Phase I/II Trial of rAAV1-hAAT Vector for Treatment of Alpha-1 Antitrypsin Deficiency

Principal Investigator: T. Flotte

Sponsor: Applied Genetic Technologies Corp

Other Funding: NHLBI R01HL69877

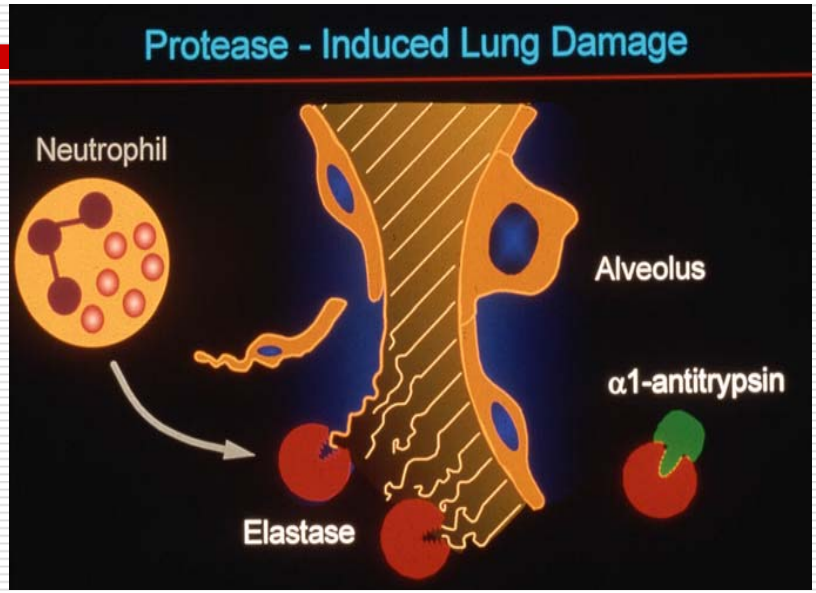
NGVL Preclinical Tox

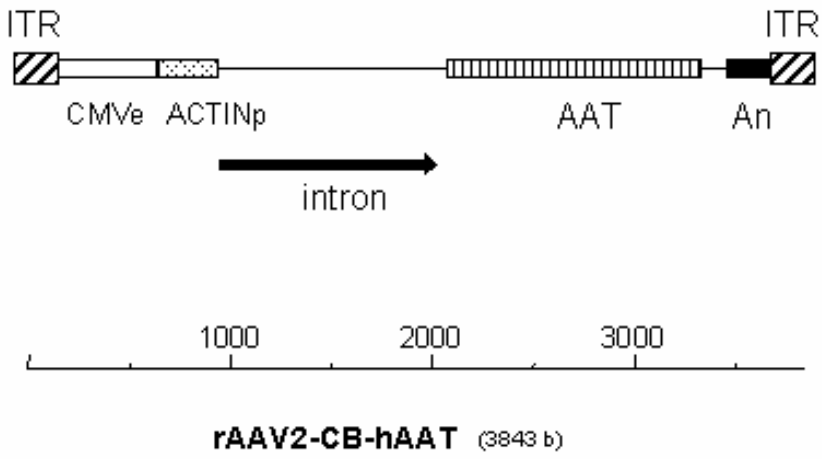
Specific Aims

- Safety of IM administration of rAAV1-CB-hAAT in AAT-deficient adults
  - General Safety
  - Biodistribution
  - Immune response
- Dose-finding pharmacokinetics of rAAV1-CB-hAAT
  - Comparison to ongoing rAAV2-CB-hAAT

