

# rAAV Gene Therapy and Peripheral Nervous System Ganglia Toxicity

**ASGCT-FDA Liaison Meeting  
November 8, 2021**

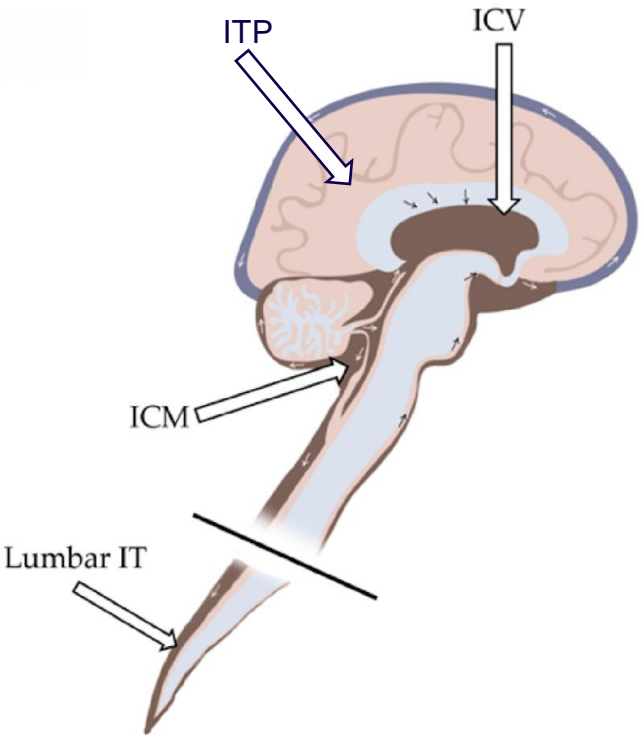
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Presented on Behalf of the ASGCT DRG Working Group

# Outline

- Anatomy of the Peripheral Ganglia and AAV Transduction
  - Sensory: Dorsal Root Ganglia (DRG) / Trigeminal Ganglia
  - Autonomic Ganglia
- Study design considerations
  - Ganglia Sampling in Toxicity Studies
  - Pathology Severity Grading
- Manifestation of DRG toxicity
- AAV DRG toxicity in different animal species
- Clinical Experience with AAV and DRG effects
- Nonclinical adversity and human risk assessment
- Discussion Questions

# Central Nervous System Routes of Administration:



Route	Pros	Cons
*Intracerebroventricular (ICV)	<ul style="list-style-type: none"> <li>Established neurosurgery protocols</li> <li>Broad central nervous system distribution</li> </ul>	<ul style="list-style-type: none"> <li>Invasive surgery</li> <li>Needle tract crosses parenchyma, risking injury and may enhanced immune response</li> </ul>
*Intra-cisterna magna (ICM)	<ul style="list-style-type: none"> <li>Good biodistribution to hindbrain structures</li> <li>Safer than ICV as does not cross parenchyma structures</li> </ul>	<ul style="list-style-type: none"> <li>Risk of medullary injury</li> <li>Not a routine clinical procedure</li> </ul>
*Intrathecal (IT)	<ul style="list-style-type: none"> <li>Non-invasive outpatient procedure – lumbar puncture<sup>2</sup></li> </ul>	<ul style="list-style-type: none"> <li>Fluid dynamics of bolus injection and distribution to brain poorly understood</li> <li>Limited understanding of dose translation and brain exposure</li> </ul>
Intraparenchymal (ITP)	<ul style="list-style-type: none"> <li>Targets specific locations in CNS</li> <li>Eliminate / reduces exposure to DRG</li> </ul>	<ul style="list-style-type: none"> <li>High Complexity</li> </ul>

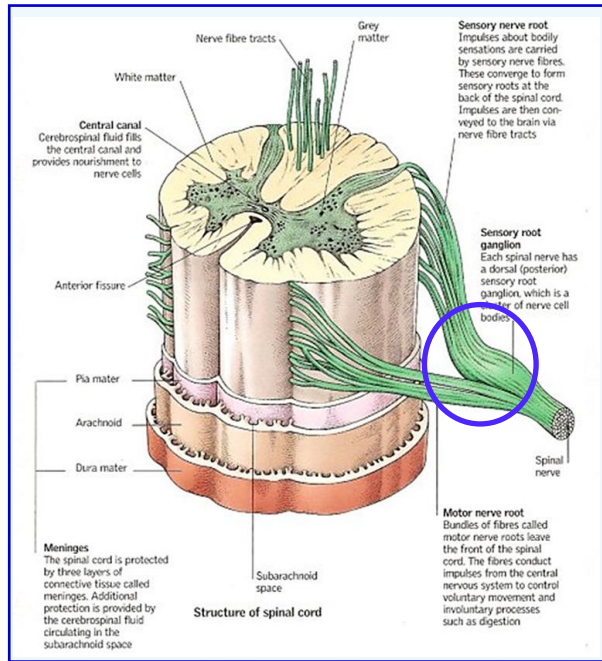
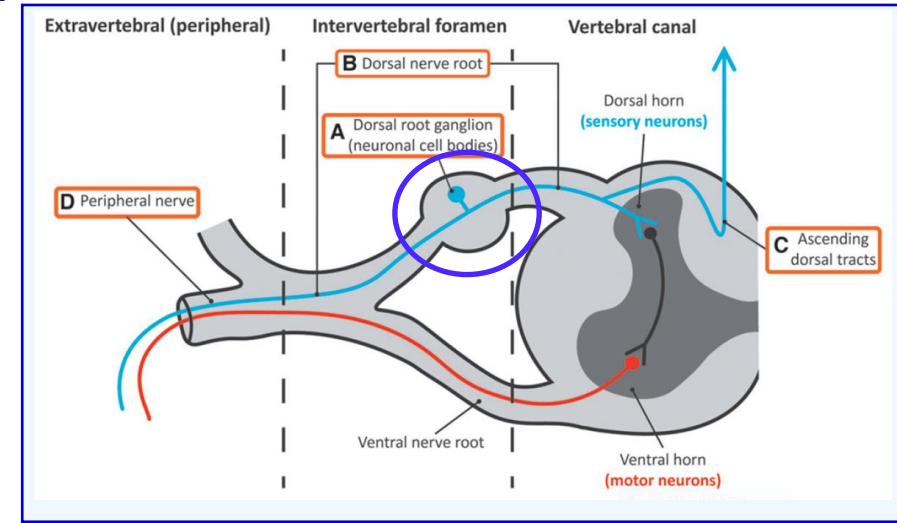
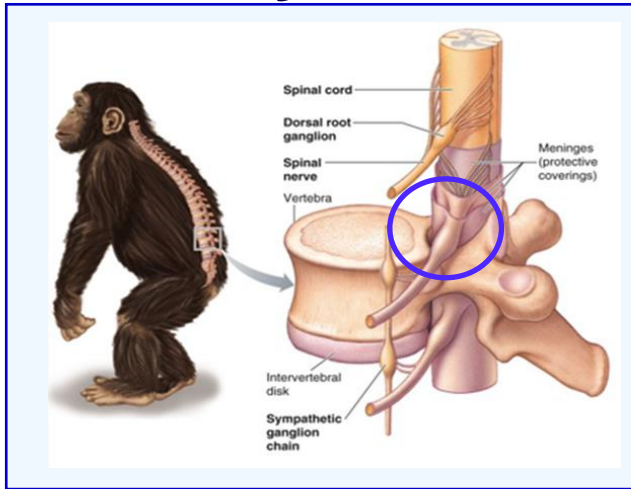
\* AAV Delivery routes associated with DRG pathology

DRG: Dorsal root ganglion / ganglia.  
 Image modified from: Perez BA, et al. *Brain Sci* 2020;10(2):119.

