



Giant Axonal Neuropathy Gene Therapy

Recombinant DNA Advisory Committee Meeting

10:30 a.m.; June 12, 2013

Lori Sames, executive director of Hannah's Hope Fund (sponsor)

Dr. Carsten Bonnemann, NINDS, NIH Clinical Center (PI)

Dr. Steven Gray, Investigator, UNC Gene Therapy Center

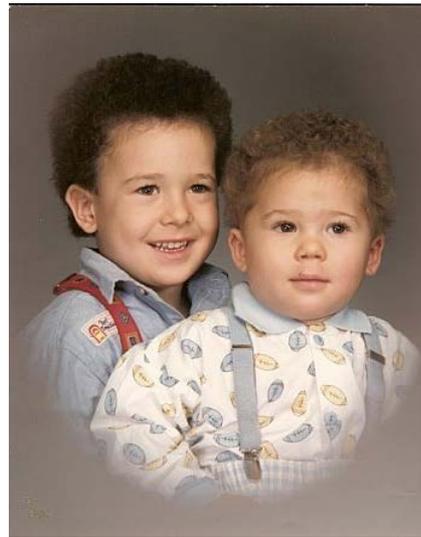
Dr. Jude Samulski, Investigator, Director of the UNC Gene
Therapy Center

Dr. Jahannaz Dastgir, Investigator, NINDS, NIH Clinical Center

Overview

- Introduction of Giant Axonal Neuropathy
- Assess the risk factors of a GAN gene transfer study
- Summary of proof-of-concept and efficacy studies
- Summary of GLP toxicology studies
- Summary of the clinical protocol
- A note from the sponsor, Hannah's Hope Fund

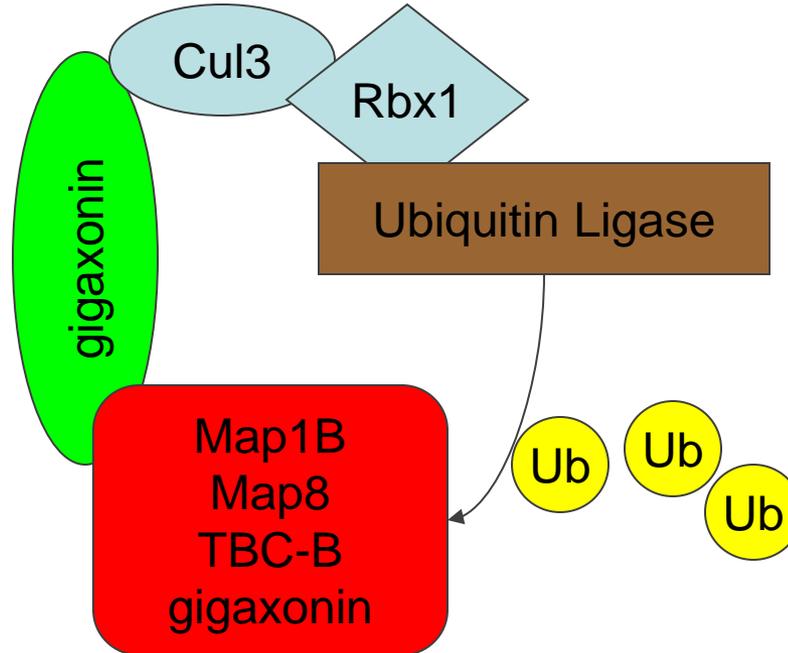
Giant Axonal Neuropathy



7/9/2013

Giant Axonal Neuropathy (GAN)

- Rare autosomal recessive disease of the central and peripheral nervous system caused by loss of gigaxonin gene (GAN)

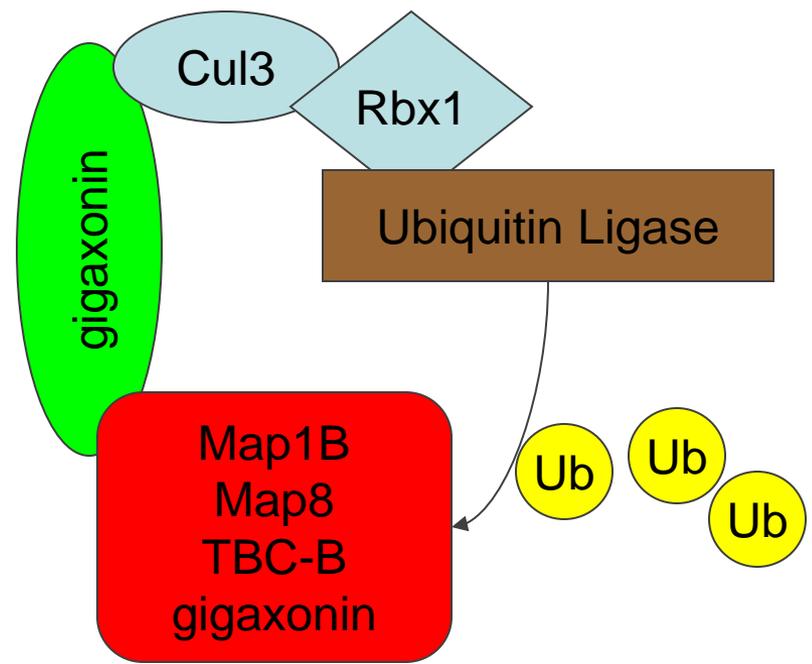


Dysregulated Proteins

- GFAP
 - Keratin
 - NF-H
 - NF-L
 - Vimentin
 - Peripherin
 - Alpha-Internexin
 - Other IFs
-
- Loss of gigaxonin protein results in intermediate filament (IF) accumulation
 - Axonal accumulation of IFs causes the most severe disease symptoms

Giant Axonal Neuropathy (GAN)

Rare autosomal recessive disease of the central and peripheral nervous system caused by loss of gigaxonin gene (GAN)



Dysregulated Proteins

- GFAP
- Keratin
- NF-H
- NF-L
- Vimentin
- Peripherin
- Alpha-Internexin
- Other IFs

- Loss of gigaxonin protein results in intermediate filament (IF) accumulation
- Axonal accumulation of IFs causes the most severe disease symptoms

