Transduction of the upper airway epithelium in humans with cystic fibrosis by an AAV6 vector that encodes human placental alkaline phosphatase

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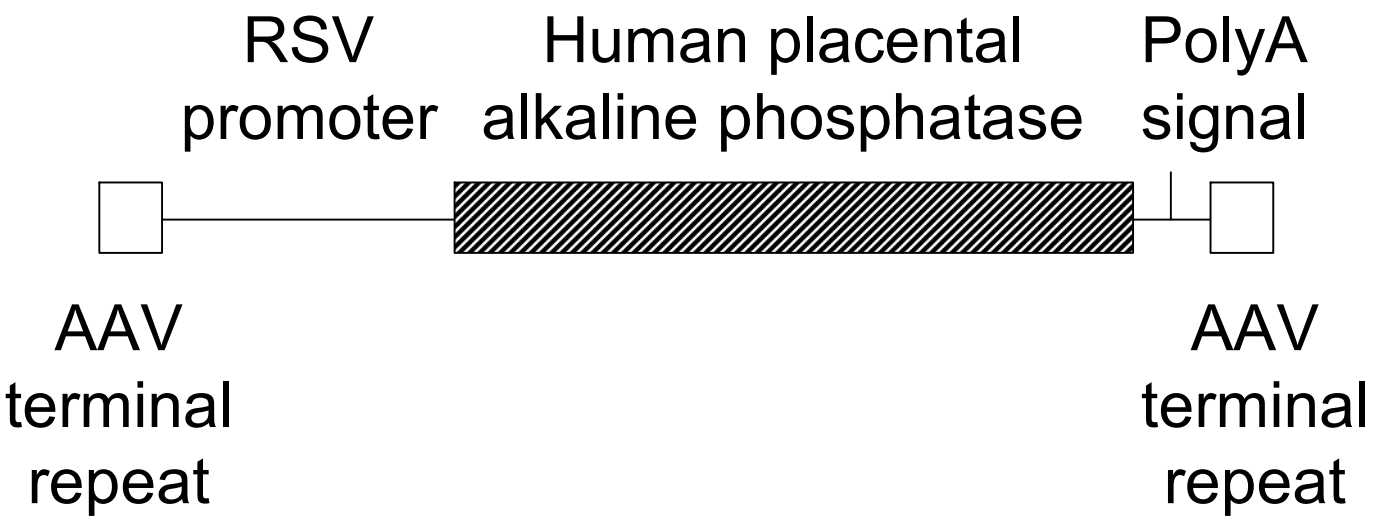
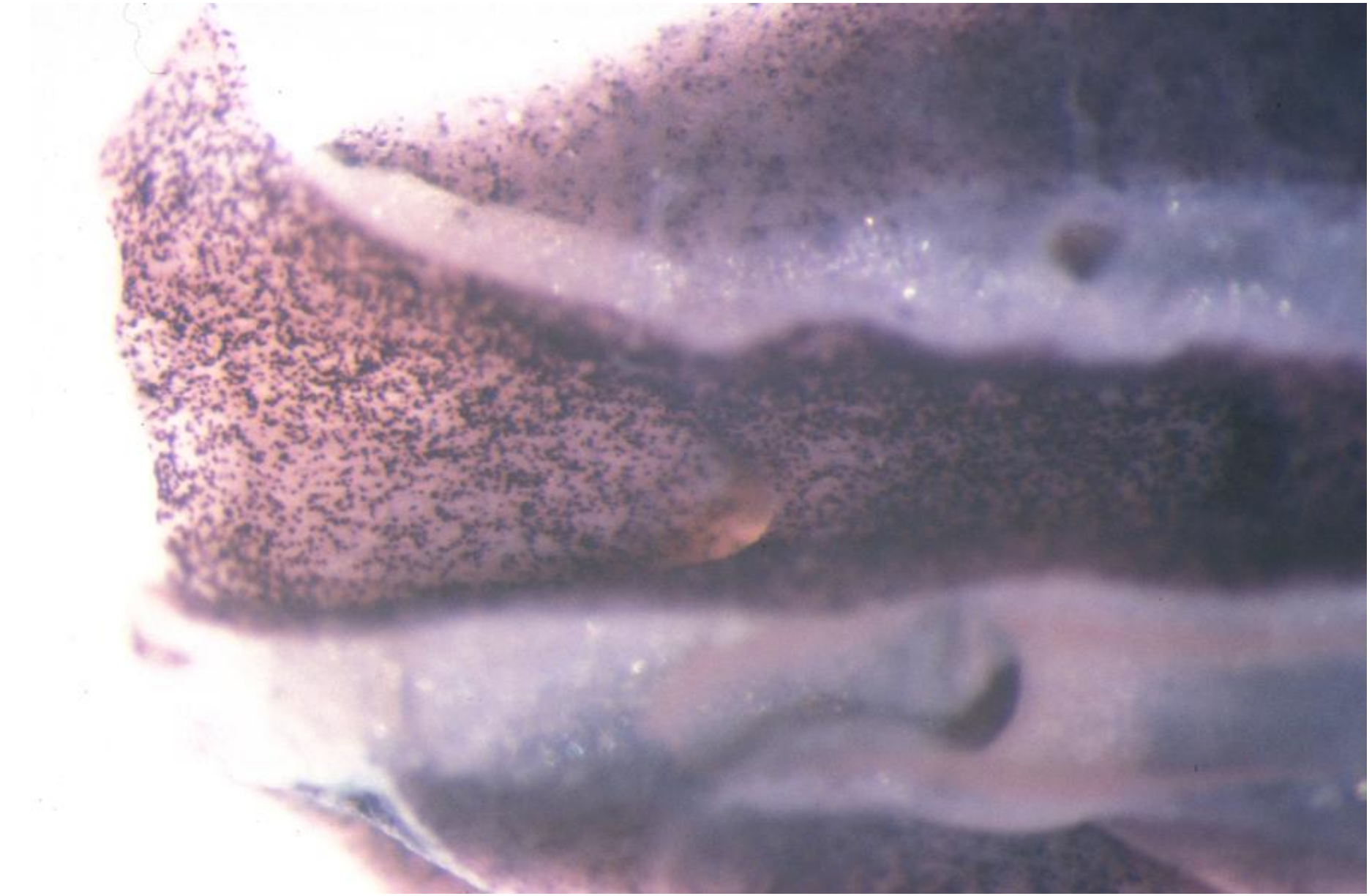
Sponsor: A. Dusty Miller Ph.D.

