Nonclinical Findings of Dorsal Root Ganglion, Spinal Cord and Peripheral Nerve Toxicities

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Cellular, Tissue and Gene Therapies Advisory Committee

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Disclosure Statement

- **Equity:** J.M. Wilson/his family hold equity in the following biotech companies that use AAV gene therapy technology: Passage Bio, Scout Bio, G2 Bio-associated asset companies, and IECure.
- **Contracts:** J.M. Wilson has sponsored research agreements relating to AAV technology with the following companies: Amicus Therapeutics, Biogen, Elaaj Bio, FA212, Janssen, Passage Bio, Scout Bio, G2 Bio, and IEcure.
- **Grants:** J.M. Wilson holds grants from NHLBI Gene Therapy Resource Program and rare disease foundations.
- **Principal Investigator:** J.M. Wilson is the PI on the above contracts and grants.
- **Employment of Relative:** Matthew Wilson (child) is employed by Scout Bio.
- **Scientific Advisor:** J.M. Wilson is a paid advisor for Scout Bio and Passage Bio.
- **Other:** J.M. Wilson is an inventor on patents that have been licensed to various biopharmaceutical companies and for which he may receive payments.

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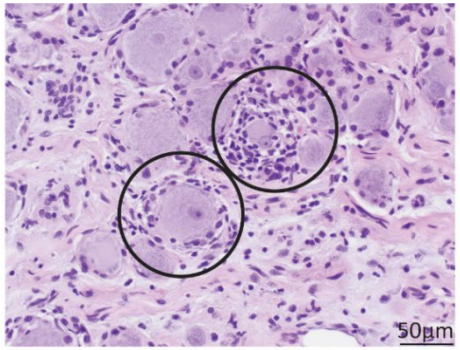
Sponsors
REGENXBIO
Janssen
Biogen
Passage
Amicus
FAST
RSRT
FA
NHLBI

Gene Therapy Program
Vector Core
Histology Core
Program in Comparative Medicine
Immunology Core
Regulatory Affairs
Project Management

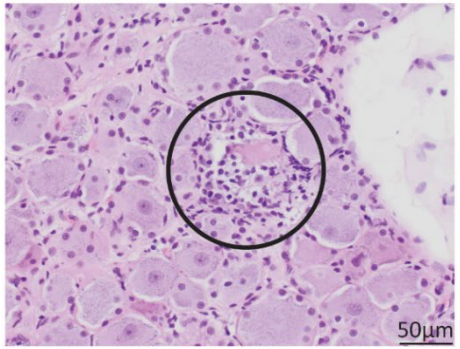
AAV-Mediated Toxicity of Dorsal Root Ganglia

Progression of DRG Pathology

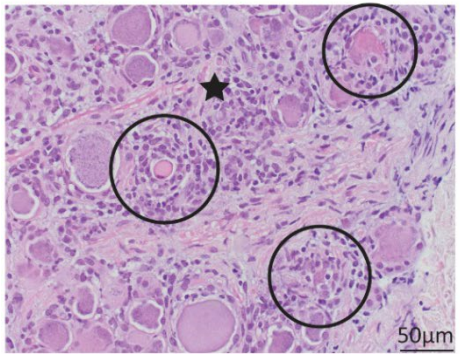
Early

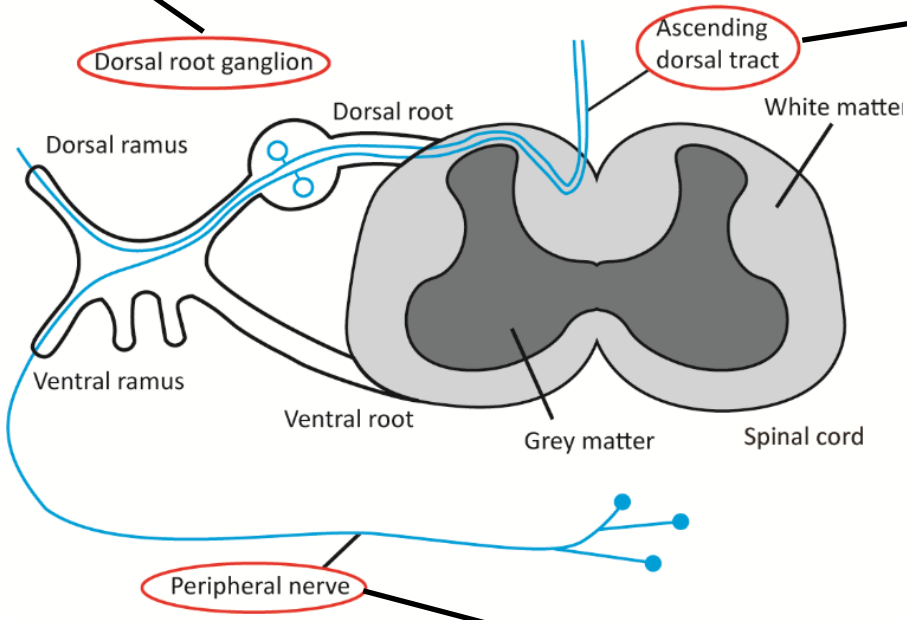


Middle

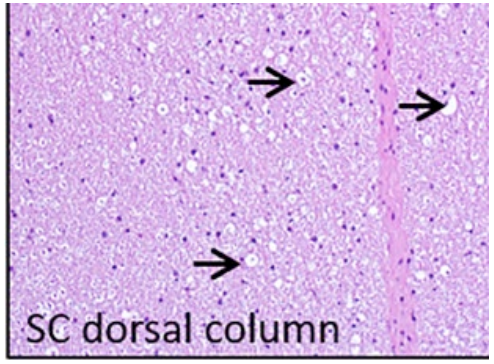


Late

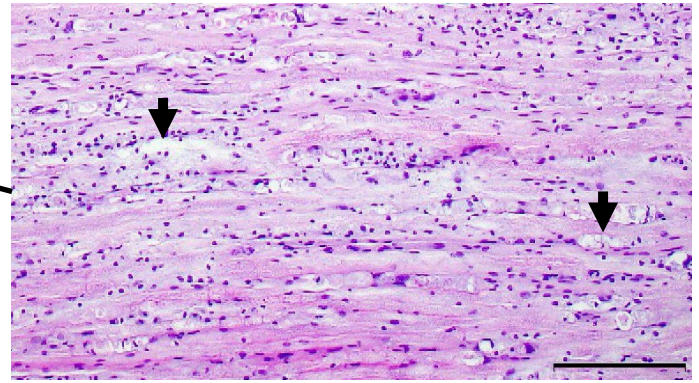




Dorsal column degeneration



Peripheral nerve mild degeneration



Grade

1 minimal (<10%)
2 mild (10-25%)
3 moderate (25-50%)
4 marked (50-95%)
5 severe (>95%)

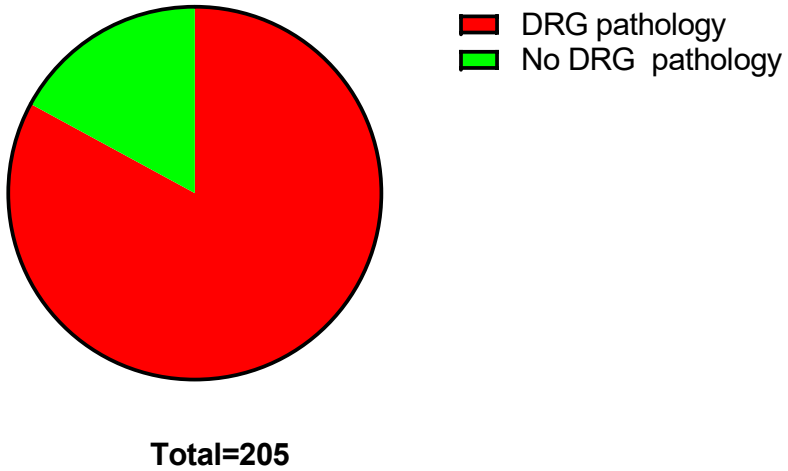
Penn
University of Pennsylvania

GENE THERAPY PROGRAM

DRG Pathology and AAV Gene Therapy in Nonhuman Primates: A Meta-analysis

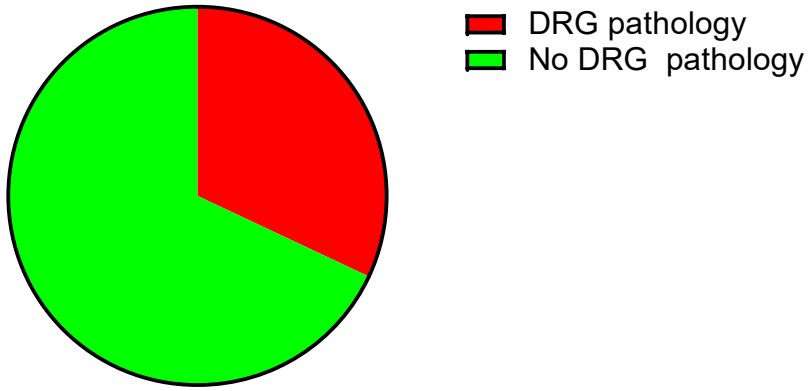
- Retrospective data collected by GTP over the last 5 years
- DRG pathology is dependent on route of administration:
  - **170/205 NHP ICM/IT: 83 % of AAV-treated animals**
  - **8/25 NHP IV: 32% of AAV-treated animals**
  - **1/17 : 6% of vehicle-treated animals**
  - **Toxicity seen with all the capsids and promoters tested (mostly ubiquitous)**

