ASGCT and FDA Liaison Meeting

1-4 PM ET
November 8, 2021

Welcome and Introductions

rAAV Gene Therapy and Peripheral Nervous System Ganglia Toxicity

ASGCT-FDA Liaison Meeting
November 8, 2021

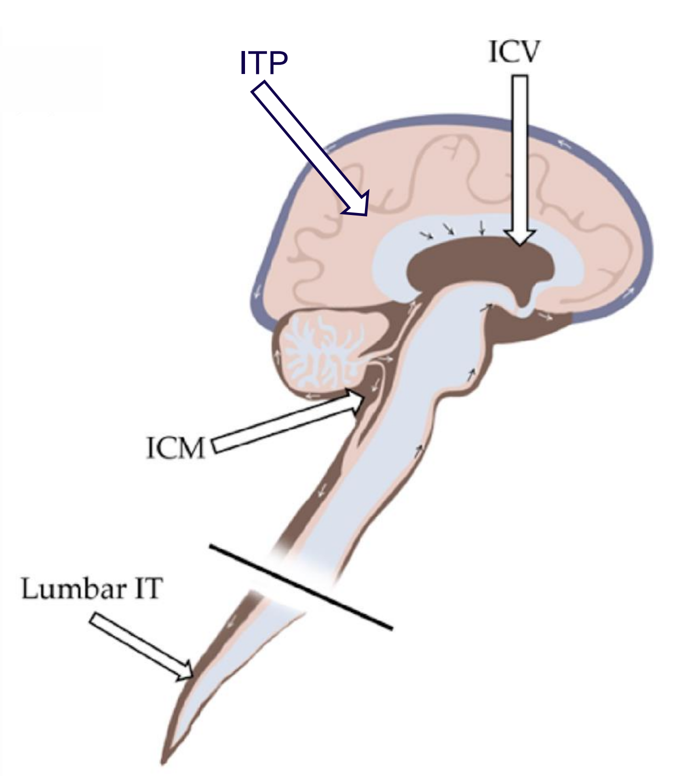
Laurence Whiteley, DVM, PhD, DACVP, Pfizer Inc.
Juliette Hordeaux, DVM, PhD, DECVP, University of Pennsylvania

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:



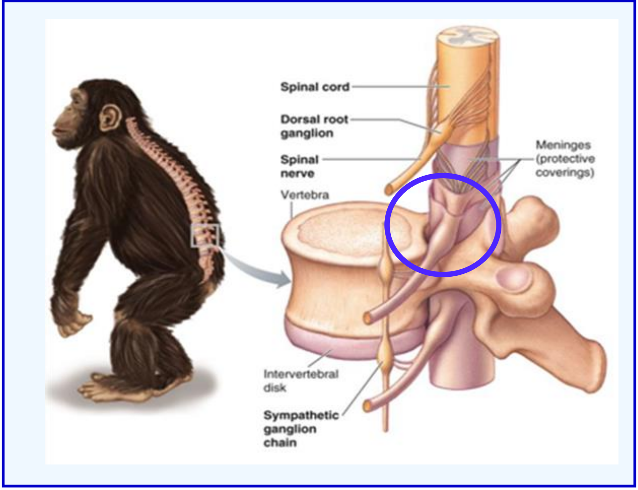
Route	Pros	Cons
*Intracerebroventricular (ICV)	• Established neurosurgery protocols • Broad central nervous system distribution	• Invasive surgery • Needle tract crosses parenchyma, risking injury and may enhanced immune response
*Intra-cisterna magna (ICM)	• Good biodistribution to hindbrain structures • Safer than ICV as does not cross parenchyma structures	• Risk of medullary injury • Not a routine clinical procedure
*Intrathecal (IT)	• Non-invasive outpatient procedure – lumbar puncture[2]	• Fluid dynamics of bolus injection and distribution to brian poorly understood • Limited understanding of dose translation and brain exposure
Intraparenchymal (ITP)	• Targets specific locations in CNS • Eliminate / reduces exposure to DRG	• High Complexity

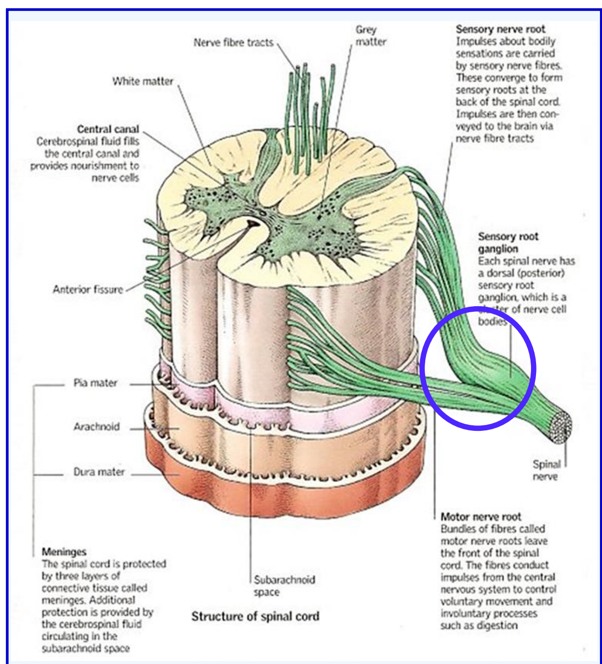
* AAV Delivery routes associated with DRG pathology

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

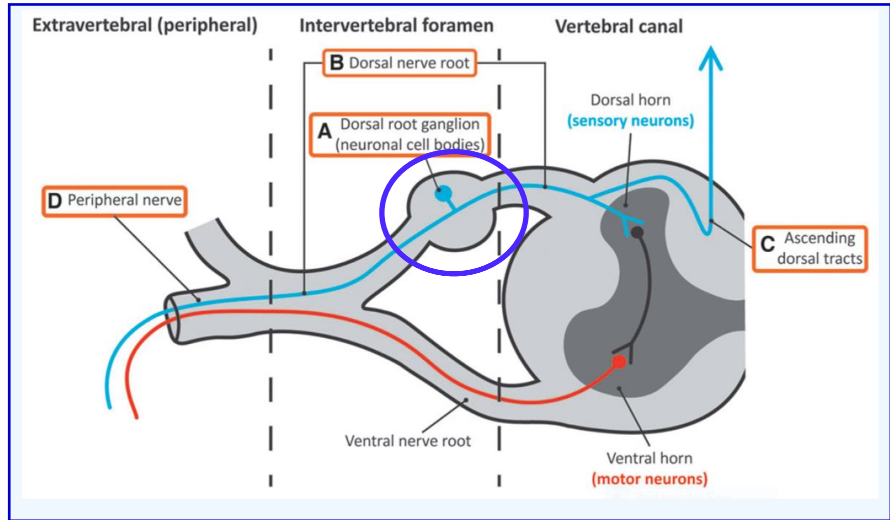
1. Perez BA, et al. *Brain Sci* 2020;10(2):119.
2. Hinderer C, et al. *Mol Ther Methods Clin Dev* 2020;17:969–74.