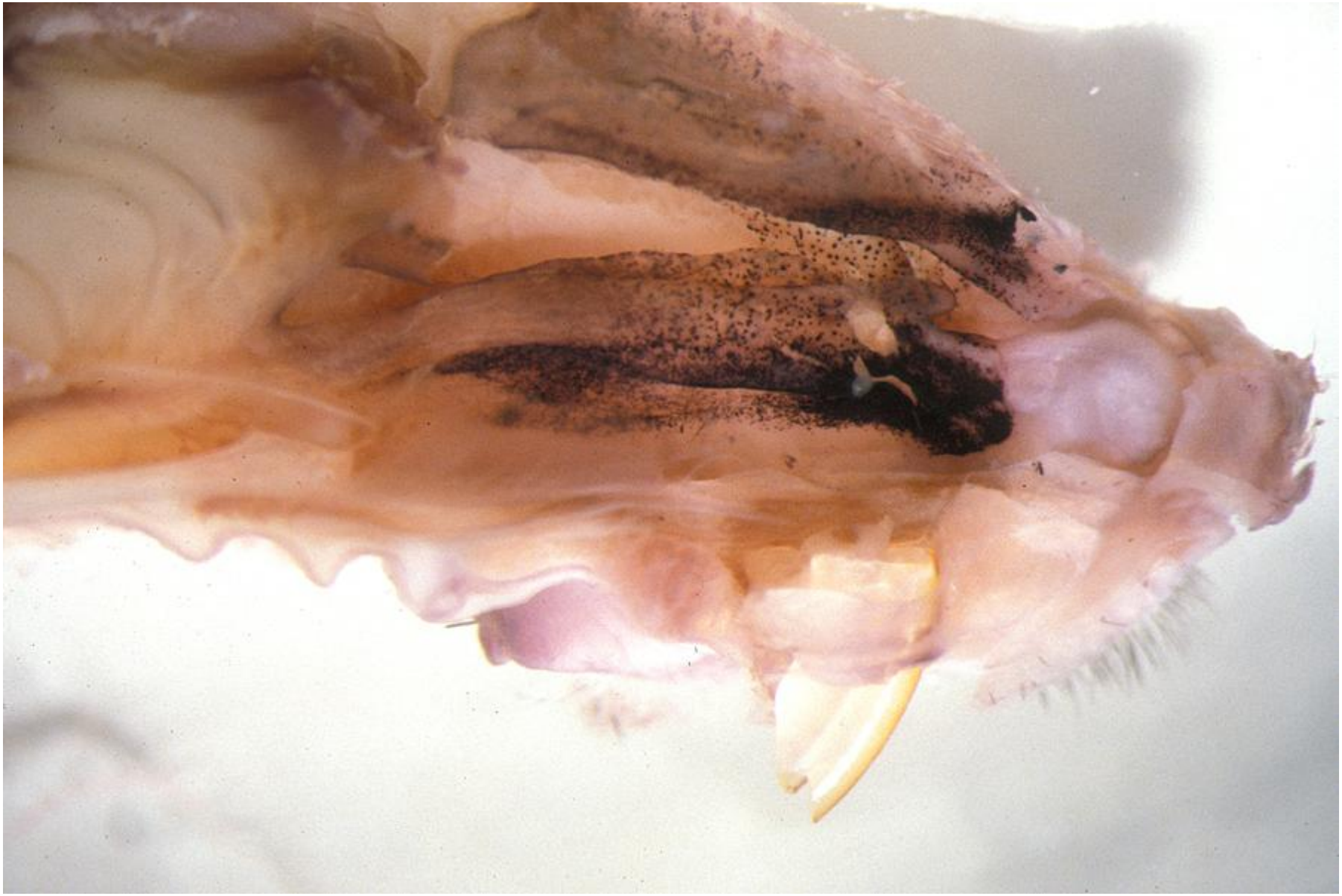


“ARAP4” AAV Vector

ARAP4(AAV6), 2 x 10^{10} vector genomes/mouse



ARAP4(AAV6), 2 x 10^{10} vector genomes/mouse



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