CSF administration (ICM / LP)



Total=205

IV administration



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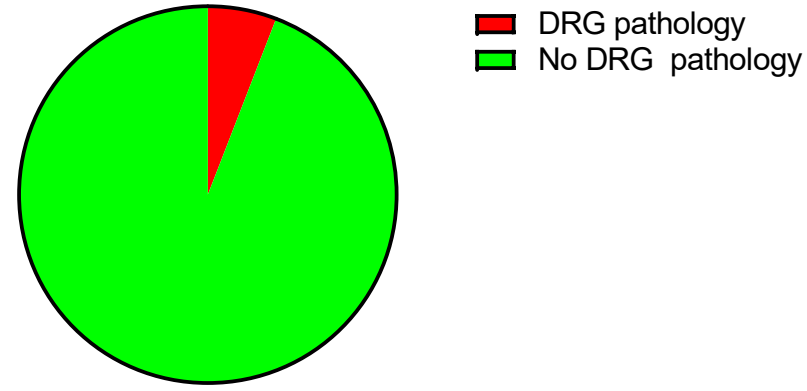
vehicle controls



Total=17

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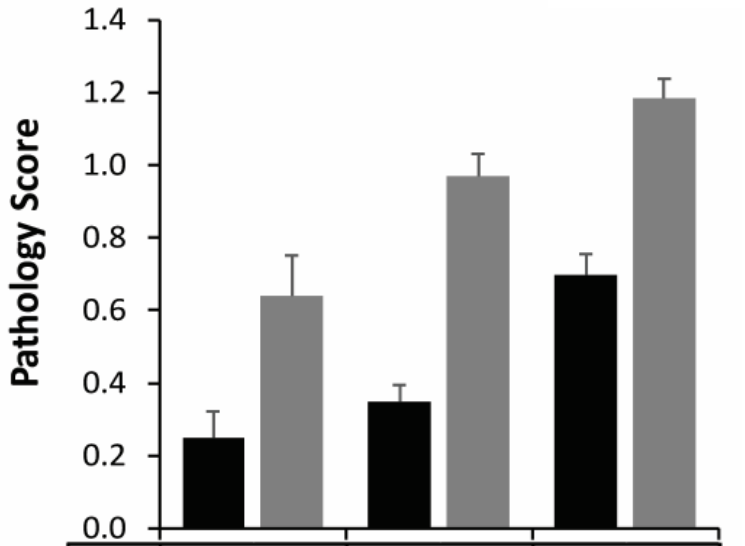
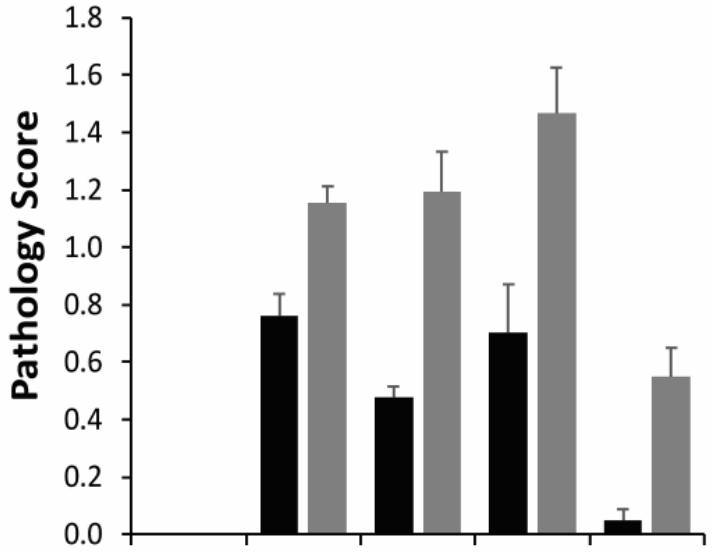
DRG and SC Pathology By Dose, Time Course, and Capsid

Dose



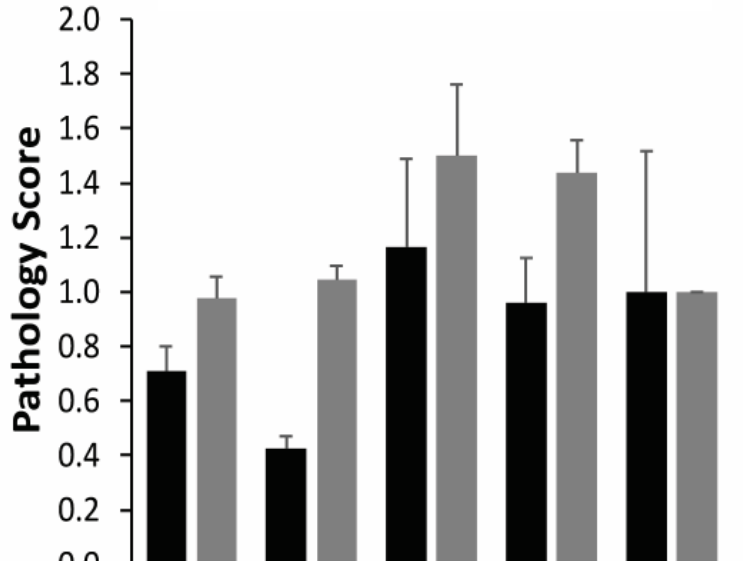
	<3E12		3E12>=1E13		>1E13	
n	12		75		117	
Count	36	36	181	225	321	351

Time Course



Days	14		21-60		90		120-169		180+	
n	12		81		79		15		17	
Count	27	36	224	243	226	45	27	45	39	51

Capsid



	AAV9		AAVhu68		AAV9-CRB3		AAV1		AAV5	
n	46		131		4		18		2	
Count	89	138	375	393	12	12	53	54	6	6

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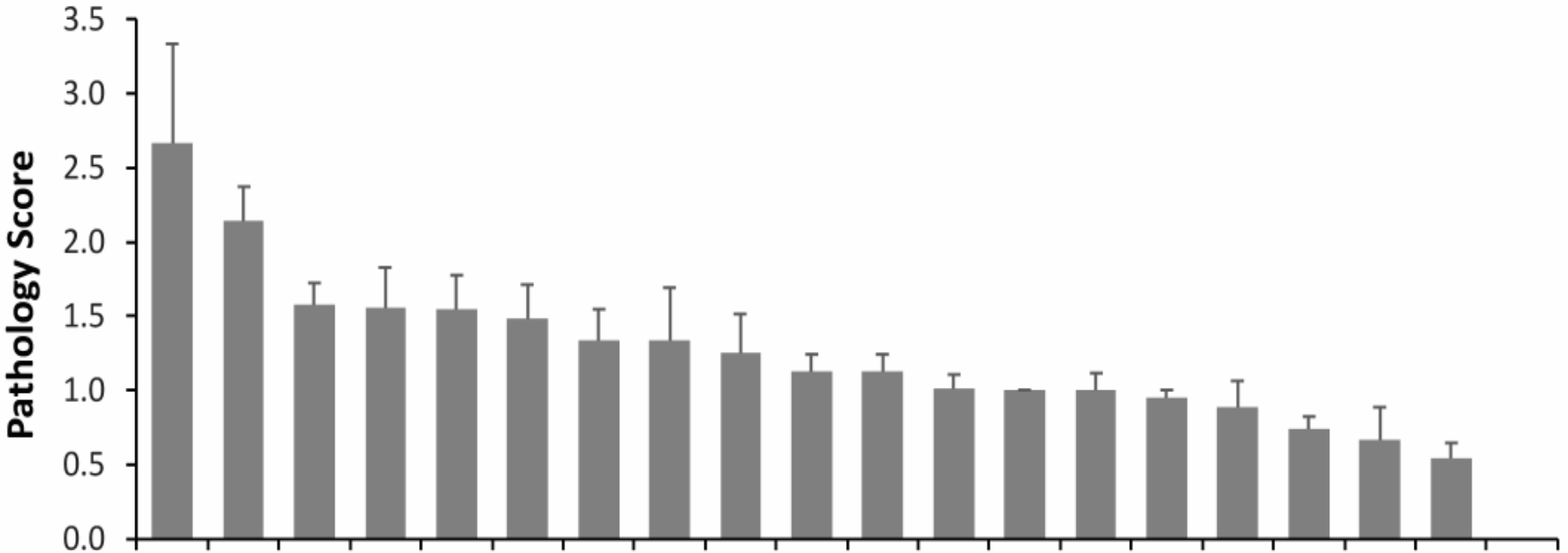
Dorsal Root Ganglion Spinal Cord