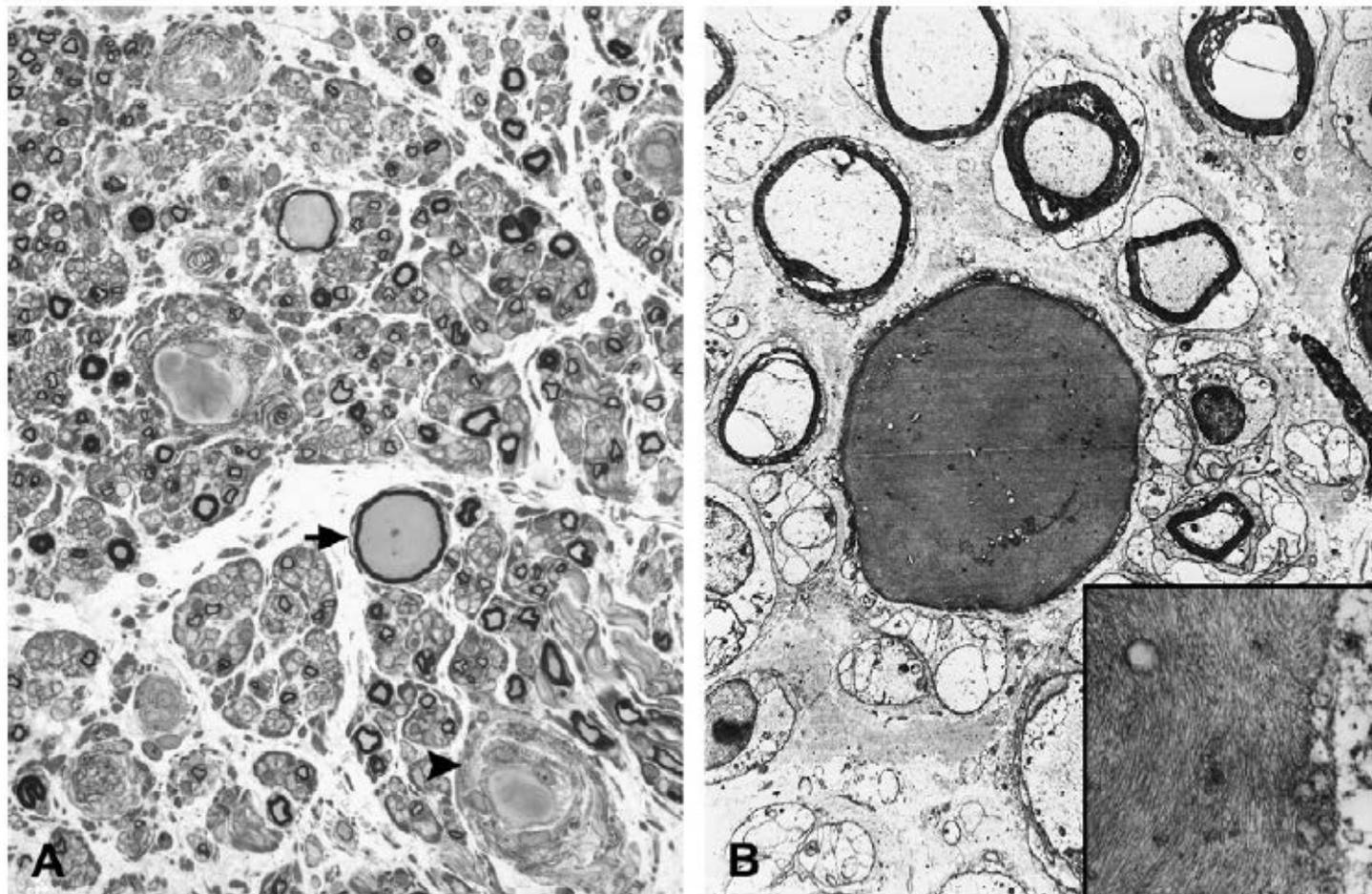


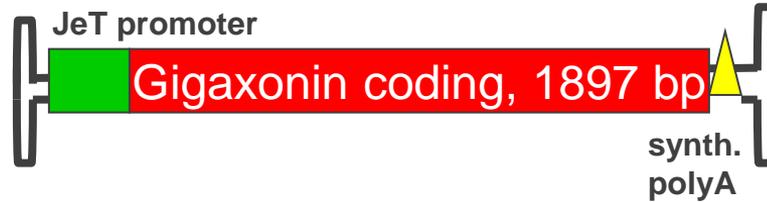
Fig. 5. (A, B) Sural nerve biopsy. (A) Light microscopy of a semithin section of plastic embedded nerve tissue shows several giant axons of varying sizes, either completely denuded of myelin or surrounded by a thinned out myelin sheath (arrow). Occasionally a large, loose cluster of 'onion bulb' Schwann cell hyperplasia is seen (arrowhead). (B) Transmission electron microscopy (TEM) shows a giant axon in the center stuffed with neurofilaments and ensheathed by thinned out myelin. The giant axon is surrounded by several smaller myelinated fibers, the axons of which show normal density of neurofilaments. Inset shows higher magnification of the closely packed neurofilaments within the giant axon dispersing the normal cellular organelles (A: Toluidine blue stain, original magnification $\times 400$; B: EM graph: $\times 8000$; Inset: $\times 28,000$).



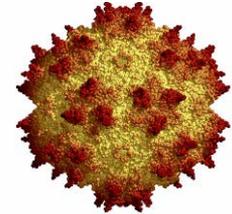
Animal Models for GAN

- GAN/Yang mouse model (exon 3-5 Δ)
 - » Ding *et al.*, 2006
 - » Mild behavioral phenotype, axon pathology
- GAN/Julien mouse model (exon 1 Δ)
 - » Dequen *et al.*, 2008
 - » Axon pathology at 6 months, intermediate filament inclusions in the CNS, no behavioral phenotype.
- GAN/Bomont mouse model (exon 3-5 Δ)
 - » Ganay *et al.*, 2011
 - » Axon pathology, weak but significant behavioral phenotype at 14 months. Tested separately on 129 and C57BL/6J backgrounds.

Proposed Approach for GAN



self-complementary genome



AAV9 Capsid

Targets:

- Primary targets will be spinal cord and brainstem motor neurons, DRG (to treat peripheral motor and sensory disease)
- Secondary targets will be the brain and peripheral tissues

Preliminary Clinical Approach

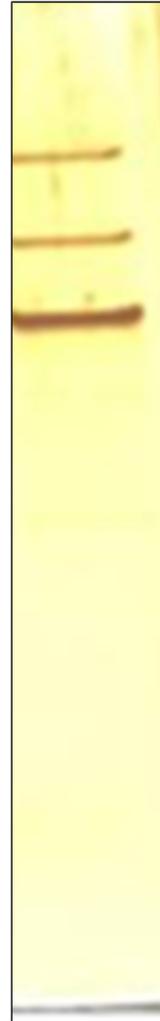
- Agent: GMP scAAV9/JeT-GANopt vector manufactured by the UNC Vector Core Human Applications Lab

