Another 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 ($< 2.5 \times$ 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 of these 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 was 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 an anti-AAV antibody titers of 1:4 at baseline which had increased to 1:128 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 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 done on samples from subjects who were not on systemic TNF antagonist therapy because it can not distinguish between the transgene product and other TNF antagonists. In eight subjects who received the highest dose (1×10^{13} DRP) 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 blood levels of TNF antagonists for subjects receiving these agents systemically. For adalimumab, which the decedent was taking systemically, 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 1 st injection	5.4
March 28, 2007	4 weeks after 1 st injection	7.5
May 29, 2007	12 weeks after 1 st injection	8.4
July 2, 2007	Prior to 2 nd 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 clinical histoplasmosis infection in patients on adalimumab is 4 per 4870 patients.

The discussion of Protocol 705 was continued by Dr. Edward Fudman, a PI on Protocol 705 from Austin Rheumatology Associates. He 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 receive a copy of the informed consent.

Case Presentation

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

The case was presented by Dr. Kyle Hogarth, Assistant Professor, Division of Pulmonary and Critical Care, Department of Medicine, University of Chicago, who was the subject's attending physician during her stay in the intensive care unit at University of Chicago. 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. Despite her disease, she led an active life as a mother, wife and was employed 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 26, 2007, she received the first injection of the active study agent, total dose 5×10^{13} DRP, into the right knee. Laboratory studies 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 2007, a private physician prescribed, by phone, a five day course of valacyclovir for a presumed recurrent HSV infection. On June 28, the subject received, by phone again, a seven day prescription for metronidazole for a gynecological infection. On June 29, and 30, the subject reported increased fatigue. On July 1, the subject reported unusual fatigue and low grade fevers. Nonetheless, she did go to work on July 2, and after work went to the office of her rheumatologist to receive the second injection of tgAAC94. A temperature of 99.6° F was recorded but her other vital signs were unremarkable, as were the results of routine laboratory studies drawn that day with the exception of an elevated c-reactive protein. 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 following her second injection (July 2), the subject complained of nausea, and had vomiting, high fevers, and chills. She 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 5, 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 was reported to be 104° F. The evaluation included a chest x-ray, interpreted as normal, blood tests, including normal chemistries and complete blood count and negative blood and urine cultures. She was given a diagnosis of viral syndrome, prescribed 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 care physician that day and she was sent home. These studies showed an elevated white blood cell count of 29.2 K/mm^3 and increased liver enzymes (aspartate aminotransferase (AST) 162 U/L, alanine aminotransferase (ALT) 125 U/L and total bilirubin 1.2 mg/dl). In addition, a number of blood tests for other viral infections, including acute hepatitis, cytomegalovirus, parvovirus, mononucleosis and ehrlichia came back 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 for abnormal 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 serum 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 biochemical evidence of a coagulopathy. 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 tests and a 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 micafungin was started. Due to the enlarging hematoma arterial embolization was attempted but a source of bleeding was not identified. Surgical evacuation of the clot was considered but due to her clinical condition this 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 radiologic 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 inotropic 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, eight days after being transferred to the University of Chicago Hospital.

Presentation of Autopsy Data

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

Dr. John Hart, Professor, Department of Pathology, 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 not all immunosuppressed patients fail to form granulomas. Indeed, granulomas formation has been seen in patients with Acquired Immunodeficiency Syndrome (AIDS) who have histoplasmosis infection. Dr. Hart also noted that there was no underlying liver disease.

Dr. Hart reviewed the other organs that showed evidence of histoplasma infection including the lungs, bone marrow, spleen, lymph nodes, kidney, and brain.

The retroperitoneal hemorrhage weighed 3.5 kilograms on autopsy 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 capsulatum*.

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 same 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.

Dr. Karen Frank, Assistant Professor, Pathology, University of Chicago 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, human immunodeficiency virus (HIV), and 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 *Candida* 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. 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.