Spinal Cord Axonal Degeneration Is Substantially Impacted By Transgene



TG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
n	2	7	13	6	6	3	2	2	8	23	18	32	2	11	3	9	38	4	12	3
Count	6	21	39	18	18	9	6	6	24	69	54	96	6	33	9	27	114	12	36	9

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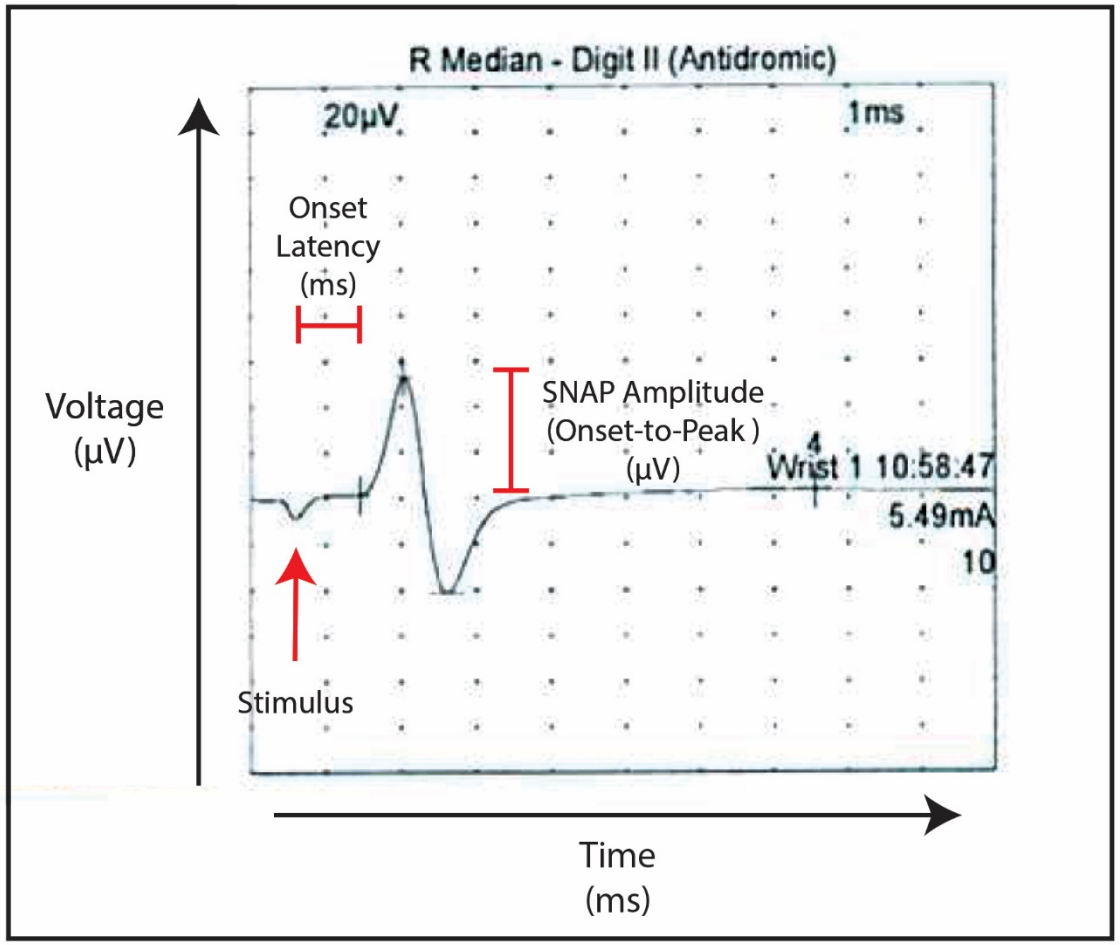
Clinical Summary

- Neuro exam
  - Cage-side: mentation, posture, and gait
  - Restrained: Cranial nerve assessment, proprioception, motor strength, sensory, and reflexes
- Clinical findings from 483 animals (LP and ICM)
  - WNL 478 animals
  - Neurological findings in 5 animals receiving AAV-GFP at >1E13 GC
    - 1 severe requiring euthanasia and 4 milder and reversible
    - Hindlimb ataxia and tremors +/- paresis (mostly asymmetric)
    - Did not measure NCVs

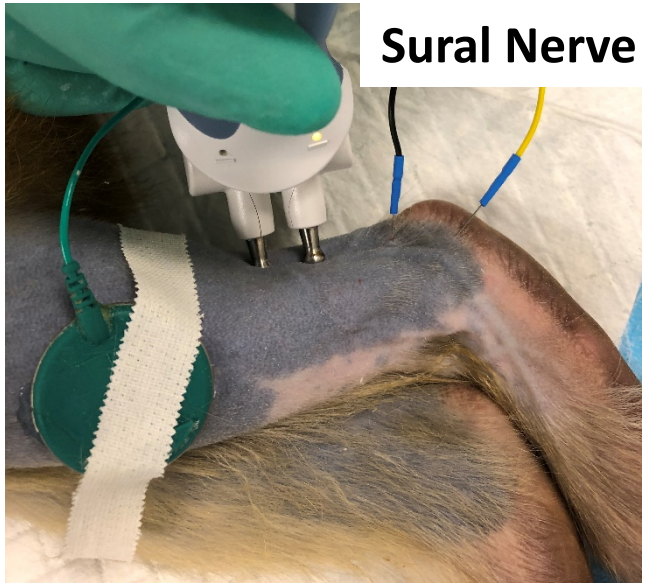
Measurement of Nerve Conduction Velocity (NCV) in Macaques