Silver Stains, Past and Present

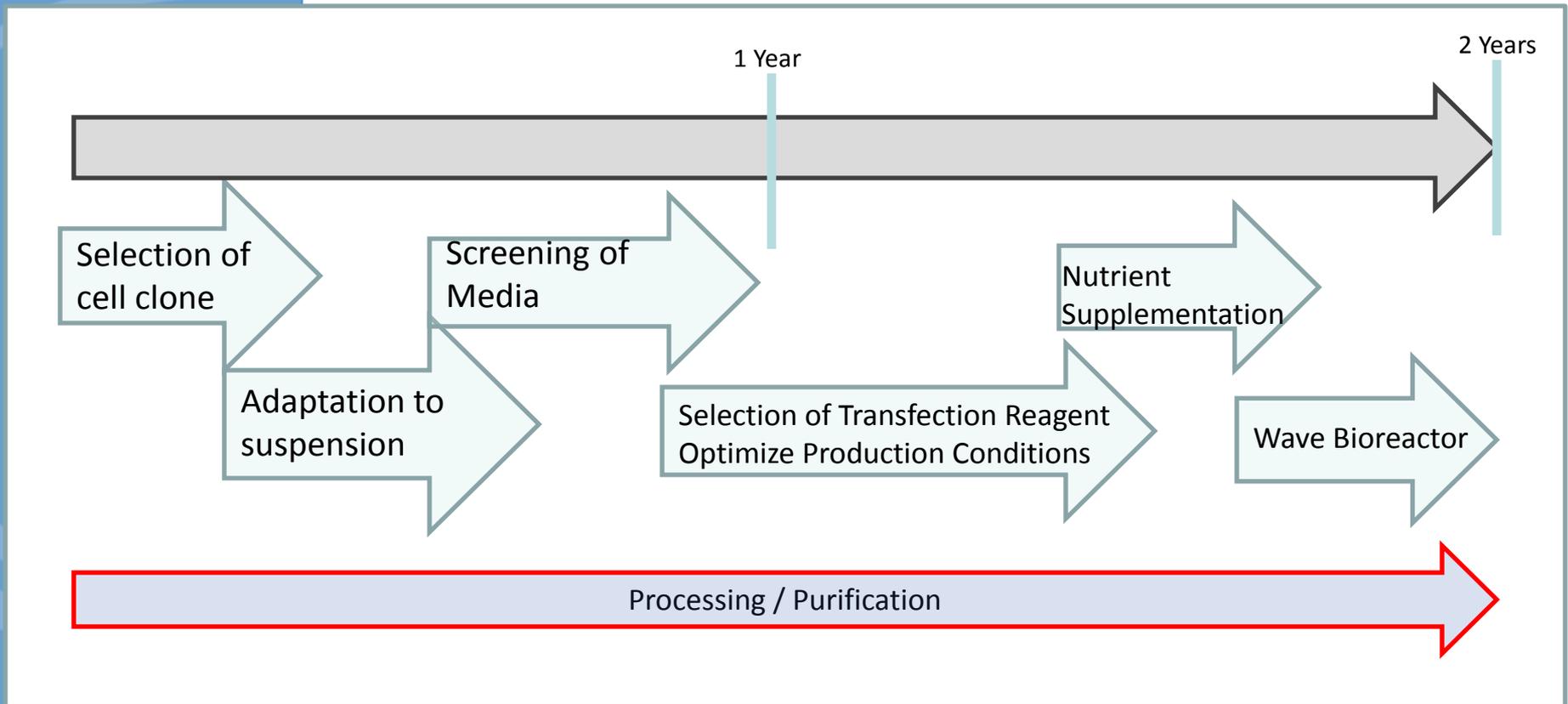
Past Technology



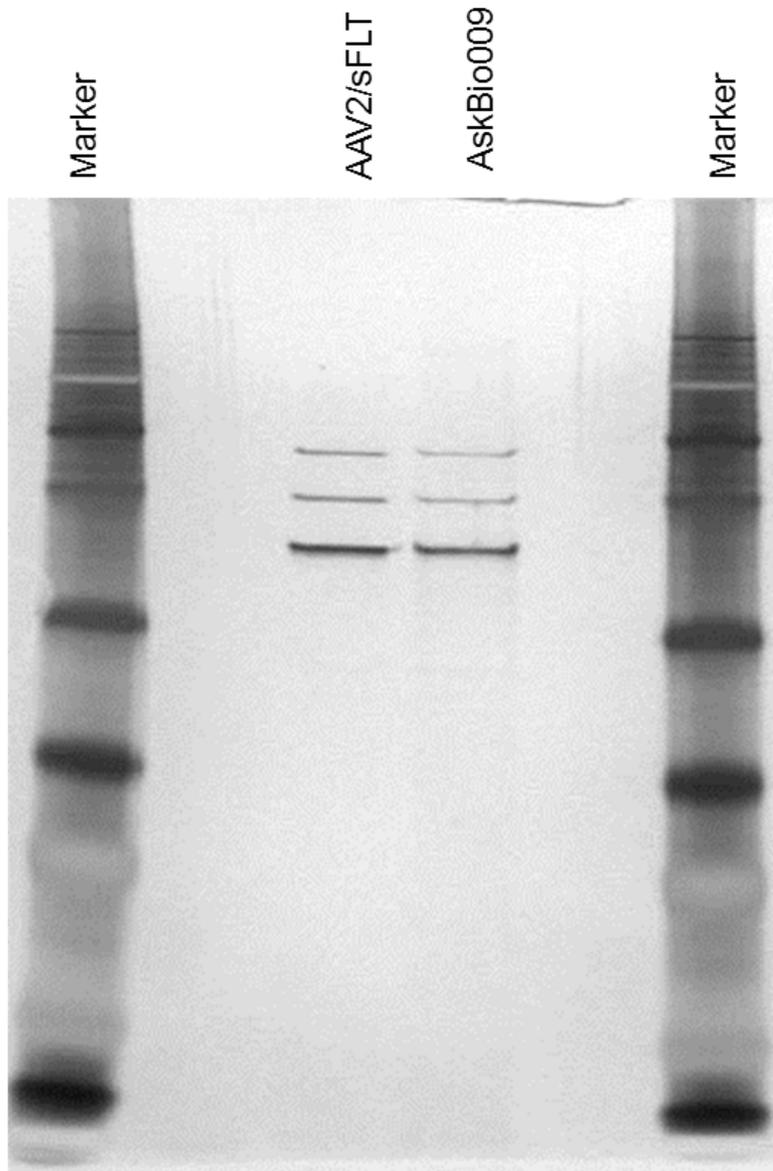
Phase I Technology



Development of Large Scale Production



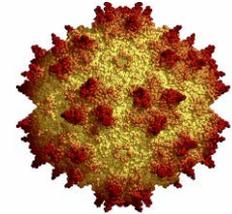
7/9/2013



Proposed Approach for GAN



self-complementary genome



AAV9 Capsid

Targets:

- Primary targets will be spinal cord and brainstem motor neurons, DRG (to treat peripheral motor and sensory disease)
- Secondary targets will be the brain and peripheral tissues

Preliminary Clinical Approach

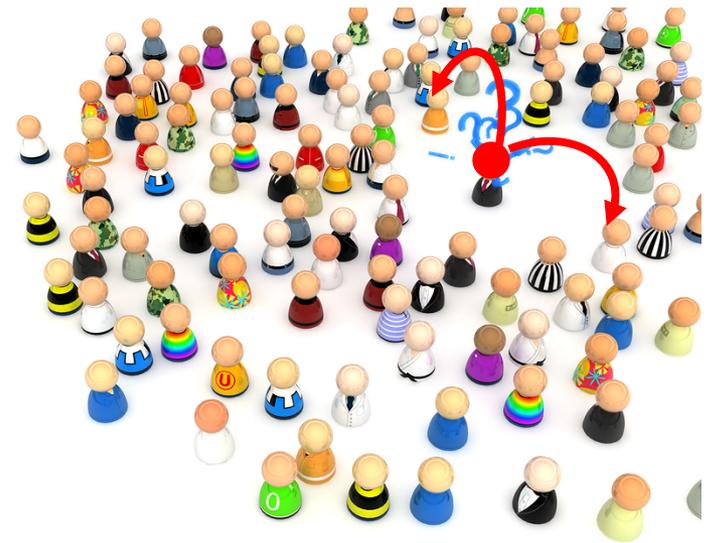
- Agent: GMP scAAV9/JeT-GANopt vector manufactured by the UNC Vector Core Human Applications Lab
- Dose = 3.5×10^{13} vg per person ($\sim 2.5 \times 10^{11}$ vg per mL CSF)
- Route = intrathecal (1 administration, lumbar puncture)
- Patient Age = 5 yrs and older
- Phase I (safety)

GAN Study Risk Factors

1. **Germ line transmission**



2. **Dissemination of vector into the public domain**



GAN Study Risk Factors

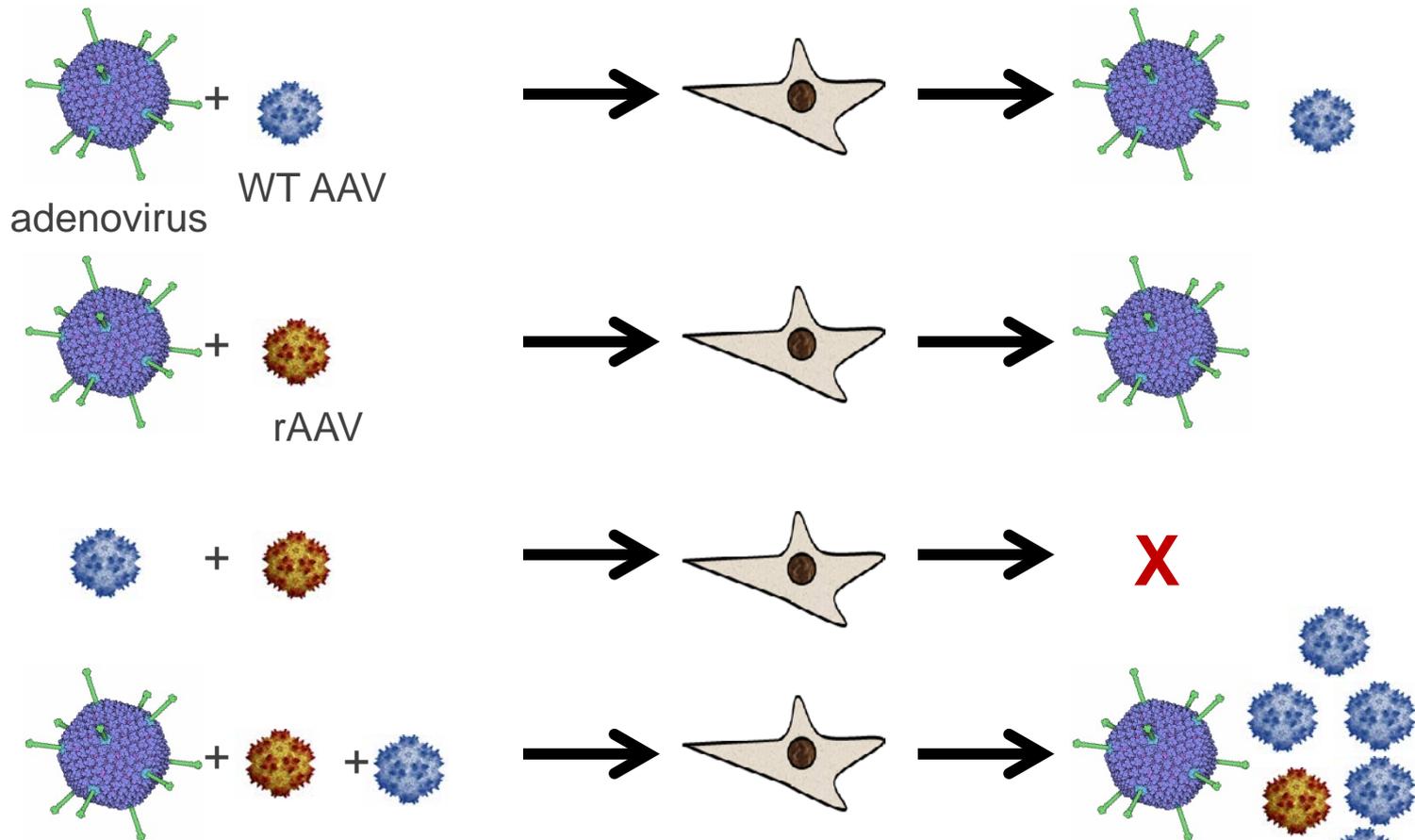
1. **Possible germ line transmission**
 - Very minor and remote concern
 - » Injection is localized to the central nervous system
 - » Patients don't retain sufficient health past puberty to reproduce

