Gene Therapy for Central Nervous System Disease

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Received patent royalties from Asklepios Biopharma
The promise of gene therapy is to fix a genetic disease at the source. If you fix the DNA, you’ve solved the problem permanently.
Adeno-associated virus (AAV) characteristics
• single-stranded DNA human parvovirus
• non-pathogenic, requires helper virus for lytic infection
• able to transduce non-dividing cells and confer long-term transgene expression
• can package up to 4.6 kb in place of viral Rep and Cap genes
• dozens of naturally-occurring serotypes identified with differing tissue tropism
• Over 100 clinical trials initiated using rAAV, no serious adverse effects related to vector. In 2012, the first gene therapy product (Glybera) received full regulatory approval in Europe.
Gene therapy for aromatic L-amino acid decarboxylase deficiency.

Hwu WL, Muramatsu S, Tseng SH, Tzen KY, Lee NC, Chien YH, Snyder RO, Byrne BJ, Tai CH, Wu RM.
Targeting AAV to the CNS

- Direct injection into brain
- i.v. injection and transcytosis of vector
- Intrathecal injection into CSF
Intracranial injection of AAV9

Proportion of cells infected (%)

>95% neuronal

NeuN +ve (neuronal)  NeuN -ve

Striatum  Thalamus  Brain stem
Hypothalamus  Motor cortex  Hippocampus

Gadalla et al, Mol Ther, 2013
Intravenous: $1 \times 10^{13}$ vg/kg AAV9/CBh-GFP

Gray et al, Mol. Ther., 2011
CONCLUSIONS:

• IV administration of AAV9 works very well in a dose-responsive manner to delivery a gene to the CNS in mice.
• AAV9 delivery to the CNS is less efficient in non-human primates, particularly in neurons.
• High doses, high peripheral organ transduction, and inhibition of transduction by anti-AAV neutralizing antibodies were identified as barriers to human translation.
Intrathecal Administration
(inject vector into CSF)
Intrathecal Delivery, AAV9

1.25x10^9 vg injected IT, 10 wk time point
Extensive brain transduction occurs following a lumbar puncture with AAV9.

*scAAV9/CBh-GFP injected into adult WT mice at a dose of 4.15 \times 10^{11} \text{ vg}*
CONCLUSIONS:

- Intrathecal injection of AAV9 in adult pigs resulted in 50-100% of spinal cord motor neuron transduction across the entire spinal cord.
Intrathecal injection in pigs leads to brain delivery and extensive cerebellum transduction

- Collaboration with Nick Boulis at Emory University
- ~15 kg pigs were injected with $3 \times 10^{12}$ vg scAAV9/CBh-GFP ($2 \times 10^{11}$ vg/kg) $[n=6]$
- Pigs were euthanized after 4 weeks and fixed tissue subjected to IHC against GFP

Note: There was minimal or no delivery to peripheral tissues.
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.
Giant Axonal Neuropathy (GAN)

- Sensory and Motor Peripheral Neuropathy, “ALS in kids”
- Cognition is mostly unaffected
- 3-4 yrs old: clumsiness, loss of coordination
- ~10 yrs old: unable to walk
- Late teens: highly reduced coordination and use of arms/hands
- ~20 yrs old: Fatal
- Ultra-rare
"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 (GAN)

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

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

**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 (to address white matter abnormalities and suspected protein aggregations/ inclusion bodies)
GAN Gene Therapy is Effective and Safe

- Demonstrated Function of Gigaxonin in cultured human GAN fibroblasts
  - By overexpression and with JeT promoter

- Demonstrated Function of Gigaxonin in iPSC-derived human GAN motor neurons
  - By overexpression and with JeT promoter

- Demonstrated Function of Gigaxonin in GAN mice (in vivo)
  - By overexpression and with JeT promoter

- Phenotypic rescue in GAN mice after intrathecal injection
  - Improved motor function (rotarod)
  - Improved nerve pathology

No toxicities observed in mice (120) or non-human primates (14) at up to a 4-fold overdose, up to 1 year post-injection. No toxicities observed in rats at a 10-fold overdose up to 6 months post-injection.
The Road to an IND

- IND = Investigational New Drug

Good lab results (proof of concept) ➔ FDA preIND ➔ toxicology ➔ PK/PD ➔ Clinical trial design ➔ IRB ➔ FDA ➔ Phase I Trial

- 2008-2011*
- 2012
- 2012-2014
- 2014-2015
- 2015+

Funded 100% from Hannah’s Hope fund up to the Phase I trial.
Intrathecal Administration of scAAV9/JeT-GAN for the Treatment of Giant Axonal Neuropathy

This study is currently recruiting participants. (see Contacts and Locations)

Verified December 2014 by National Institutes of Health Clinical Center (CC)

Sponsor:
National Institute of Neurological Disorders and Stroke (NINDS)

Information provided by (Responsible Party):
National Institutes of Health Clinical Center (CC) (National Institute of Neurological Disorders and Stroke (NINDS))

ClinicalTrials.gov Identifier:
NCT02362438

First received: February 12, 2015
Last updated: NA
Last verified: December 2014
History: No changes posted

Purpose

Background:
- The Gigaxonin gene lets the body make a protein chemical called Gigaxonin. Nerves need Gigaxonin to work properly. Giant Axonal Neuropathy (GAN) is a rare condition in which Gigaxonin is not made or is made improperly. This causes problems with walking and sometimes with eating, breathing, and many other activities. GAN has no cure. Over time, gene transfer treatment may help people with GAN.

