Follow-up on Serious Adverse Event in OBA Protocol 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 with and without Concurrent TNF- α

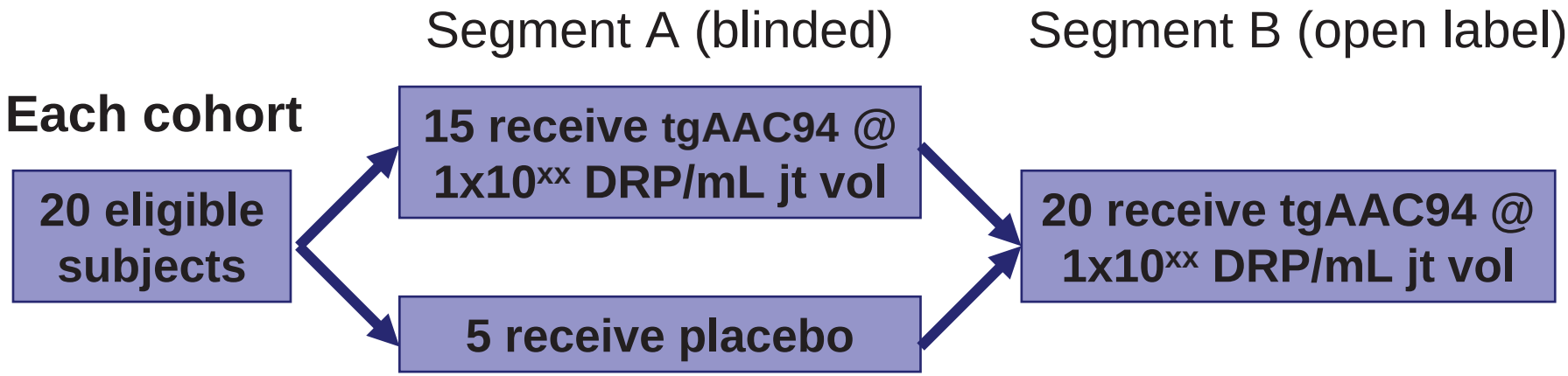
NIH Office of Biotechnology Activities
December 3, 2007

rAAV human TNFR:FC Vector (tgAAC94)

- Single stranded DNA encoding huTNFR:Fc
- huTNFR:Fc cDNA sequence identical to the cDNA used for etanercept production
- Packaged in AAV2 capsid
  - Lacks viral coding sequences
  - Based on wtAAV virus which is considered non-pathogenic
  - Vector non-replicative even in presence of helper virus
  - Persist for life of host cell, primarily as episomal concatemers
- Extensively purified
  - Testing includes safety, identity, purity and sterility

Slide provided by Dr. Philip Mease, M.D. University of Washington

Phase I/II Clinical Study: Safety of Repeat Local AAV2-TNFR:Fc Administration



Two doses

- **First dose in Segment A**
- **Second dose 12 to 30 weeks later, based on predetermined criteria**

Dosing based on joint volume

Knee, 5mL **Wrist, 1mL** **Elbow, 1.5 ml**
Ankle, 2mL **MCP, 0.5 mL**

Slide provided by Dr. Philip Mease, M.D. University of Washington

Phase I/II Clinical Study Agent Exposure

- First dose: Blinded study agent – 127 subjects
- Second dose: Open label study agent – 74 subjects
  - One dose of tgAAC94, preceded by placebo – 22 subjects
  - Two doses of tgAAC94 – 52 subjects

	Placebo	Low $1x10^{11}$ DRP/mL	Mid $1x10^{12}$ DRP/mL	High $1x10^{13}$ DRP/mL
Blinded study agent	31	33	32	31
One dose of tgAAC94 preceded by placebo	n/a	8	7	7
Two doses of tgAAC94	n/a	18	17	17

Targeted Genetics
Phase I/II Clinical Study Safety Overview

Study unblinded for safety analyses

Adverse Events	Placebo	Low $1x10^{11}$ DRP/mL	Mid $1x10^{12}$ DRP/mL	High $1x10^{13}$ DRP/mL
After 1st dose (n=127)	20 (65%)	26 (79%)	20 (63%)	20 (65%)
After 1 active dose preceded by placebo (n=22)	n/a	4 (50%)	6 (86%)	5 (71%)
After 2 active doses (n=52)	n/a	10 (56%)	12 (71%)	15 (88%)

Pattern of adverse events among subjects on tgAAC94 was not dose-dependent and was similar to the placebo group.

