EVALUATION OF AAV9 GENE THERAPY FOR SMARD1/CMT2S IN DIFFERENT MOUSE MODELS REVEALS DIFFERENCES IN EFFICACY DEPENDENT ON PROMOTER CHOICE

Julieth Andrea Sierra Delgado[1], Shibi Likhite[1], Vicki McGovern[2] , Sarah Holbrook3, Amy Huffenberger[1], Shrestha Sinha Ray[1], Deepti Chung[2], Leah S. Stefanik[1], Megan Baird[1], Maura Schwartz[1], Amy Hicks[3], Monica Nizzardo[4] , Stefania Corti[4], Arthur Burghes[2], W. David Arnold[2], Greg Cox[3], Kathrin Meyer[1,2]

1. The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA. 2. College of Medicine, The Ohio State University, Columbus, Ohio, USA. 3. The Jackson Laboratory, Bar Harbor, Maine, USA. 4. University of Milan, Milan, Italy

Background

- Mutations in IGHMBP2: rare autosomal recessive neurodegenerative disorders, ranging from distal muscle weakness with fatal respiratory distress/failure (**SMARD1**) to milder neuromuscular issues without respiratory symptoms (**CMT-2S**).
- **NO** clear phenotype-genotype correlation
- IV delivery of AAV9.IGHMBP2 rescues the disease phenotype in an intermediate IGHMBP2 mouse model (Nizzardo et al, Science advances, 2015).
- Further optimization in delivery and promotor needed to advance towards clinical applications.

The focus of this project was to evaluate the efficacy of two different AAV9.IGHMBP2 constructs in multiple in vivo disease models prior to IND-enabling studies.

Methods

C.

Model	Genotype	Phenotype
Em3	L362del	Severe SMARD1-like, paralysis. Dies ~21 days.
nmd-2J	Splicing variant Reduced protein	Intermediate SMARD1-like, paralysis, Dies ~60-100 days
Em5	Y918C (CMT2S patient allele)	CMT2S, Sensory and motor neuropathy. Lives >10 months

Figure 1. Experimental design *in vivo* study A) Two vector constructs and one empty capsid were randomly assigned as virus A, B or C. B) Scheme of ICV injections and mouse models used. C) Table of mouse models and mutations. HW = Hanging Wire Test; NCS = Nerve conduction studies.

Results

Severe Em3 Mouse Studies (JAX)

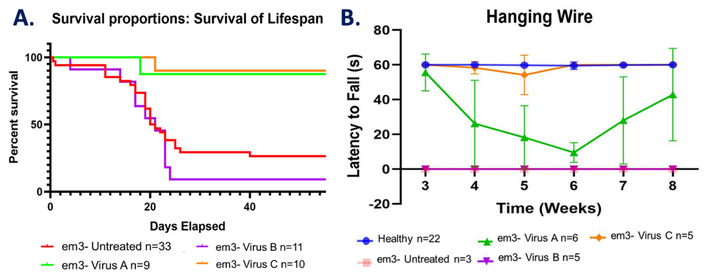
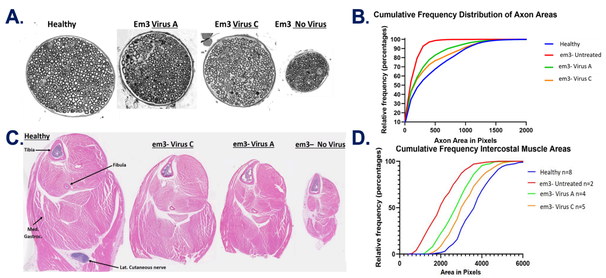


Figure 2. Virus A and C Improve Survival But Show Differences in Strength in Em3 Mice.
A) Survival plot for the severe Em3 mouse model. Both virus A and C show strong improvement on survival. Virus B showed no effect and was not tested in the other models. B) Hanging wire test shows Difference In Strength Between Virus A And C Treated Mice. Virus C performance on the hanging wire test shows a complete rescue of strength on par with healthy mice at all time points. Virus A shows a delayed rescue of strength. Virus B did not have an effect.

Results, cont.



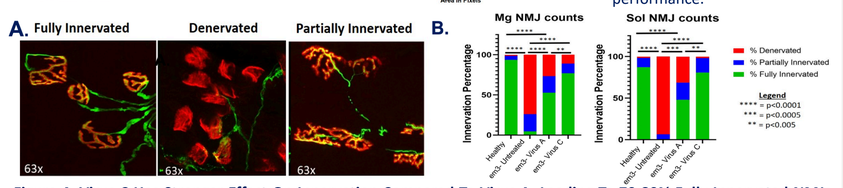


Figure 4. Virus C Has Stronger Effect On Innervation Compared To Virus A, Leading To 70-80% Fully Innervated NMJs. A) Representative images of fully innervated, denervated and partially innervated NMJs. B) Counts of NMJ on Medial gastrocnemius and soleus show increasing of fully innervated NMJ with both virus A and C, with virus C showing a higher % of fully innervated NMJs.