GAN Study Risk Factors

- 1. Possible dissemination of vector into the public domain**
 - Injection is localized to the central nervous system

GAN Study Risk Factors

1. Possible dissemination of vector into the public domain



7/9/2013

Hewitt et al., J. Virology, 2009

GAN Study Risk Factors

- 1. Possible dissemination of vector into the public domain**
 - Injection is localized to the central nervous system
 - **There is no evidence for concern from AAV gene transfer trials for hemophilia, alpha 1 antitrypsin deficiency, lipoprotein lipase deficiency, or heart disease where up to 1.4×10^{14} vg was delivered systemically.**

Risk Factors to Patient

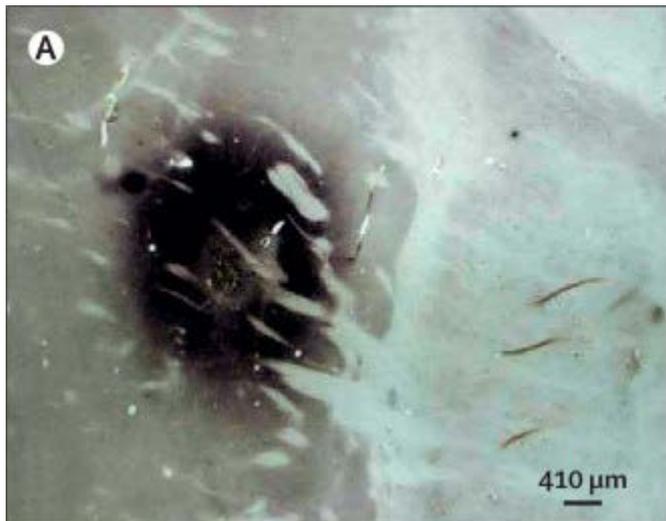
1. Route of Administration
 - » Lumbar intrathecal injection into CSF
2. AAV9
 - » Human-derived capsid
3. Novel promoter / transgene
 - » JeT promoter (weak, ubiquitous expression)
 - » Gigaxonin transgene

Risk Factors to Patient

1. Route of Administration

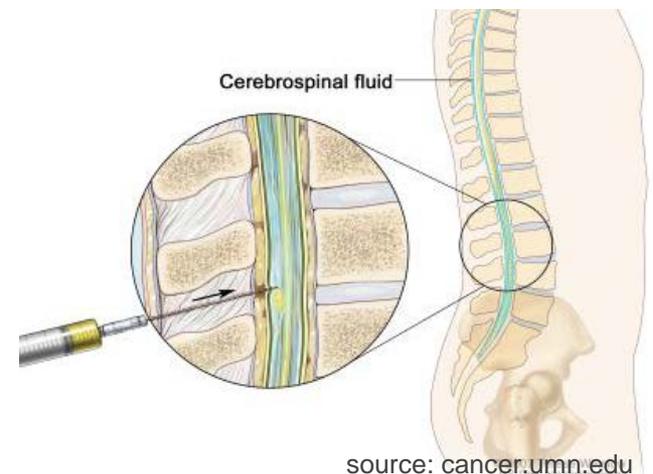
Direct Injection

- Canavan trial, LINCL, AADC, Parkinson's
- Highly invasive neurosurgery



Intrathecal lumbar puncture

- Routine outpatient procedure



Risk Factors to Patient

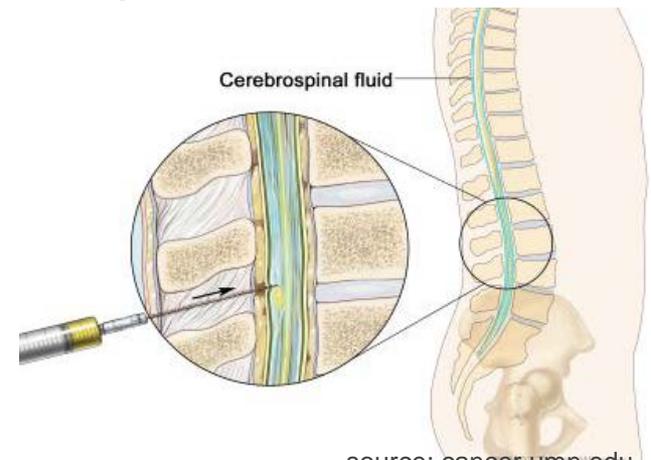
1. Route of Administration

Intravenous

- Hemophilia, heart disease
- AAV1, AAV2, AAV8
- Doses up to $\sim 1.4 \times 10^{14}$ vg per patient (2×10^{12} vg/kg)

Intrathecal lumbar puncture

- Routine outpatient procedure
- Dose of 3.5×10^{13} vg per patient



source: cancer.umn.edu

Risks of AAV9 (cont.)

SPINAL MUSCULAR ATROPHY (proposed)

- Reviewed and approved by the RAC in December 2012.
- Patient population is infants (type I) with short life expectancy
- Lowest dose = 6.7×10^{13} vg/kg
- Highest dose = 3.3×10^{14} vg/kg ($3\text{kg} = 1 \times 10^{15}$)
 - » *Approximately 30-fold higher than our proposed dose*
- IV route of administration with scAAV9 vector

- *A key recommendation from the RAC in Dec 2012 was to explore intrathecal administration as an alternative.*

Risk Factors to Patient

2. AAV9 – unknown risk

- AAV1 – administered IV and IM (full regulatory approval of Glybera in Europe at 1×10^{12} vg/kg)
- AAV2 – administered by direct intracranial to the brain, IV, IM, subretinal, at up to 2×10^{12} vg/kg
- AAV8 – administered IV at up to 2×10^{12} vg/kg
- AAVrh10 – direct intracranial (Batten trial)
- Chimeric AAV2.5 for DMD
- Pubmed search with “AAV9” identifies 143 citations of animal studies.

Route	Rodent	Large Animal
Intravascular	59	13
Intra-CSF	4	5
Direct brain	8	-
Other	12	2

Risk Factors to Patient

3. Novel Promoter / Transgene

- First-in-human use of the synthetic JeT promoter
- JeT was chosen partly because of its small size to allow packaging of the large gigaxonin transgene
- Provides weak and ubiquitous expression to mimic endogenous (very low) gigaxonin expression levels.

Gigaxonin is expressed at very low levels in most if not all cells

Human Molecular Genetics, 2009, Vol. 18, No. 8 1384–1394
doi:10.1093/hmg/ddp044
Advance Access published on January 24, 2009

Gigaxonin controls vimentin organization through a tubulin chaperone-independent pathway

Don W. Cleveland¹, Koji Yamanaka^{1,2,3} and Pascale Bomont^{1,4,5,*}

¹Ludwig Institute for Cancer Research, Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA 92093, USA, ²Yamanaka Research Unit, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan, ³CREST, Japan Science and Technology Agency, Japan, ⁴INSERM, U901, INMED, Marseille 13009, France and ⁵Aix Marseille Université, Faculté des Sciences, Marseille F-13000, France

Gigaxonin is normally expressed at very low levels, approximately 7500 molecules per cell.

Risk Factors to Patient

3. Novel Promoter / Transgene

- First-in-human use of the synthetic JeT promoter
- JeT was chosen partly because of its small size to allow packaging of the large gigaxonin transgene
- Provides weak and ubiquitous expression to mimic endogenous (very low) gigaxonin expression levels.