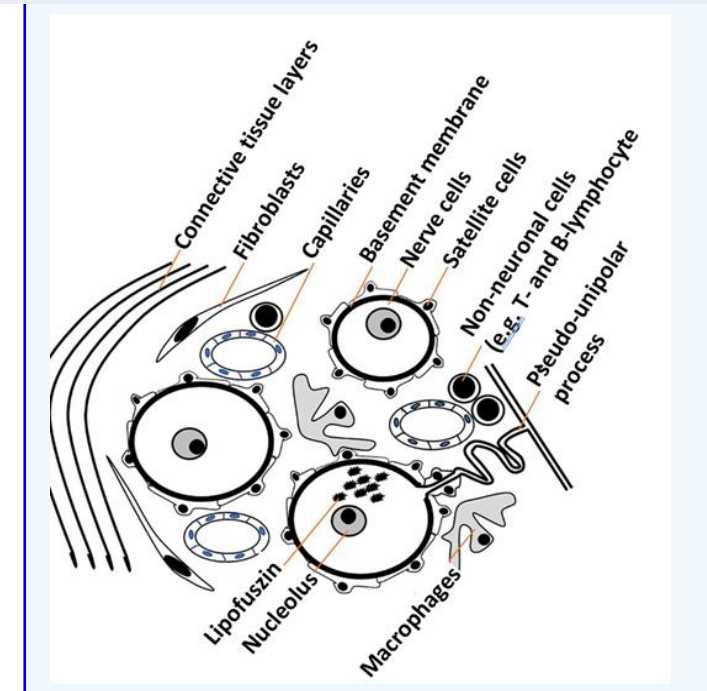
1. Perez BA, et al. *Brain Sci* 2020;10(2):119.  
 2. Hinderer C, et al. *Mol Ther Methods Clin Dev* 2020;17:969–74.

# Anatomy of the Dorsal Root Ganglion (DRG)

## DRG Connections to Nerve Pathways



## Multiple Cell Types Make Up the DRG



Krames ES. *Pain Medicine* 2014;15:1669–85. 2. David Darling Encyclopedia.

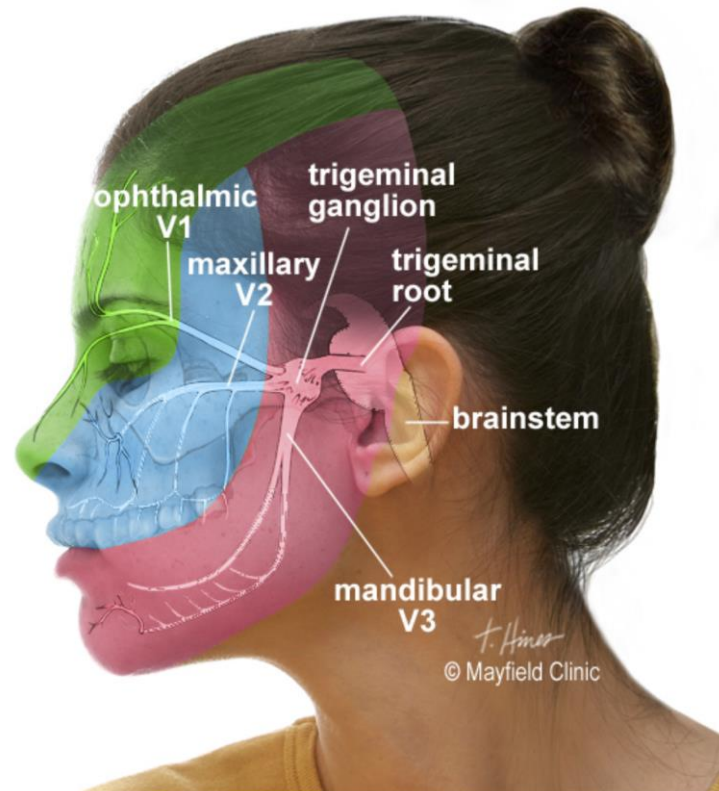
Spinal cord: [www.daviddarling.info/encyclopedia/S/spinal\\_cord.html](http://www.daviddarling.info/encyclopedia/S/spinal_cord.html)

Haberberger RV, et al. *Front Cell Neurosci* 2019;13:271.

Hordeaux J, et al. *Hum Gene Ther* 2020;31(15-16):808–18. 2. Abaira VE, Ginty DD. *Neuron* 2013;79(4):618–39

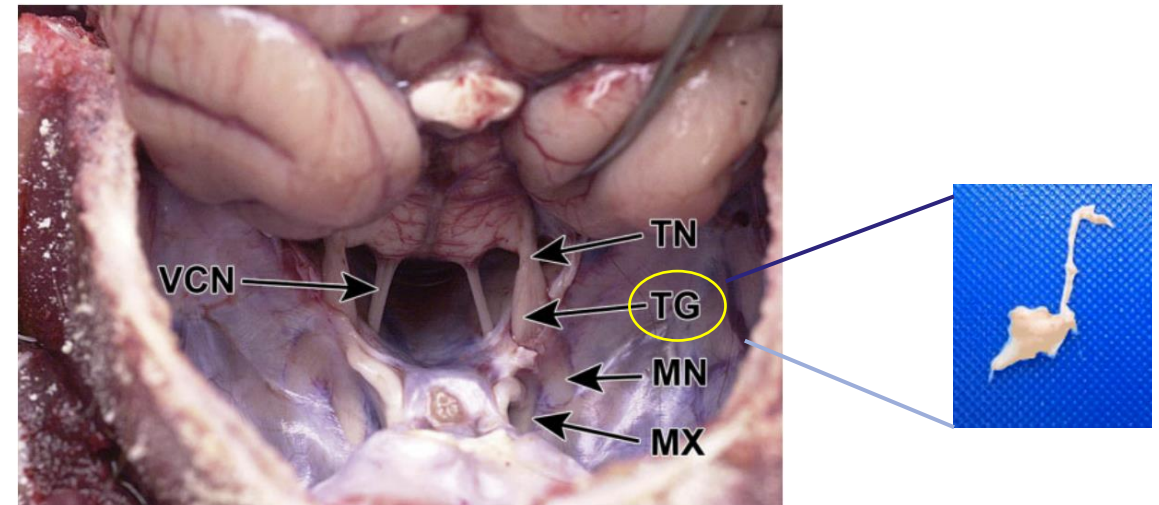
# Other Ganglia to Consider: Trigeminal Ganglia

Trigeminal Ganglia and Branches of Trigeminal Nerve Provide both Sensory and Motor Innervation to the Head



<https://www.facepain.org/understanding-facial-pain/cranial-nerves/>

## Trigeminal Ganglia in Cynomolgus Monkey Collected when Brain is Removed



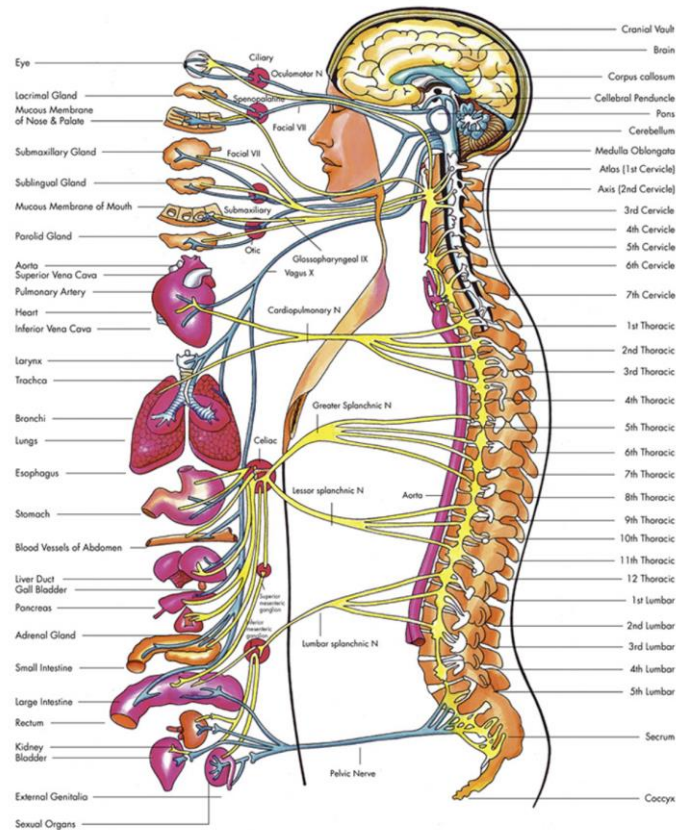
Toxicologic Pathology 2020, Vol. 48(1) 30-36