Anatomy of the Dorsal Root Ganglion (DRG)

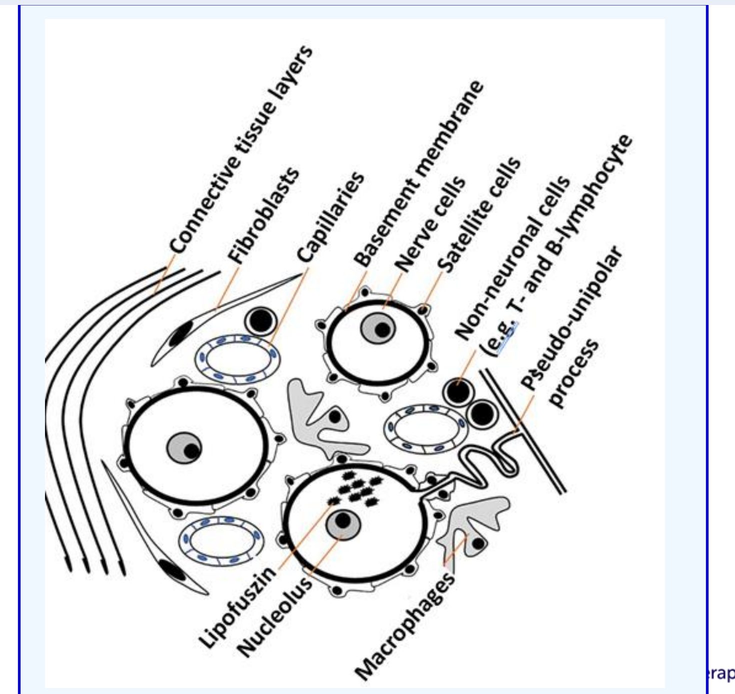




DRG Connections to Nerve Pathways



Multiple Cell Types Make Up the DRG

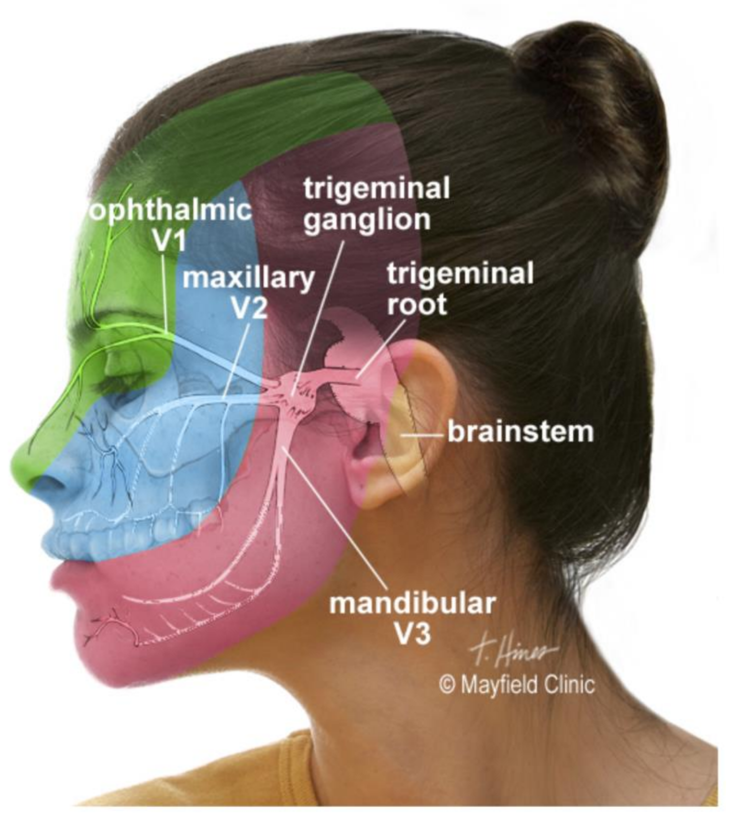


Krames ES. *Pain Medicine* 2014;15:1669–85. 2. David Darling Encyclopedia.

Spinal cord: www.daviddarling.info/encyclopedia/S/spinal_cord.html
Haberberger RV, et al. Front Cell Neurosci 2019;13:271.
Hordeaux J, et al. Hum Gene Ther 2020;31(15-16):808–18. 2. Abraira VE, Ginty DD. Neuron 2013;79(4):618–39

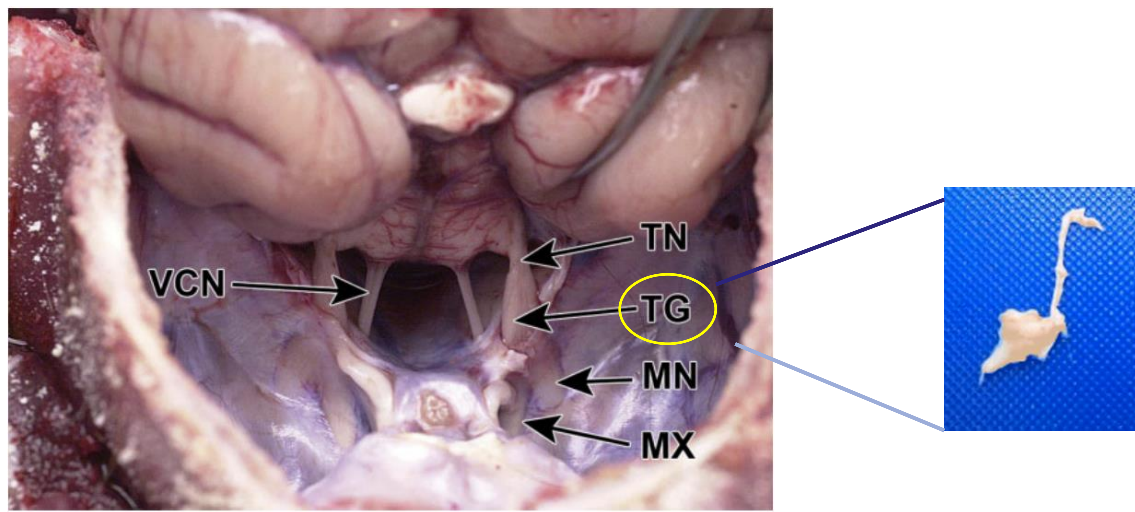
Other Ganglia to Consider: Trigeminal Ganglia

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

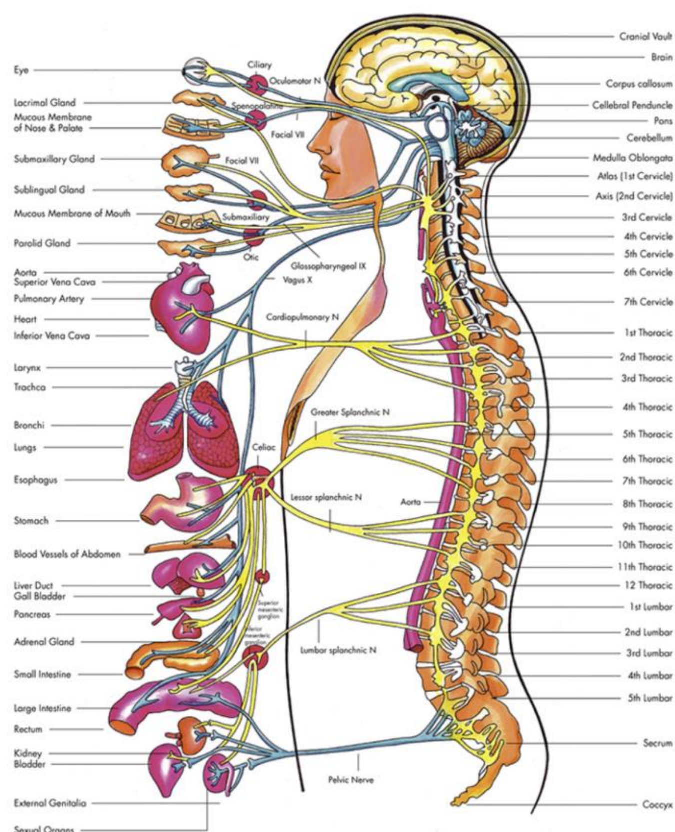


Toxicologic Pathology 2020, Vol. 48(1) 30-36

Other Ganglia to Consider: Autonomic Nervous System

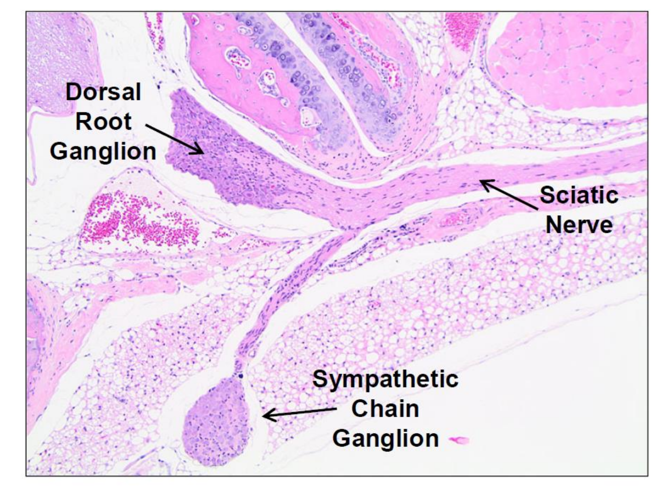
Paravertebral Autonomic Trunk and Visceral Organ Ganglia