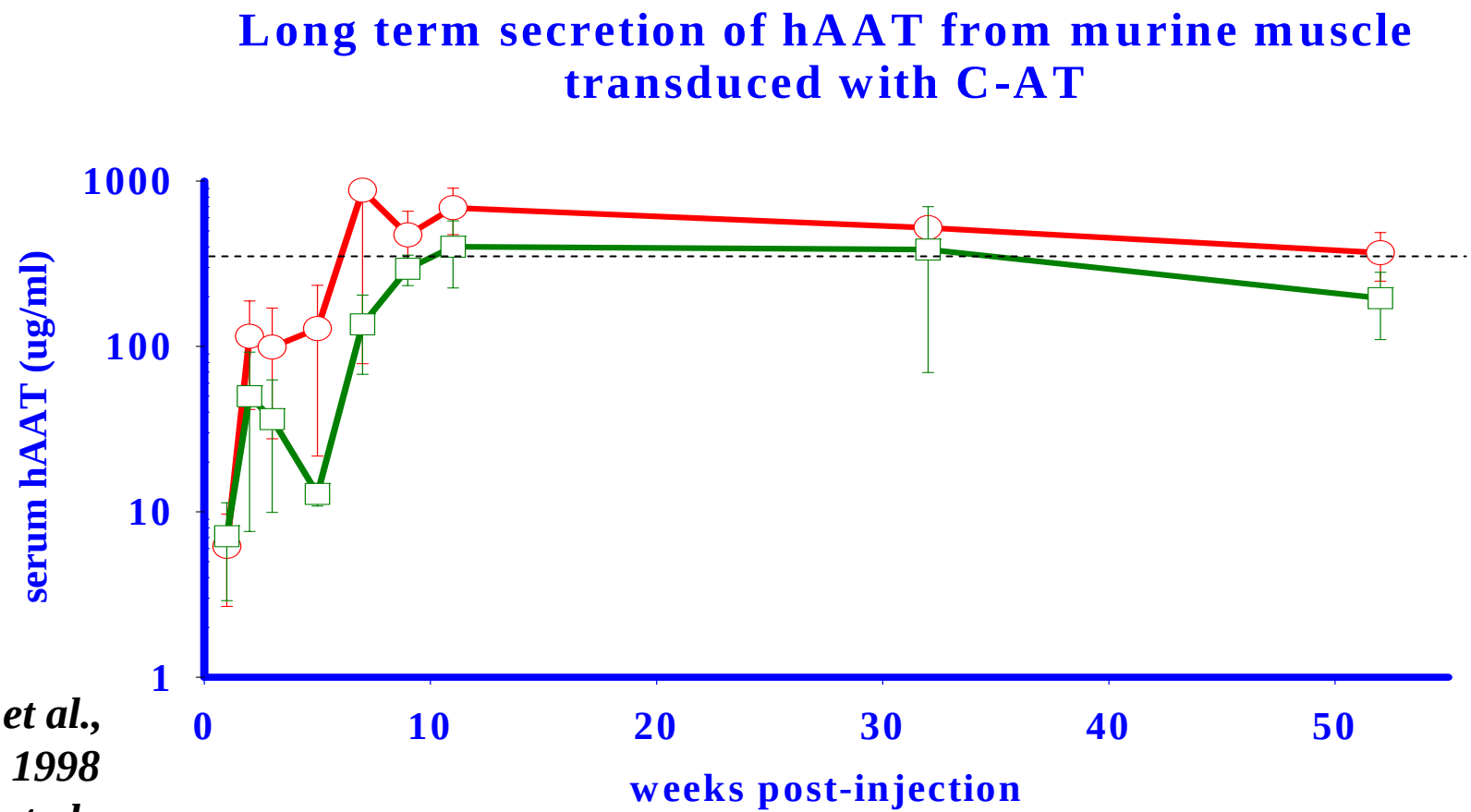
Background: Alpha 1-antitrypsin Deficiency

- Mutations in AAT gene (esp. PiZ)
  - Defective secretion
  - Lack of antiprotease defense from this 52kD serpin
- Lung disease: unopposed action of NE and other white blood cell products on interstitial elastin
  - 11 microM is protective
- Liver disease: only 10% of cases, due to PiZ in hepatocytes