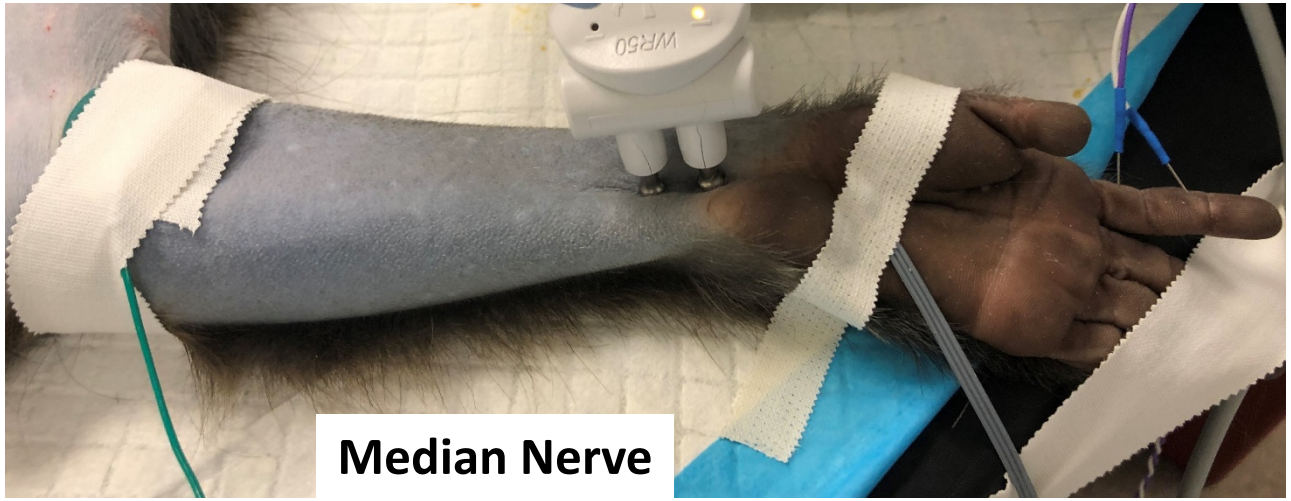
Unpublished data

Summary of NHP experience

- 23 studies
- 183 animals
- 714 measurements



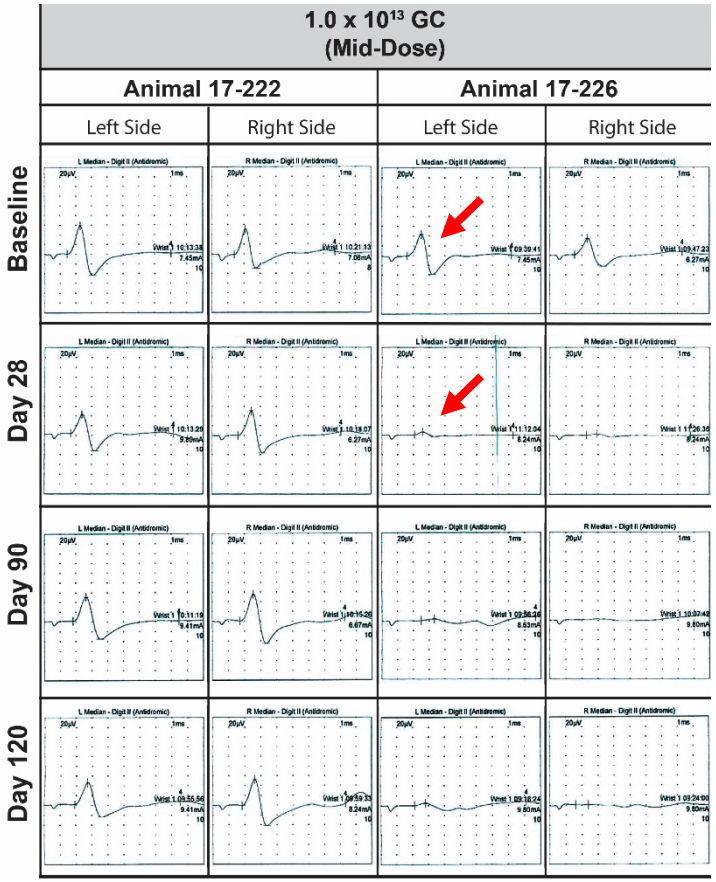
Sural Nerve

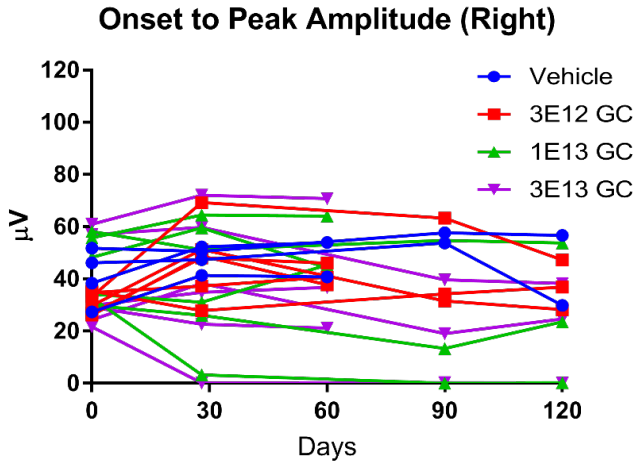


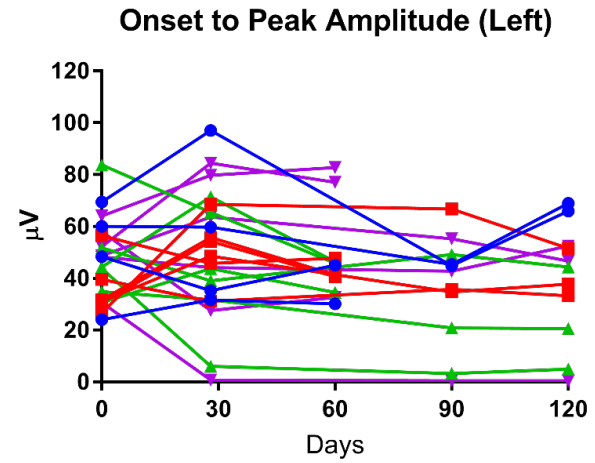
Median Nerve

Severity of Axonal Degeneration and Peri-axonal Fibrosis Correlates with NCV Abnormalities

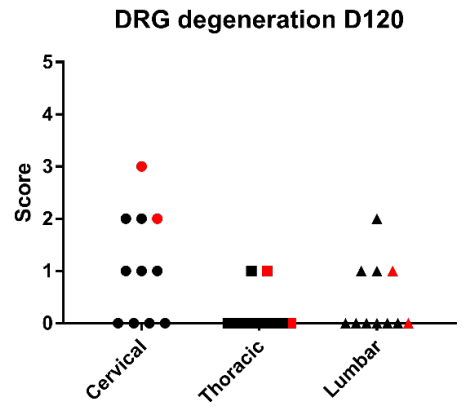
- GLP toxicology and biodistribution study
- Juvenile rhesus macaques (n = 22)
- Image-guided ICM injection of AAV-X
- 3 doses + vehicle control

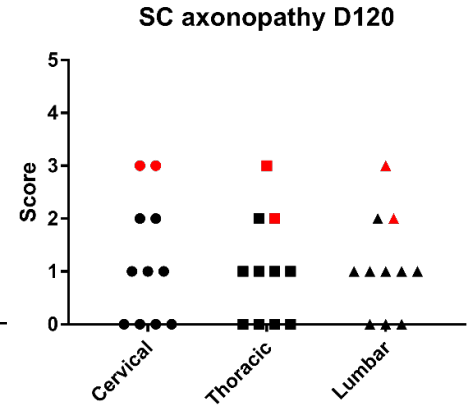


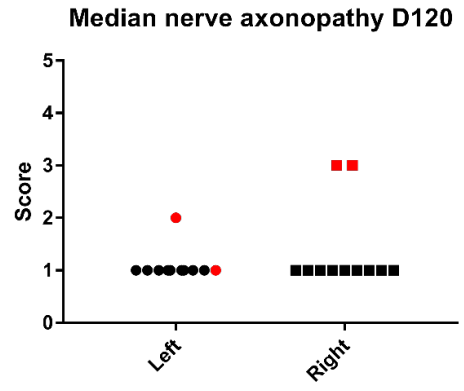


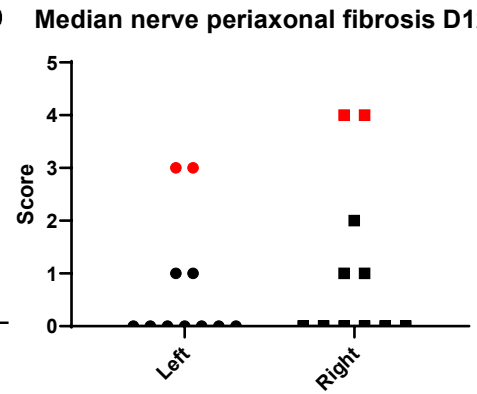


Histopathology









Red indicates the two animals with NCV abnormalities

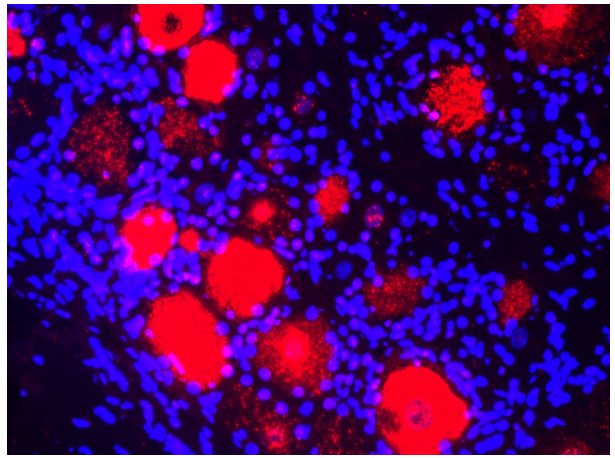
Unpublished data

Widespread Transduction But Variable Transgene Expression in NHP DRGs

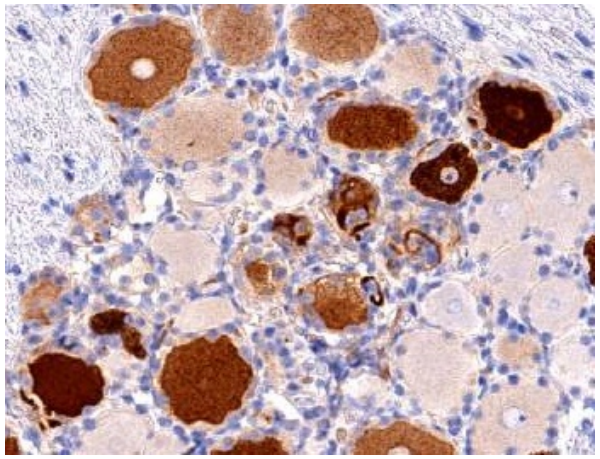
Variation of transgene expression within a DRG