Autonomic Nervous System



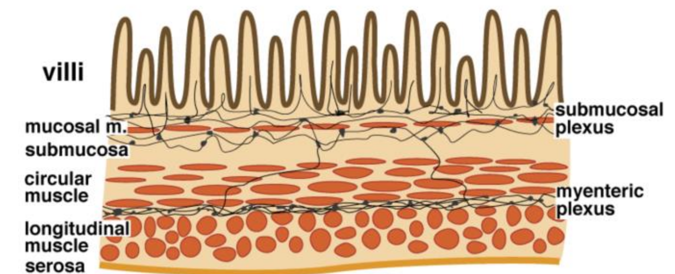
https://www.dyansys.com/products-applications/product-technology/ans-monitor-technology

DRG and Paravertebral Autonomic Ganglia are Closely Associated



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

Autonomic Ganglia in the Intestine

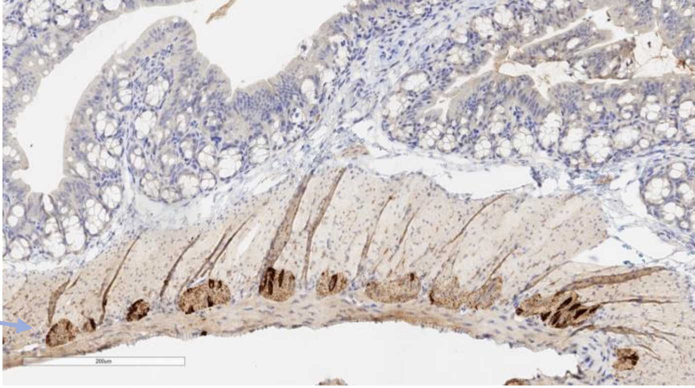


Transverse Section of Intestinal Wall

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).

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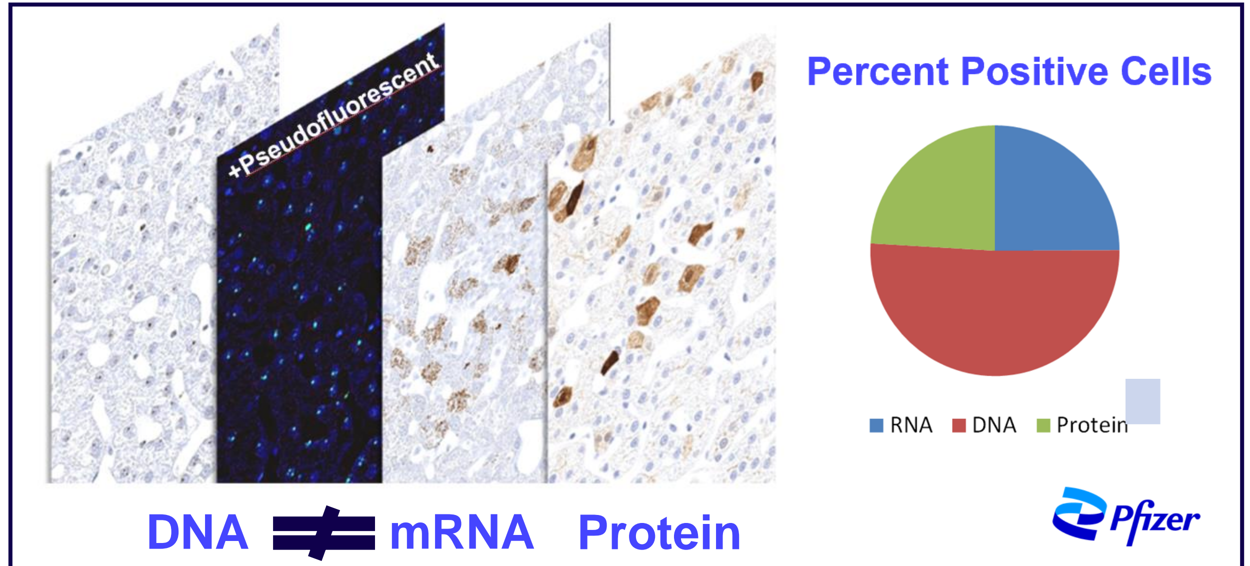
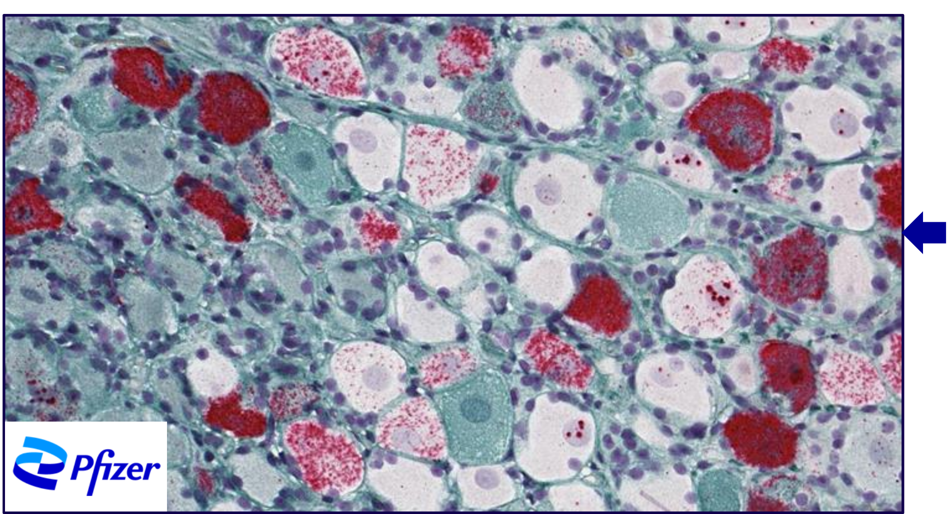
AAV Transduction is Complex