Background:
Proof of Concept with AAV2-AAT IM



Song, et al., PNAS 1998
Song, et al., PNAS 2001

Dose = 1.3×10^{13}vg/mouse = 5×10^{14}vg/kg

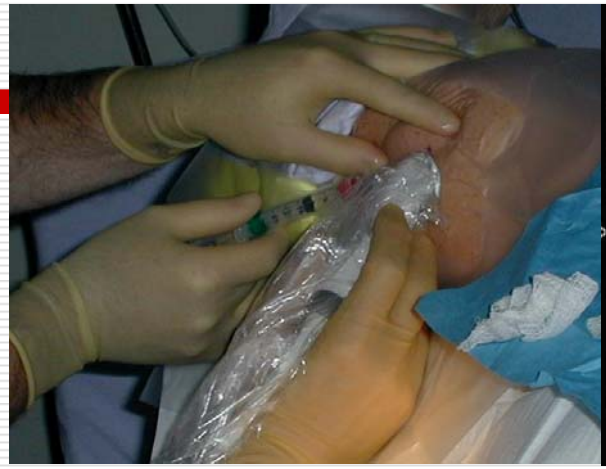
Phase I rAAV2-CB-hAAT HL69877

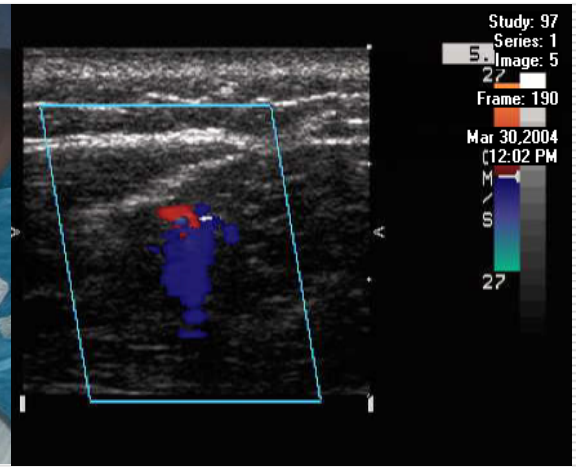
- Single site (UF)
- Open label
- Single dose
- Dose escalation between subjects
- Intramuscular administration with ultrasound guidance to avoid vascular structures
- N = 12 (4 cohorts of 3 subjects)

Cohort #	**1**	**2**	**3**	**4**
Dose, vg	2.1×10^{12}	6.9×10^{12}	2.1×10^{13}	6.9×10^{13}
N	3	3	3	3

First participant: 101

- 59 year old Male
- Caucasian
- Protein replacement prior to study entry





	Baseline	Day -1	Day 0 Dosing	Day 3	Day 14
hAAT uM/ IEF	10.1/ M1Z	3.67/ ZZ		3.56/ ZZ	3.34/ ZZ
FEV1 %	34.8	33.3		31.7	
CK U/L	63	69			82
GGT	19	17		19	17

Phase I rAAV2-CB-hAAT Trial: Cohort 1 Demos and Biodistribution

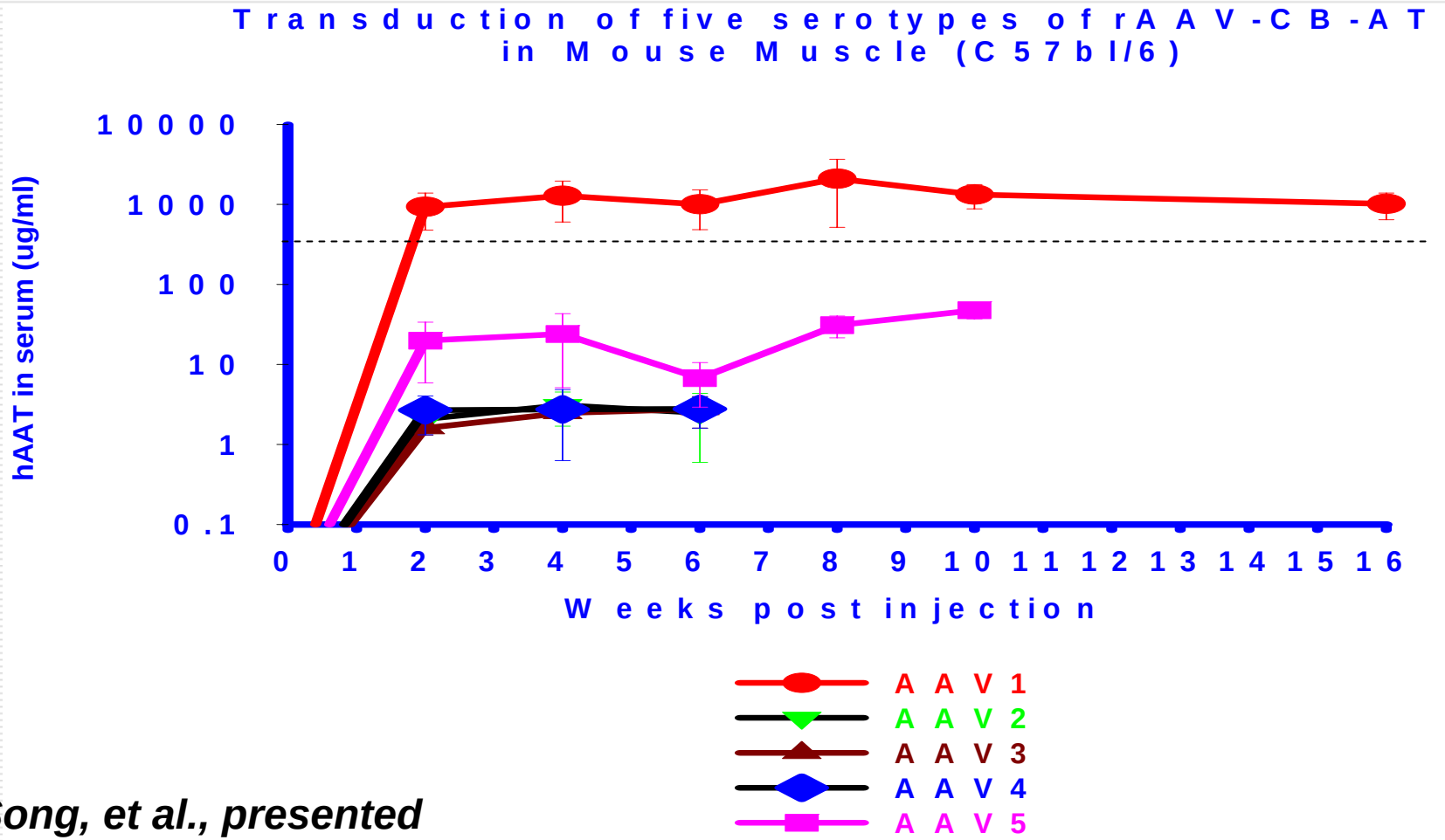
ID	Age	Sex	FEV1 %Pred	DNA-PCR			
				Day -28 S/B	Day 1 B	Day 3 S/B	Day 14 S/B
101	59	M	34.8	-/**	-	-/**	-/**
102	45	M	54.8	-/-	-	-/-	-/-
103	62	M	53	-/***	Pend	Pend	Pend