Brain-directed AAV gene transfer human studies:

- Batten (LINCL, 2 trials): strong ubiquitous promoter
- Parkinson's (multiple trials): strong ubiquitous promoter
- Canavan: strong ubiquitous promoter
- AADC deficiency: strong ubiquitous promoter

- GAN (proposed): weak ubiquitous promoter

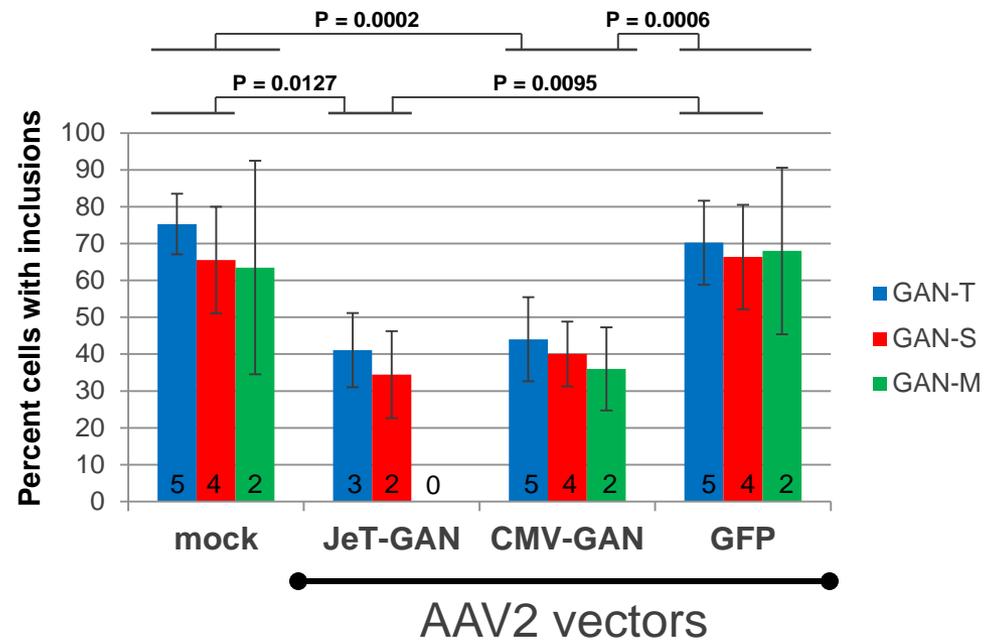
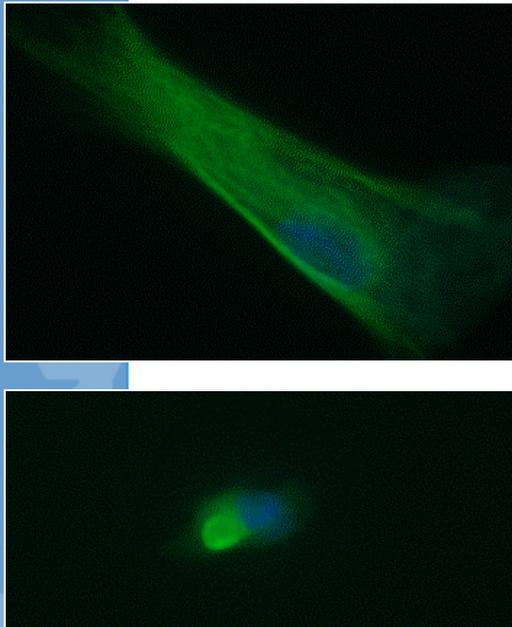
Major Points Raised

- **Questions regarding whether very low expression from the novel JeT promoter was appropriate.**
 - » Gigaxonin is normally expressed at very low levels
 - » The provided level of gigaxonin expression was enough to rescue cellular and behavioral phenotypes, in cultured cells and mice.
- **Questions regarding whether patients could expect a benefit, to offset possible risks.**
 - » Successful widespread CNS gene transfer achieved in mice, pigs, and NHPs
 - » Demonstration that introduction of low levels of gigaxonin expression rescue the cellular phenotype within days
 - » Improvement in motor function of symptomatic mice

Proof-of-Concept Studies

- Demonstrated Function of Gigaxonin in culture human GAN fibroblasts
 - *By overexpression (CMV) and with the JeT promoter*
 - *No benefit found associated with overexpression*
- Demonstrated Function of Gigaxonin in iPSC-derived human GAN motor neurons
 - *By overexpression (CMV) and with the JeT promoter*
 - *Both approaches effectively treated the cells*
- Demonstrated Function of Gigaxonin in GAN mice (*in vivo*)
 - *By overexpression (CMV) and with the JeT promoter*
 - *Both expression levels efficiently cleared pathological aggregates*
- Improved the phenotype of GAN KO mice (*in vivo*)
 - *JeT promoter – improvement in motor function*
 - *JeT promoter – preservation of peripheral nerve ultrastructure*

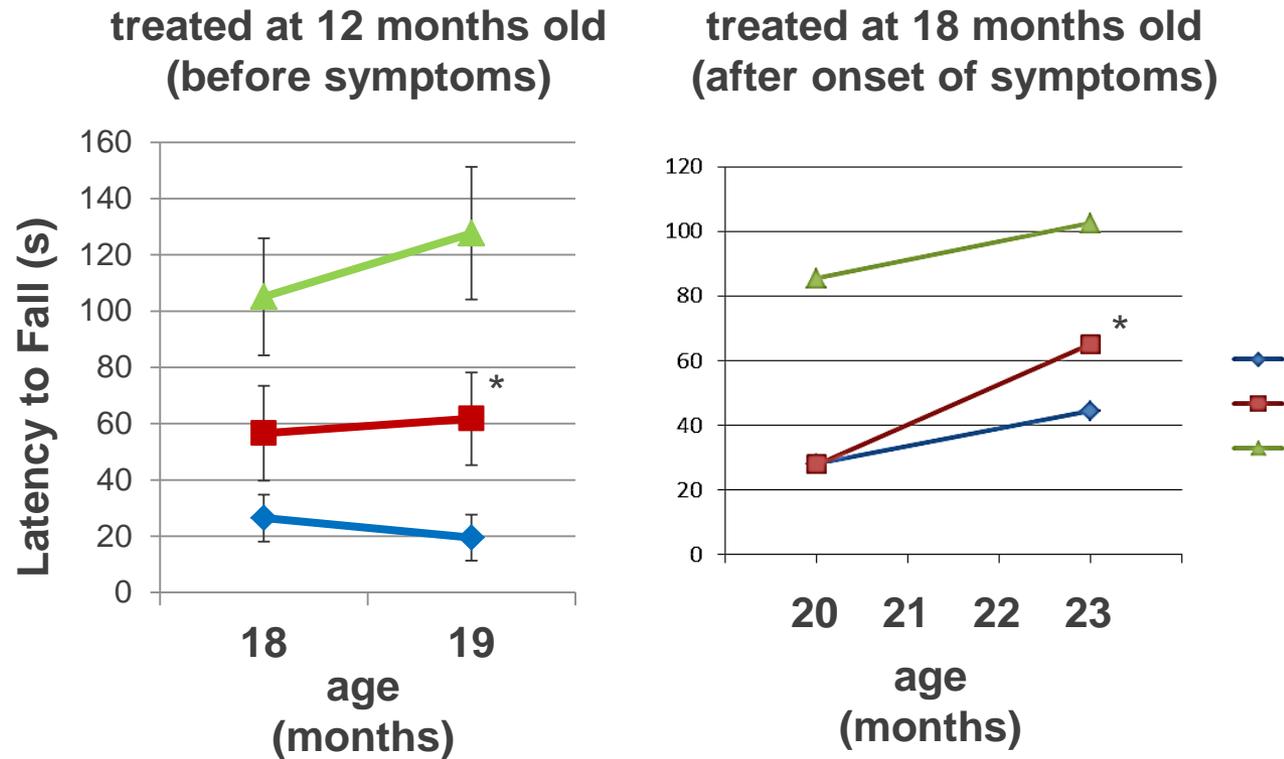
Rescue of Vimentin Aggregates in Cultured GAN fibroblasts