NHP Liver : 30 days post AAV treatment

ISH for DNA and mRNA **IHC for Protein**



DNA ≠ mRNA Protein

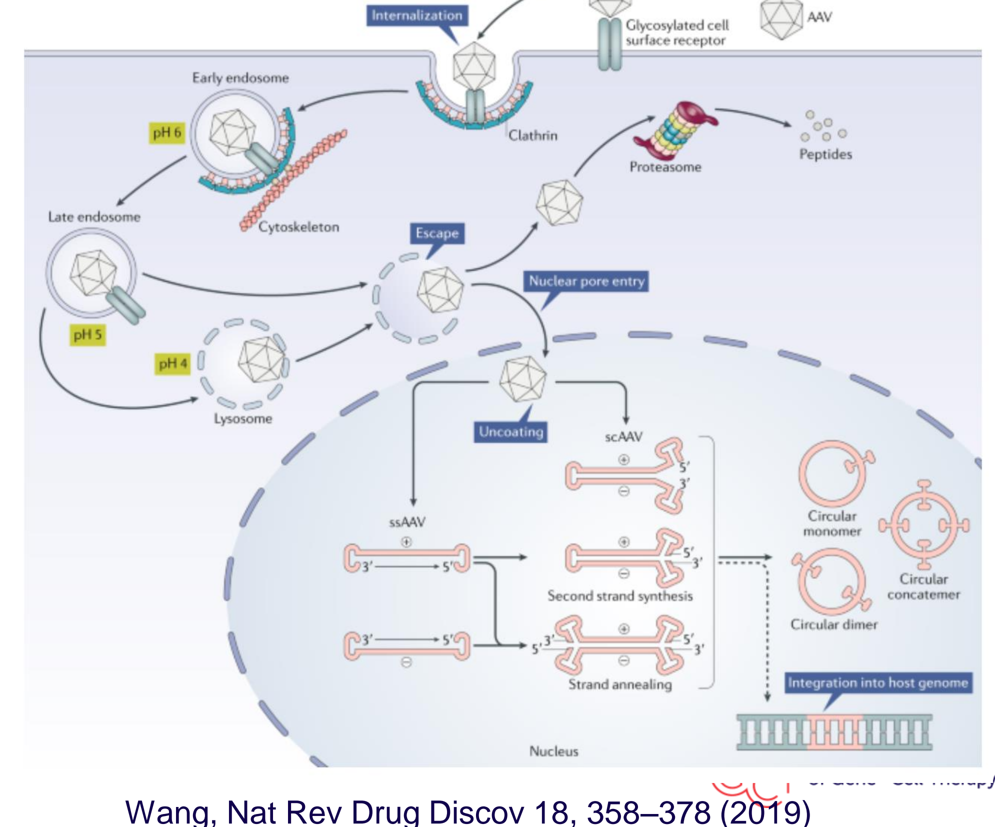


NHP DRG 48 days post AAV treatment

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



Wang, Nat Rev Drug Discov 18, 358–378 (2019)