** Unable to produce
*** Insufficient quantity

Rationale for proposed trial

- rAAV2-CB-hAAT vector currently undergoing phase I clinical trial
  - Dose: 2.1×10^{12} to 6.9×10^{13} vg/patient
- rAAV1-pseudotyped vectors show 500- to 1000-fold potency advantage over rAAV2 in mouse muscle *(Chao, et al., Blood 2000)*
- Identical cassette to be pseudotyped into AAV1 capsid
  - Same Dose Range
  - Safety and Pharmacokinetic profile

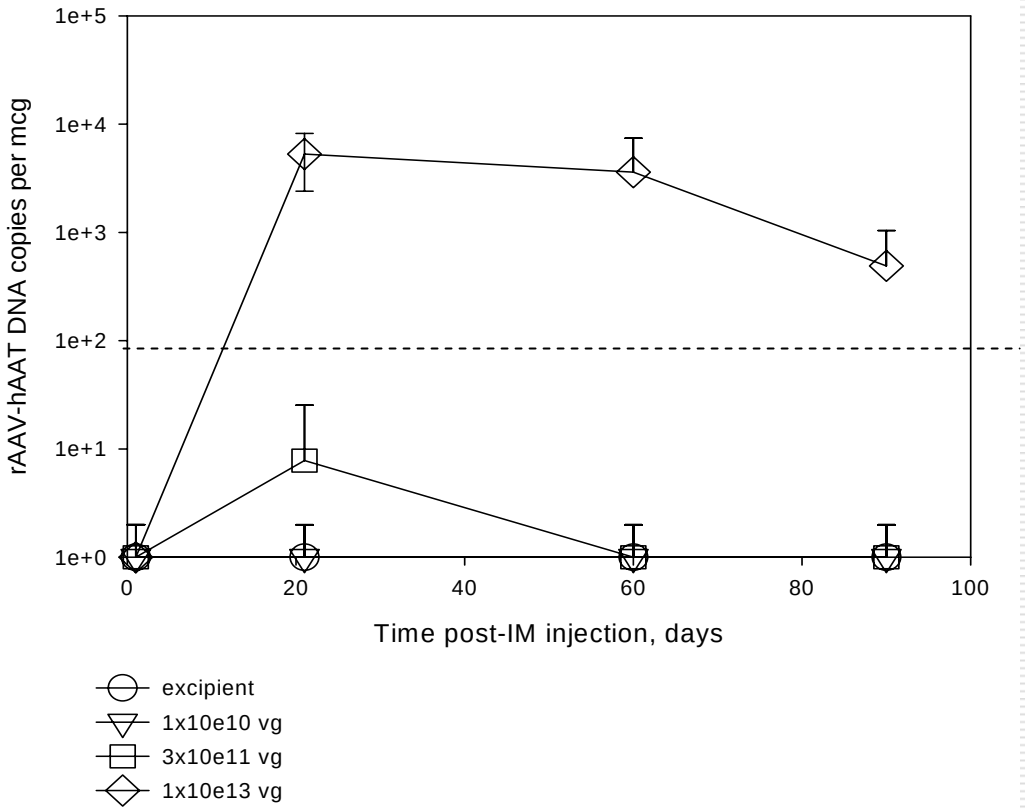
Background: Proof of Concept rAAV1-CB-hAAT IM [10^{11}vg=4x10^{12} vg/kg]



