Langen, September 18, 2007

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Report on NIH RAC meeting September 17, 2007,
Discussion of a Serious Adverse Event on a Human Gene Transfer Trial (OBA Protocol #0504-705) Using an Adeno-Associated Viral Vector: Analysis of Its Scientific and Safety Implications (see attached agenda)

Summary and conclusion
On the basis of currently available data (see below) there are two main factors which seem to have caused the death of patient Jolie Mohr: (1) disseminated histoplasma capsulatum infection due to immune suppression mediated by antirheumatic treatment with methotrexate, adalimumab and corticosteroids, (2) abdominal hematoma. It was considered unlikely that the AAV injection was a significant contributing factor to the patient´s death.

The quality of the gene therapy medicinal product was fine and there was also no indication of more than expected AAV biodistribution, e.g., mediated by the Herpes simplex virus infection.

1. Clinical trial protocol.

Title: :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-Alpha Antagonists

The 13G01 clinical trial is a phase I/II dose escalation study designed to be conducted in adults with inflammatory arthritis who have persistent moderate or severe swelling in one or more joints, without a disease severe enough to warrant a change in regimen for the next three months. Total enrolment: 120 patients.
The study will permit inclusion of subjects who are concurrently on anti-tumor necrosis factor (TNF)-alpha antagonists. For subjects on disease-modifying antirheumatic drugs (DMARDs), a stable regimen for inflammatory arthritis for the previous three months, with no changes in doses in the four weeks prior to screening will be required.
The primary objectives are:

1. to evaluate the safety of intra-articular administration of tgAAC94 in subjects currently taking TNF-alpha antagonists, and
2. to evaluate the safety of repeat intra-articular administration of tgAAC94 (gene therapy vector

Medicinal product: tgAAC94 is a recombinant adeno-associated virus serotype 2 (AAV2) vector genetically engineered to mediate transcription and subsequent translation of the cDNA encoding human tumor necrosis factor receptor (TNFR)-immunoglobulin (IgG1) Fc fusion (TNFR:Fc) gene. The DNA sequence of TNFR:Fc in tgAAC94 codes for a protein identical to etanercept (Enbrel®). TNF-alpha has been strongly implicated as a major participant in the inflammatory cascade that leads to joint damage and destruction in diseases such as rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS).
Design: Dose-finding had been performed in a preceeding phase I study in 20 patients who did not receive comcomitant systemic anti-TNF treatment. In the current study patients are randomly assigned in groups of 20 subjects to placebo, 10^{11}, 10^{12}, 10^{13} DRP/ml tgAAC94 of

joint volume, respectively. In the open treatment segment repeat injection is performed 12 to 36 weeks after the first administration at the dose level of the originally assigned cohort.
Status: 127 patients have been enrolled in 20 study centres in the US, 74 patients have received a second injection. Most patients are on at least one DMARD. Anti-AAV neutralising antibody titers increase with increasing dose. There is no information on serum levels of the gene product in this trial, as the assay can not distinguish between systemically administered anti-TNFs and the gene product. In the preceeding phase I trial where patients did not receive concomitant anti-TNFs the gene product was undetectable in 8/8 subjects indicating a localized expression of the gene product. Adverse events after the first dose were not significantly different from placebo: mild to moderate erythema at injection site, infections in about 30 % of patients, elevated liver function tests (LFT) in about 20 % of patients. There was no significant dose-relationship of AEs. Of 8 SAEs, 6 were unlikely related to the study medication, 1 probably related case was the case of patient Mohr, who died on July 24, 2007.

2. Medical history of the patient.

Mohr was a 36 year-old Caucasian female with a history of rheumatoid arthritis (RA) starting in 1992. From 2002 to 2004 she received etanercept (recombinant human tumour necrosis factor (TNF) receptor-p75Fc fusion protein), since 2004 she was treated with adalimumab in combination with methotrexate and prednisone. Her history was remarkable for bilateral toe surgery and repeated intra-articular knee injection of corticosteroids: 10 injections, 6 of these between 2005 and 2006.
She was randomized to the highest dose group and received her first dose on February 26, 2007. She had normal LFT prior to the first and to the second dose.
On May 22nd 2007, acyclovir and metronidazole were prescribed. On July 29 and 30th she complained of fatigue and on July 1st she had a low grade fever. Recent boating on fresh water was reported.