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

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

SACRAL	S3	RNA
	S4	Histology

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

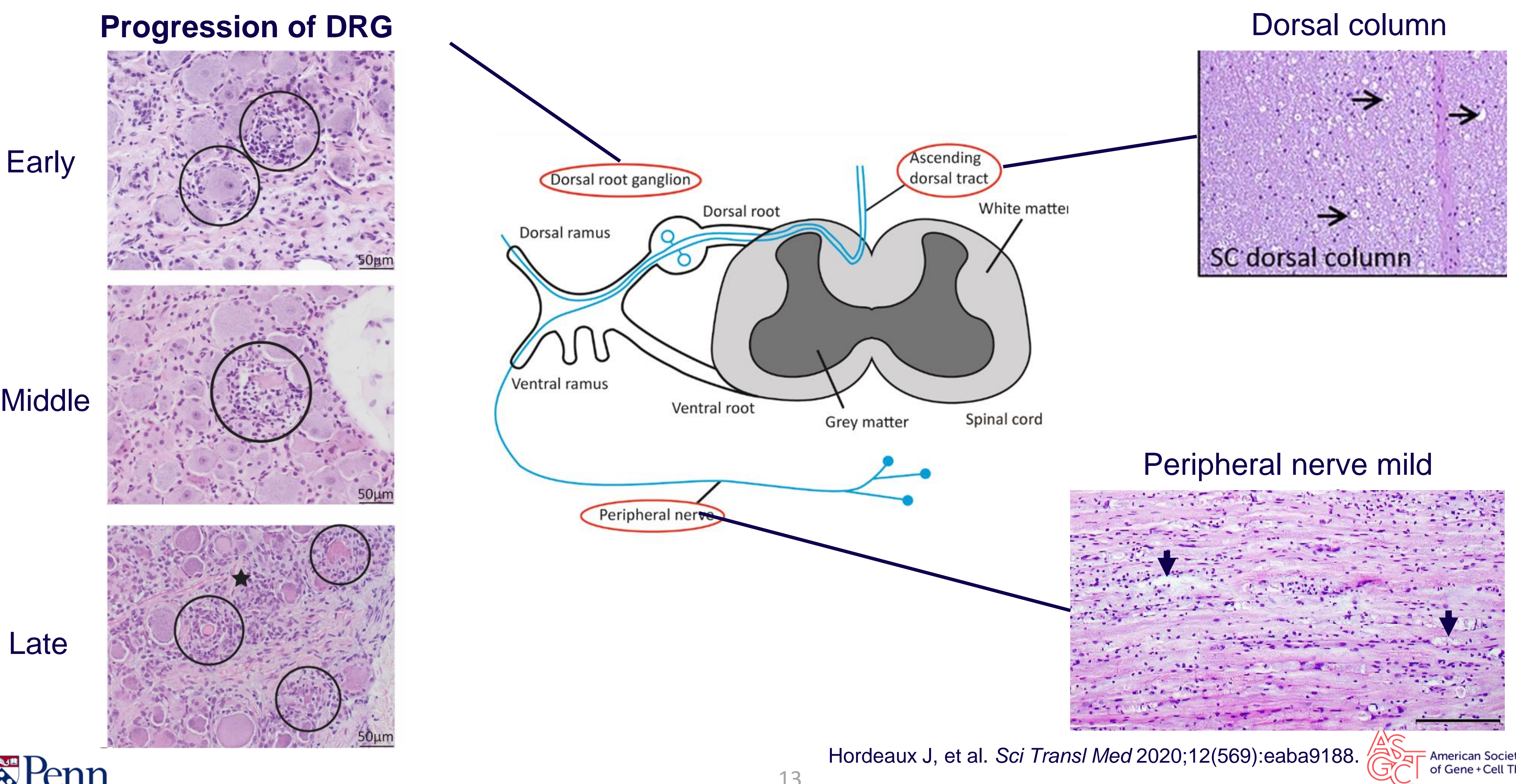
Autonomic Ganglia

Ensure histologic evaluation of visceral ganglia in GI track

Collection of paravertebral ganglion may be considered

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

AAV-Mediated DRG Toxicity and Secondary Axonopathy



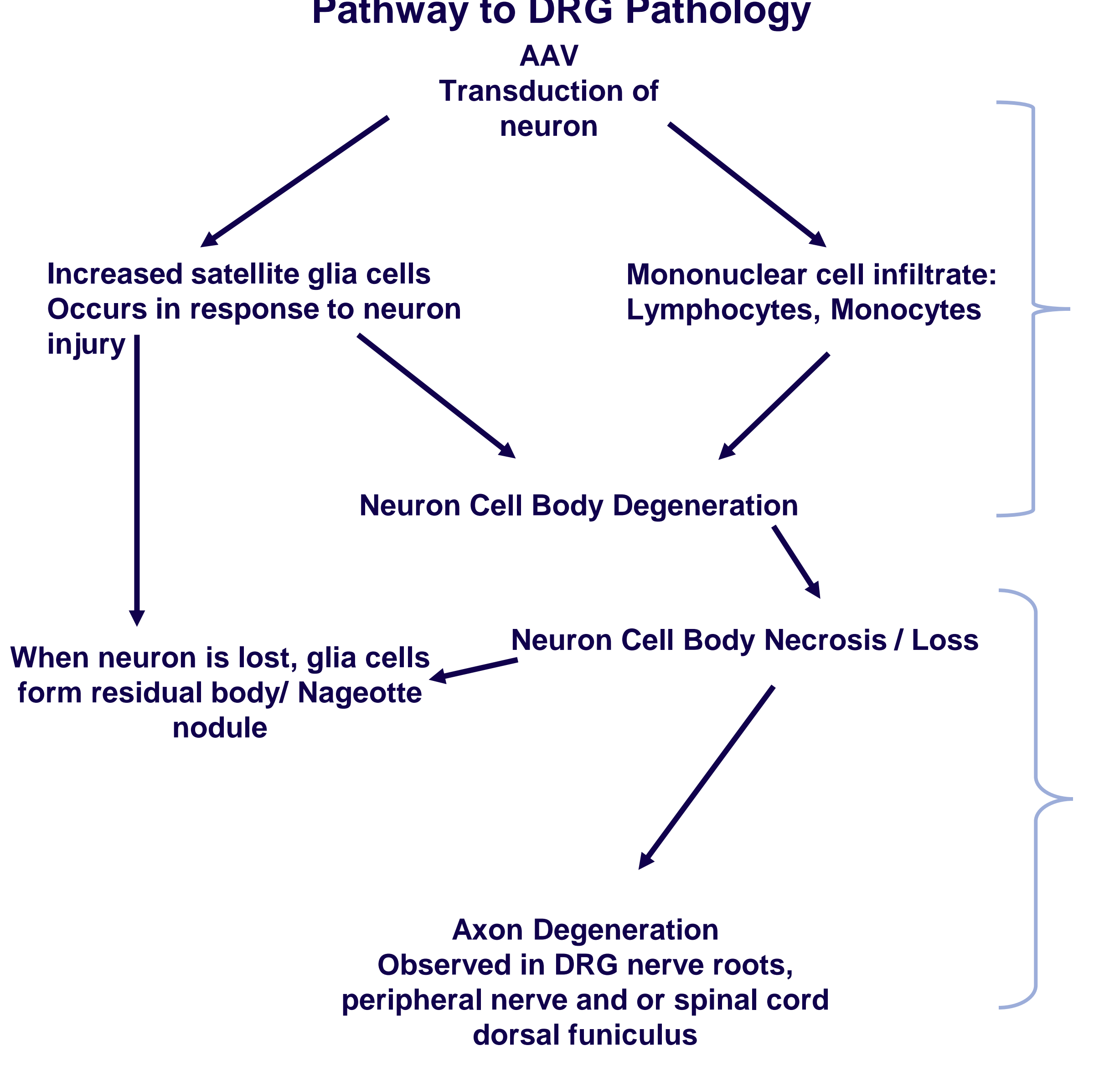
Hordeaux J, et al. *Sci Transl Med* 2020;12(569):eaba9188.

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

Penn University of Pennsylvania

ASGCT American Society of Gene + Cell Therapy

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



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

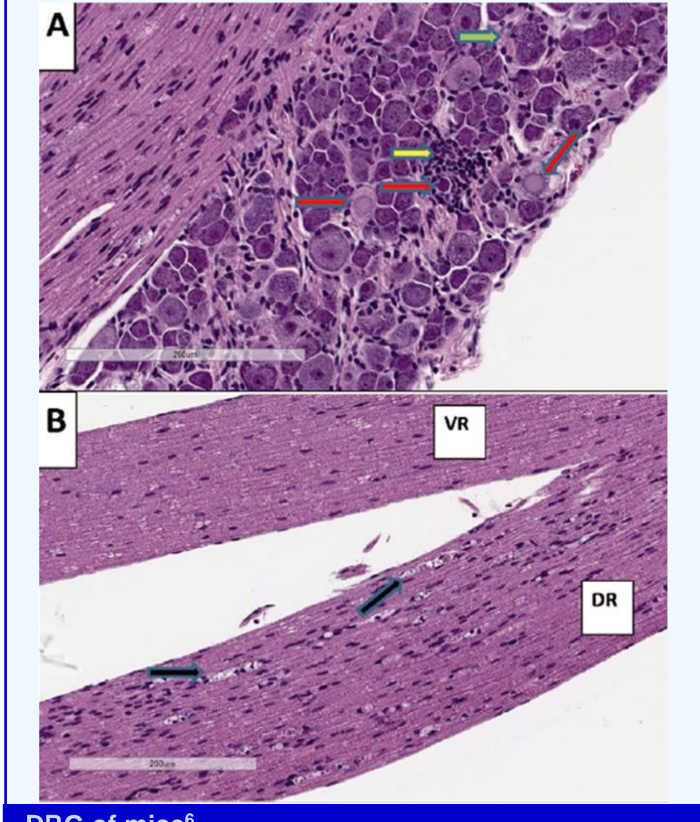
*Assuming transgene is expressed in DRG

- 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[1–4]
  - NOAEL in NHPs is below $1x10^{13}$ vg/kg (IV)[5]
- Although DRG toxicity has not generally been reported in mice or rats,[3] there is data suggest that rodents can exhibit DRG-related toxicity[6]
  - DRG toxicity matching that in monkeys has been observed in C57BL/6J mice[7]
  - Relatively high dose ($1x10^{14}$ vg/kg) of rAAV vector with DRG tropism and a promoter that is active in the central nervous system [6]
  - DRG toxicity similar to that seen in monkeys has been observed in Wistar rats (Pfizer publication accepted)
- Neurological phenotype (hind limb clasping) correlated with dysfunction of the proprioceptive neurons in C57BL/6J mice and SMA mice treated with AAV9-GUSB-SMN[7]
  - Reduced amplitude of H-reflex (dysfunction of proprioceptive synapses)
  - Dose-dependent loss of proprioceptive neurons (L5 DRG)

AAV Induced Effects in Mouse DRG



DRG of mice[6]
(A) Neuronal degradation **(red arrow)**, satellitosis **(green arrow),** and mononuclear infiltration **(yellow arrow)** in mouse DRG administered AAV-based vector dose of $1x10^{14}$ vg/kg, IV. **(B)** Secondary axon degeneration with multiple axonal digestion chambers **(black arrows)** in the DR; VR is not affected

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.
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).

*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

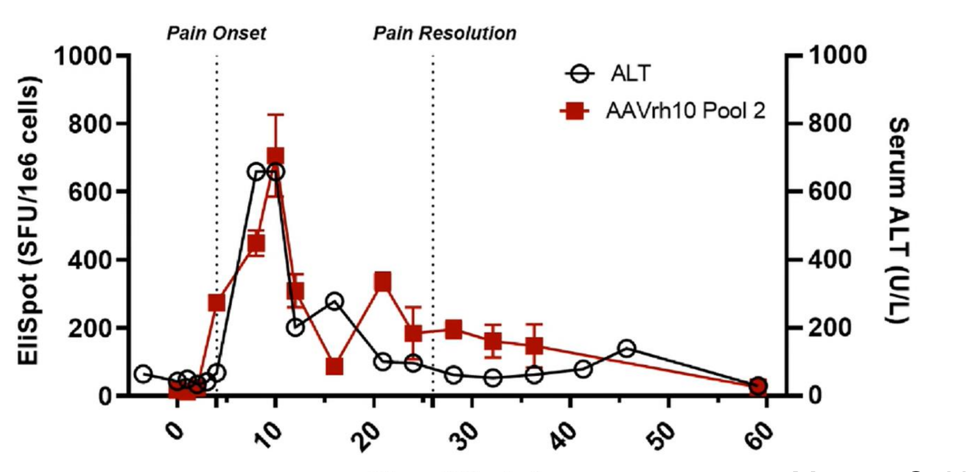
Patient 1: Prophylactic prednisolone/ prednisone treatment.