Intermediate nmd-2J Mouse Model Studies (OSU/NCH)

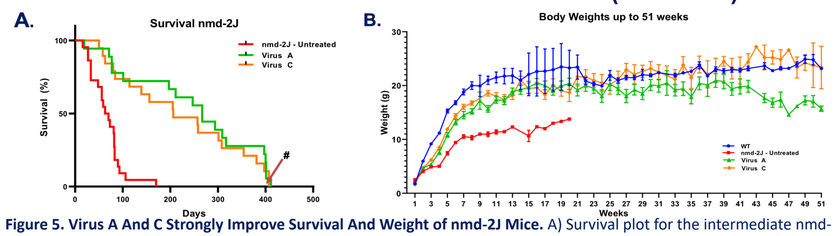


Figure 5. Virus A And C Strongly Improve Survival And Weight of nmd-2J Mice. A) Survival plot for the intermediate nmd-2J mouse model. Both virus A and C show strongly improved on survival. Median survival for virus A: 260 days, Virus C: 207 days. Median survival untreated: 66 days. Survival curves of virus A and C are not significantly different between them. #Arrow indicates end of study (~400 days). B) Weight curves were analyzed using the CGGC permutation test. Virus A and Virus C treated nmd-2J mice showed a statistically significant weight gain when compared to untreated nmd-2J mice. Adj P value <0.001.

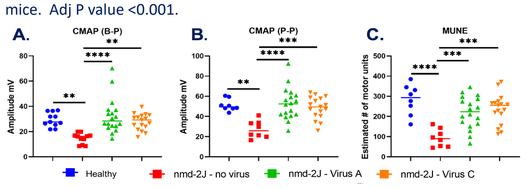


Figure 6. EMG Measures As Potential Clinical Biomarker For Efficacy Show Clear Difference Between Untreated And Treated Animals. There is a significant increase in CMAP (A-B) and MUNE (C) with both virus A and C. Virus A and C are not different from each other CMAP: Measures strength of innervation. MUNE: Estimates # of Neurons innervating a muscle.

Results, cont.

Intermediate nmd-2J Mouse Model Studies (MILAN)

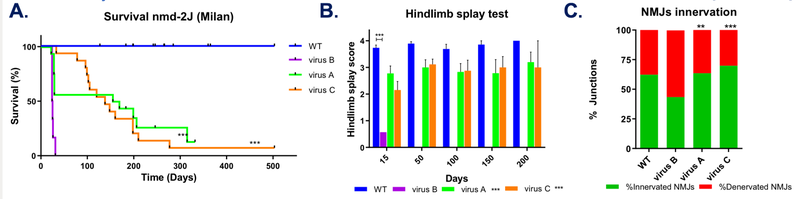


Figure 7. Efficacy of Virus A and Virus C on nmd-2J Model Is Consistent Among Multiple Centers. A) Virus A and Virus C show a strong improve of survival on nmd-2J mice showing reproducible results similar to Figure 5. B) Both virus significantly improved hindlimb strength as seen on the Hindlimb Splay test, as well as C) NMJ innervation on Medial Gastrocnemius. Both Virus A and C were significantly different from the Virus B treated nmd-2J.

Mild Em5 Mouse Model (JAX)

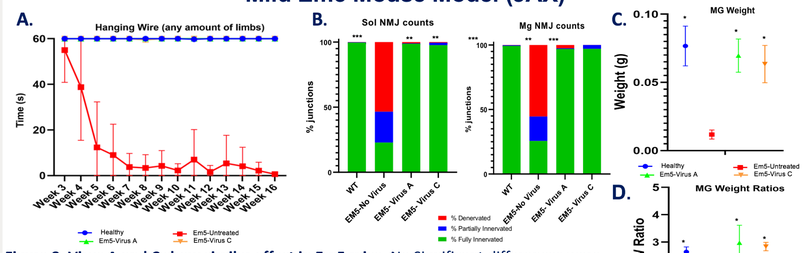


Figure 8. Virus A and C show similar effect in Em5 mice. No Significant differences were found between Healthy, Virus A, and Virus C treated animals in A) Hanging wire test or B) NMJ innervation. All groups are significantly different from untreated Em5. C) Virus A and C show increased muscle weight on Medial gastrocnemius D) Gastrocnemius weight in relation to total body weight. There is no difference between treated and healthy animals.

Conclusions

- CSF delivery of AAV9.IGHMBP2 rescues the disease phenotype in multiple IGHMBP2 mouse models.
- Studies in severe animal model (Em3) reveal differences in efficacy of the two promoter constructs that are less evident in the milder disease forms.
- Independent multicenter studies corroborate the robustness of CSF-delivered AAV9.IGHMBP2 gene therapy.

Collectively, the data provides a strong foundation for the development of phase I/IIa clinical trials for SMARD1/CMT2S.

Acknowledgements

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