Gene Therapy for the Neurologic Manifestations of Friedreich's Ataxia by Delivery of Therapeutic AAV Vectors to the Cerebellum: Comparison of Direct Cerebellar Delivery to Cerebral Spinal Fluid Administration Via the Cisterna Magna

Dolan Sondhi[1], Michael G. Kaplitt[1], Bishnu P. De[1], Jonathan B. Rosenberg[1], Andreane Syra Cruz[1], Meghan Herlitzka[1], Alicia Cowern[1], Shaili Lakhotia[1], Kimberly Castaneda Hernandez[1], Stephen M. Kaminsky[1], Heather R. Martin[1, 2], Sebastian Carrasco[1, 2] and Ronald G. Crystal[1]

[1] Weill Cornell Medicine and [2] Memorial Sloan Kettering Cancer Center, New York, NY

Gene Therapy for Friedreich's Ataxia

Background

- Friedreich's Ataxia (FA) is an autosomal recessive disorder caused by a GAA trinucleotide expansion within intron 1 of the frataxin (FXN) gene resulting in transcriptional silencing and reduced levels of frataxin protein
- Following the development of neurological disease, FA homozygotes develop cardiac dysfunction which is the cause of death in two-thirds of patients (average age of death 37.5 yr)
- While corrective gene therapy for the cardiac disease can be delivered to the heart by systemic administration of a cardiotropic AAV vector, gene therapy to the cerebellum is challenging

Aim

- The aim of this study was to determine the ideal route of delivery of an AAV gene therapy vector delivering the human frataxin gene to the cerebellum of nonhuman primates
- This was done in the context of presence or absence of pre-existing immunity, which would mimic the scenario of a clinical trial where subjects were treatment naive had previously undergone gene therapy for the cardiac manifestation of FA

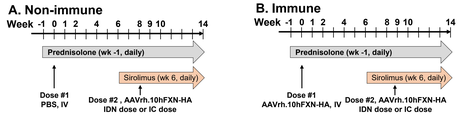
Strategy

- To identify the optimal route of delivery of an AAV vector to the cerebellum, we administered AAVrh.10hFXN-HA (normal human FXN cDNA + 3' HA tag) to nonhuman primates (NHP), directly to the cerebellum (n=6) compared to administration to the cerebral spinal fluid (CSF) via the cisterna magna (n=2)
- The studies were carried out in NHP with no pre-existing immunity to AAVrh.10 compared to NHP with elevated anti-AAVrh.10 antibodies evoked by intravenous administration of AAVrh.10hFXN-HA 8 weeks prior to administration to the cerebellum or CSF
- The doses, $3.2x10^{10}$ vg for direct cerebellar administration (intradentate nucleus)[1] and $5x10^{13}$ vg for CSF administration[2,3], were determined based on prior data of the maximum doses that can be safely delivered via each route
- All NHP were immunosuppressed throughout the study with prednisolone plus sirolimus for last 8 weeks

[1] Sondhi D et al, Transl. Med 2020; 12:eabb5413
[2] De BP et al, Hum Gene Ther 2023; 34:905
[3] Rosenberg JB et al, Hum Gene Ther Clin Dev 2018; 29:24

Study Design

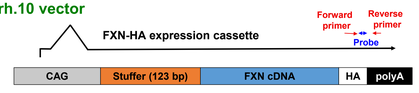
Timeline



Doses (AAVrh.10hFXN-HA)

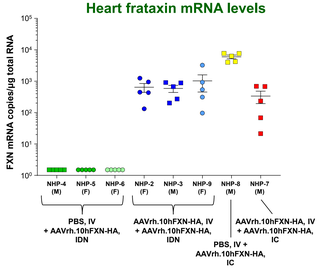
- Intravenous (IV) $5.6x10^{11}$ gc/kg
- Intradentate nuclei (IDN, bilateral) $1.6x10^{10}$ gc/site ($3.2x10^{10}$ gc/NHP)
- Intracisternal (IC) $5.0x10^{13}$ gc