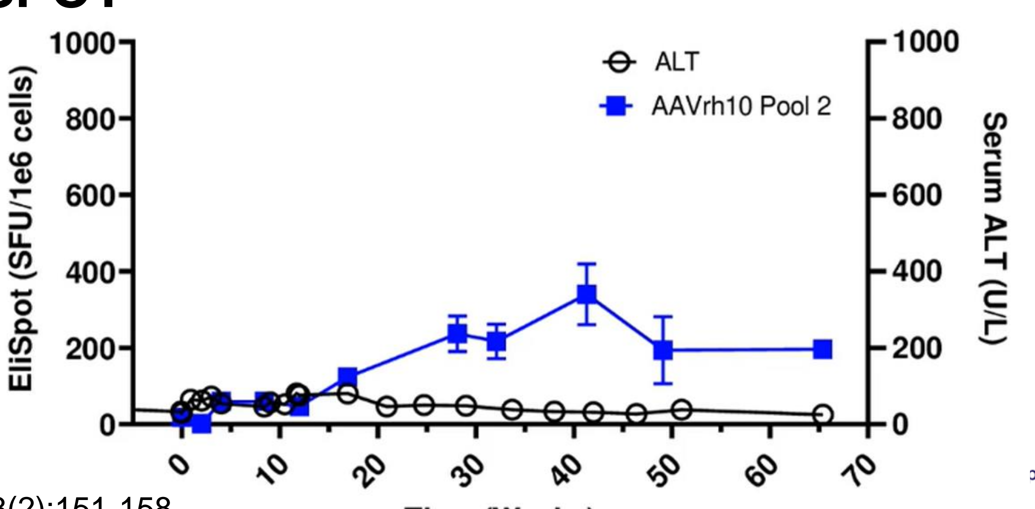
- Positive capsid ELISPOT,
- ALT increase
- Peripheral pain
- CSF pleocytosis
- MRI: contrast enhancement in the cauda equina and some dorsal root ganglia consistent with inflammation
- Equivocal transient benefit in leg strength

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





Mueller C, N Engl J Med. 2020 Jul 9;383(2):151-158

DRG toxicity in NHPs with IT Onasemnogene Abeparvovec (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[1]

The clinical relevance of the DRG findings in NHP studies associated with IT administration of AAV vector gene therapies remains unknown[2]

In two patients who received a single IV infusion of Zolgensma® (1.1×10^{14} vg/kg)[1]

- 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[3]

- **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"*[4]

1. European Medicines Agency. Zolgenmsa® Assessment report: www.ema.europa.eu/en/documents/product-information/zolgensma-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 (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

American Society of Gene + Cell Therapy

Acknowledgements: DRG Toxicity work group members

- S. Kaye Spratt, PhD, BridgeBio Gene Therapy *(work group chair)*
- Juliette Hordeaux, DVM, PhD, DECVP University of Pennsylvania
- Laurence Whiteley, DVM, PhD, DACVP Pfizer
- Nicholas Buss, PhD, REGENXBIO
- 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 assessmnet

Talk by RegenxBio 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[1]

- 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[2]

- An initial low-grade neuronal injury may induce the secretion of cytokines by satellite glial cells and neurons[3,4]
- 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

OR

DRG pathology is due to primary immune-mediated toxicity
T-cell response to AAV capsid protein or transgene protein products in NHPs[5]

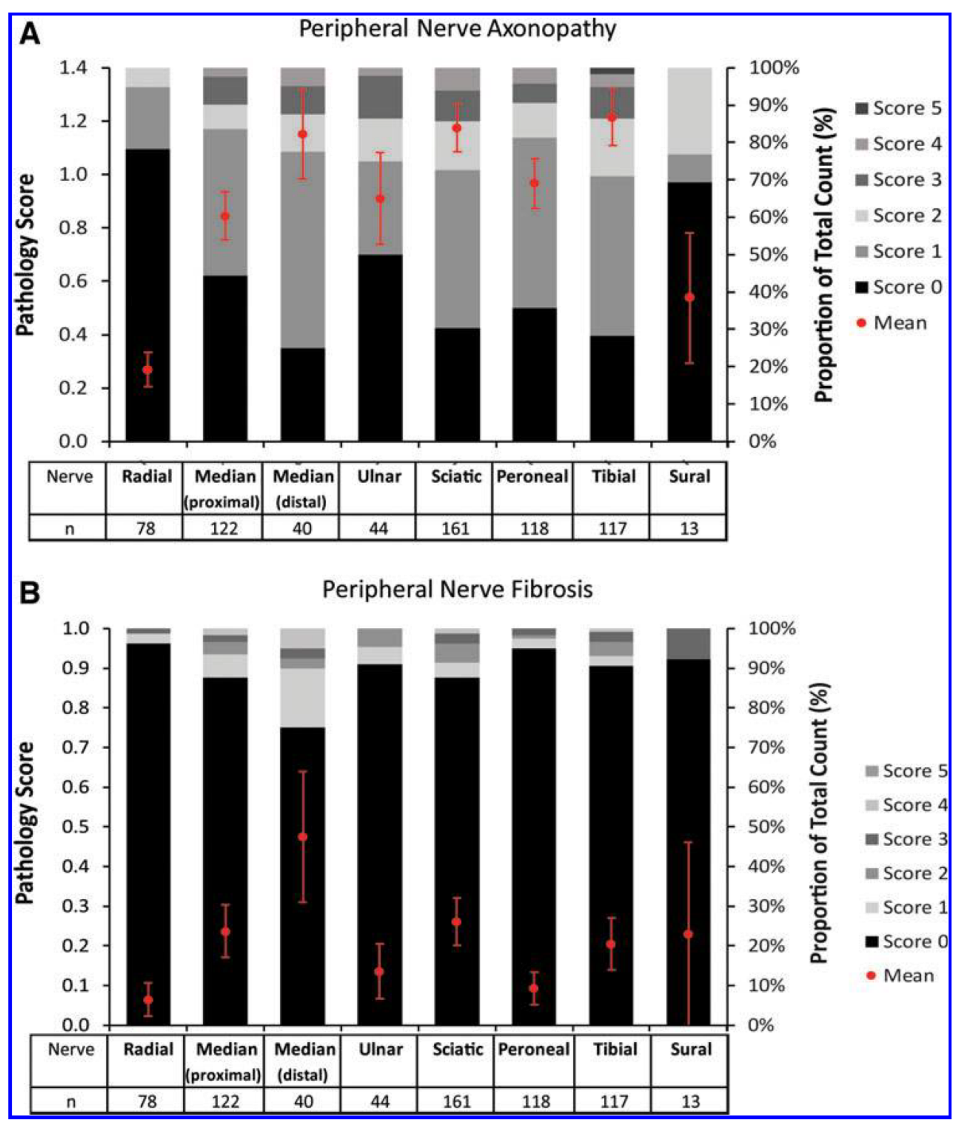
- Mycofenolate mofetil and rapamycin did not prevent DRG toxicity in toxicity studies, nor did steroids[2,5,6]
- The time course of delayed but non-progressive DRG degeneration did not support the notion that adaptive immunity played a role[5]

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.