3. Course of disease:

She received her second intra-articular right knee injection on July 2ndand in the evening of the same day complained of high fever and diarrhea. Due to persistent fever (101°F) she received a prescription of levofloxacin on July 5th. On July 7th she had nausea and vomiting, fever of 104°F.White blood cell count (WBC) and creatinine were within normal limits, blood and urine cultures negative. Her treating physician suspected a viral infection.
Histoplasma antigen testing: stored blood tests were negative on May 29th, 2007 and low positive (<0.6ng/ml) on July 2nd 2007.
On July 9th she developed tachycardia and a tender right knee. WBC was 29.000/mm^3 with lymphocytosis, AST 162, ALT 123 U/l, bilirubin 1,2 mg/dl. Serological tests including CMV were negative. Up to July 15 she remained febrile despite of various antibiotic regimens.
On July 16/17 her haemoglobin began to drop, LFTs increased further and she developed hypotension and respiratory distress. Coagulopathy and retroperitoneal hemorrhage was diagnosed, and her haemoglobin dropped further to 4.6g/dl despite repeated rbc transfusions
She was transferred to the University of Chicago Medical Centre with the question of a potential liver transplantation. She presented with a tender abdomen, no swelling of knees, a low central venous pressure, and was intubated and sedated. Chest X-ray revealed bilateral infiltrates and effusion, CTt scan revealed a massive retroperitoneal hematoma and displacement of left kidney and spleen. She was started on antifungal treatment based on yeast in a blood smear, on antibiotics and on renal replacement therapy. Repeated imaging did not reveal the source of bleeding, surgery for evacuation of the hematoma was considered too risky.

On July 17 to 19th serology and microbiology was negative for histoplasma, and positive for candida (tracheal smear). HSV-1 serology showed 300 copies/ml on July 18th, and positive nasopharyngeal cultures on July 19th. The liver biopsy from July 19th showed small areas of acute necrosis, inflammatory infiltrates, positivity for histoplasma. Electron microscopy showed histoplasma, but no viral particles. Clinically she deteriorated further, her mean arterial blood pressure decreased and oxygenation worsened despite oxygen supply and high PEEP. Her antemortem culture for histoplasma was positive. She died on July 24th.

4. Main autopsy findings:

Autopsy revealed disseminated histoplasma capsulatum in all organs and absence of granuloma which are typical for histoplasmosis in immunocompetent patients, a retroperitoneal hematoma of 3,5 kg, no anatomic source of bleeding, minimal swelling of knees bilaterally. Histoplasmosis was at all sites in the bone marrow, extensive spleen infarction and necrosis were present. No mycobacteriae.
Cultures: Viral cultures of brain and knee were positive for HSV-1.

5. Possible role of gene transfer

The drug product was without infectious material as known to date. The drug product was tested negative for wild type AAV. DNA sequences for vector were detected in liver and spleen and were negative in lung. In all samples rep gene was negative, indicating that there was no replication of vector genome in tissues. HSV has been shown to be a poor helper virus for AAV. In this case it is considered highly unlikely that HSV-1 served as a helper virus for wild-type AAV.
As shown in the previous study anti-AAV antibody titers are known to rise in a dose-dependent fasion (patient was at highest dose group), but here, kinetics are not known. There is only one anti-AAV antibody titer of 1/128 at second injection on July 2nd.
Concerning the production of transgene product it was discussed that AAV2 vectors show a significant delay (1-2 months) following vector delivery before expression of the transgene takes place. No significant expression is to be expected the same day. So the symptoms of fever and diarrhea on the day of injection on July 2nd are unlikely related to transgene product. Serum level of gene product in this patient is unknown as serum assay for anti-TNFs does not distinguish between anti-TNFs. However, in phase I there was no serological detection of drug product in 8/8 patients at the high dose level. This allows to draw the conclusion that a localised expression of the gene product is likely. Whether the transgene product contributed to the immune suppression of the patient remains to be elucidated.
Concerning the immune response to AAV leakage of AAV to liver was discussed. In principle AAV infection may lead to rise of transaminases as sign of an immune reaction, but this would not be fatal. It is uncertain which role the gene therapy played with regard to transaminase increase, due of the concomitant histoplasma infection and focal necrosis of the liver**.**