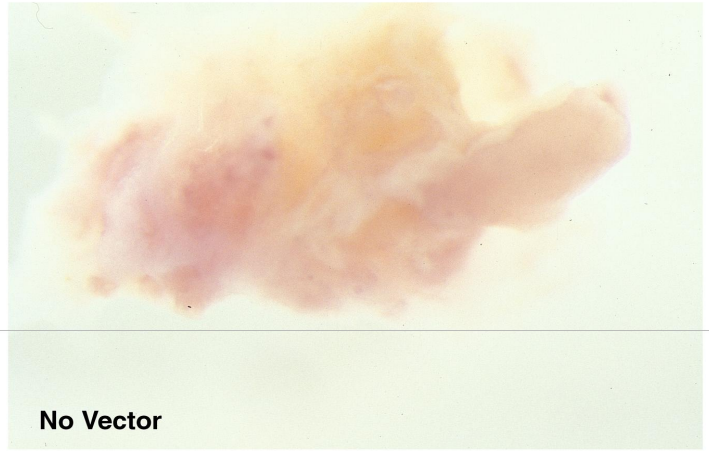
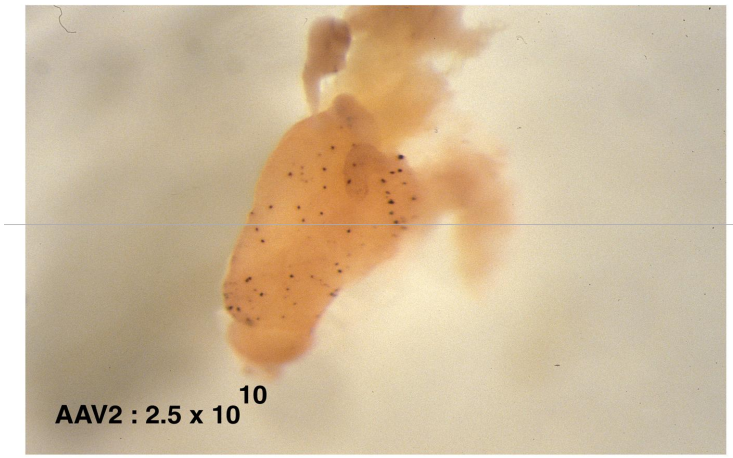
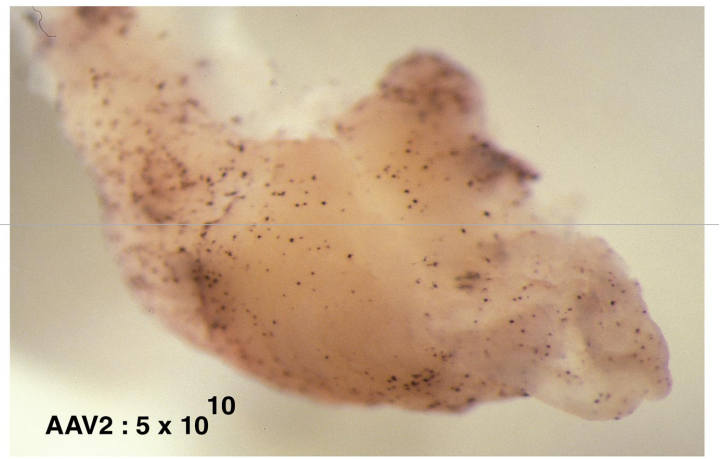
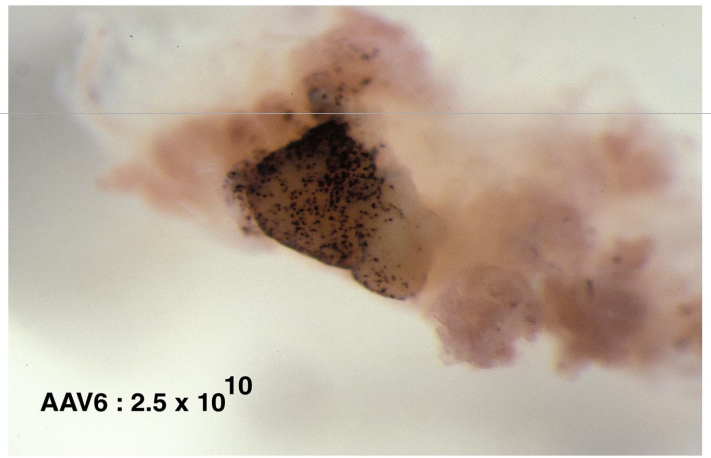
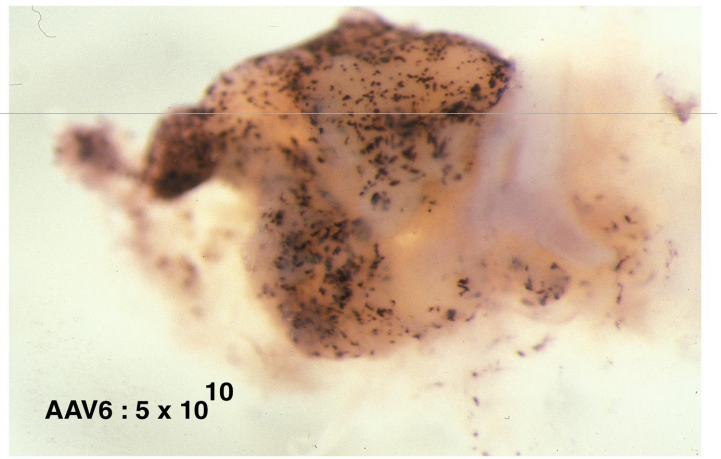