Objectives:
- To see if a gene transfer is safe and shows potential to help people with GAN.

Eligibility:
MILESTONE: May 2015
First-in-human intrathecal gene transfer to broadly treat a CNS disorder for any disease

Pictured: Diana Bharucha, Lori Sames, Carsten Bonnemann, Steven Gray
GAN Trial Summary

• 8 subjects dosed, ranging from 6-14 years old
  – 2 @ 3.5x10^{13} vg
  – 4 @ 1.2x10^{14} vg (3.3x)
  – 2 @ 1.8x10^{14} vg (5x)

• Overall, well-tolerated (no treatment-related SAEs)
Other trials with AAV9 vectors

- SMA (IV, started in 2014)
- GAN (intrathecal, started in 2015)
- CLN6 (intrathecal, started in 2016)
- MPS IIIA (IV, started in 2016)
- MPS IIIB (IV, anticipated 2017)
- CLN3 (IV, anticipated 2018)
- CLN1 (intrathecal, anticipated 2018)
Disease Applications Within My Lab (intrathecal or IV gene transfer using AAV9)

- Giant Axonal Neuropathy
- Rett Syndrome
- Krabbe Disease
- Tay-Sachs Disease
- Batten Disease, Infantile (INCL)
- Batten Disease, Late Infantile (LINCL)
- Aspartylglucosaminuria (AGU)

- Clinical Trial
- Discovery
- IND-enabling
- IND-enabling
- IND-enabling
- Discovery
- Advanced preclinical
Improved Survival and Reduced Phenotypic Severity Following AAV9/MECP2 Gene Therapy in Neonatal and Juvenile Male MeCP2 Knockout Mice

Kamal KE Gadalla, Mark ES Bailey, Rosemary C Spike, Paul D Ross, Kenton T V Saha, Sahana Nagabhushan Kalburgi, Lavanya Bachaboina, Jie V Deng, Anne E West, Steven J Gray, and Stuart R Cobb

Original Article

Intrathecal Administration of AAV/GALC Vectors in 10–11-Day-Old Twitcher Mice Improves Survival and Is Enhanced by Bone Marrow Transplant

Subha Karumuthil-Melethil, Michael S. Marshall, Clifford Heindel, Beverly L. Bongarzone, and Steven J. Gray

HUMAN GENE THERAPY 24:209–219 (February 2013)

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DOI: 10.1089/hum.2012.107

Restoration of Cytoskeleton Homeostasis After Gigaxonin Gene Transfer for Giant Axonal Neuropathy

Silke Mussche, Bart Devreese, Sahana Nagabhushan, Jonathan C. Fox, Hung-Jui Shih, and Rudy Van Coillie

Molecular Therapy Methods & Clinical Development

Original Article

Novel Vector Design and Hexosaminidase Variant Enabling Self-Complementary Adeno-Associated Virus for the Treatment of Tay-Sachs Disease

Subha Karumuthil-Melethil, Sahana Nagabhushan Kalburgi, Patrick Thompson, Michael Troupak, Michael D. Kavitor, John G. Keimmel, Brian L. Mark, and Don Mahuran

Improved MECP2 Gene Therapy Extends the Survival of MeCP2-Null Mice without Apparent Toxicity after Intracisternal Delivery

Sarah E. Sinnett, Ralph D. Hector, Kamal K.E. Gadalla, Clifford Heindel, Daphne Chen, Violeta Zoric, Mark E.S. Bailey, Stuart R. Cobb, and Steven J. Gray
Supporting via External Collaborations

Charcot-marie Tooth 4j
Batten (cln7)
Pitt-hopkins
Charcot-marie Tooth 6
Batten (cln6)
Batten (cln5)
GM2A deficiency
Niemann-pick C
Spastic Paraplegia Type 7
Mucopolysaccharidosis Type I/III
Retinitis Pigmentosa (impg2)
Epilepsy
H-abc
Key Points

• Gene therapy is permanent, for *better* or *worse*

• With current technology, this would be a *treatment*, not a *cure*

• AAV9 is an exciting *platform technology* that has a positive translational track record across many diseases, *including human trials*
Proposed pilot gene therapy approach for Angelman

*In Collaboration with Ben Philpot (UNC)*

**Approach**
- Neonatal ICV injections in AS mice to assess possible phenotypic rescue
- Neonatal ICV injections in WT mice to assess possible toxicity
- If efficacy and lack of toxicity is observed, move to dose-ranging studies in older AS mice.
Research positions are available

Not pictured: Dr. Emma Hoffman
Acknowledgements

**Past contributing lab members**
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Diana Bharucha (NIH/NINDS)

**CIDD (UNC)**
Sheryl Moy (Mouse behavioral studies)

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