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*)

Histoplasmosis

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 histoplasmosis antigen data presented by the protocol sponsor (Targeted Genetics Corporation) does not establish infection prior to dosing. The value on July 2, 2007, 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 this result. The clinical course, however, is strongly suggestive that she had an active histoplasmosis at the time of injection on July 2.

The course was fulminant sepsis with disseminated intravascular coagulation. The case reports for patients on TNF antagonists include some severely ill patients who required care in an intensive care unit 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 TNF antagonists agents. Such a fulminant course has also been described in patients with 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.

Herpes Simplex Virus

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. Since, she had a positive nasopharyngeal culture for HSV Type 1 and history of HSV-1 infections demonstrated by the pre-existing antibody, this positive HSV blood test likely represents 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.

Liver

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 evidence for liver dysfunction was primarily from the abnormal liver blood tests. There was a striking rise in the AST at around the same time as the hematoma and a progressive rise in bilirubin. He postulated that the acute rise in 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 tests was likely a result of multiple factors, including 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 that drug induced liver injury was the etiology of the liver problems. Finally, he concluded that the liver dysfunction was not a contributor to her death.

Retroperitoneal Bleed

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, 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 dilute Russell's viper venom time (dRVVT). 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.

RAC Discussion: Assessment of the Possible Role of Gene Transfer

The RAC first addressed whether there was any evidence of contamination of the product by an infectious agent. Targeted Genetics Corporation provided product lot release data that demonstrated that the agent given to the subject 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 of vector DNA 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 quantification 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 wtAAV 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 wtAAV. 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 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 systemic TNF antagonists, there was no detection of the transgene product in the serum using these same assays at four and twelve weeks. Targeted Genetics 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. Dr. Bartlett added 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 RAC then considered whether there could have been an immune response to the AAV vector that contributed to the liver disease or overall clinical course. Immune responses to AAV vectors was the discussion of a day long symposium at the June 19, 2007, RAC meeting. At that meeting, data from a hemophilia trial were presented on two subjects who received hepatic artery injection of an AAV vector. These subjects experienced a transaminitis starting approximately four weeks after administration of the AAV vector and simultaneously had 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, at an earlier stage, significantly more vector was present in these organs. An immune reaction against the vector could have led to some 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.

Dr. Bartlett noted, however, 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.

RAC Discussion: Informed Consent and Subject Selection

Dr. Kodish framed the discussion on research ethics by stating that the key ethical concepts that drive 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 in clinical trials: (1) how an informed consent document can clearly explain the theory behind the intervention without creating a therapeutic misconception and (2) how the overall goal of a trial can be described in a way that does not imply that the long-term goals are the goals of the current phase of the study (particularly for early-phase studies). 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, so the tendency of such patients is 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 the injection interventions were based on clinical symptoms; administration of the active agent was timed, in part, on clinical symptoms in the target joint, as opposed to being scheduled at a specified time. 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 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 highlighted wording from the *NIH Guidance on Informed Consent for Gene Transfer Research* (a guidance that is available to investigators, sponsors, institutional review boards, potential participants, and the public <<http://www4.od.nih.gov/oba/rac/ic/>>) that specifically discusses and offers sample language regarding disclosure of the competing roles of investigators and physicians and 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. Nancy King suggested that perhaps consideration should be given to reimbursement to subjects to reinforce the message that research is to benefit future patients and not current research participants. It was also noted that investigators also suffer from therapeutic

misconception and that the informed consent document and process could reflect the investigator's unconscious bias thereby affecting the potential subject's belief.

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 the subject.
- The GTSAB should help 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 the subject.
- 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 3-5, 2007 RAC meeting.

Committee Motion

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.

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. 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 appear to lead to 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 before the Committee, 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.

Public Comment

Mr. Robert Mohr, the subject's husband, 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 to not 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 Professor, St. Jude Children's Research Hospital; L. Joseph Wheat, M.D., President and Director of MiraVista Diagnostics, and several members of the press.