No Vector


AAV2 : 2.5 x 10^10


AAV2 : 5 x 10^10


AAV6 : 2.5 x 10^10


AAV6 : 5 x 10^10

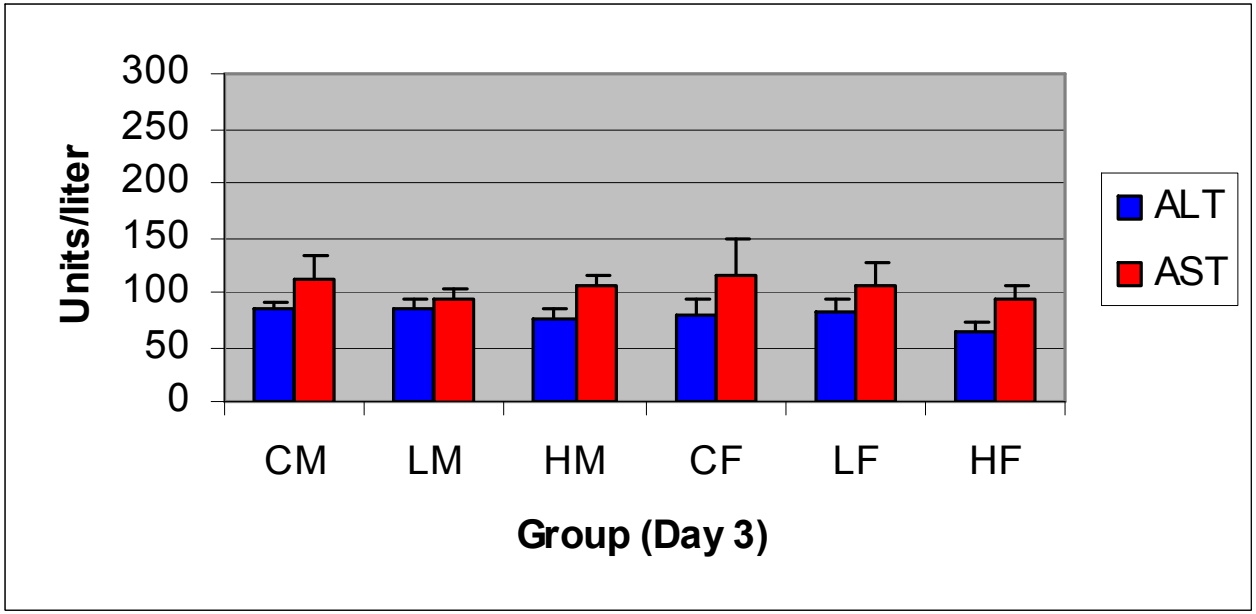
Preclinical Safety Study

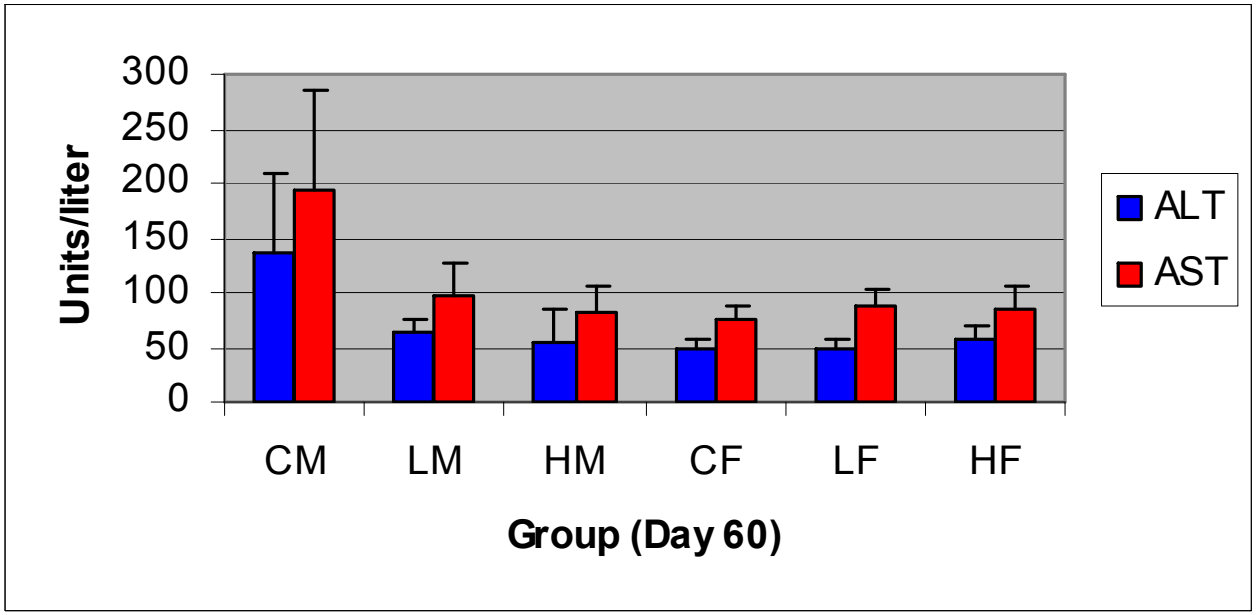
- 150 g rat: 0, 3.3×10^9, 3.3×10^{10} vg.

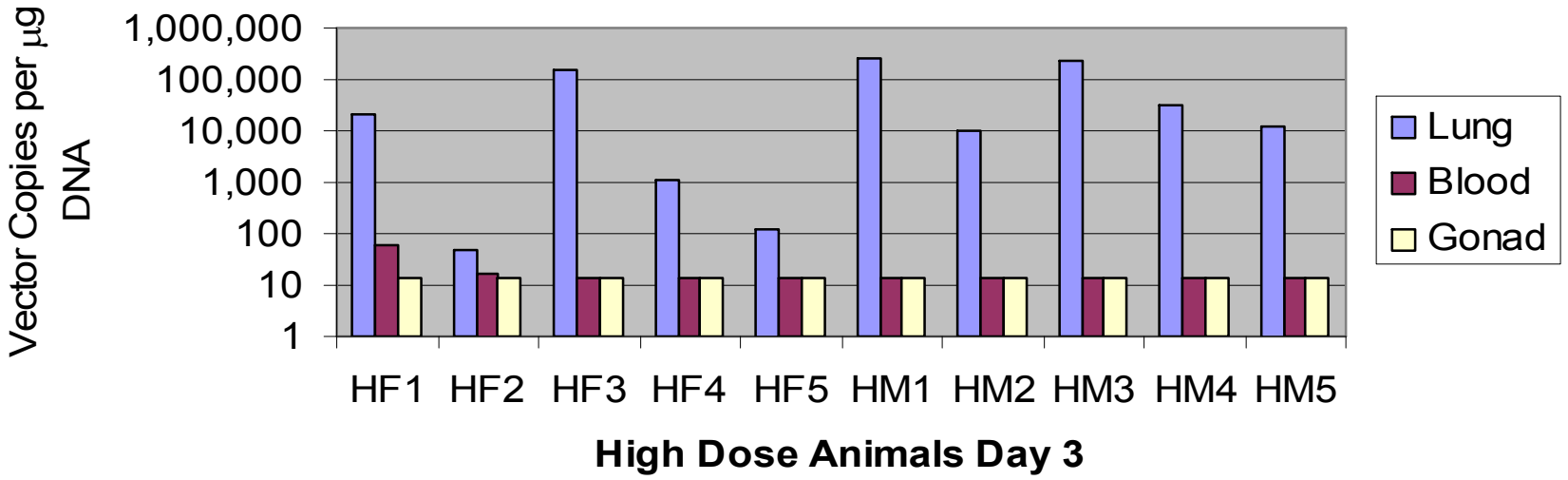
  Max = 2.2×10^8 vg/g
- 50 kg human: 0, 10^{11}, 10^{12} vg.

  Max = 2×10^7 vg/g.
- Examined at 3 and 60 days after nasal instillation of vector: CBC, serum chemistry, DNA in lung, blood and gonads, histology.