Song, et al., presented at ASGT 2004

Mouse Tox/Biodis Studies: rAAV1-CB-hAAT

- Vector prepared in GLP lab, study performed GLP tox lab
- Dose: up to $4x10^{14}$ vg/kg (400X)
- No clinical, heme, chem abnormalities
- Mild focal Inflammation at injection site with 400X top human dose
- hAAT levels confirmed
- Biodistribution in Blood, other organs, Gonads ->



Rabbit Semen Study

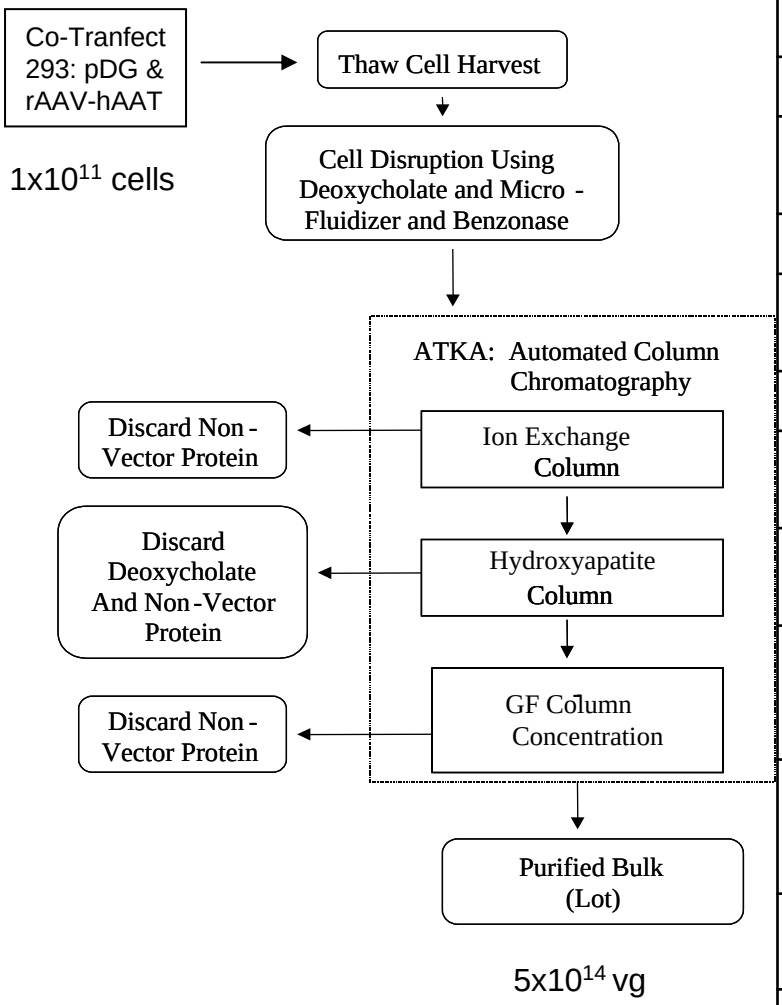
- 4kg NZW rabbits
- IM injection with ultrasound
- Blood and semen
  - D1, 3, 7, 14, sac
- Semen fraction-ated (motile sp)
- Taqman PCR (triplicates, 100 copies per mcg)

Grp	Fold over top dose	21d	60d	90d
4e13 AAT	10X	N=2	N=2	N=2
1e13 AAT	2.5X	N=2	N=2	N=2
4e12 AAT	1X	N=2	N=2	N=2
4e12 GFP	1X	N=2	N=2	N=2