Targeted Genetics
Adverse Event Analysis

Study unblinded for safety analyses

- Administration site reactions in 10% of subjects
  - Increases in target joint tenderness & swelling
    - Occurred within 15 days of study agent administration
    - Sometimes accompanied by warmth, redness or itching
  - More common after administration of high dose tgAAC94
  - Rates varied with the joint injected, ranging from 4.3% (MCP) to 15.9% (wrist)
  - Similar incidence in subjects on and off TNF-α antagonists
- No dose-dependent increase in infection or liver enzyme abnormalities

Targeted Genetics Analysis of Serious Adverse Events

- One SAE prior to the case at hand considered probably related
  - **Culture-negative septic arthritis**, 15 weeks after 1st injection of $1x10^{12}$ DRP/mL, in subject on leflunomide
    - Considered probably related by investigator
- Other SAEs, considered unrelated by investigators
  - Infected incision after repair of traumatic ankle fracture (non-target joint)
  - Abdominal pain from constipation
  - Cellulitis & ulcer left calf (target joint = wrist)
  - Acute pyelonephritis
  - Myocardial infarction
  - Pulmonary emboli
  - Syncope from coronary artery disease

Subject 1209

- Subject was a 36 y/o Caucasian female with a 15 year history of rheumatoid arthritis (RA).
- Treated with disease modifying anti-rheumatic drugs (DMARDs) since the early 1990s, including TNF inhibitors since 2002. Also treated with methotrexate and prednisone.
- Subject enrolls in the Phase I/II study for Intra-Articular Administration of tgAAC94 in Inflammatory Arthritis in February 2007:
  - She met all inclusion criteria
  - Screening labs were within normal limits (WNL)

Subject 1209

- Subject received first injection of active study agent into right knee on February 26th (Dose= $5x10^{13}$ DNase resistance particles (DRP)).
- Uneventful course and met criteria for second dosing in June 2007 due to persistently swollen knee.
- About 10 days prior to second dose prescribed acyclovir by phone by private physician for presumed HSV infection.
- About 5 days prior to second dose receives phone prescription for antibiotic for gynecologic infection.

Clinical Course

- Prior to injection on July 2nd reported several days of increased fatigue and low grade fevers.
- Temperature day of dosing was 99° F.
- Receives second dose of tgAAC94 on July 2, 2007.
- Relevant laboratory findings on July 2, 2007
  - Elevated C-reactive protein
  - Anti-AAV titer 1:128

Clinical Course Continued

- Within 12 hours of injection experiences fevers, chills, and abdominal pain.
- Progression of symptoms leads to hospitalization on July 12th.
- Initial laboratory values significant for thrombocytopenia, elevated white blood cell count and abnormal liver tests.

Clinical Course Continued

- Treated with broad spectrum antibiotics and imaging done.
- Etiology of continued fevers not determined.
- Experienced episode of hypotension, is intubated and develops acute renal failure.
- Discovered to have large retroperitoneal bleed.
- Worsening liver enzymes leads to transfer to University of Chicago for possible liver transplant.

University of Chicago

- Remains intubated with alteration of respiratory mechanics due to large retroperitoneal hematoma.
- Liver biopsy reveals liver injury is reversible.
- Antibiotics continued and micafungin started due to yeast on blood smear; blood cultures remain negative but urine culture positive for candida.
- Continues to require multiple transfusions of blood products.
- Continues on renal replacement therapy.

University of Chicago, cont…

- Unable to control the retroperitoneal bleed despite massive transfusion of products. Hematology consult fails to discover etiology and attempt at arterial embolization was unsuccessful; source of bleed not found.

- Subject becomes progressively more hemodynamically unstable with evidence of possible acute respiratory distress syndrome.

University of Chicago
July 24, 2007

- Worsening status leads to decision to provide comfort care.
- Subject passed away approximately 3 weeks after second dose.
- Blood cultures drawn on 7/24 turn positive two days post-mortem: *Histoplasma capsulatum.*

Histoplasma Capsulatum

- Seen in pre and post-mortem blood cultures.
- Pathologic evidence of histoplasmosis in multiple organs including liver, lungs, bone marrow, spleen, lymph nodes, kidney and brain.
- Absence of granuloma formation is consistent with systemic immunosuppression from TNF-antagonist.