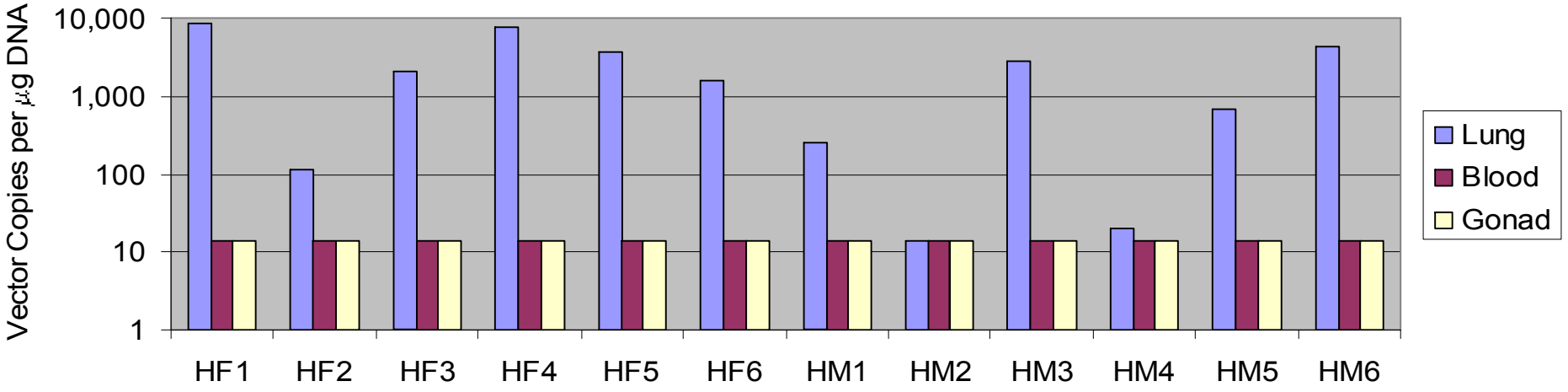
ALT and AST Enzyme Levels in Mice





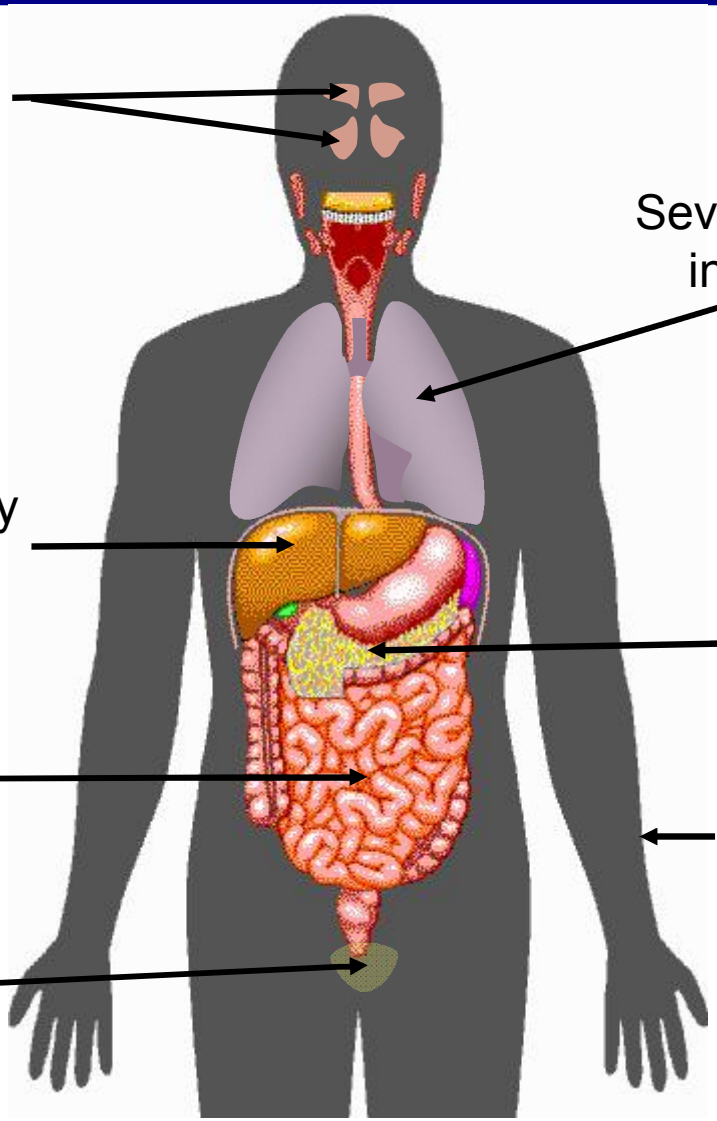


High Dose Animals Day 3



High Dose Animals Day 60

Cystic Fibrosis: Multi-organ disease



Chronic Sinusitis

Severe chronic bacterial infection of airways

Severe hepatobiliary disease (5-10% of cases)