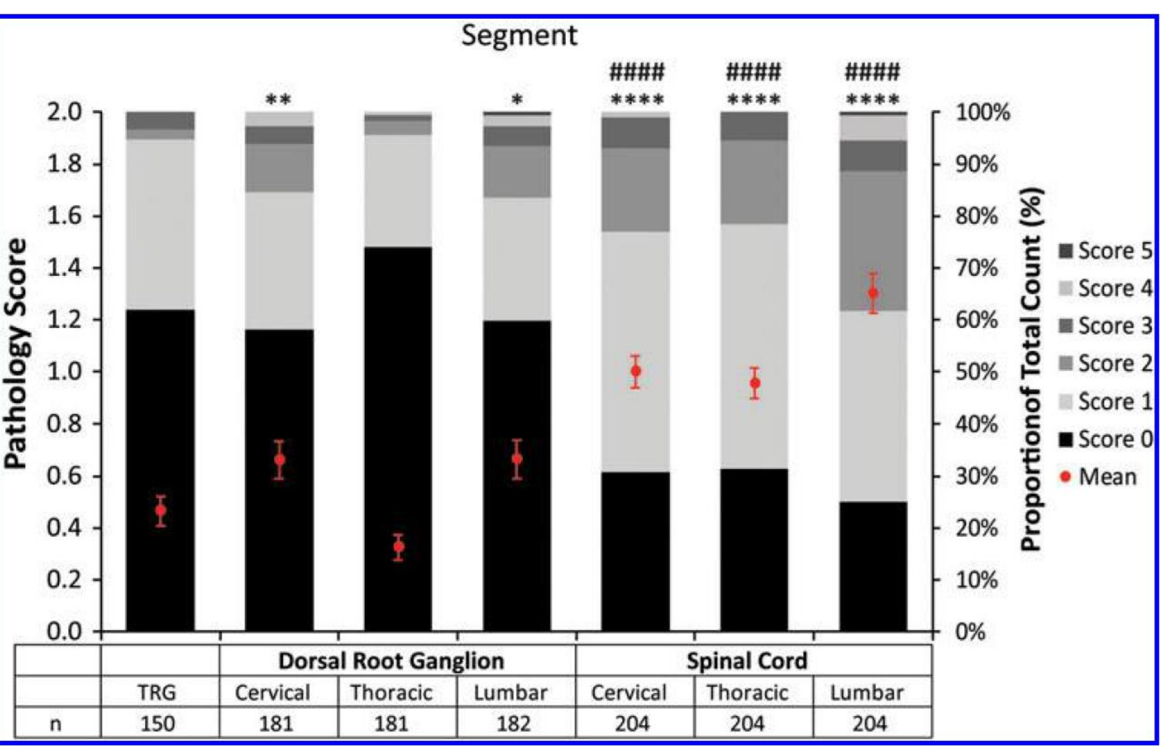
Adeno-Associated Virus-Induced Dorsal Root Ganglion Pathology in NHP
DRG vs Peripheral Nerve and Spinal Cord Findings

Hordeaux et al, Human Gene Therapy 2020

Peripheral Nerve Pathology Score



DRG and Spinal Cord Pathology Score



Discussion
1:35 – 1:50 pm ET

Integration/Insertion Considerations for AAV-based Gene Therapy Vectors

ASGCT Recommendations based on the Society's AAV Integration Roundtable event

ASGCT-FDA Liaison Meeting
November 8, 2021

Kevin Eggan, PhD, BioMarin Pharmaceutical
Markus Grompe, MD, Oregon Health & Science University

ASGCT AAV Integration Roundtable

ASGCT convened **a roundtable of multi-stakeholder experts** in the field on August 18, 2021 to discuss AAV integration, findings from non-clinical research, and implications for drug development and clinical trials.

ASGCT AAV Integration Roundtable Experts	
Kevin Eggan, Ph.D., BioMarin Pharmaceutical	**Markus Grompe, M.D, Oregon Health & Science University**
Randy Chandler, Ph.D., NIH, NHGRI	Mark Kay, MD, Ph.D, Stanford University School of Medicine
Ronald Crystal, M.D., Weill Cornell Medicine	David Lillicrap, M.D, Queens University
Ricardo Dolmetsch, Ph.D., UniQure	Eugenio Montini, Ph.D., San Raffaele Telethon Institute for Gene Therapy
Guangping Gao, Ph.D., Umass Medical School	Denise Sabatino, Ph.D., Children's Hospital of Philadelphia
Irene Gil-Farina, Ph.D, GeneWerk	Dinah Sah, Ph.D., Voyager Therapeutics
Fred Bushman, Ph.D, University of Pennsylvania School of Medicine	Jing Yuan, Ph.D., Pfizer

Discussion from FDA's September 3, 2021, CTGTAC meeting on AAV toxicity-integration was considered in preparation for this liaison meeting.

Learnings and recommendations regarding AAV integration will be compiled in a **whitepaper issued by ASGCT in collaboration with multi-stakeholder experts**.

American Society of Gene + Cell Therapy

rAAV Integration Background and Current Thinking

rAAV Integration Mechanism

Takeaways:

- AAV genomes uncoat within the nucleus and present as linear DNA molecules with hairpin inverted terminal repeat (ITR) structures.
- Free DNA ends are recognized by DNA damage repair pathways leading to conversion to double-stranded DNA (dsDNA) and ligation of free ends to form circles, concatemers, and chromosomally integrated forms.
- The repair processes are frequently imprecise, leading to aberrant junctions and deletions and rearrangements within the vector genome and/or the chromosome.
- We do not yet have a full understanding of the factors that determine the distribution of species that result from repair processes in mammalian cells, or how they might be influenced by parameters of the design and production of the vector and the tissue being transduced.

ASGCT Position

- As a field, we need to further our understanding of the molecular fate of recombinant AAV (rAAV) DNA and the factors that influence these outcomes.

rAAV Integration Frequencies

Takeaways

- rAAV integration is generally random in terms of DNA sequence, but favored in actively transcribed regions, DNA hairpin structures (or other features that are prone to dsDNA breaks), or ribosomal RNA genes in those AAV serotypes that traffic to the nucleolus.*
- In rodents, these processes are largely complete within 4-6 weeks post-administration in liver and muscle, and the likelihood of integration after that point is greatly reduced.**
- Estimates of rAAV integration rates in hepatocytes range from 0.1 to 10% of vector genomes. Recent estimates in humanized mouse models suggest 1 to 3%. Recent analysis of patient samples from UniQure suggest 0.01 to 0.1%.
    - The integration rate is likely dependent on the quantity of rAAV entering the nucleus.
    - rAAV vectors bearing significant homology to chromosomal sequences have been shown to lead to targeted recombination at those loci, but the relative frequency of these events are difficult to predict.

ASGCT Position

- Estimates of integration frequencies remain highly variable, illustrating a need for better assays to quantitate and characterize integration events in nonclinical and clinical samples.

*Nakai et al., AAV serotype 2 vectors preferentially integrate into active genes in mice. Nat Genet. 2003 Jul;34(3):297-302. [PMID 12778174]
**Nakai et al., Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo. J Virol. 2001 Aug;75(15):6969-76 [PMID: 11435577]

Impact of Vector Product Quality

Takeaways

- Known features of rAAV vector design including potential hairpin structures, repetitive and GC rich regions, and excessive genome size can contribute to aberrantly packaged genomes.
- The process whereby rAAV products are manufactured may have an influence on the quantity and characteristics of sequence contaminants and the form of vector genome termini.

ASGCT Position

- The scientific community must remain committed to understanding the integrated vector forms associated with HCC and how vector design and quality attributes contribute
- Additional research in the field is needed to determine whether AAV vectors without termination signals pose an increased risk and also to further our understanding of what design features are needed to mitigate.

Risk Assessment of rAAV Integration

Theoretical Risk of Oncogenesis Associated with rAAV Chromosomal Integration

Takeaways:

- To date, the only observed cancer associated with recombinant AAV (rAAV) integration has been hepatocellular carcinoma (HCC) in mice. Transduction in muscle, CNS, pancreas and other organs does not reach the copy number achieved in the liver and has not led to measurable tumor incidence in mouse models.
  - Systemically administered products with transduction in liver may have different considerations than locally administered products, e.g., in the eye.
- Given the large number of hepatocytes transduced with rAAV in many gene therapy applications, even a small percentage of integrated genomes would result in a large number of integration events.
- Random integration patterns could result in integration events near or within proto-oncogenes.
- Although rAAV chromosomal integration is likely to be complete within weeks of vector administration, longer-term data will inform any impact of integration events in humans. Humans treated with AAV and followed for up to 8 years have shown no signs of oncogenesis.