# Other Ganglia to Consider: Autonomic Nervous System

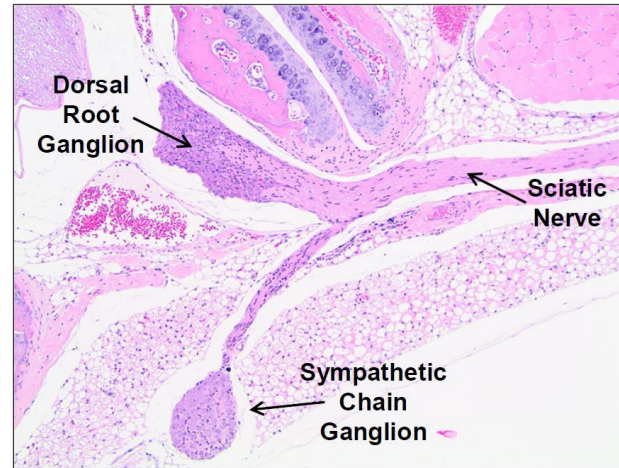
## Paravertebral Autonomic Trunk and Visceral Organ Ganglia

### Autonomic Nervous System



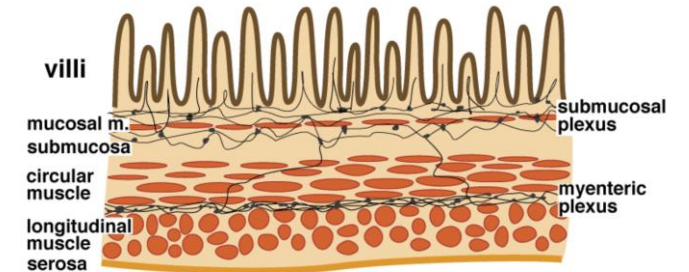
<https://www.dyansys.com/products-applications/product-technology/ans-monitor-technology>

### DRG and Paravertebral Autonomic Ganglia are Closely Associated



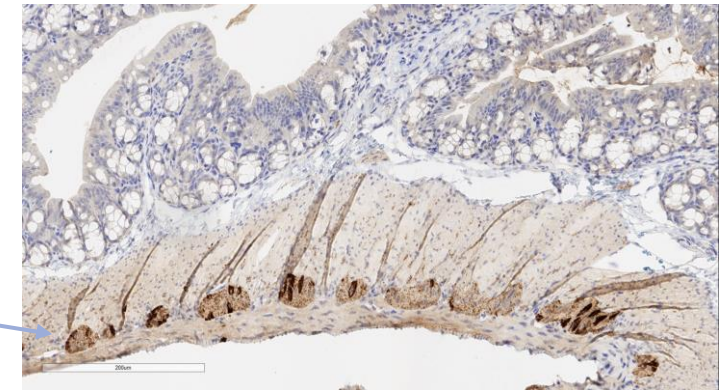
Comparative Anatomy and Histology: A Mouse, Rat, and Human Atlas, 2<sup>nd</sup> ed., Ch. 20, 2017

### Autonomic Ganglia in the Intestine



Transverse Section of Intestinal Wall  
<http://vanat.cvm.umn.edu/ans/pages/General3.html>

### AAV Can Transduce Autonomic Ganglia HC for Transgene Expression in Mouse Colon



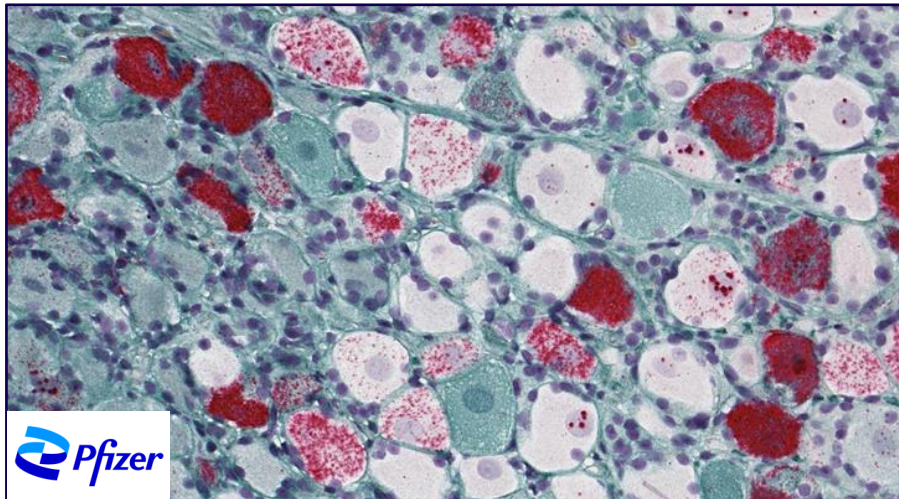
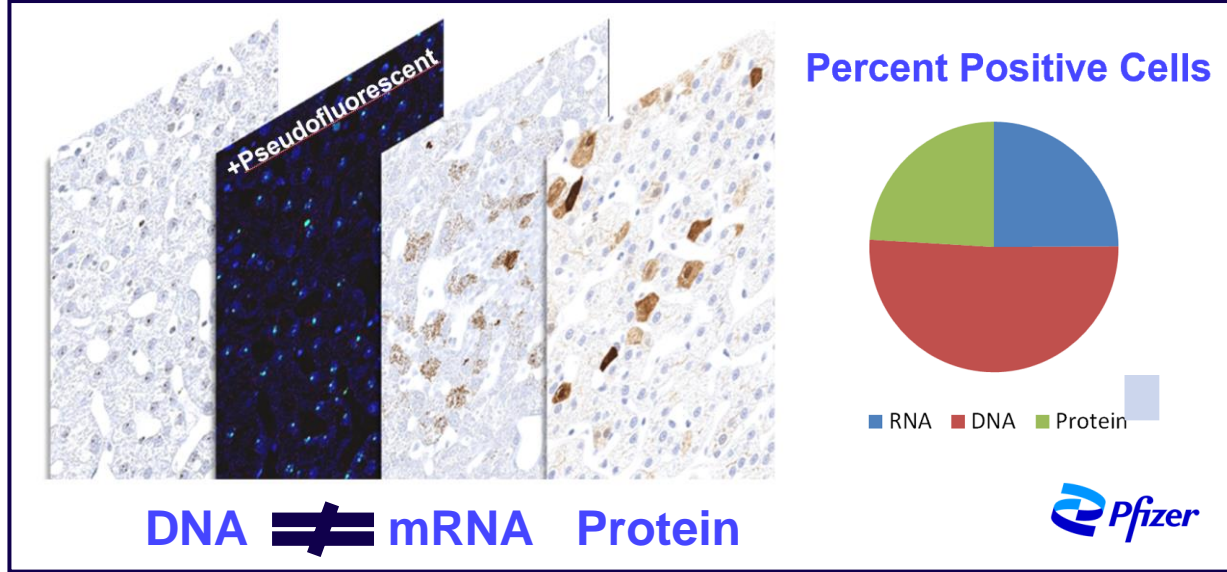
Autonomic ganglia :  
 myenteric plexus

Hutt J et al. Scientific and Regulatory Policy Committee Points to Consider: Nonclinical Research and Development of In Vivo Gene Therapy Products, Emphasizing Adeno-Associated Virus (AAV) Vectors. accepted Toxicologic Pathology (2021).