ICM AAV 2 months pi

Transgene mRNA in **DRG**

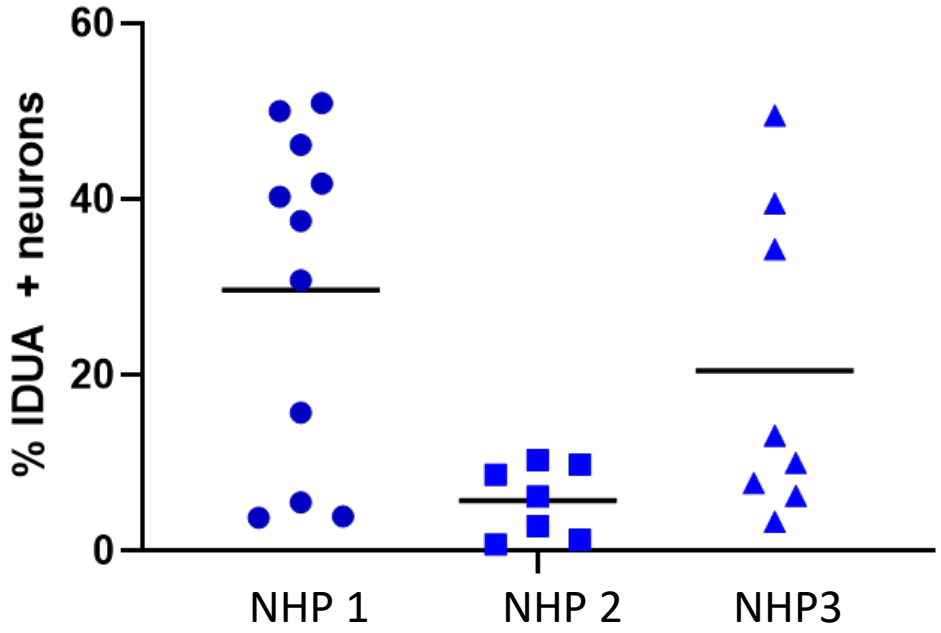


Transgene protein in **DRG**



Variation of transduction efficiency between DRGs

ICM AAV 3 months pi



Unpublished data

No Impact of Immune Suppression on DRG Toxicity

- AAV.hIDUA +/- IS
- 1e13 GC ICM

→

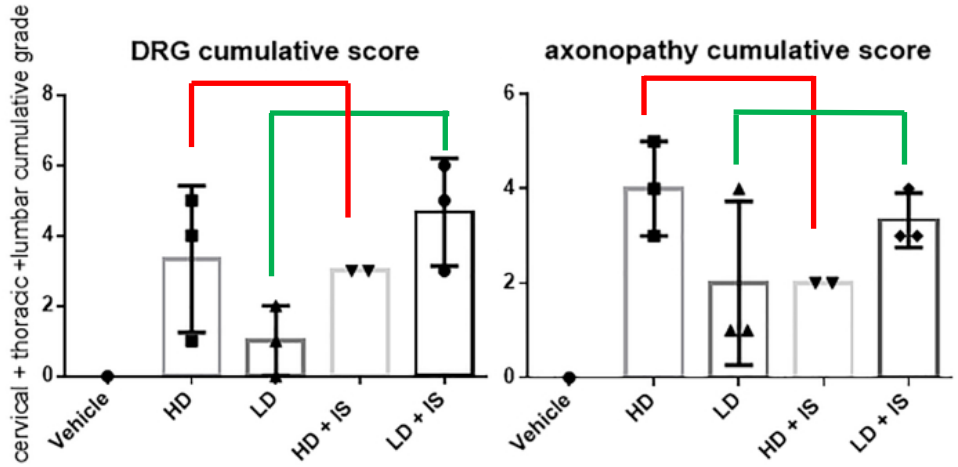
- 90/180 days pi
- **hIDUA quantification IF, IHC, ISH**
- Histopathology

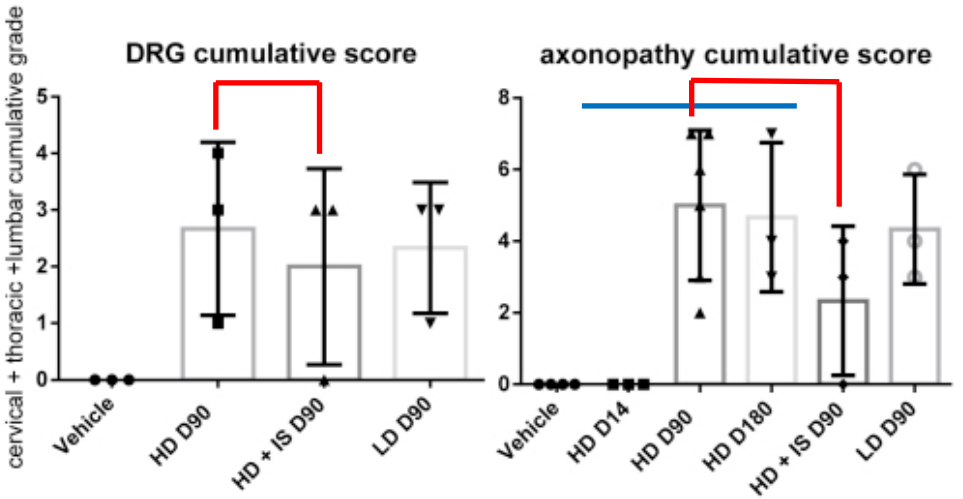
Immune suppression

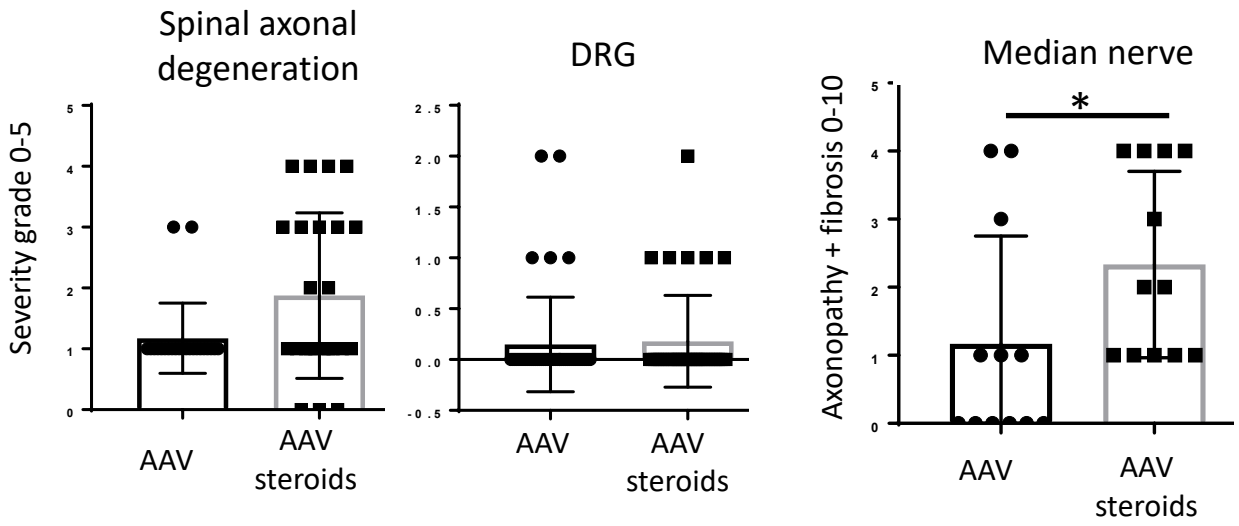
- Prednisolone qd -1 to 30 D, then taper