PCR for detection of Vector in Rabbit samples (2.5X top human dose)

Day	0	1	3	7	14
Blood	0/3	3/3	3/3	0/3	
Semen	0/3	2/3	1/3	1/3	
Motile sperm fraction	0/1	0/1	0/1	na	

cGMP Manufacturing at UF: Production Scheme and Lot Release



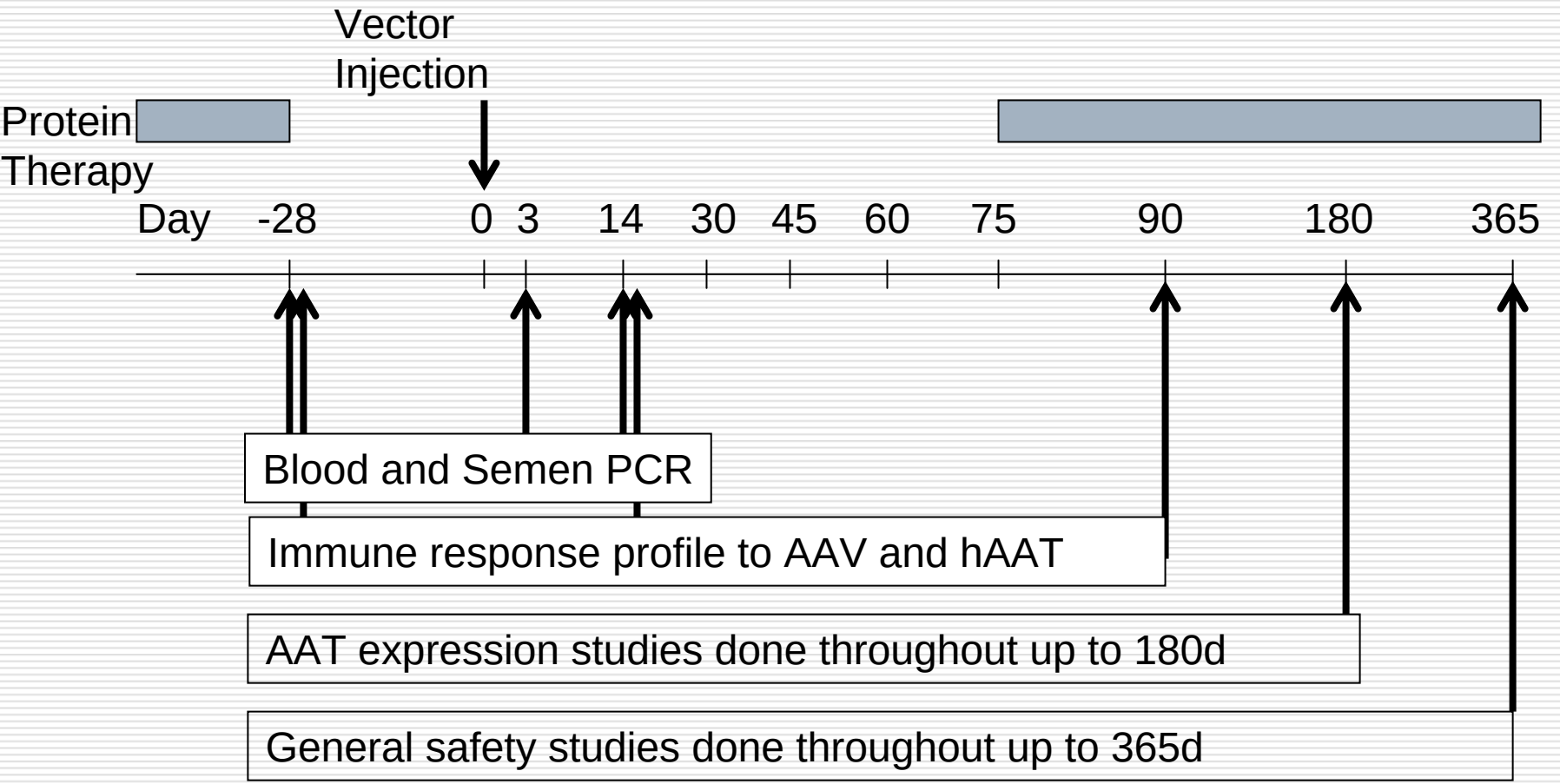
Test	Method	Specification
Sterility	Direct Inoculation	No growth
Endotoxin	LAL	< 50 EU/mL
Titer		
Infectious Titer	ICA	>1X10e11 IU/ml
Vector Genome Titer	Dot Blot	>1X10e13 vector genomes/ml
Capsid Titer	ELISA	Report Results
Infectivity Ratio	ICA/Dot Blot	Report Results
Purity		
Protein purity	PAGE and coomassie blue stain for protein	>90% pure
293 Cell contaminating DNA	Hybrization	< 100 ng per dose
Benzonase residual	ELISA	Report Results
rcAAV	Infectious Center assay	<1 in 1X10e8 IU rAAV