Herpes Simplex Virus

- **Hospital Data (July 18th -19th)**
  - Herpes Simplex virus (HSV) detected in blood (300 copies/ml).
  - Nasopharyngeal aspirate culture HSV 1 +
  - IgG Antibody for HSV 1 + and HSV 2 neg.
- **Autopsy Data**
  - Herpes virus cultured from brain, trachea and right and left knee
  - No evidence of cytopathic effect in these tissues
  - Immunohistochemical stains on these tissues negative
  - PCR performed on extracted DNA on brain and liver negative

Retroperitoneal Hematoma

- Hematoma weighed 3.5 Kilograms at autopsy.
- It caused significant displacement of the internal abdominal organs (upward and right) and displaced the diaphragm upward leading to compression of lungs.
- Enveloped left kidney leading to focal infarction.
- **No anatomic source of bleeding was found.**

RAC Conclusions with Respect to Clinical Presentation

September 2007

- Immediate cause of death was most likely due to large retroperitoneal hemorrhage and disseminated histoplasmosis.
- Subject's use of systemic TNF-antagonists placed her at risk for disseminated histoplasmosis.
- Etiology of the large retroperitoneal bleed was not determined.

RAC Conclusions with Respect to Clinical Presentation

September 2007

- HSV infection likely did not play a role in clinical course.
- There was no evidence of fulminant liver failure.
- Liver injury was likely caused by a combination of factors, including infection and hypotension but was not contributor to cause of death.

Role of Gene Transfer?

- Was there contamination of the product?
- Was there evidence of replication competent AAV in liver and other tissue?
  - Could HSV have played a role as helper virus for AAV?
- Was there overproduction of transgene product leading to excessive systemic TNF-antagonist?
- Could an immune response to the AAV vector have contributed to the clinical course?

Retesting of Product*

- Sterility test: No evidence of contamination.
- Adenovirus not detected.
- HSV-1 culture negative (unaudited results from day 14).
- Histoplasmosis culture results pending.
- No functional TNF-alpha binding activity (no functional etanercept) was detected.

* **Data as of September 17, 2007**

Quantitative Real-Time PCR Test Results

Targeted Genetics Corporation Clinical Study Protocol Number 13G01
Preliminary Data
14-Sept-07

WARNING: Data subject to change following QA audit

Target Sequence	Tissue Sample	Mass of DNA Analyzed Per Well (µg)	Spiked Well Status	Un-Spiked Replicate Well Information			Designation of Test Sample
				C_T Value*	Quantity (copies/µg)	Status	
tgAAC94	Liver	1.00	Positive	36.10	5.67	Amp.	Positive, < 22 copies/µg
				38.50	1.03	Amp.	
	Lung	1.00	Positive	45.00	0.00	No Amp.	< LLOQ
				45.00	0.00	No Amp.	
	Spleen	0.42	Positive	38.36	2.58	Amp.	Positive, < 9.46 copies/µg
				39.07	1.46	Amp.	
wt AAV2 Rep gene	Liver	1.00	Positive	45.00	0.00	No Amp.	< LLOQ
				45.00	0.00	No Amp.	
	Lung	1.00	Positive	45.00	0.00	No Amp.	< LLOQ
				45.00	0.00	No Amp.	
	Spleen	0.42	Positive	45.00	0.00	No Amp.	< LLOQ
				45.00	0.00	No Amp.	

*a numerical C_T value of 45 (the terminal cycle of the PCR protocol) was assigned to wells that did not show amplification and that were designated as "Undetermined" by the instrument.
Amp. = exponential amplification

Interpretation of PCR data

- Low level of vector present in the liver and spleen is consistent with pre-clinical biodistribution studies.

- The absence of AAV rep gene makes it highly unlikely there was widespread replication of the virus systemically.

Role of Transgene Product?

- Data presented that serum level of TNF-antagonist on July 2nd was not above the expected steady state.
- Validated assay to distinguish transgene product from systemic TNF-antagonist was unavailable.
- In 8 subjects who received the same dose but who were not taking systemic TNF-antagonists, the transgene product was <u>not</u> detectable in their serum at 4 and 12 weeks post administration of vector.

Possible Immune Reaction?

- An immunologic response to the AAV vector capsid has been postulated to have been the cause of an asymptomatic elevation in liver enzymes in a gene transfer trial for hemophilia in which the AAV vector encoding factor IX was injected into the hepatic artery.
- No data available in this case to comment on whether there was a CD8+-mediated T-cell response against the vector capsid.

Possible Immune Reaction?