Immune suppression

- MMF qd -21 to +60 D
- Rapamycin bid -21 to +90 D

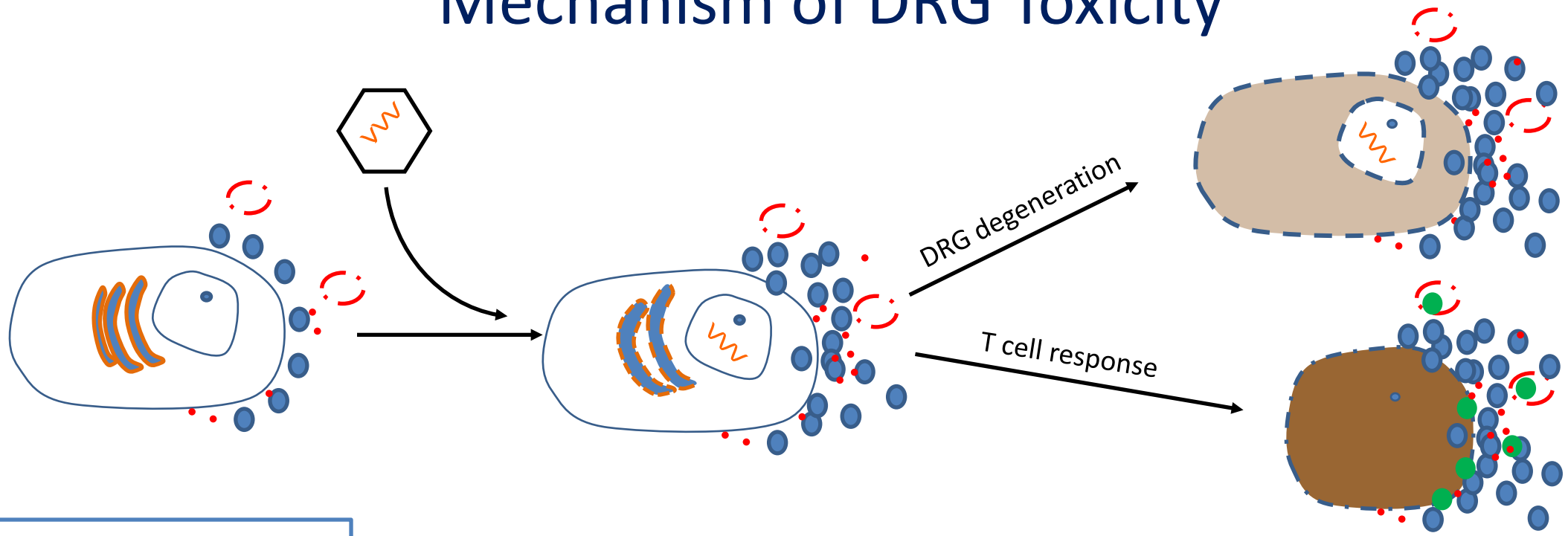






Hordeaux et al Science Translational Medicine 2020
Hordeaux et al Molecular Therapy Methods & Clinical Development 2018
Hordeaux et al Molecular Therapy Methods & Clinical Development 2018

Mechanism of DRG Toxicity



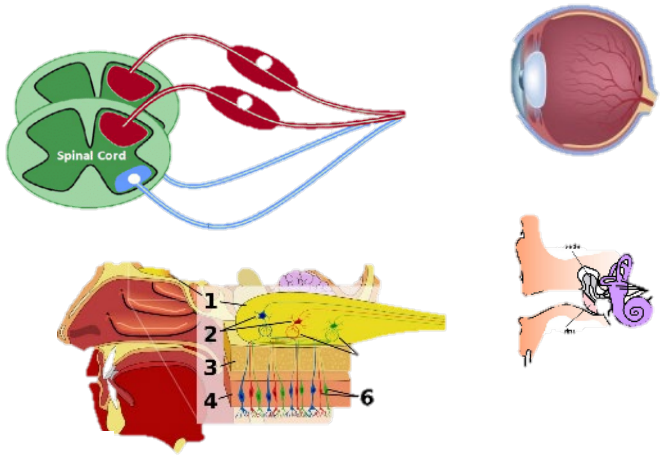
- AAV genome
- Satellite cells
- Fenestrated capillary: no BBB
- Inflammatory cytokines
- T lymphocytes

Direct injury of transgene overexpression <u>NOT</u> T cells

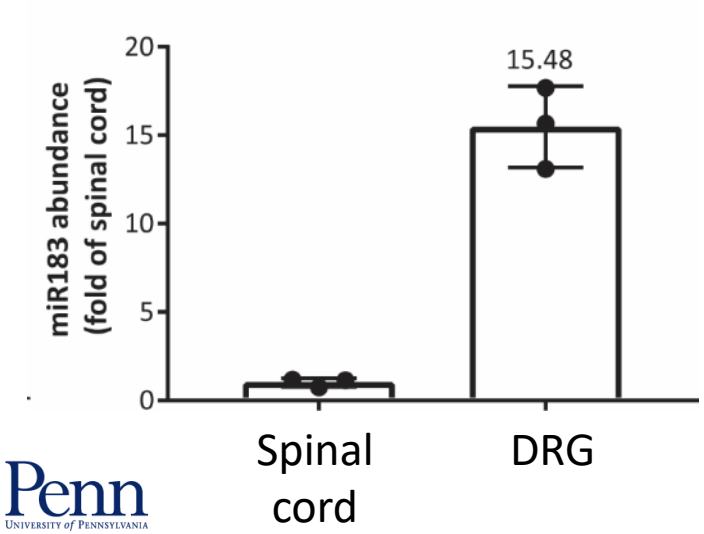
- Self limited
- Insult more likely transgene RNA or protein
- Substantial impact of transgene
- Not significantly reduced with immune suppression

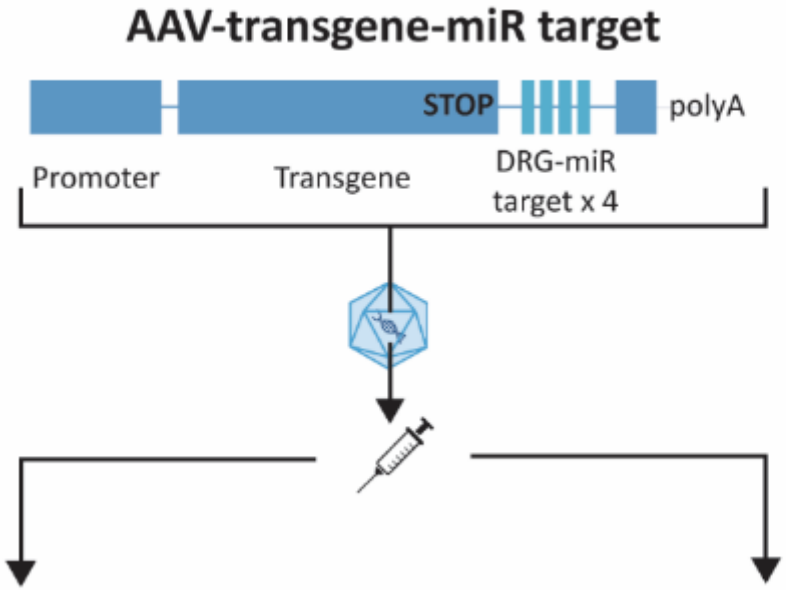
miR183

miR183 cluster is conserved across species and selectively expressed in sensory neurons: DRG, retina, inner ear, olfactory receptors