# AAV Transduction is Complex

NHP Liver : 30 days post AAV treatment  
 ISH for DNA and mRNA      IHC for Protein

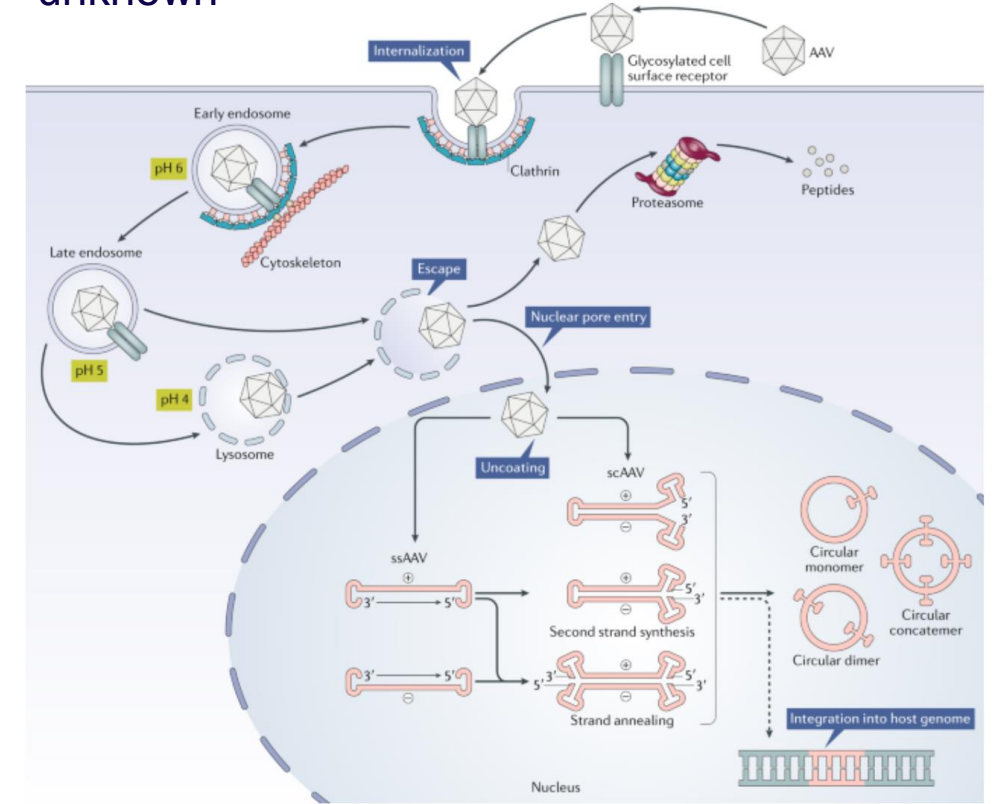


NHP DRG 48 days post AAV treatment

Red stain is ISH probe that recognizes vector DNA and mRNA

Green is Parvalbumin IHC

- The cellular pathway leading from AAV entry to transgene expression is complex and poorly understood
- Why two cells of the same type immediately adjacent to each other contain vector DNA but only one translates this to mRNA and protein is unknown



# Animal studies



# Species Selection and Study Design Consideration for Toxicity Evaluation of Ganglia

## Species Selection considerations related to DRG toxicity assessment

- 1) Permissive to vector transduction and biologic activity of transgene
- 2) Immunologically naive to capsid allowing transduction ( Relevant for IV delivery not CSF as nAbs do not cross BBB)
- 3) Comparable transduction efficiency to humans
  - May be unknown, but is an important consideration in developing an understanding of therapeutic index / safety margin
  - Nonclinical program should develop an understanding of comparable transduction across species to aid in species selection and developing strategy to translate efficacious dose range and safety margin
- 4) Sensitivity to DRG toxicity
  - Monkey, pig, rat, mouse and dog have all been shown to be susceptible to AAV induced DRG toxicity
  - However, relative sensitivity or relevance to humans is poorly understood.
  - At this time monkeys are considered most relevant due to the preponderance of studies conducted in this species
- 5) Potential for Immune response to transgene with cell mediated immunity causing DRG toxicity
  - Immune response may not be relevant to human

## Study Design

- 1) Two time points: One at expected peak transgene expression and one after steady state exposure  
Single time point may be justifiable with supporting data
- 2) Sufficient number of animals to provide identification of a relevant toxicologic response
- 3) Include neurofunctional assessments with appropriate sensory assessment  
Other biomarkers or imaging modalities may be also be include if scientifically justified.

# \*Spinal Cord, DRGs, Trigeminal Ganglion, Autonomic Ganglia

## Multi –Endpoint Sampling Schema

### Adapted to Study Specific Goals

## Recommendation for Collection

### DRG

Representative number from each anatomic region (cervical , thoracic, lumbar and sacral) for histology

### Trigeminal Ganglion

Recommended if delivery is into CSF

One side collected for histology opposite collected for DNA/RNA/Protein

**DRG collection for DNA, RNA and / or Protein as needed for study requirements**

### DRG Axon Evaluation

Nerve roots associated with DRGs and peripheral nerve from rear and / or fore limb collected  
bilateral but examine unilateral

Dorsal funiculus of spinal cord

### Spinal cord




Cervical, Thoracic and Lumbar


Multiple cross section or one cross and longitudinal /oblique from each level

### Autonomic Ganglia

Ensure histologic evaluation of visceral ganglia in GI track

Collection of paravertebral ganglion may be considered

Dorsal Root Ganglia	Location	End-point
	C1	Histology
	C2	DNA
	C3	RNA
	C4	Histology
	C5	Protein
	C6	RNA
	C7	Protein
	T1	DNA
	T2	RNA
	T3	Histology
	T4	RNA
	T5	Protein
	T6	Histology
	T7	DNA
	T8	RNA
	T9	Histology
	T10	Protein
	T11	RNA
	T12	Histology
	L1	DNA
	L2	RNA
	L3	Protein
	L4	Histology
	L5	RNA

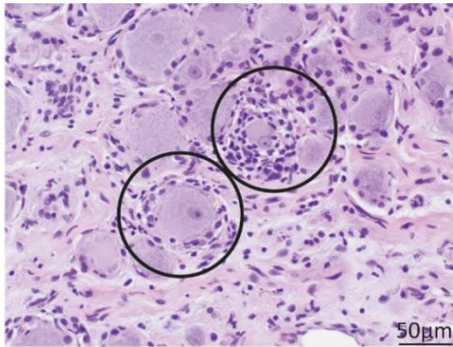
	S3	RNA
	S4	Histology

\* Study that is intended for DRG risk assessment. Adapted as needed for rodent or non rodent species.