- **The systemic anti-AAV titer of 1:128 might make systemic dissemination of the vector less likely and therefore make an immune response less likely.**
- **If such an immune reaction occurred it was unlikely to have played a significant role in the clinical course and was not the primary cause of the patient's death.**

Data Received since September Meeting

- Updated product testing data
- qPCR for vector and wtAAV rep gene in multiple tissues and in blood sample from July 24th
- Assay for TNF-antagonist in serum samples from July 9th,16th 22nd and 24th
- Coagulation studies

Testing of Product

- **Final results:**
  - Sterility test: No evidence of contamination.
  - Adenovirus not detected.
  - Culture for HSV-1 remained negative at 28 days.
  - *Histoplasma capsulatum* culture negative.
  - TNF-alpha activity remains negative.

qPCR for tgAAC94 (LLOQ 22copies/ug)

- **Blood sample 7/24** <23 copies/ug
- **Positive autopsy tissues** < 22 copies/ug
  - Spleen, Liver- right and left lobe, Brain
- **Positive Tissues Quantifiable**
  - Tonsil 29.14 copies/ug
  - Right knee (soft tissue) 4.45×10^5 copies/ug
  - Right knee (soft tissue) 3.61×10^4 copies/ug
- **Negative Tissues**:
  - Left knee, kidney, lymph nodes - mesenteric and porta hepatis, bladder, trachea, small bowel, heart, adrenal

qPCR for wtAAV2 rep gene

(LLOQ 4.3 copies/ug)

- **Blood sample 7/24** Negative
- **Positive tissues** < 4.3 copies/ug
  - Heart, trachea
- **Positive Tissues Quantifiable**
  - Right knee (soft tissue) 435 copies/ug
  - Right knee (soft tissue) 19.24 copies/ug
- **Negative Tissues**:
  - Left Knee, kidney, brain, liver, R. Lung, lymph nodes- mesenteric and porta hepatis, bladder, trachea, small bowel, tonsil, adrenal

Tissue	PCR tgAAC94	PCR wtAAV2 rep. gene
Blood (7/24)	+ (not quant.)	Negative
Spleen	+ (not quant.)	Negative
Liver (right/left lobe)	+ (not quant.)	Negative
Brain	+ (not quant.)	Negative
Right Knee	Quantifiable	Quantifiable
Tonsil	Quantifiable	Negative
Heart	Negative	+ (not quant.)
Trachea	Negative	+ (not quant.)

Assay for TNF-antagonist

- Validated assay to distinguish transgene product (etanercept) from adalimumab not available.
- Decision made to determine overall level of TNF-antagonist using BioMonitor Assay.
- Per Targeted Genetics, assay verified to be able to detect both TNF-antagonists if present in the same serum sample.

Serum Levels for Systemic TNF-antagonists

Systemic Administration of TNF Antagonists in Humans	Mean Steady State Drug Concentration (µg/mL)
Adalimumab, 40 mg SC every other week with concomitant methotrexate	**8-9**
Infliximab, 3-10 mg/kg IV every 4-8 weeks	**0.5-6**
Etancercept, 25 mg SC twice a week	**2**