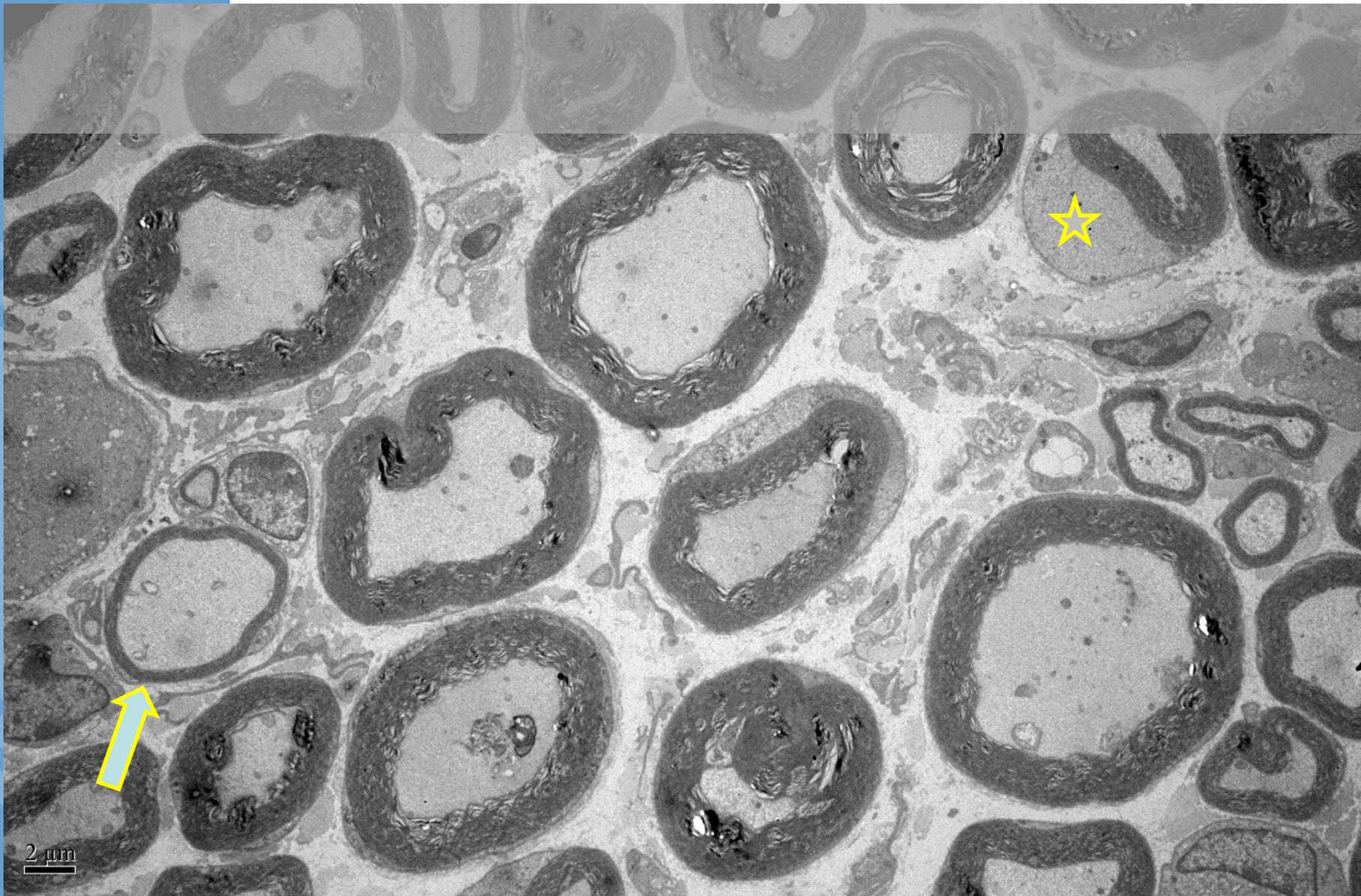
Transduction efficiency was approximately 50% in these experiments, suggesting that nearly all targeted cells were completely rescued within 3-4 days.

- CMV data published Mussche et al., HGT, 2013.

KO mice treated with GAN vector show significant improvement vs GFP vector



GAN KO mice injected with scAAV9/JeT-hGANopt at the same dose proposed for humans, scaled by CSF volume



240583LD-2

X2500

12/28/2012



UNC
SCHOOL OF MEDICINE

Enlarged axon with thin myelin sheath (arrow). Schwann cell cytoplasm with filamentous accumulation (star). (untreated GAN KO)



240471LP-25

X8000

12/28/2012

Summary of Efficacy Studies

- The scAAV/JeT-hGANopt vector provides sufficient gigaxonin expression to correct intermediate filament disorganization in GAN KO cells
- Mice treated with scAAV9/JeT-hGANopt have improved motor function at least 6 months post-treatment, even if treated after the onset of symptoms
- Symptomatic mice treated with scAAV9/JeT-GANopt show preservation of the sciatic nerve ultrastructure at 6 months post-injection.
- *If the JeT-GAN construct is delivered to a cell, it is capable of correcting that cell.*

Major Points Raised

- Questions regarding whether very low expression from the novel JeT promoter was appropriate.
 - » Gigaxonin is normally expressed at very low levels
 - » The provided level of gigaxonin expression was enough to rescue cellular and behavioral phenotypes, in cultured cells and mice.
- **Questions regarding whether patients could expect a benefit, to offset possible risks.**
 - » Demonstration that introduction of low levels of gigaxonin expression rescue the cellular phenotype within days
 - » Improvement in motor function of symptomatic mice
 - » Successful widespread CNS gene transfer achieved in mice, pigs, and NHPs

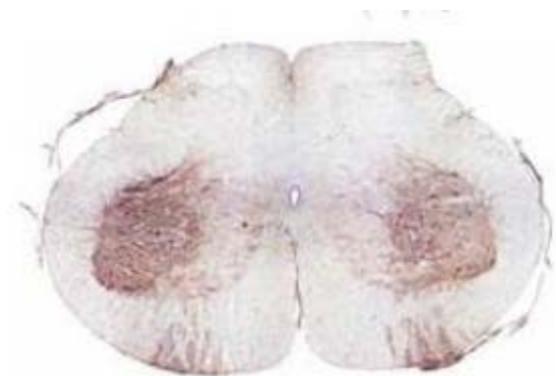
ORIGINAL ARTICLE

Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs

T Federici¹, JS Taub¹, GR Baum¹, SJ Gray², JC Grieger², KA Matthews¹, CR Handy¹, MA Passini³, RJ Samulski² and NM Boulis¹

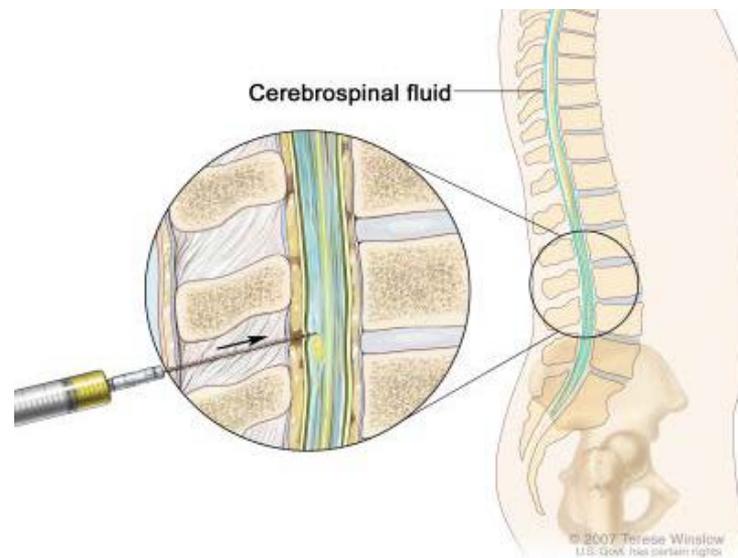
CONCLUSION:

- Intrathecal injection of AAV9 in adult pigs resulted in 50-100% of spinal cord motor neuron transduction across the entire spinal cord.





Lumbar intrathecal injection of AAV9 into non-human primates gave widespread gene delivery



source: cancer.umn.edu

ORIGINAL ARTICLE

Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates

SJ Gray, S Nagabhushan Kalburgi, TJ McCown and R Jude Samulski

Gene Therapy, 2013

CONCLUSIONS:

- IT administration of AAV9 leads to global vector distribution and transgene expression to the CNS.
- IT administration overcomes many of the barriers associated with IV administration, including a lower dose, avoidance of NAbs, and reduced peripheral organ biodistribution.

IT Biodistribution Summary

- Intrathecal AAV-mediated gene transfer effectively distributes the transgene throughout the CNS, particularly spinal cord motor neurons and DRG
- Peripheral organ biodistribution is considerably lower than an IV route
- Pre-existing AAV neutralizing antibodies are avoided
- The dose used in the pigs and NHPs, scaled according to CSF volume, equates to $\sim 3.5 \times 10^{13}$ vg in a human ≥ 5 years old. This is considerably lower than doses used in multiple trials systemically and approximately 30 times lower than the high dose proposed for SMA.