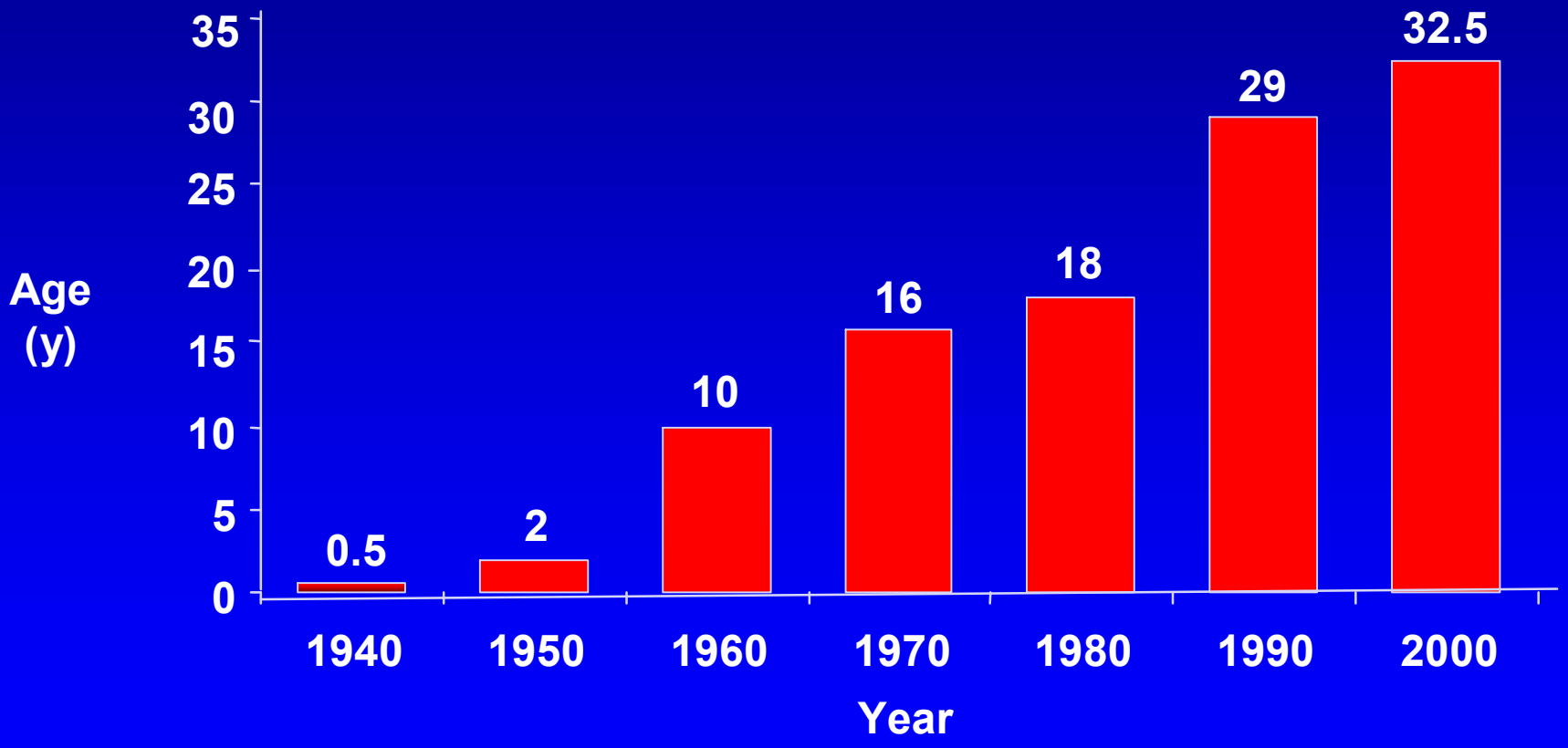
Pancreatic exocrine insufficiency

Meconium ileus at birth (15-20% of cases)

Sweat chloride value 60mmol/L or more

Obstructive azoospermia

Survival in CF improved to 35 years with conventional treatments



Ref: Cystic Fibrosis Foundation, Bethesda, MD.

Protocol Synopsis 1

- **Objectives** To determine the safety, transduction efficiency and immune response associated with AAV6-AP delivery

- **Study Design** A phase I, double-blind, placebo-controlled, randomized trial. Two dose levels will be studied. Escalation to the second level will occur only if there are no safety concerns at the first dose level.

Protocol Synopsis 2

- **Sample Size**
  - Two dose cohorts with four subjects per cohort ie total of eight subjects if both levels are studied
- **Subjects**
  - Patients with documented cystic fibrosis age 18-50 years.
- **Drug dosage** The first cohort will receive 10^{11} vector genomes with subsequent cohort to receive 10^{12} vector genomes.

Protocol Synopsis 3

- **Outcomes**

1) **Safety** outcome measures will include: adverse events profile, physical examination, vital signs, oxygen saturation, spirometry, serum chemistries, CBC with differential, blood and nasal fluid for vector shedding, **serum neutralizing antibodies to AAV6 and hpAP.** Additional safety measures include:

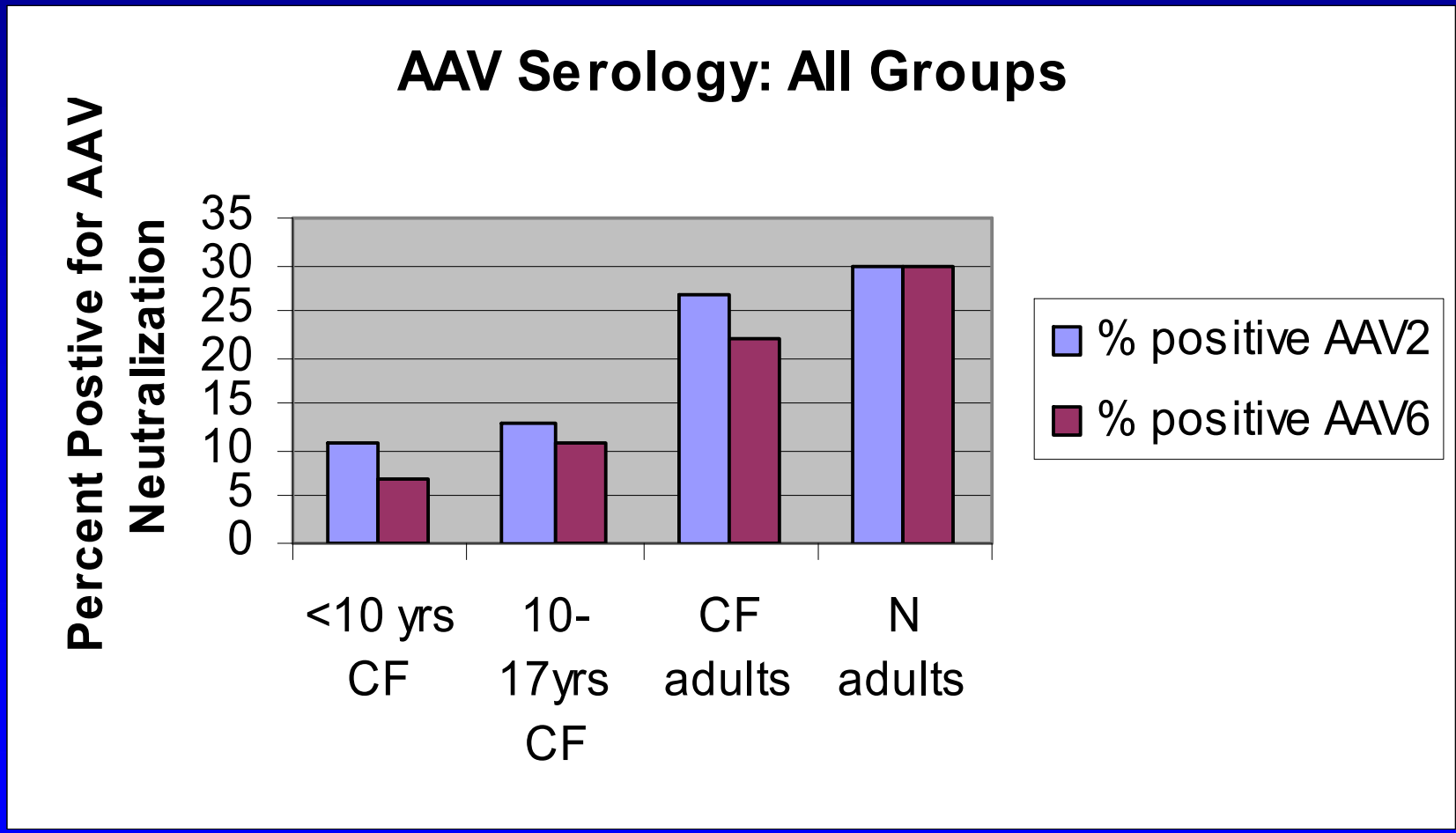
   – endoscopic inspection of nasal mucosa; cell counts with differentials and cytokines in nasal fluid

2) The **efficacy** outcome measure will be expression of AP in the nasal epithelium. Expression of AP will be determined on the basis of AP staining of samples obtained by nasal biopsies.