Levels of TNF-antagonist in Serum for Subject 1209

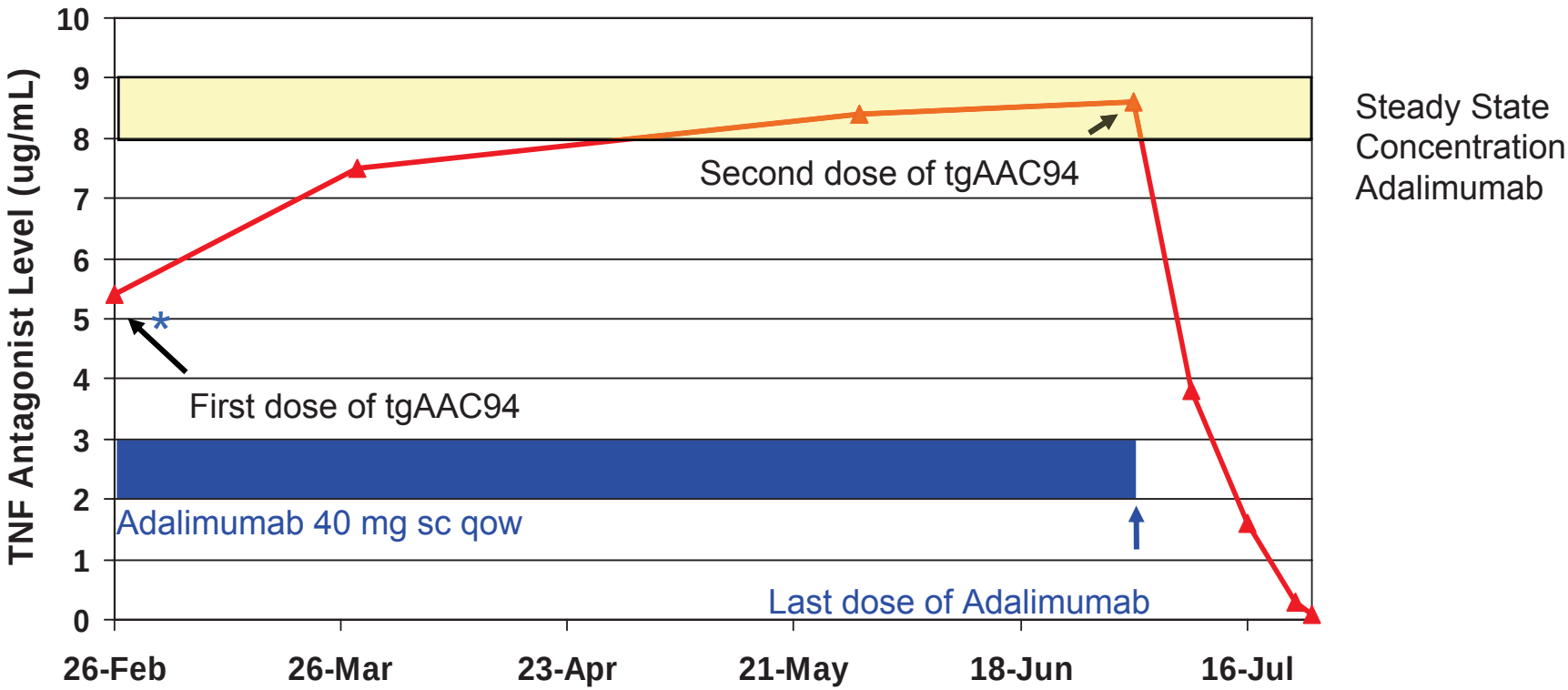
Date of collection	Timing	Result (ug/ml)
Feb. 26, 2007	Prior to 1st injection	5.4*
Mar. 28, 2007	4 wks after 1st injection	7.5
May 29, 2007	12 wks after 1st injection	8.4
July 2, 2007	Prior to 2nd injection	8.6
July 9, 2007	1 wk after 2nd injection	3.3-4.3**
July 16,2007	2 wks after 2nd injection	1.2-2
July 22,2007	3 wks after 2nd injection	<0.2-0.3

* Per TG subject missed dose of adalimumab in February

** Methotrexate likely withdrawn which may account for faster clearance of adalimumab

Systemic TNF Antagonist Levels

Subject 1209



* *Subject missed a dose of adalimumab in February*

- Levels measured using a radioimmunoassay that detects ^{125}I-TNFα:anti-TNF complexes precipitated after addition of anti-human IgG antibodies
- Standard curve constructed based on specific anti-TNF construct being assayed
- Limit of detection 0.01 µg/mL

Systemic Levels of TNF Antagonists after Intra-articular Gene Transfer

Intra-articular delivery of tgAAC94	Systemic Levels of TNFR:Fc Protein (µg/mL)
$1x10^{11}$-$1x10^{13}$ DRP/mL in 102 rats	Up to 0.01 in 4 rats
$1x10^{11}$-$1x10^{13}$ DRP/mL in 6 monkeys	Up to ~0.07
$1x10^{13}$ DRP/mL in 16 humans*	Not detectable * < 0.01