GLP Toxicology in WT Mice

Study was contracted to MPI Research

Study Design:

- » Vehicle, normal dose, high dose groups.
- » N=15 male and 15 female in each group
- » 5 males and 5 females sacrificed at 7, 28, and 180 days post-injection

Results:

- » no study-related changes in clinical chemistry
- » no study-related histopathological findings
- » 2 vehicle-treated and 1 high-dose animals died prior to the study endpoint. Examination did not indicate any vector-related toxicity.

GLP Toxicology in NHPs

Study was contracted to MPI Research

Study Design:

- » 2 males and 2 females injected at high dose (4x proposed human dose)
- » Termination at 2 weeks (n=2), 8 weeks (n=2)
- » 1 year cohort in progress (n=2 vehicle, n=4 normal dose, n=4 high dose)

Results (2 and 8 weeks):

- » no study-related changes in clinical chemistry
- » no study-related histopathological findings except for rare and focal infiltrating T-cells
- » 1 animal in the 1-year cohort was euthanized for self-mutilation behavior. Physical and histological examination concluded that this was not vector-related.

Major Points Raised

- Questions regarding whether very low expression from the novel JeT promoter was appropriate.
 - » Gigaxonin is normally expressed at very low levels
 - » The provided level of gigaxonin expression was enough to rescue cellular and behavioral phenotypes, in cultured cells and mice.
- Questions regarding whether patients could expect a benefit, to offset possible risks.
 - » Successful widespread CNS gene transfer achieved in mice, pigs, and NHPs
 - » Demonstration that introduction of low levels of gigaxonin expression rescue the cellular phenotype within days
 - » Improvement in motor function of symptomatic mice

Clinical Protocol

- SITE: NIH Clinical Center in Bethesda, MD
- Single administration of 3.5×10^{13} vg scAAV9/JeT-hGANOpt by lumbar puncture in patients > 4 years old.
- Major Exclusion Criteria:
 - » Night and day time ventilator-dependency
 - » Absence of any detectable gigaxonin
- Sample collection / procedures
 - » CSF sampling, MRI, skin biopsy, peripheral nerve biopsy, blood draws, urine, saliva, excrement
- Timeline:
 - » 1, 2, 4 and 8 weeks; Months: 3, 6, 9 and 12; Annually, years 2-5
- Outcome Measures
 - » Primary: safety
 - » Secondary: GMFM, FARS, MRI, CSF Biomarkers, NCV, BAER, peripheral nerve biopsy

	Screening Visit	Treatment Visit		Follow-up visits				
Procedure	14 -28 days prior to day 0	Day 0	Day 1-6	Weeks 1/2/4/8 ± 1 day	Months 3/6/9 ± 3 days	Year 1 ± 1 month	Years 2 to 5 ± 1 month	Years 6 to 15± 1 month
Informed consent	X							
Demographics	X							
Inclusion/exclusion criteria	X							
Pregnancy test	X	X			X	X	X	X
Outpatient clinic	X			X	X		X	X
Inpatient admission		X	X			X		
Intensive care unit admission		X						
Routine vital signs	X		X	X	X	X	X	X
Continuous vital signs		X						
Record Height & Weight	X	X		X	X	X	X	X
Medical history	X	X		X	X	X	X	X
Concomitant medication	X	X		X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
12-lead ECG	X	X	X	X	X	X	X	X
PFT	X	X		X	X	X	X	X
Echocardiogram	X	X			X	X	X	X
Safety lab testing: Hematology/serum chemistry/urinalysis	X		X	X	X	X	X	X
Coagulation parameters	X	X						
Screening labs	X							
Serum neutralizing antibody for AAV9	X			X	X	X	X	X
Serum AAV9 and gigaxonin Ab, and PBMCs to monitor immune responses	X			X	X	X	X	X
Blood, saliva, urine samples for AAV9 shedding analysis	X	X	X	X				
Assessments of acute and adverse events		X	X	X	X	X	X	X
Distribute diary cards			X	X	X	X	X	X
Collect diary cards				X	X	X	X	X
Physical Therapy testing	X				X	X	X	X
Imaging studies	X				X	X	X	X
Neuropsychological evaluation	X				X	X	X	X
Ophthalmology	X				X	X	X	X
Electrophysiology testing	X				X	X	X	X
Administer vector genome		X						
Measurement of infusion site erythema and induration		X						
Lumbar puncture / CSF labs		X		X (week 4)	X (month 6)	X		
Telephone contact	X	X	X	X	X	X	X	X

Inclusion Criteria

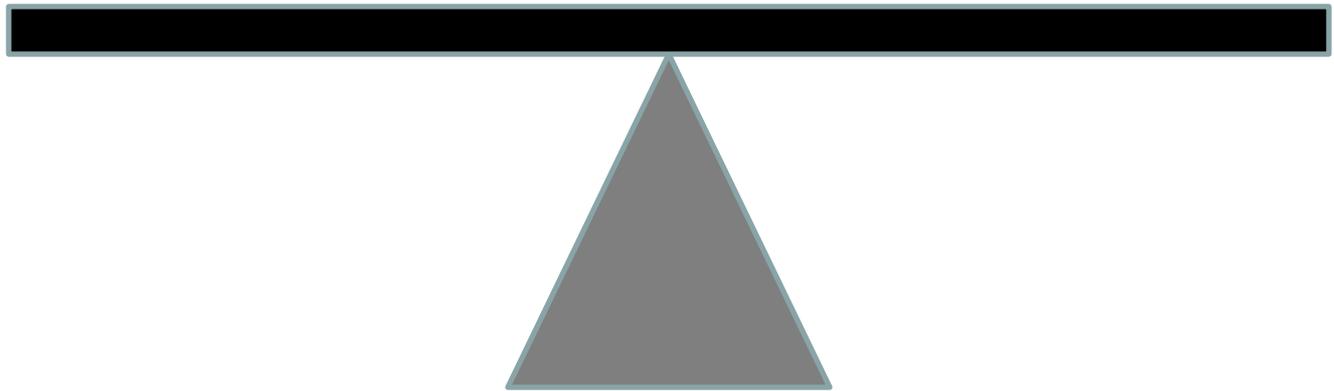
- **Age > 4 years**
- **Genetic diagnosis of GAN: Genotype to include are patients who are homozygous for a missense mutation, compound heterozygous for two missense mutations, or compound heterozygous for a missense and a functional null mutation.**
- Men capable of fathering a child must agree to use barrier contraception (combination of a condom and spermicide) or limit activity to post-menopausal, surgically sterilized, or contraception-practicing partners, for 3-6 months after administration of investigational product. Women over the age of 9 must agree to have urine human chorionic gonadotropin testing performed to rule out the possibility of pregnancy at each visit. Those women who are sexually active must also agree to use barrier contraception as well or limit activity to surgically sterilized or contraception-practicing partners for 3-6 months after the administration of the investigational product. Pregnant or lactating women will not be included in this study.
- **Willing and able to give informed consent if >17 years of age and assent if >7 years of age. For patients ages 7-17, parents or guardians must also consent to the child's participation in the study.**
- Forced vital capacity \geq 50%
- **No history of daily daytime assistive ventilation**
- Willingness to undergo a baseline nerve biopsy if no such diagnostic study has been done in the past.
- Willing to undergo a baseline and follow-up lumbar punctures
- Normal echocardiogram
- Normal ECG
- No history of adverse reaction to anesthesia