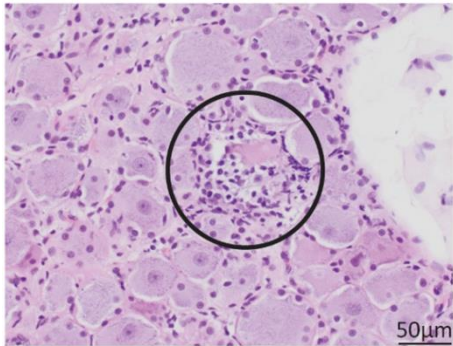
# AAV-Mediated DRG Toxicity and Secondary Axonopathy

## Progression of DRG

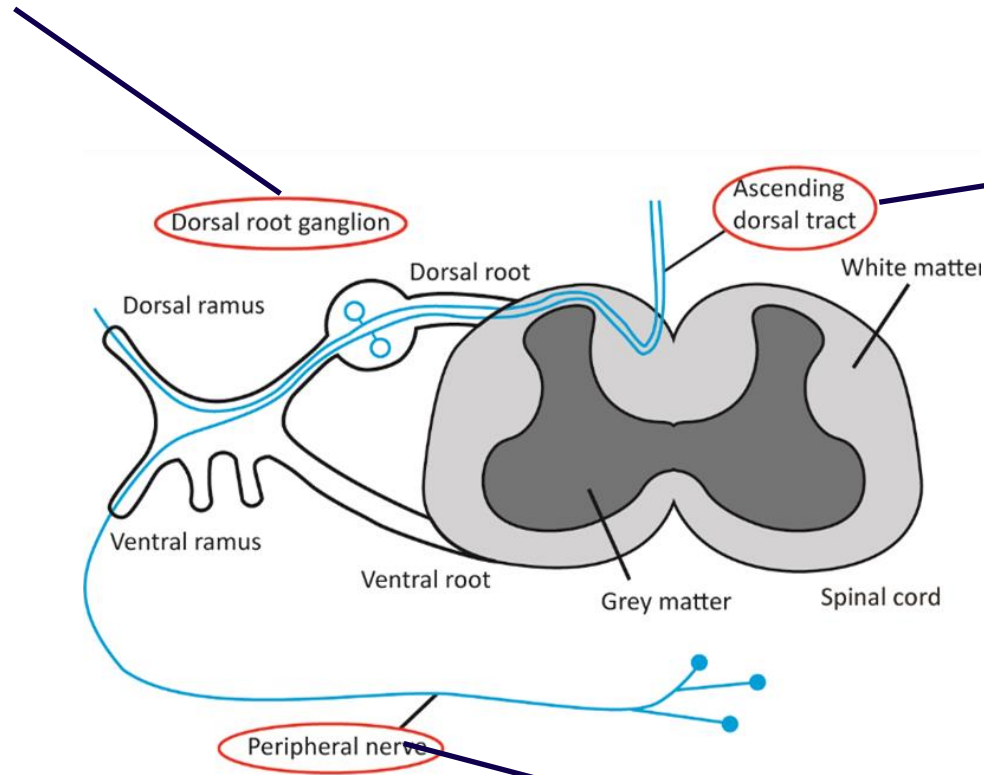
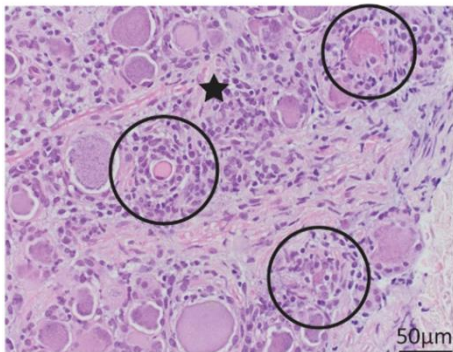
Early



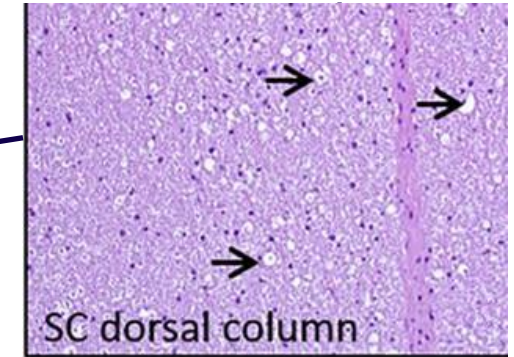
Middle



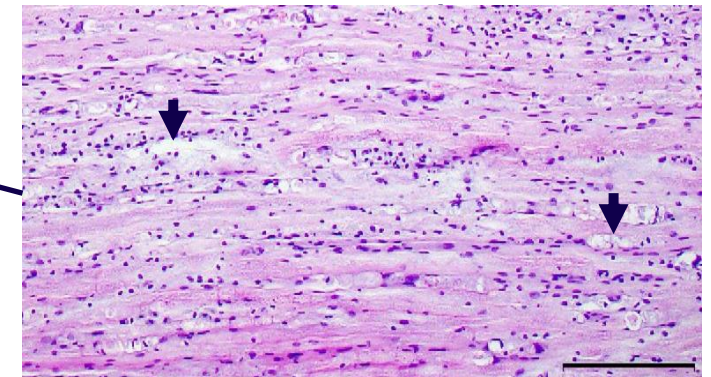
Late



## Dorsal column



## Peripheral nerve mild



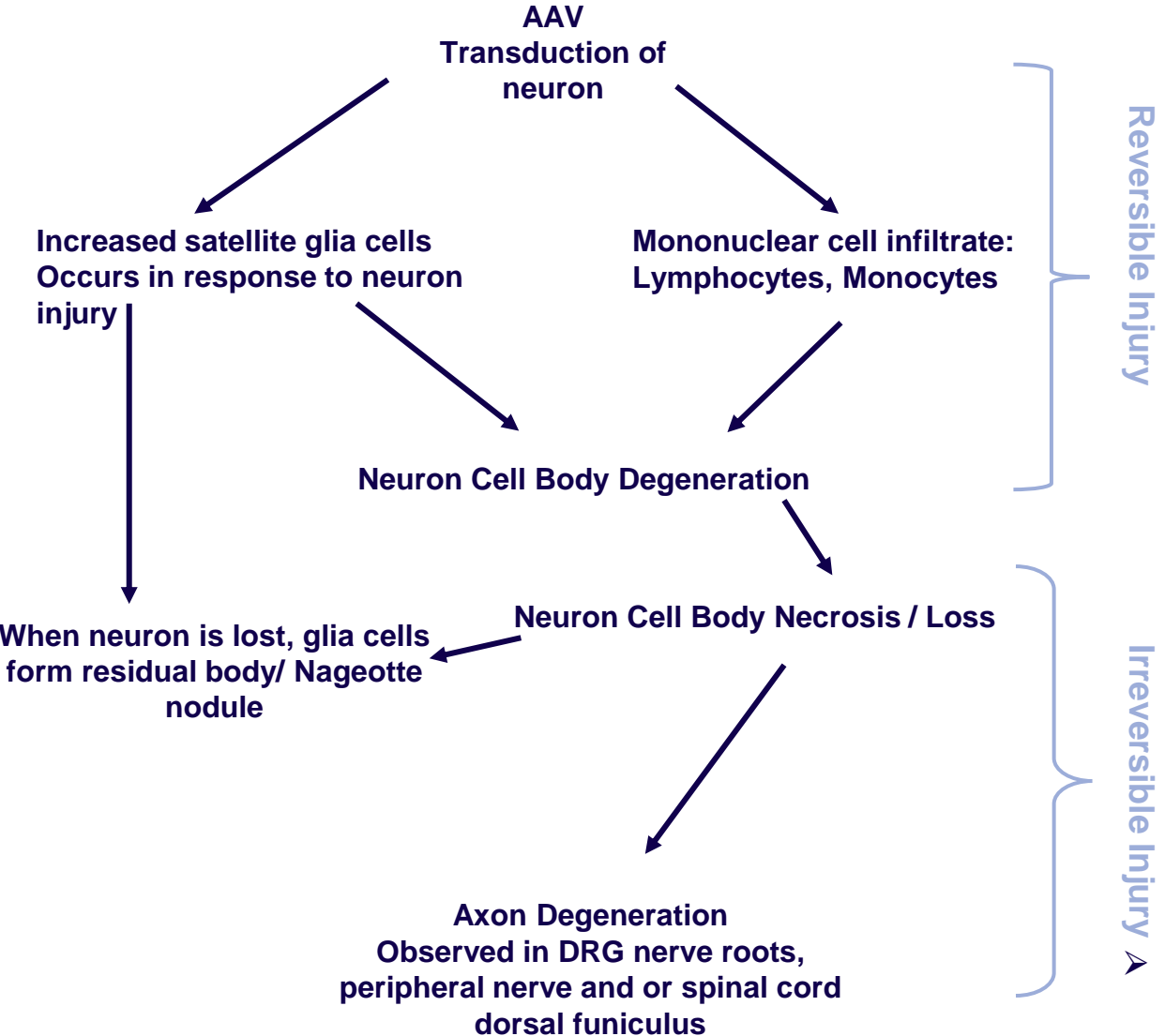
Hordeaux J, et al. *Sci Transl Med* 2020;12(569):eaba9188.