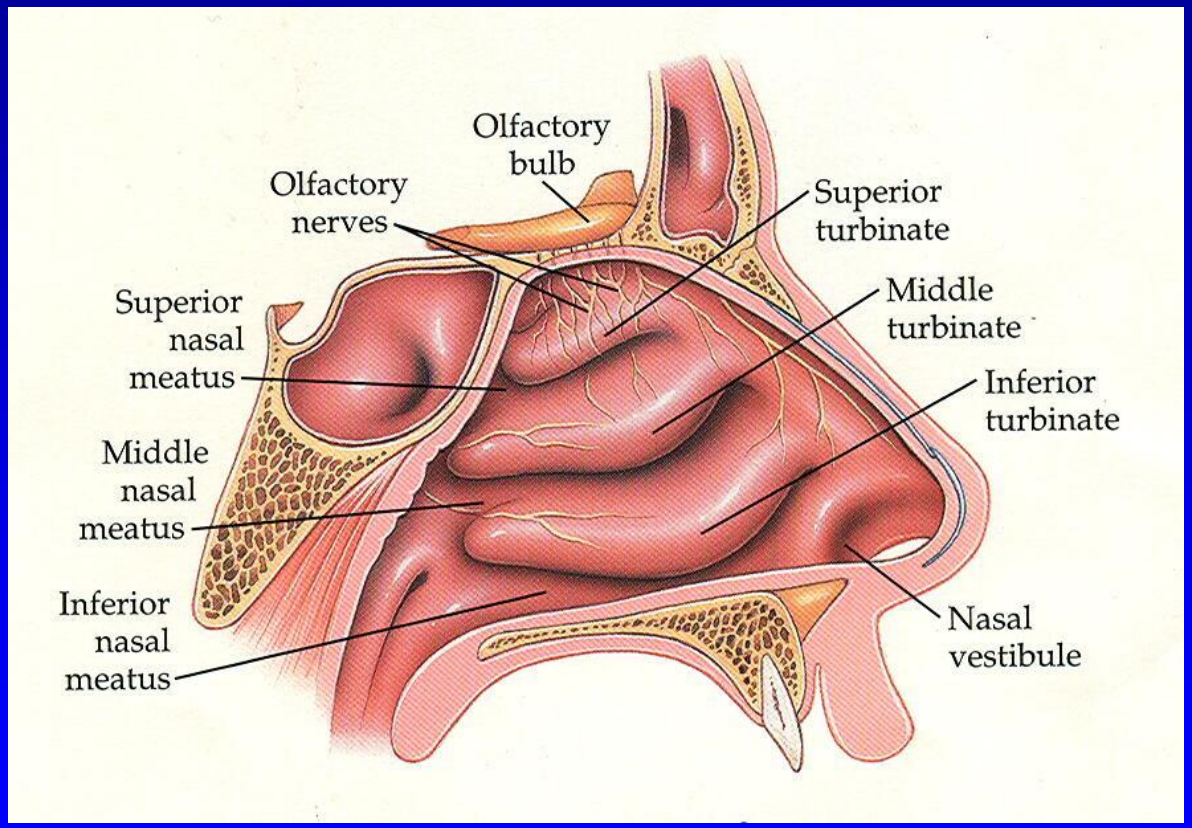
Subject Inclusion Criteria

- Age 18-50 years
- Diagnosis of CF confirmed
- FEV1 > 40% predicted at screening
- Oxygen saturation > 90% at screening
- Sero negative for AAV6
- Use contraception for the duration of the study
- Informed consent provided