* These patients were not on systemic Anti-TNF therapy

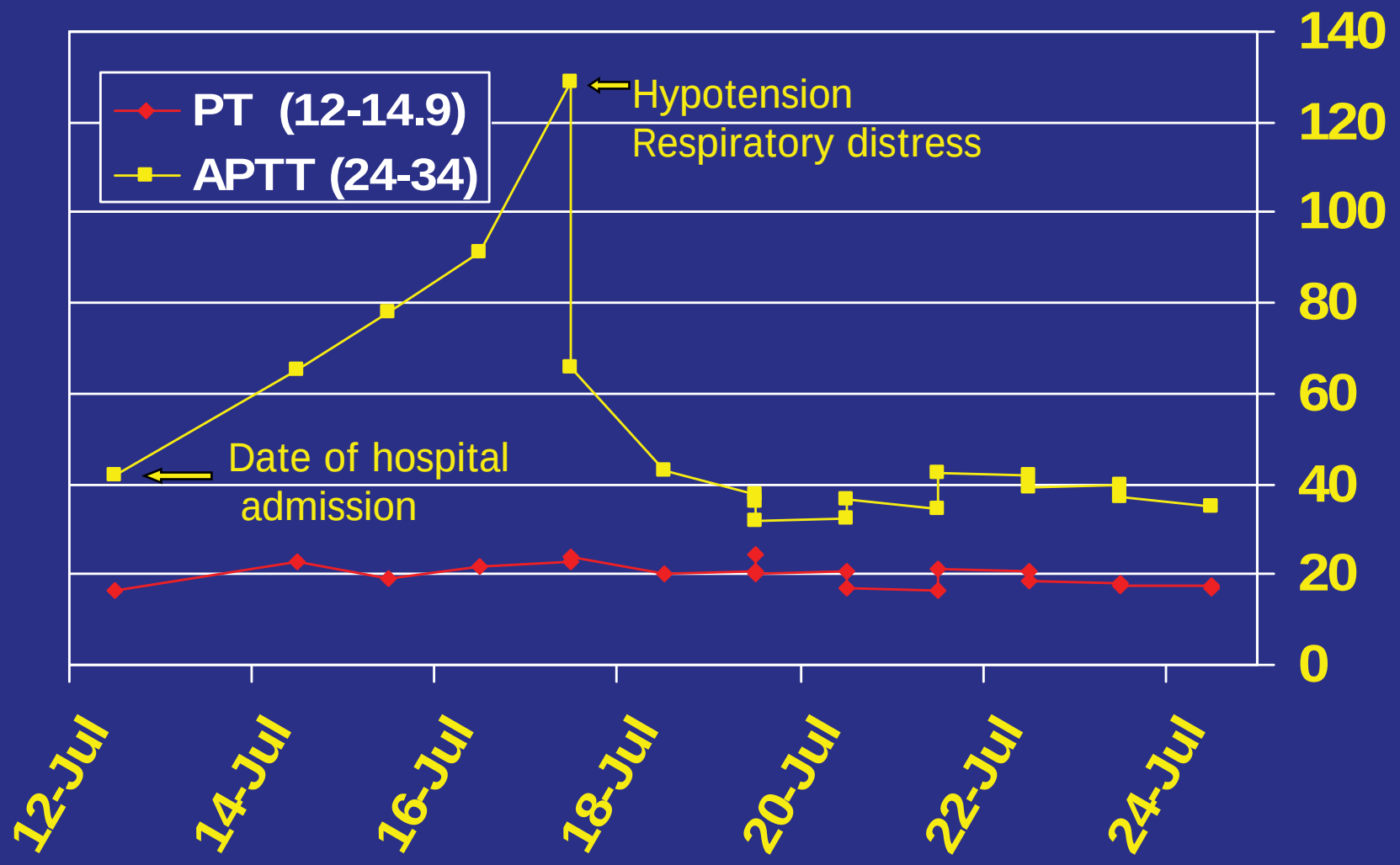
* Measurements done at 4 and 12 weeks

*Slide Provided by Dr. Philip Mease and Targeted Genetics

Coagulopathy and Retroperitoneal Hemorrhage

- Subject had evidence of coagulopathy: elevated prothrombin time and partial thromboplastin time.
- Subject had acute decline in platelets and evidence of disseminated intravascular coagulation.
- These abnormal hematologic parameters did not seem to completely explain the degree and location of hemorrhage seen in this case.

Coagulation



Results of Coagulation Studies

- **Factor II, X, and XI levels were approximately 20-25% of normal**
- **Factor IX was approximately 30% of normal**
- **Factors V and VII levels were approximately 50% of normal**
- **Fibrinogen was low: 90 (nl 180-409 mg/dl)**
- **Factor VIII and vWF were elevated:**
  - **VIII 163 -175 (nl 57-152)**
  - **vWF 269**
- **D Dimer was elevated at 14 (nl <0.42)**
- **There was evidence of a weakly positive lupus anticoagulant**