Hordeaux J, et al. *Hum Gene Ther* 2020;31(15–16):808–18.



# Sequence of Possible Events in Evolution of DRG Toxicity and Severity Grading

## Pathway to DRG Pathology



Grading Schema related to DRG  
Neuron Cell Body degeneration and / or necrosis

- Normal: No grade
- 1 Minimal < 10% of DRG is affected
- 2 Mild 10-25% of DRG is affected
- 3 Moderate 26-50% of DRG is affected
- 4 Marked 51-75% of DRG is affected
- 5 Severe > 75% of DRG is affected

- Cellular infiltrate /inflammation, glial cell reactivity are graded separately using a similar grading schema. Use of IHC markers to differentiate infiltrating cell types may be beneficial
- Nerve fiber degeneration / fragmentation in spinal cord and peripheral nerve are also graded using similar grading schema

# Effect of rAAV Route of Administration, Dose, Age and Study Duration on DRG Pathology

Aggregated data from 33 non-clinical studies in 256 NHPs for meta-analysis of severity of DRG pathology, to assess: ROA, dose, time course, study conduct, age, sex, capsid promoter, purification method, and transgene

- All \*ROAs except IM led to significant pathology in DRG and Spinal Cord vs vehicle controls. DRG pathology observed in:
  - 83% for CSF (ICM or IT; 170/205 animals), 32% for IV (8/25 animals), 100% for the combination of ICM + IV (4/4 animals,) and 0% for intramuscular (0/4 animals)
- Dose and age at injection significantly affected the severity, whereas sex had no impact
  - Intra-CSF maximal dose range ( $>1E+13$  GC) led to significantly worse pathology scores ( $p=0.04$  in DRG;  $p=0.001$  in SC) than both lower dose ranges ( $<3E+12$  GC and  $3E+12-1E+13$  GC), while IV doses showing pathology were as low as  $1E+13$  GC/kg
  - Juvenile animals had less severe DRG degeneration vs adults but similar SC axonopathy; four animals treated as infants showed no signs of DRG or SC pathology
    - Results should be interpreted with caution due to the small sample size
- Severity of lesions was consistently reduced at 6 months post AAV administration

Based on current literature DRG pathology is almost universal\* after AAV gene therapy in non-clinical NHP studies and is affected by route of administration, dose, and age at time of injection; sex has no impact on DRG pathology

\*Assuming transgene is expressed in DRG

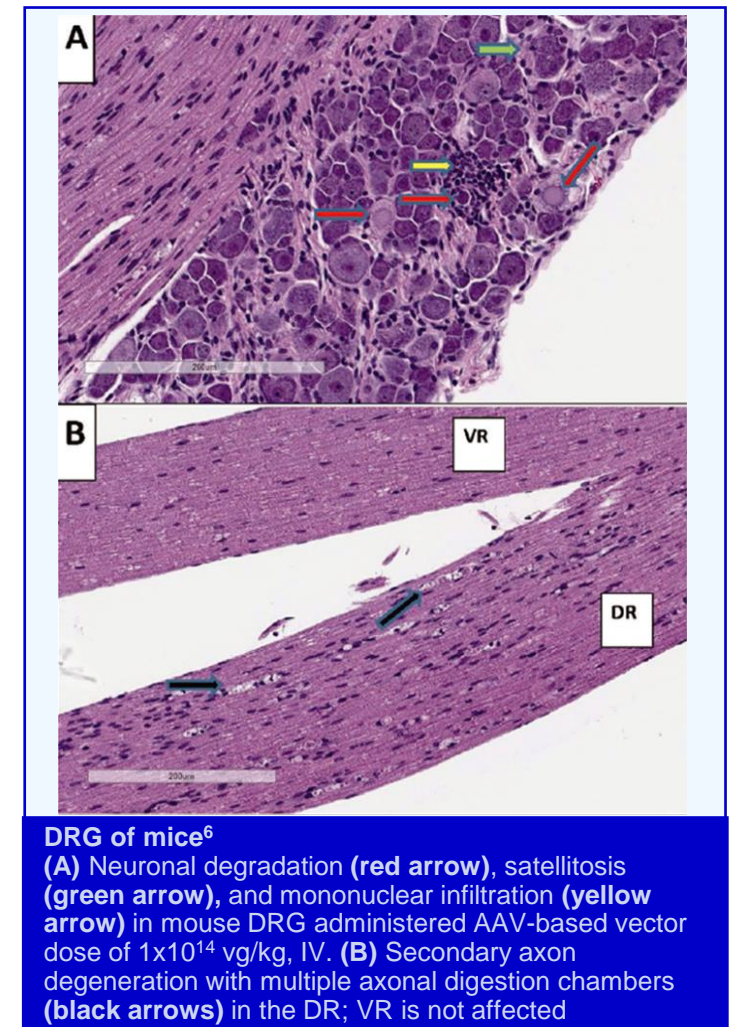
- CSF: Cerebrospinal fluid; DRG: Dorsal root ganglion / ganglia; GC: Genome copies; ICM: Intra-cisterna magna; IT: Intrathecal; IV: Intravascular; NHP: Non-human primate; ROA: Route of administration; SC: Spinal cord.
- \*Hordeaux J, et al. *Hum Gene Ther* 2020;31(15-16):808–18.

# DRG Toxicity in Different Animal Species\*

- Toxicity in the DRG have been reported in NHPs and piglets<sup>1–4</sup>
  - NOAEL in NHPs is below  $1 \times 10^{13}$  vg/kg (IV)<sup>5</sup>
- Although DRG toxicity has not generally been reported in mice or rats,<sup>3</sup> there is data suggest that rodents can exhibit DRG-related toxicity<sup>6</sup>
  - DRG toxicity matching that in monkeys has been observed in C57BL/6J mice<sup>7</sup>
  - Relatively high dose ( $1 \times 10^{14}$  vg/kg) of rAAV vector with DRG tropism and a promoter that is active in the central nervous system <sup>6</sup>
  - DRG toxicity similar to that seen in monkeys has been observed in Wistar rats (Pfizer publication accepted)
- Neurological phenotype (hind limb claspings) correlated with dysfunction of the proprioceptive neurons in C57BL/6J mice and SMA mice treated with AAV9-GUSB-SMN<sup>7</sup>
  - Reduced amplitude of H-reflex (dysfunction of proprioceptive synapses)
  - Dose-dependent loss of proprioceptive neurons (L5 DRG)

1. Hinderer C, et al. *Hum Gene Ther* 2018; 29(3):285–98.
2. Hordeaux J, et al. *Mol Ther Methods Clin Dev* 2018;10:68–78.
3. Hordeaux J, et al. *Mol Ther Methods Clin Dev* 2018;10:79–88.
4. Hordeaux J, et al. *Hum Gene Ther* 2019;30(8):957–66.
5. Hordeaux J, et al. *Hum Gene Ther* 2020;31(15-16):808–18.
6. Bolt M, et al. *J Toxicol Sci* 2021;46(2):57–68
7. Van Alstyne M, et al. *Nat Neurosci* 2021; doi: 10.1038/s41593-021-00827-3 (Epub ahead of print).