AAVrh.10 vector

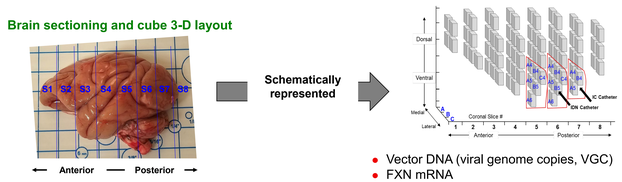


Systemic Delivery to Heart

- All animals that received intravenous delivery of AAVrh.10hFXN-HA had significant levels of vector-generated frataxin mRNA in the heart
- The animals that received PBS prior to intradentate delivery of AAVrh.10FXN-HA had no vector-generated frataxin mRNA in the heart, while the animal that received PBS prior to intracisternal delivery had significant levels

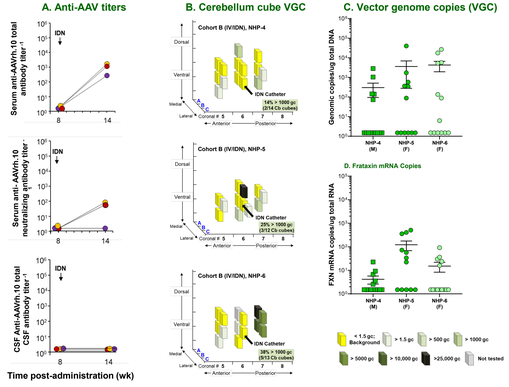


Analysis of the Cerebellum: Vector DNA, mRNA



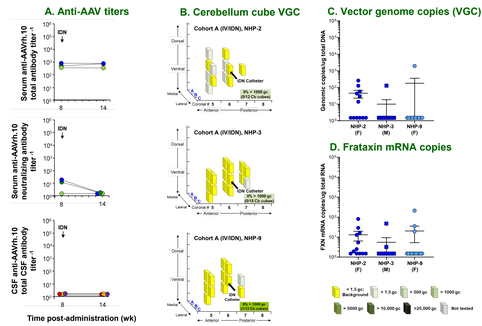
- Vector DNA (viral genome copies, VGC)
- FXN mRNA

Cerebellum Levels of Vector DNA and Vector Generated Frataxin mRNA 6 weeks After Delivery of AAVrh.10hFXN-HA to the Intradentate Nuclei of Nonhuman Primates with No Anti-vector Systemic Immunity



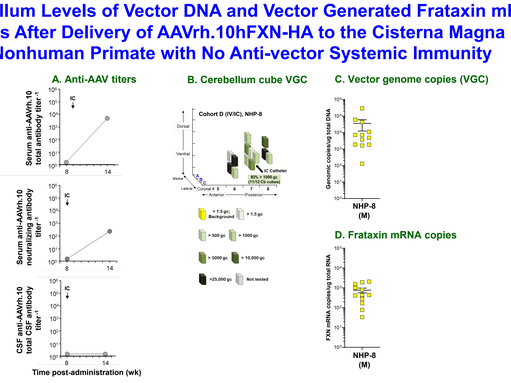
- In the absence of systemic anti-capsid antibodies, direct intradentate administration of AAVrh.10FXN-HA results in low levels of cerebellar vector distribution and expression

Cerebellum Levels of Vector DNA and Vector Generated Frataxin mRNA 6 weeks After Delivery of AAVrh.10hFXN-HA to the Intradentate Nuclei of Nonhuman Primates with Serum Anti-vector Antibodies from Prior Systemic Gene Therapy to Treat the Cardiac Manifestations of Friedreich's Ataxia



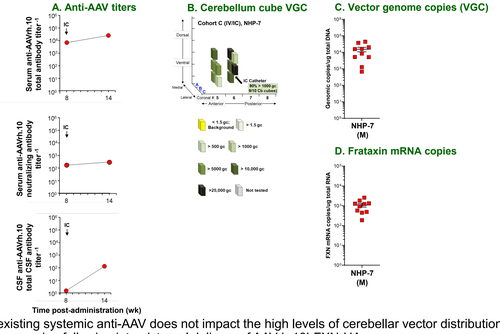
- Pre-existing systemic anti AAV immunity resulted in decreased cerebellar uptake following intradentate delivery of AAVrh.10hFXN-HA despite no apparent immunity in the CSF. This warrants further study