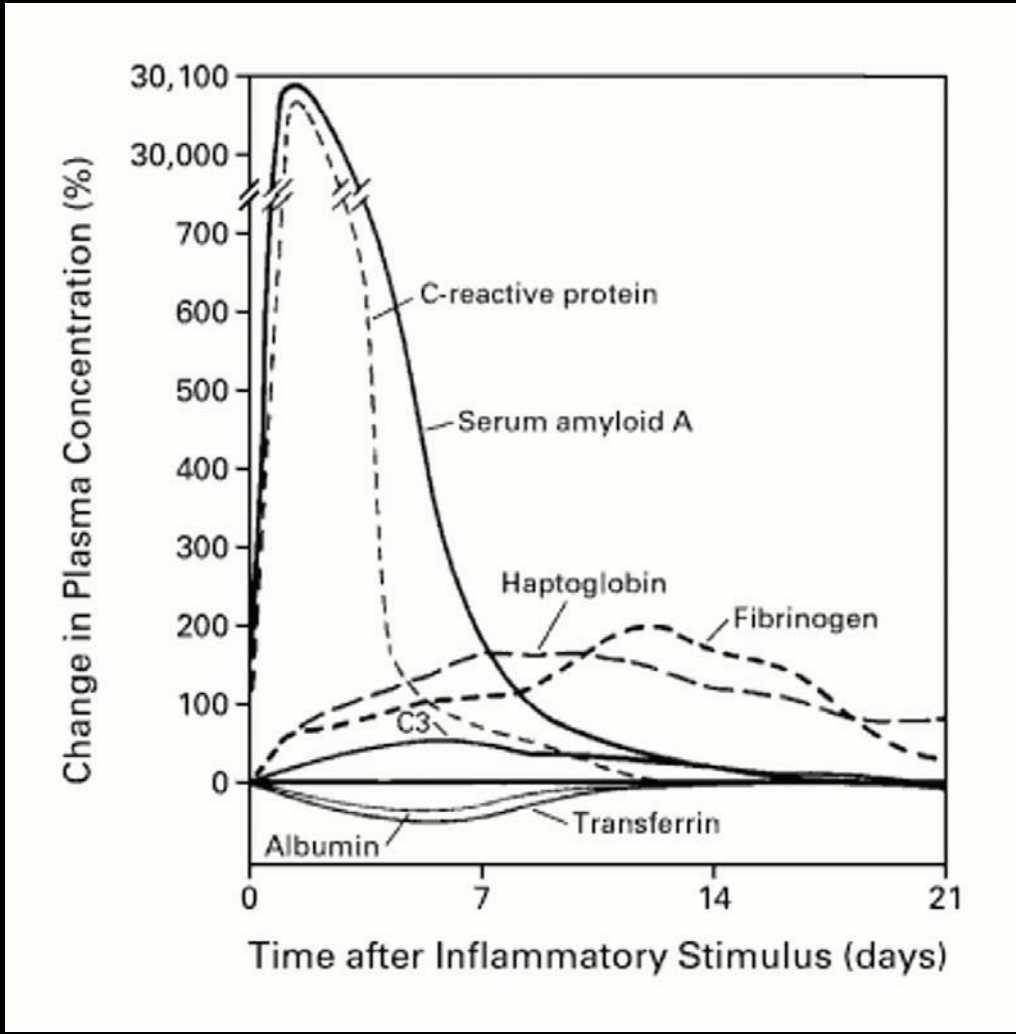
Coagulation Data continued

- There was a global decrease in clotting factors.
- The exception was an elevation in factors VIII and vWF which can be explained in part by the fact these are also acute phase reactants.
- The low levels of clotting factors partly explain the type and degree of hemorrhage seen this patient.
- There may have been other anatomic factors such as aneurysm that was not identified.
- Finally, the weakly positive lupus anticoagulant, together with the low factor XI may explain some of the striking elevation of the aPTT.

Coagulation Data Continued

- The low level of fibrinogen, which is also an acute phase reactant likely indicates some role for a consumptive coagulopathy in explaining the low levels of coagulation factors.
- A decrease in liver synthesis may have also played a role.
- However, given both the histopathology of the liver on the biopsy and autopsy and the levels of transaminases, in particular the ALT, one would not expect such a decrease in synthetic function.

Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus



Gabay C and Kushner I. N Engl J Med 1999;340:448-454

The NEW ENGLAND JOURNAL of MEDICINE

DRAFT*
RAC CONCLUSIONS: ASSESSMENT OF THE ROLE OF THE GENE TRANSFER

*THE PROPOSED RAC CONCLUSIONS WERE NOT FORMALLY VOTED UPON. VOTING DEFERRED IN ORDER TO GIVE RAC MEMBERS TIME TO ADEQUATELY REVIEW AND COMMENT ON THE SPECIFIC LANGUAGE.