## AAV Induced Effects in Mouse DRG



\*Relative species sensitivity and relevance to man is not well understood.



# DRG toxicity in human clinical trials

# DRG Toxicity In Familial ALS Patient

## Intrathecal AAVrh10 miRNA-SOD1

Likely a different mechanism of toxicity (immune response) compared to DRG toxicity in Monkey

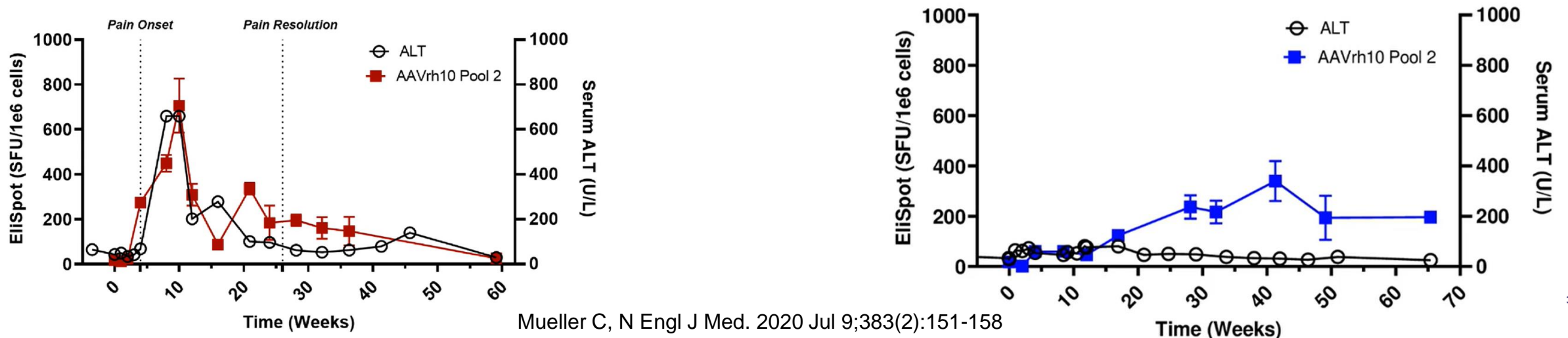
**Patient 1:** Prophylactic prednisolone/ prednisone treatment.

- Positive capsid ELISPOT,
- ALT increase
- Peripheral pain
- CSF pleocytosis
- MRI: contrast enhancement in the cauda equina and some dorsal root ganglia consistent with inflammation
- Equivocal transient benefit in leg strength

**Patient 2:** Aggressive immune suppression (rituximab, prednisolone/ prednisone, sirolimus)

- Decrease in capsid ELISPOT and anti-AAV antibodies
- No ALT increase
- No peripheral pain
- No CSF pleocytosis
- No MRI abnormalities in cauda equina or DRG
- No clinical benefit

### ALT and Capsid ELISPOT



# DRG toxicity in NHPs with IT Onasemnogene Apeparvovec (Zolgensma®) Results in Clinical Hold: Translatability and Significance for Humans Remains Unknown

DRG toxicity was observed with IT but not IV administration of Zolgensma® to NHPs<sup>1</sup>

The clinical relevance of the DRG findings in NHP studies associated with IT administration of AAV vector gene therapies remains unknown<sup>2</sup>

In two patients who received a single IV infusion of Zolgensma® (1.1x10<sup>14</sup> vg/kg)<sup>1</sup>

- DRGs from one patient appeared unremarkable
- DRG abnormalities with ganglion cell loss, excess small round cells, and some inflammatory cells were reported in the other patient
  - It is not known whether the observed DRG toxicity was due to SMA disease phenotype, secondary to hypoxic/ischemic injury in the terminal illness of these patients, or secondary to treatment with Zolgensma®

The FDA has placed a partial clinical hold on IT administration of Zolgensma® (AVXS-101 IT) in a clinical trial for subjects with SMA Type 2, until further investigation is complete<sup>3</sup>

- **The FDA lifted clinical hold in 2021 based on additional animal data**
  - *2020 Novartis statement: “FDA is open to either a six-month or a one-month data readout in our NHP study. We have taken the decision to go to the one-year readout of the NHP study just to ensure that we have a very robust data package so that when we move to a hopeful filing in next year, we’ll have the best possible data to support our filing”<sup>4</sup>*

1. European Medicines Agency. Zolgenmsa® Assessment report: [www.ema.europa.eu/en/documents/product-information/zolgenmsa-epar-product-information\\_en.pdf](http://www.ema.europa.eu/en/documents/product-information/zolgenmsa-epar-product-information_en.pdf)

2. Hinderer C, et al. *Hum Gene Ther* 2018;29(3):285–98.

3. Novartis Press Release. September 23, 2020: [www.novartis.com/news/media-releases/novartis-provides-update-avxs-101-intrathecal-clinical-development-program](http://www.novartis.com/news/media-releases/novartis-provides-update-avxs-101-intrathecal-clinical-development-program) (Accessed July 2021).

4. Novartis AG’s (NVS) CEO Vas Narasimhan on Q2 2020 results – earning call transcript: <https://seekingalpha.com/article/4359749-novartis-ags-nvs-ceo-vas-narasimhan-on-q2-2020-results-earnings-call-transcript> (2021).



# Translational biomarkers

# DRG toxicity: Translational Biomarkers and Clinical Monitoring Strategies to Consider

- Patient communication of symptoms
- Age-appropriate neurological exam in patients
- MRI (for altered fluid composition)
- Nerve conduction velocity (NCV) testing
  - May not assess small poorly myelinated nerve fibers as well as large well myelinated fibers
- Intradermal Nerve Fiber Counts
  - Can be done in animals and humans but has high variability and hard to interpret
- Emerging data on soluble biomarkers such as neurofilaments

# Risk assessment and progressing to clinical trials

# ASGCT Positions

- DRG lesions are recognized as part of the spectrum of AAV-related toxicities observed in monkeys. Nonclinical findings indicate that transgene expression is important in the induction of neuronal toxicity, but it is unclear what level of expression is required to induce toxicity. To date nonclinical findings indicate that maximum severity occurs at early time points with subsequent resolution of active neuron injury.
- A 13-week study in monkey with interim necropsy at 4-6 weeks post dose to assess maximum severity is adequate to enable DRG risk assessment and clinical dose selection.
- **The absence of a NOAEL for DRG toxicity should not preclude clinical development in the context of appropriate risk / benefit considerations and incidence / severity of DRG findings.**  
*Clinical data is needed to understand the relevance of nonclinical findings*
- Nonclinical risk assessment for AAV DRG pathology when intended to treat disease indications that are considered severely debilitating or life-threatening or have high unmet medical need, may be based on the concepts in ICH S9 *Nonclinical Evaluation for Anticancer Pharmaceuticals*.

***S9 Nonclinical Evaluation for Anticancer Pharmaceuticals Questions and Answers Guidance for Industry***  
**Q21. Is use of the highest non-severely toxic dose ((HNSTD\*), Note 2) to select an appropriate starting dose applicable to biopharmaceuticals? (3.2)**

The HNSTD may be appropriate in determining a starting dose of a biopharmaceutical (e.g., when a drug is not an immune agonist) taking into consideration differences in binding affinity between animals and humans and pharmacological properties of the biopharmaceutical (including ADCs).