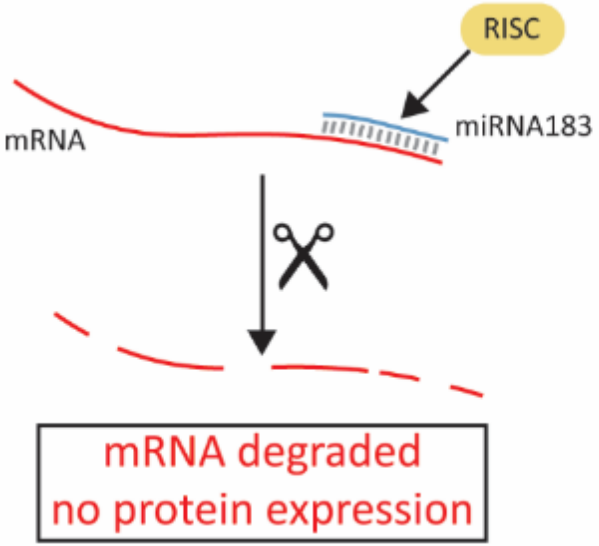


miR183 abundance, human tissue

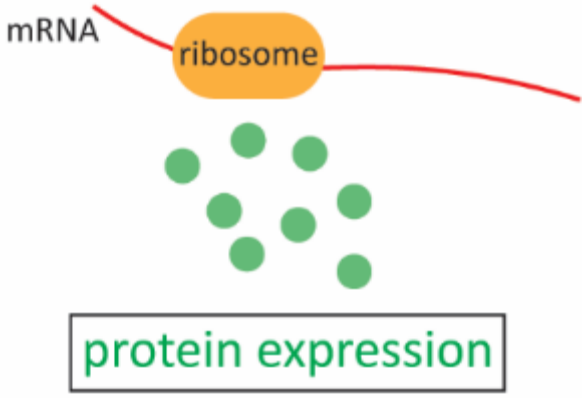




DRG neurons:



CNS neurons, other cells:



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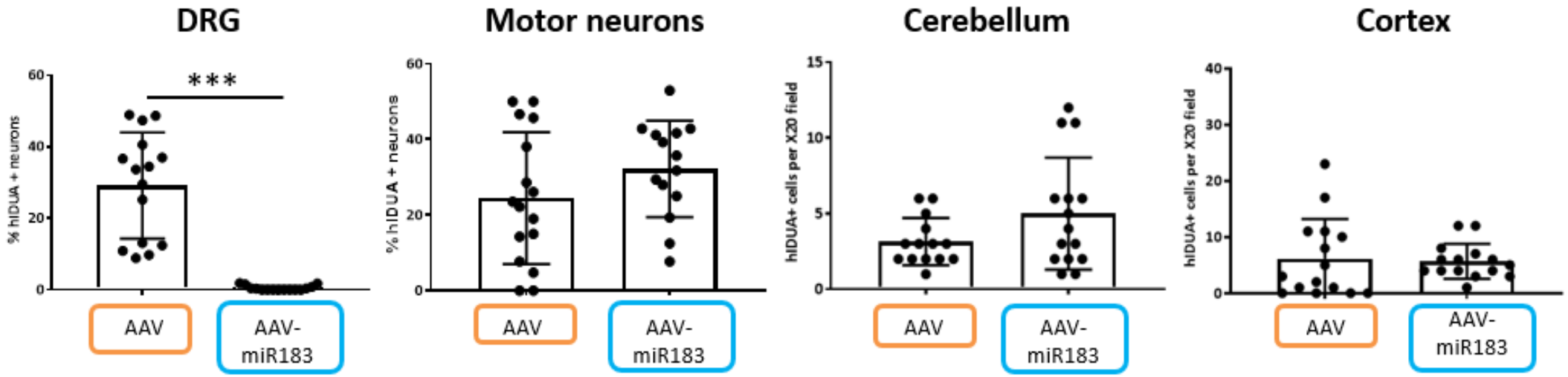
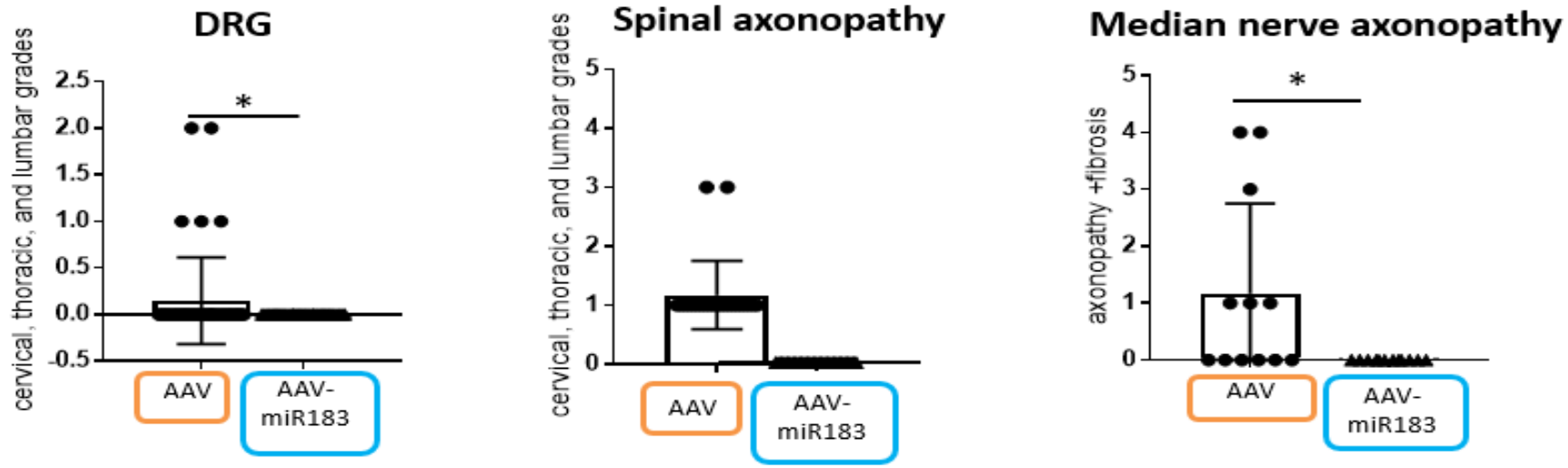
Impact of miR183 on IDUA Expression and DRG Toxicity