Exclusion Criteria

- **Age ≤ 4 years**
- **Complete absence of any full-length gigaxonin protein (functional or non-functional). Thus, patients with function stop mutations on both alleles in the gigaxonin gene deletion are excluded (stop codon mutations, out of frame deletion, out of frame splice site mutations, whole gene deletions)**
- **Pregnant or lactating patients.**
- **Ventilator dependence to include daytime use of assisted ventilation.**
- Current clinically significant infections including any requiring systemic treatment.
- Any prior participation in a study in which a gene therapy vector was administered.
- Participation in a clinical study with an investigational drug in the past six months.
- History of or current chemotherapy, radiotherapy or other immunosuppressive therapy.
- Immunizations of any kind in the month prior to the study.
- Current use of medication (e.g. levothyroxine, vitamin A supplementation, oral contraceptive use, tetracycline, Diamox) that could potentially lead to changes in intracranial pressure.
- Known sensitivity to medications planned for use in the peri-operative period.
- GAN subjects without quantifiable weakness or functional loss.
- Cardiomyopathy based on clinical exam
- Echocardiogram with evidence of cardiomyopathy or ejection fraction less than 55%
- Abnormal electrocardiogram.
- History of diabetes
- Active viral infection (Human immunodeficiency virus, Hepatitis A, B, or C, Varicella zoster virus, HTLV-1)
- Positive purified protein derivative testing for tuberculosis.
- **Abnormal laboratory values considered clinically significant**
 - » Platelet count $< 100,000 / \text{mm}^3$
 - » Persistent leukopenia or leukocytosis (Total white blood cell count $< 3,000/\text{mm}^3$)
 - » Significant anemia
 - » Abnormal prothrombin (PT) or partial thromboplastin time (PTT).
 - » Abnormal liver function tests
 - » Abnormal pancreatic enzymes
 - » Patients with renal impairment defined as urinary protein concentration $\geq 0.2 \text{ g/L}$
- Pleocytosis noted in cerebrospinal fluid evaluation.
- Failure to thrive, defined as: Falling 20 percentiles (20/100) in body weight in the 3 months preceding Screening/Baseline In patients below the 3rd percentile, any further drop in body weight percentile in the 3 months preceding
- Screening/Baseline weight less than 17kg at baseline
- Morbidly obese or grossly overweight (≥ 86 percentile BMI in children)

RISK

BENEFIT



RISK of gene transfer



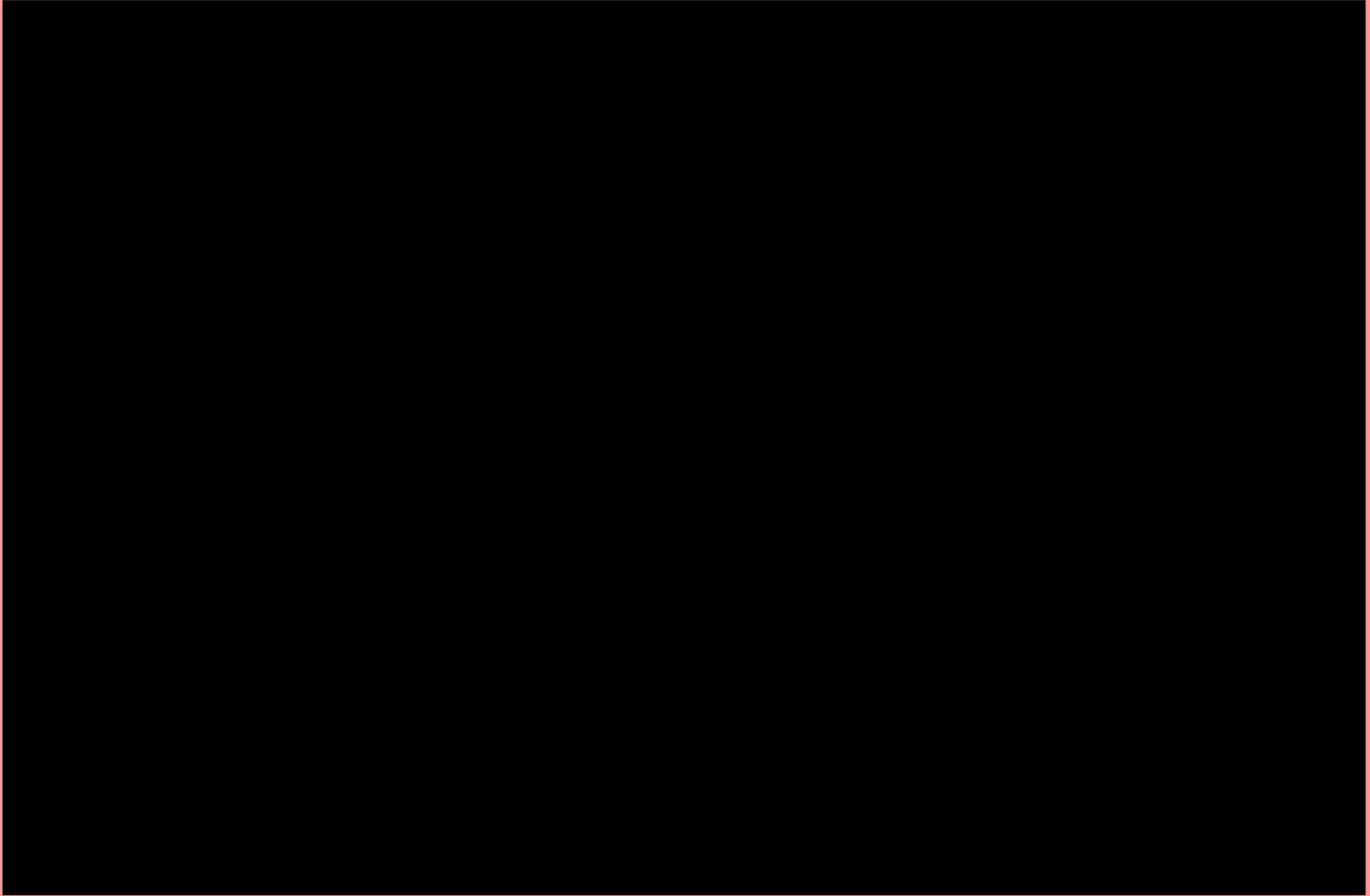
RISK of unaltered disease course

Potential BENEFIT of gene transfer

Faces of GAN



4 years old



7 years old



8 years old



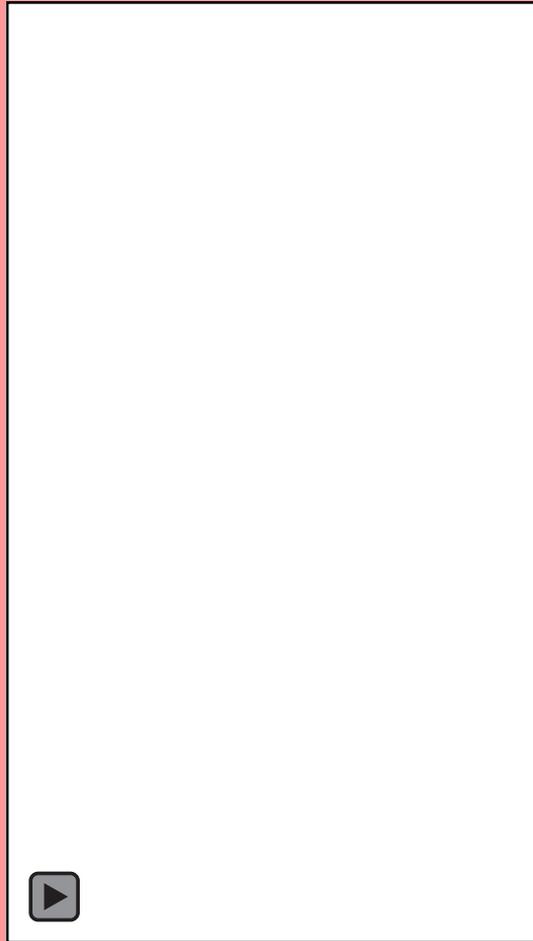
13 years old



18 years old



20 years old



Brothers, ages 2 & 4



Same individuals, Ages 22 & 24





"Never doubt that a small group of thoughtful, committed citizens can change the world. Indeed, it is the only thing that ever has." - Margaret Mead



Giant Axonal Neuropathy Gene Therapy

Lori Sames, executive director of Hannah's Hope Fund (sponsor)	
Carsten Bonnemann, MD	Principal Investigator, NINDS, NIH
Steven Gray, PhD	Co-Investigator, UNC at Chapel Hill
R Jude Samulski, PhD	Co-Investigator, UNC at Chapel Hill
David Dismuke, PhD	Co-Investigator, UNC Vector Core
Jahannaz Dastgir, DO	Co-Investigator, NIH
Diana X. Bharucha-Goebel, MD	Co-Investigator, NIH
Sandra Donkervoort, MS, CGC	Co-Investigator, NIH
Kenneth Fischbeck, MD	Co-Investigator, NIH
Minal Jain, DPT	Co-Investigator, NIH
Diane Damiano PT, PhD	Co-Investigator, NIH
Avi Nath, MD	Co-Investigator, NIH

Hannah