*\*HNSTD= highest non-severely toxic dose (nonclinical studies for oncology compounds are not assigned adversity, but rather the dose levels are deemed Severely Toxic or Not Severely Toxic to allow determination of the HNSTD)*

<https://www.fda.gov/media/73161/download>

<https://www.fda.gov/media/100344/download>



# Acknowledgements: DRG Toxicity work group members

- S. Kaye Spratt, PhD, BridgeBio Gene Therapy (*work group chair*)
- Juliette Hordeaux, DVM, PhD, DECVP University of Pennsylvania
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- Timothy MacLachlan, PhD, DABT Novartis
- Kathleen Meyer, PhD, DABT Sangamo Therapeutics
- Adora Ndu, PharmD, JD, BioMarin
- Julie Rider, PhD, BioMarin
- David Scott, PhD, BridgeBio Gene Therapy
- Angela Whatley, PhD, BridgeBio Gene Therapy
- Megan Zoschg Canniere, PharmD, Spark Therapeutics

Back Up

# Is the Observed DRG Toxicity Capsid mediated?

AAV9 null:  
Lacks transcriptional activity  
due to mutated start codon  
and lack of promoter

## Limited animal studies have investigated the cause of DRG toxicity to date

RegenerexBio shared data from an animal model at a recent meeting to suggest that DRG toxicity is not capsid mediated

- The study compared an AAV9 vector with a null AAV9 vector
- DRG pathology, spinal nerve roots and SC degeneration were assessed
  - Neuronal degeneration was observed in 1/4 control animals, 3/4 AAV9-treated animals, and 0/4 null AAV animals
  - Increased cellularity and degeneration of spinal nerve roots was observed in 4/4 vector-treated animals and 0/4 of the null AAV9-treated animals
  - Degeneration of the dorsal tracts in the spinal cord was seen in 3/4 of the vector-treated animals and 0/4 of the null vector-treated animals
- DRG toxicity was not present following administration of the null AAV9 vector

Further investigation is required to determine the cause of DRG toxicity following rAAV gene therapy  
This is not considered needed for current human risk assessment

Talk by RegenerxBio at Biosafe Meeting 2020.

# Mechanisms for AAV-induced Sensory Neuropathy Remain Undefined

**Over-expression of the transgene product in highly transduced DRG leads to neuronal injury, and degeneration of the cell body and associated axons in NHPs<sup>1</sup>**

- DRG are highly accessible to AAV regardless of route of administration
  - The capillaries are highly fenestrated so there is no blood–ganglion barrier
  - The axons of DRG neurons are directly exposed to cerebrospinal fluid in the dorsal roots
  - AAV transduction through peripheral axon targeting followed by retrograde trafficking to the cell body
- Different hypotheses for the potential cellular mechanisms of AAV-mediated injury

**Primary DRG neuronal degeneration may lead to a secondary T-cell-mediated immune response and contribute to DRG toxicity in NHPs<sup>2</sup>**

- An initial low-grade neuronal injury may induce the secretion of cytokines by satellite glial cells and neurons<sup>3,4</sup>
- Along with the expression of a foreign transgene protein product, the secretion of cytokines may be capable of triggering an adaptive immune response that would worsen the initial overexpression-related injury

**DRG pathology is due to primary immune-mediated toxicity  
T-cell response to AAV capsid protein or transgene protein products in NHPs<sup>5</sup>**

**OR**

- Mycophenolate mofetil and rapamycin did not prevent DRG toxicity in toxicity studies, nor did steroids<sup>2,5,6</sup>
- The time course of delayed but non-progressive DRG degeneration did not support the notion that adaptive immunity played a role<sup>5</sup>

1. Hordeaux J, et al. *Hum Gene Ther* 2020;31(15–16):808–18.
2. Hordeaux J, et al. *Mol Ther Methods Clin Dev* 2018;10:68–78.
3. Austin P, Moalem-Taylor G. *J Neuroimmunol* 2010;229:26–50.
4. Moalem G, Tracey D. *Brain Res Rev* 2006;51:240–64.
5. Hordeaux J, et al. *Sci Transl Med* 2020;12(569):eaba9188.
6. Hordeaux J, et al. *Mol Ther Methods Clin Dev* 2018;10:79–88.

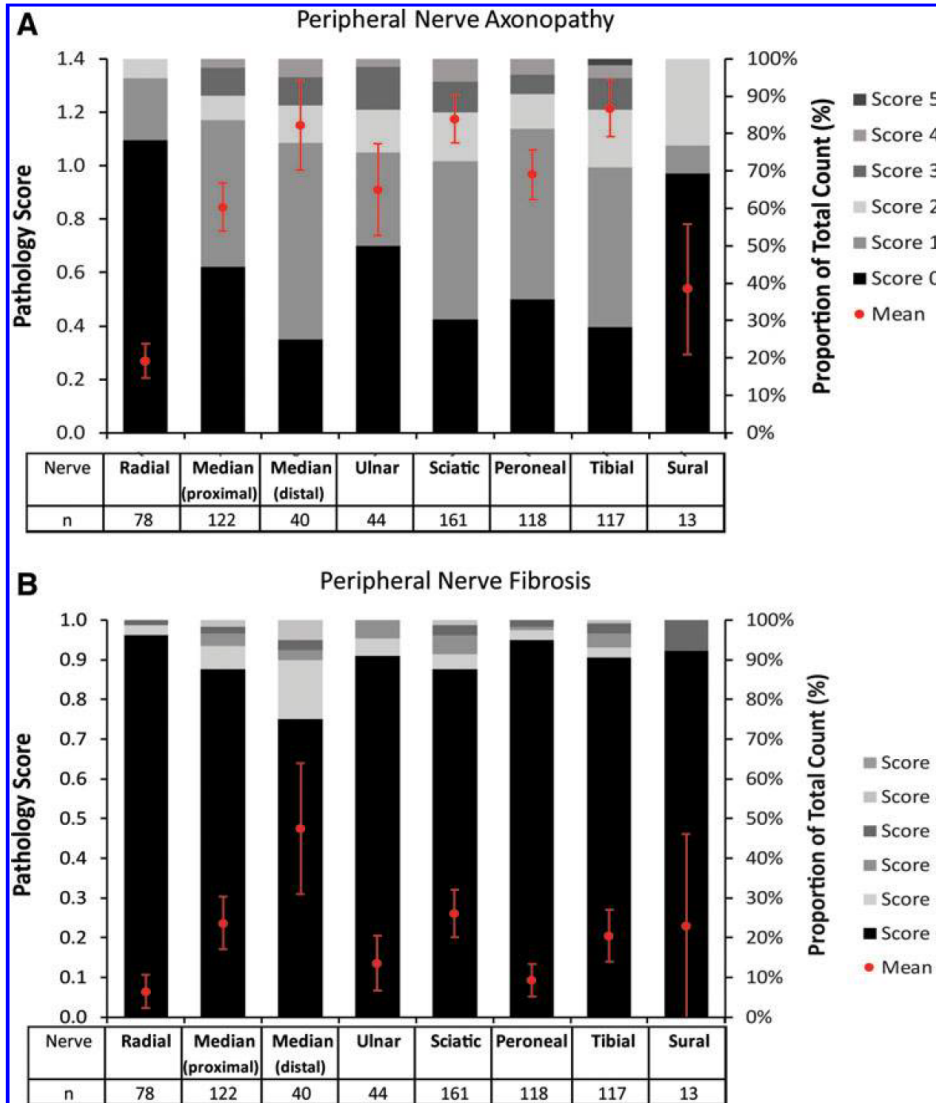


# Adeno-Associated Virus-Induced Dorsal Root Ganglion Pathology in NHP

## DRG vs Peripheral Nerve and Spinal Cord Findings

Hordeaux et al, Human Gene Therapy 2020

### Peripheral Nerve Pathology Score



### DRG and Spinal Cord Pathology Score