70% of CF adults are sero-negative to AAV6



Delivery to the lateral wall of human nose

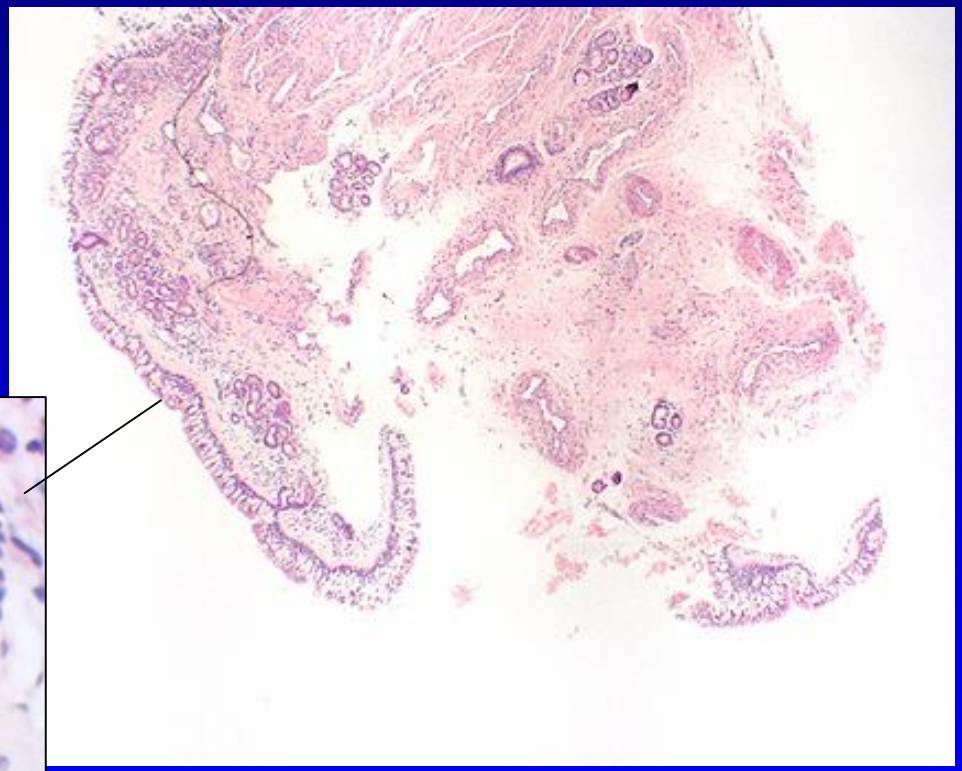


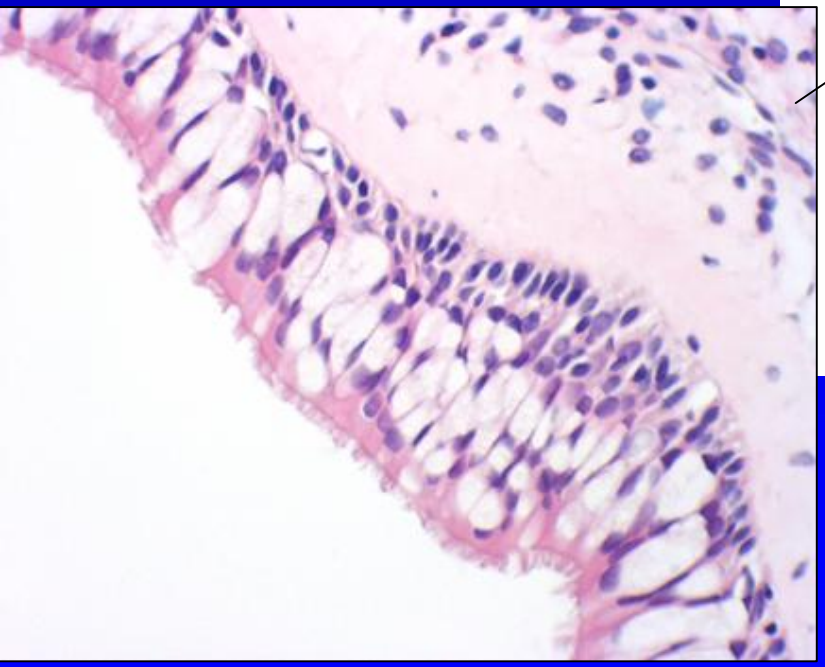
2-5 cm in inferior turbinate

CFTR is expressed in epithelium of inferior turbinate

Clinical trial preparations: nasal biopsies

No endogenous AP staining





Normal human inferior turbinate biopsy

N = 5 non CF subjects

Sample size justification

Proof of concept study

-to determine if AP can be delivered to the upper airway

Pilot study to guide AAV6 CFTR trials

Mechanistic study

-not powered to produce a statistically significant result

-AAV6 AP is not a therapeutic vector

- limit the number of subjects used

In summary, the number chosen is a compromise of scientific data and minimizing the subject number in a non therapeutic vector

Response to RAC comments

AP expression

Predict that expression will increase over time

Transduction rates will depend on vector dose

Transduction rates will be higher at higher dose

AP expression

Outcome % AP-positive epithelial cells

Strong positive response if any of three nasal biopsies ≥10% transduction

Dose escalation will NOT depend on the lower dose transduction rates

PROPOSED AMENDMENT

If 2/3 subjects at the higher dose are strongly positive the study will be considered a success and no further patients will be enrolled

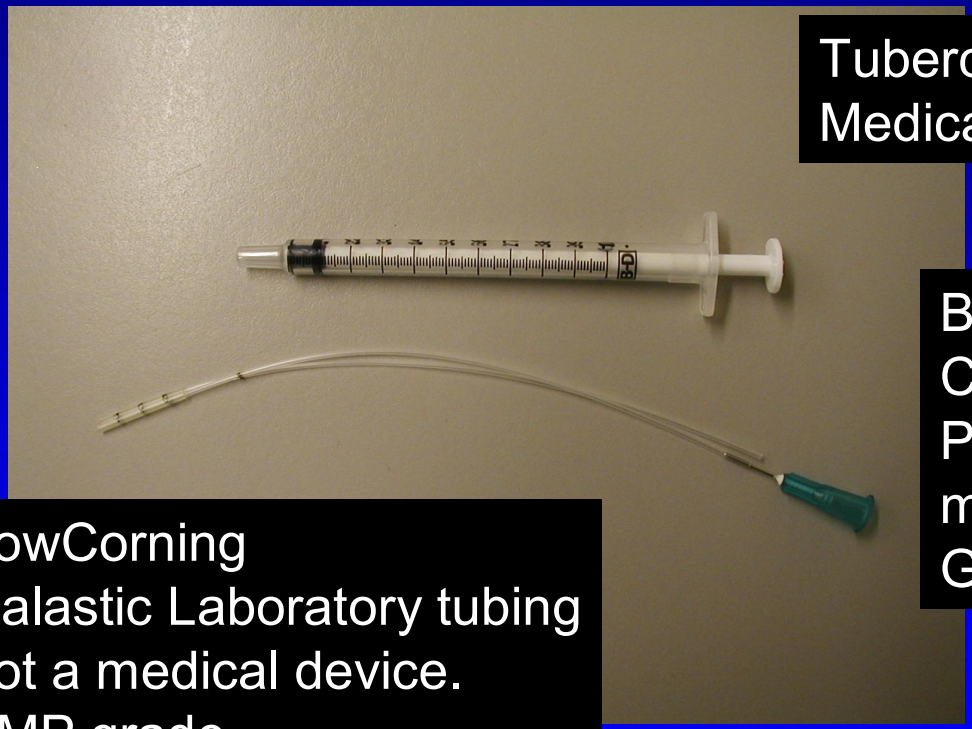
If 0 or 1 subject is strongly positive at the higher dose then we wish to recruit 3 additional subjects, i.e. a total of n=6, at the higher dose level

Transduction of the Upper Airway Epithelium in Humans with Cystic Fibrosis by an AAV6 Vector that Encodes Human Placental Alkaline Phosphatase

Future studies

- AAV6 AP to the lower airway
- AAV6 CFTR to the nasal epithelium
- AAV6 CFTR to the lung

Drug delivery “device”



Tuberculin syringe
Medical device

Benton Dixon/Intramedic
Clay Adams brand
Polyethylene tube. Not listed as medical device. Used in surgery.
GMP grade.

DowCorning
Sialastic Laboratory tubing
Not a medical device.
GMP grade.

20% drug loss

Device Regulators: John Beemer B.S., Morty Cohen Pharm D.
CFF TDN