DRAFT

- **There was no evidence of contamination of the product.**
- **As expected, the majority of vector remained in the knee at the injection site with only extremely low levels of vector present in tissues outside the knee.**
- **AAV2 rep gene, which may indicate replication competent AAV, was only found in very low levels in the heart and trachea but gene transfer vector (tgAAC94) was not detected in these sites.**
- **The evidence does not support the theory that a helper virus led to widespread dissemination of replication competent AAV.**

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RAC Conclusions Continued

- The declining serum levels of TNF antagonist in the serum do not support the theory that transgene production led to excessive systemic levels of TNF-antagonist.
- The fact that this does not appear to have been a factor in this case does not diminish the importance of having an assay to specifically detect levels of the transgene product.
- The degree of functional TNF inhibition cannot be determined.

DRAFT
RAC Conclusions continued

- The absence of significant vector in the liver and spleen at autopsy and the high anti-AAV titers does not exclude an immune response.

- Whole blood samples from before and after administration of the product were not available for CD8+ capsid specific T-cell assays.

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RAC Conclusions continued

- In the absence of these data an immune response cannot be *definitively* ruled out.
- If such a response occurred, it may or may not have been contributory, but it was not a *primary factor* in this patient's death.
- The inability to exclude an immune reaction to the vector underscores the importance of obtaining samples for T-cell assays.

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RAC Final Assessment

- A possible role of the gene transfer in the clinical course can not definitively be ruled out due to a lack of data.

- If the intra-articular injection of the gene transfer vector did play a role, it was very unlikely to have had been a significant contributor, if any, to the subject's clinical course and was not the cause of her death.

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RAC Final Assessment

- It is the Committee's opinion that this patient's unfortunate death was primarily a result of an opportunistic infection, disseminated histoplasmosis with subsequent bleeding complications and multi-organ failure. Her apparent risk factor for such an infection was her systemic RA therapy, chiefly the TNF-antagonist adalimumab.

Update on Status of Protocol 705

- FDA has removed trial from clinical hold
- Enrollment complete, but not all subjects have received second dose
- Protocol revised as follows:
  - Study product will not be administered to subjects with temperature >98.6˚F, localizing signs and symptoms or unexplained fatigue or malaise on day of administration
    - Considered sensitive indicators of possible infection
  - Patients with a history of opportunistic infection excluded
  - Patients required to have failed at least one DMARD

Protocol Revisions Continued

- Subjects who will receive a second dose will first sign a revised informed consent
- **Additional monitoring**
  - Blood drawn at additional time-points after administration of study agent for CBC, serum chemistry, vector DNA, TNFR:Fc protein
  - Blood drawn at multiple time-points to obtain data on potential T-cell responses to AAV2 capsid

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RAC Recommendations on Trial Design and Lessons Learned

- This case underscores the importance of developing assays to distinguish the gene transfer product from other treatments the subject is receiving.
- The RAC recommends that AAV trials monitor for anti-capsid T cells since this may help both in interpretation of adverse events and shed light on the safety of AAV vectors generally.
  - The FDA currently recommends such monitoring for AAV trials
- The potential role of immunosuppression in altering the risks to subjects enrolled in gene transfer trials needs to be carefully considered.

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RAC Recommendations Continued

- Clinical criteria for the timing of second dosing, especially in safety trials, should be thought out in advance and articulated in the protocol.

- To enhance the safety for participants in gene transfer trials, consider developing a medical card that would include the following:
  - A brief description of the vector and a web-based link to find out more information
  - 24 hour contact numbers for the study investigators
  - A list of samples that should be collected upon admission to a hospital

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RAC Recommendations Continued

- Protocols should plan for the additional blood and other samples that may need to be collected in event of a serious adverse event and think through the logistics of collection should the subject be under the care of physicians who are not involved with the trial.

- The logistics of an autopsy, including a detailed protocol that could be shared with an outside institution as well as considering mechanisms for transfer from an outside institution to the institution conducting the trial should also be developed in advance.

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RAC Recommendations Continued

- With respect to the informed consent:
  - In early phase trials it is critical to take steps to actively prevent therapeutic misconception in the informed consent process, especially in the situation where the investigator is also the subject's physician
    - Investigators need to recognize that their own belief in their study may also lead to therapeutic misconception
  - A discussion of the importance of an autopsy is critical in clinical trials and it may be prudent to involve the family, if possible, in that discussion
  - The *NIH Guidance on Informed Consent for Gene Transfer* provides a potential resource for information on regarding these difficult consent issues http://www4.od.nih.gov/oba/rac/ic/