Cerebellum Levels of Vector DNA and Vector Generated Frataxin mRNA 6 weeks After Delivery of AAVrh.10hFXN-HA to the Cisterna Magna of a Nonhuman Primate with No Anti-vector Systemic Immunity



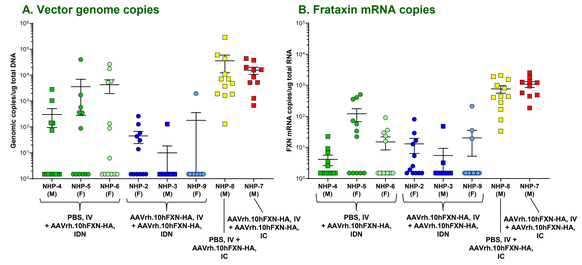
- In the absence of systemic anti-capsid antibodies, intracisternal delivery of AAVrh.10hFXN-HA results in high levels of cerebellar vector distribution and expression

Cerebellum Levels of Vector DNA and Vector Generated Frataxin mRNA 6 weeks After Delivery of AAVrh.10hFXN-HA to the Cisterna Magna of Nonhuman Primates with Serum Anti-vector Antibodies from Prior Systemic Gene Therapy to Treat the Cardiac Manifestations of Friedreich's Ataxia



- Pre-existing systemic anti-AAV does not impact the high levels of cerebellar vector distribution and expression following intracisternal delivery of AAVrh.10hFXN-HA

Summary: DNA and the Vector-Generated FXN mRNA in the Cerebellum



- Without systemic immunity intradentate administration results in low level cerebellar distribution, which is further reduced by systemic immunity
- With or without systemic immunity, intracisternal administration generates high cerebellar distribution of AAV and FXN mRNA

Summary

- Administration of the vector directly to cerebellum ($3.2x10^{10}$ vg) in NHP (n=3) with no pre-existing immunity with assessment 6 weeks after administration resulted in a mean vector level of 2,641 vg/µg genomic DNA (46.5% of the cerebellum cubes >background vg/µg and 25.9% of cubes >1000 vg/µg)
- In marked contrast, administration of the AAVrh.10 vector to the CSF ($5x10^{13}$ vg) to NHP (n=1) with no pre-existing immunity lead to vector level of 35,940 vg/µg DNA (100% of the cerebellum cubes >background vg/µg and 92% of cubes >1000 vg/µg), a level 13.6-fold higher than that achieved by the maximum tolerable dose that can be given by the direct intraparenchymal route
- Even though all NHP were immunosuppressed, intravenous administration of the AAVrh.10hFXN-HA vector prior to CNS delivery (as might be the case where there was prior AAV therapy for the heart disease), evoked total serum anti-AAVrh.10 IgG antibodies ($1.2x10^{3} \pm 1.4x10^{3}$ titer)
- In the immune NHP, while the systemic anti-AAVrh.10 antibodies had no effect on the cerebellar vector genome copies following CSF administration (p>0.4), the systemic immunity, despite immunosuppression, reduced cerebellar vector genome copies following direct delivery to the cerebellum (p<0.03)

Conclusion

- CSF (intracisternal) delivery of the AAVrh.10 vector achieves superior cerebellar biodistribution compared with direct delivery to the intradentate nuclei due to higher safely tolerated doses
- Pre-existing systemic anti-AAV immunity impairs direct cerebellar delivery but does not limit CSF administration, despite immunosuppression
- CNS delivery via IDN or IC minimally impacts the heart FXN expression, beyond prior IV dosing
- These data support a sequential cardiac → CNS gene therapy strategy for Friedreich's ataxia
- CSF delivery may enable effective neurologic gene therapy in patients previously treated systemically for cardiac disease

Disclosures

- This study was funded, in part, by LEXEO Therapeutics
- MGK, BPD, JBR, ASC, MH, AC, SL, KCH, HRM and SC have nothing to disclose
- SK and DS have equity in LEXEO
- RGC is a consultant to and has equity in LEXEO