6. Conclusion:

The fulminant histoplasma infection played a significant role in the fatal outcome and is considered the main cause of death. A substantial contributing factor was the enormous retroperitoneal hematoma. The positive blood PCR showing 300 copies/ml of HSV-1 on July 18th points at an activation of latent HSV-1 infection, but HSV-1 is likely no major factor in the course of the disease. Which role, if any, the gene therapy played, will be further analysed when more data are available. A follow-up RAC meeting is planned for December 2007.
The patient lives in an area which is endemic for histoplasma capsulatum. In the days preceding July 2nd the patient had most probably either a newly acquired histoplasma

infection or a re-activation of a preceding infection with unspecific symptoms. Usually histoplasma causes pulmonary disease that responds well to antifungal agents. Fulminant clinical courses and fatal outcome is reported for immunosuppressed patients, e.g., with AIDS or in patients with RA on combined immunosuppressive therapy including anti-TNFs. The combination of antirheumatic drugs that the patient received, i.e. methotrexate, humira and corticosteroids are known to cause a fourfold increased risk of serious infections in the first months of use. Most cases of histoplasmosis during anti-TNF treatment occur during the first 6 months of therapy, but the occurrence after several years of treatment is not unusual, as was the case for Mrs. Mohr. Clearly addressed was the problem that there is no guidance regarding the use of anti-TNFs in areas where histoplasma is endemic.

7. Further planned investigations:

These include

- ongoing analysis of AAV vector and wild typeAAV in blood,
- antibody against coagulation factors, as hematoma is not explained by mild coagulation disorder.
- assay verification on tissue AAV
- additional tests for serum level of gene product.
- further analysis of HSV-1 in brain

8. Criticism of study design and bioethical considerations

Questions on the design and ethics of the study was raised. For example it was citizised that the primary endpoint “safety of gene therapy” was difficult to assess in patients with concomitant systemic anti-TNFs. The indication for intraarticular administration in this patient was questioned, as the autopsy revealed absence of inflammatory signals in both knees. In general the benefit-risk evaluation of clinical gene therapy research in non-life threatening diseases was discussed.

9. Press release (examples)

Targeted Genetics press release

http://ir.targen.com/phoenix.zhtml?c=84981&p=irol-newsArticle&ID=1052409&highlight=

Science magazine

http://sciencenow.sciencemag.org/cgi/content/full/2007/917/1

UK:

http://www.the-scientist.com/news/home/53593/

Attachment **NATIONAL INSTITUTES OF HEALTH**
RECOMBINANT DNA ADVISORY COMMITTEE (RAC)

109th Meeting

NIH Campus
Natcher Building Auditorium

September 17, 2007
***Agenda**

Monday, September 17, 2007

8:00 AM **Call to Order and Opening Remarks**

Howard Federoff, M.D., Ph.D., Chair, NIH RAC

Tab 2466 For Your Information
Notice of Meeting
Conflict of Interest Guidance

8:10 AM **Discussion of a Serious Adverse Event on a Human Gene Transfer Trial (OBA Protocol #0504-705) Using an Adeno-Associated Viral Vector: Analysis of Its Scientific and Safety Implications**

RAC Reviewers: Steven Albelda, M.D.
Stephen Dewhurst, Ph.D.
Hildegund Ertl, M.D.
Howard Federoff, M.D., Ph.D.
Scott Strome, M.D.

Tab 2467 Background Materials

10:15 AM **BREAK**

10:30 AM **Discussion of Serious Adverse Event (continued)**

11:45 AM **Public Comment**

12:30 PM **LUNCH**