Phase I Protocol

- Single site (UF)
- Open label
- Single dose
- Dose escalation between subjects
- Intramuscular administration with ultrasound guidance to avoid vascular structures
- N = 12 (4 cohorts of 3 subjects)

Cohort #	**1**	**2**	**3**	**4**
Dose, vg	2.1×10^{12}	6.9×10^{12}	2.1×10^{13}	6.9×10^{13}
N	3	3	3	3

Time Line: Phase I/II rAAV1-AAT



Subject Selection

- Inclusion criteria
  - Male or female ≥ 18
  - Diag of AAT def by level

  (< 11uM) and genotype (Z)
  - Willing to discontinue AAT replacement 4 weeks prior and resume 11 after injection

Subject Selection

- Exclusion criteria
- Recent (15d) oral/IV antibiotics or oral corticosteroids
- LFTs > 2x ULN; Bilibrubin, GGT > 1.2x ULN
- CK > 3xULN; INR > 1.3
- Platelet dysfunction; Platelet count < 75,000/cumm
- Other investigational drug
- Pregnant or nursing
- Fertile and not using contraception
- Cig smoker or substance abuse
- Immune response to AAT replacement
- Other conditions at discretion of PI

Outcomes: Safety

- Safety
  - Injection site (Deltoid muscle)
    - Inspection
    - Arm Circumference
    - Baseline CT
  - General
    - Blood work, PFTs, EKG
    - Baseline Chest CT
  - Immunology
    - Anti-AAV: antibody by ELISA, CTL-like assay (ASR)
    - Anti-AAT: antibody by ELISA, CTL-like assay (ASR)
  - Biodistribution/Germline
    - Blood PCR
    - Semen PCR
  - Long-term Followup Plan

Outcomes: Bioactivity
Patients off from d -28 to d +75

- Total AAT by nephelometry
- M-specific IEF Western
- M-specific ELISA

Stopping Rules

- If 0 of 3 subjects experiences a serious, unexpected AE at least possibly related to vector, proceed to next dosage.
- If 1 subject experiences such an AE, plan to add 2 additional subjects to that dosage level cohort (total of 5)
- As soon as any later subject at that same dosage level experiences such an AE, stop the dose escalation
- the MTD is defined as the next lowest dose

Study day	**-28***	**-1***	**0***	**1***	**2***	**3***	**14***	**30**	**45**	**60**	**75**	**90***	**180**	**270**	**365**
Informed Consent	X														
Complete History	X														
Physical Exam	X	X	X	X	X	X	X					X			
Abbreviated History based on prior visit		X	X	X	X	X	X					X			
EKG (electrocardiogram)	X														
Pregnancy test [1]	X	X										X			
Serum hAAT, & IEF[2]	X	X				X	X	X	X	X	X	X	X		
CBC, PT/PTT, Chemistries, UA	X	X		X		X	X					X			
Creatine Kinase	X	X					X					X			
Platelet Function Assy	X														
Pulmonary Function Tests[3]	X	X				X						X			
Chest[4] and Arm CT Scan[4a]	X														

Study day	**-28***	**-1***	**0***	**1***	**2***	**3***	**14***	**30**	**45**	**60**	**75**	**90***	**180**	**270**	**365**
AAV antibody and ASR testing [5]	X						X					X			
AAT antibody and ASR testing[5]	X						X					X			
Semen test[6]	X					X	X+								
Vector in peripheral blood[7]	X			X		X	X++								
Study Agent Administration			X												
Mid-arm Circumference[8]		X		X	X	X									
Adverse Events[9]			X	X	X	X	X	X	X	X	X	X	X	X	X
AAT protein replacement product Discontinued[10]	X														
AAT protein replacement product Resumed[10]											X				