IDUA Expression



Histopathology



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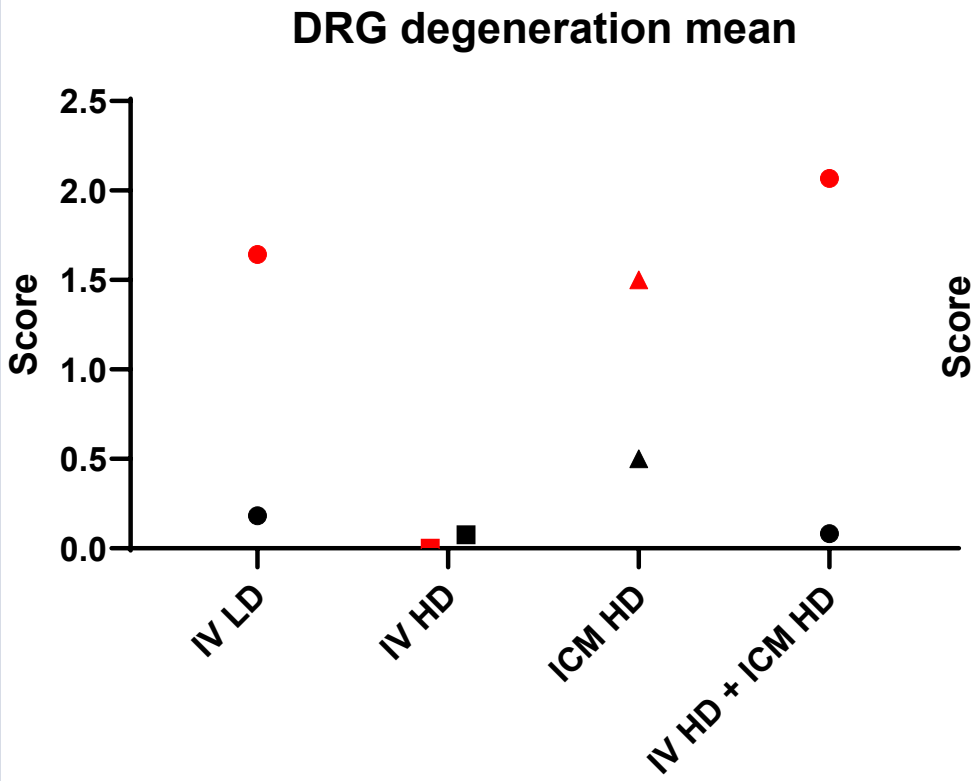
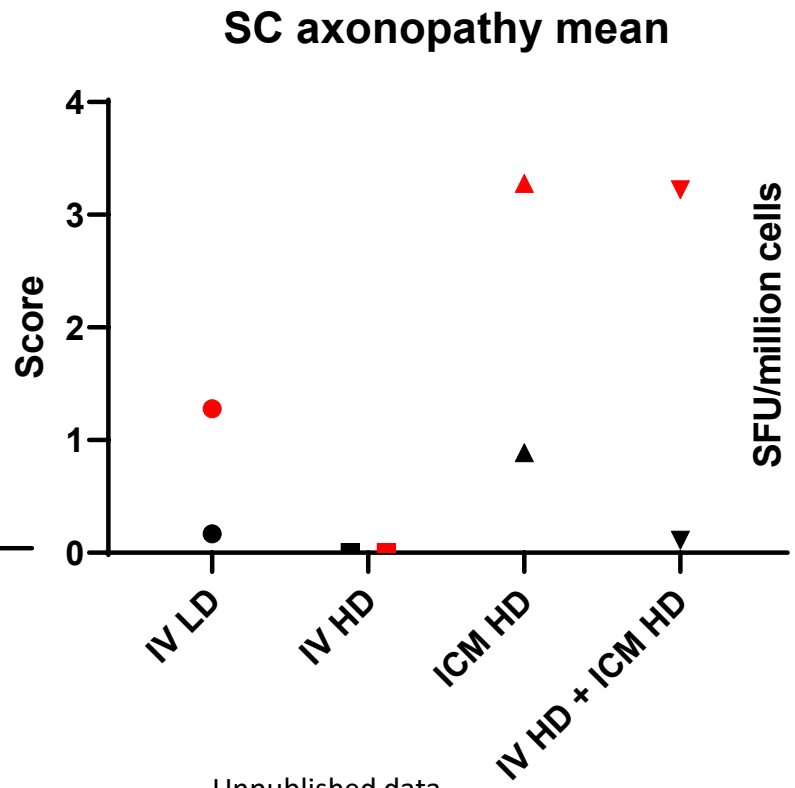
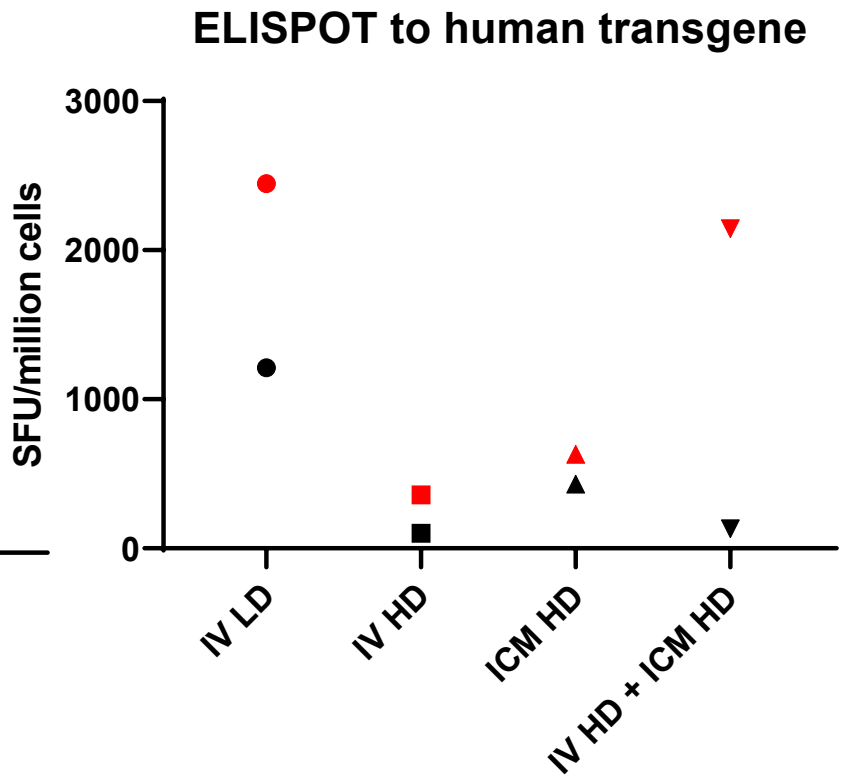
Adaptive Immunity as a Potentiator of Severe DRG Toxicity:

DRG toxicity in a NHP study following a combination of ICM/IV delivery



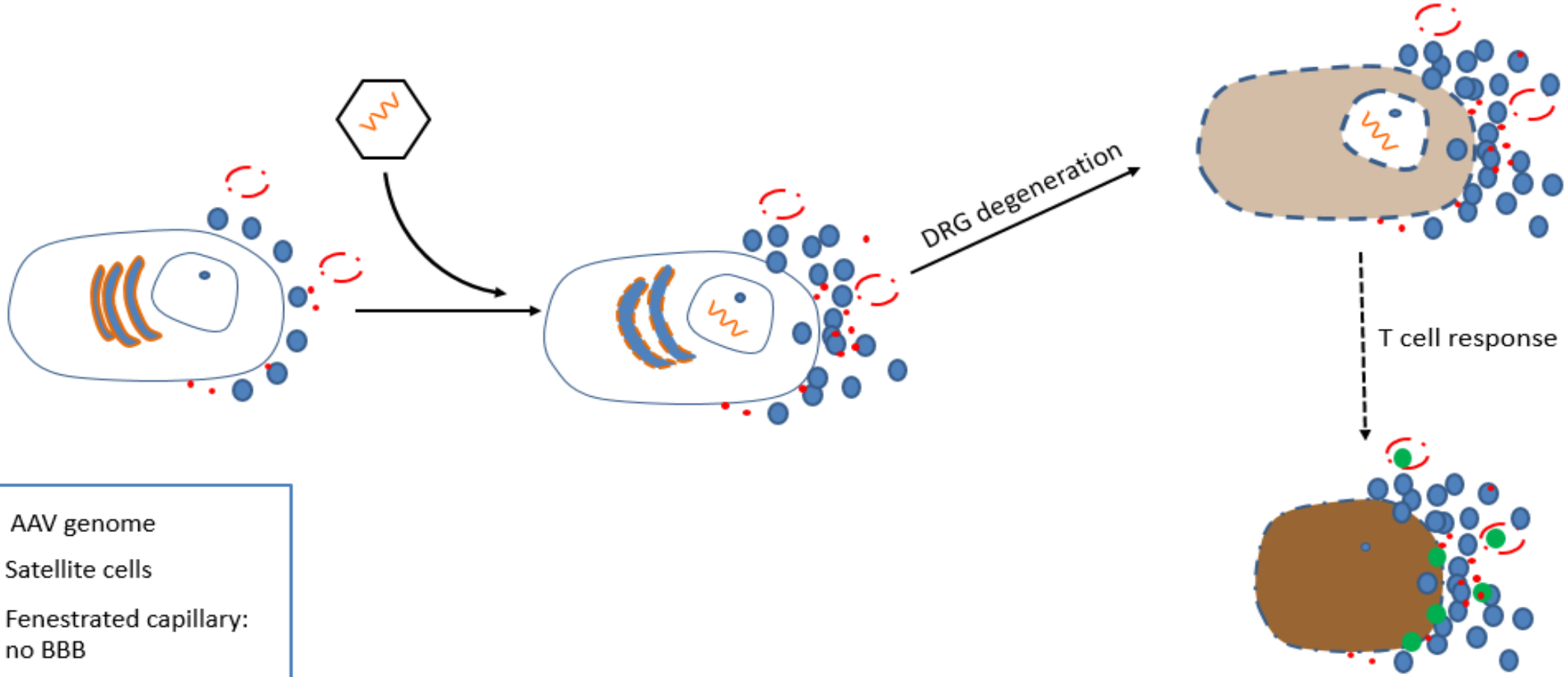




Unpublished data

Red symbols: animals with MHC I haplotype A002a

Mechanism of DRG Toxicity



Conclusions

- Very high levels of DRG neuron transduction are observed following IT/ICM delivery and high dose IV delivery.
- A consequence of high DRG neuron transduction is a toxic cellular insult caused by transgene over-expression followed by degeneration of central and peripheral axons.
- Vector-induced DRG pathology is more consistent with human sensory ganglionopathies than sensory neuropathies.
- This is an AAV platform problem with multiple vector and host factors contributing to its severity.
- The problem is greatly potentiated when destructive T cells are generated against the transgene protein.
- In most cases, DRG pathology occurs in the absence of clinical sequelae.
- Non-human primates are the most sensitive animal model for studying vector-induced DRG toxicity.
- NCV is a sensitive and specific non-invasive method for detecting more severe occurrences of sub-clinical vector-induced DRG toxicity.
- Methods to specifically reduce transgene expression in DRGs may help reduce pathology.