ASGCT Position

- The risk of oncogenesis associated with rAAV chromosomal integration in humans is theoretical based on current data and information.

Applicability of Animal Models to Predicting Risk in Humans

Takeaways:

- Although rAAV has unequivocally enhanced occurrence of HCC in laboratory mice, several aspects of these models suggest that they have enhanced sensitivity to these events relative to humans:
  - HCC occurs as a background tumor in most mouse strains
  - Mice have longer telomeres, which allows increased proliferation prior to crisis and associated apoptotic events that prevent tumor formation
- Vector designs that reduce HCC risk in mice (e.g., lacking strong enhancer/promoters that are active in the liver), would be likely to reduce the risk of HCC in humans.
- Large animal models such as canines and NHP may more accurately predict oncogenic risk in humans, although frank tumor formation may require one to two decades.

ASGCT Position and/or Recommendations

- Additional studies assessing the oncogenesis of strongly transactivating vectors in large animals could be investigated through public/private partnerships to address these issues but are likely impractical as a near term solution for individual program development as this may require one to two decades of research.
- Basic AAV vector platform research may find use of sensitive mouse models of oncogenesis to provide information about the relative risk of different vector designs, however, the translatability of any murine oncogenic events to humans remains unclear.

Canine Studies

Takeaways

- In canines treated with rAAV-FVIII, there was evidence of clonal expansion of hepatocytes with integrated rAAV approximately 4-9 years after vector administration, associated with increased expression of FVIII, but without histological evidence of HCC or other cancerous lesions.
    - Clonal expansion of hepatocytes containing integrated rAAV was confirmed through sequencing.
    - The significance of this clonal expansion with regard to interactions with integrated rAAV is difficult to interpret due to a general lack of knowledge regarding clonal expansion with age in normal liver tissue in canines.
- Observation of high variability in measured rAAV copy numbers within single liver tissue samples suggest that data from liver biopsies may not be representative of the liver as a whole.
- **Findings are variable** - In one study there was modest enrichment for integrations near oncogenes or growth control genes among clonal expansions. In the other study clustering of integrations in 3 specific chromosomal regions were not associated with proto-oncogenes, though some integration sites associated with suspected proto-oncogenes were identified.
    - Some of the observed differences in integration patterns noted between the canine studies may be due to methodologies including sequencing methods and genome annotation.

ASGCT Position

- Recent observations of clonal expansions in canine hepatocytes in the absence of tumor formation warrant additional studies but may reflect natural clonal dynamics in aging animals.

Methodologies for Assessing rAAV Integration

Takeaways

- Newer sequencing methods are improving our ability to sensitively detect rAAV DNA integration events and may reveal that integration frequencies of rAAV vector genomic DNA is higher than previously determined.
- Sequencing methodologies that incorporate steps to minimize interference from episomal rAAV genomes should be emphasized, although separate measurement of episomal copy number provides a critical perspective for integration rates.
- Differences between methodologies for sequencing methods make standardization and rigorous quantitation challenging.

ASGCT Position

- Need to improve sequencing methods for integrated rAAV genomes and genome fragments.

In Vitro Models

Takeaways

- **Features needed in cell cultures to reflect patterns of rAAV integration *in vitro*:**
  1. Non-dividing or slowly dividing cultures that are dominated by non-homologous end-joining pathways for DNA repair
  2. Transcription/expression patterns that are representative of hepatocytes or other target tissues. Tissue architecture may also be important
  3. Sufficiently infectable with relevant AAV serotypes to achieve vector genome copy numbers approximating those observed in vivo, recapitulating concatemeric populations (this has been difficult to achieve with liver organelle models)
  4. The ability to measure clonal expansion over time
  5. The ability to detect signals of transformation or interactions with proto-oncogenes
  6. Without the ability to report signals of transformation, *in vitro* models are unlikely to reveal anything beyond the typical quasi random integration patterns

ASGCT Position

- Further research is needed on development and potential predictability of *in vitro* models on integration and associated risks; however, limitations in primary hepatocyte culture are currently a substantial barrier to the development of such models.

Clinical Considerations

Takeaways

- No clinical adverse events related to integration have been observed thus far in the many patients treated with AAV vector-based gene therapies followed for up to 8 years to date,* however, it was noted that the overall clinical database is small, both in size and in duration.

ASGCT Position and/or Recommendations

- While there is a theoretical risk that rAAV integration could lead to insertional oncogenesis in patients, the paucity of rAAV-associated HCC in large animals and humans suggests that this risk is small and does not likely outweigh the potential benefit from AAV vector-based gene therapies, especially for serious and life-threatening conditions.
- As with other classes of therapeutics, it is important to communicate the relative risk benefit equation to patients and their physicians; specifically highlighting what we know and emphasize the theoretical versus observed / documented risks (e.g., risk demonstrated pre-clinically vs. those observed in clinic). Product labeling and risk management plans will be a critical element of communicating risks.
- It is view of ASGCT that given the current diseases targeted with AAV gene therapy, and the actual documented risks, the risk/benefit ratio would suggest that trials should be able to proceed in parallel with additional investigation, and in the post-market setting. This is particularly true for disorders in which the liver is not the target.

*Nathwani et al., Adeno-associated mediated gene transfer for hemophilia B: 8 year follow up and impact of removing "empty viral particles" on safety and efficacy of gene transfer. Blood. 2018 Nov;312(Suppliment 1):491. [doi: 10.1182/blood-2018-99-118334]

Long-Term Follow-Up Monitoring of Subjects

Takeaways

- Long term monitoring for development of HCC will likely be a key component of clinical plans, particularly for therapies with liver-directed expression, constitutive expression, or strong liver enhancers.
- Routine biopsies for monitoring/follow-up after administration are invasive and of limited utility. Given the heterogeneity within liver samples noted in the canine studies, and the low probability of sampling an incipient clonal expansion, biopsies are likely to reflect random rAAV integration patterns.
- Cell-free DNA (cfDNA) could be a potential safety biomarker that may allow for early identification of expanding clones growing in solid organs, but further research and validation is needed.

ASGCT Recommendations

- Duration of long-term follow-up monitoring should be risk-based and depend on the product-specific risk factors (e.g., promoter, route of administration, dose) and patient population's background risk.
- Monitoring should focus on non-invasive methods such as ultrasound, liquid biopsies, biomarkers through blood work, etc.
- Biopsies of neoplastic nodules and surrounding non-tumor tissue should be conducted to further investigate any positive signal picked up in non-invasive methods.
- Cell-free DNA (cfDNA) should be further researched and validated in the field as a potential safety biomarker.

ASGCT Recommendations

Summary: ASGCT Recommendations

- **Theoretical Risk**
  - The risk of oncogenesis associated with rAAV chromosomal integration in humans is theoretical based on current data and information.
  - While the risk of leading to insertional oncogenesis in patients is theoretical, the paucity of rAAV-associated HCC in large animals and humans suggests that it is small and does not likely outweigh the potential benefit from AAV vector-based gene therapies, especially for serious and life-threatening conditions.
  - Given the current diseases targeted with AAV gene therapy, and the actual documented risks, the risk/benefit ratio would suggest that trials should be able to proceed in parallel with additional investigation
- **Animal Models**
  - The use of sensitive mouse models of oncogenesis may provide information about the relative risk of different vector designs; however, translatability of any murine oncogenic events to humans remains unclear.
    - If longer term PD studies are conducted, the background rates of HCC must be taken into account and risks must be understood.
  - While additional studies assessing the oncogenesis of strongly transactivating vectors in large animals will be needed to better understand these issues, these studies are impractical as a near-term solution for specific development programs and may require one to two decades of research.
  - Recent observations of clonal expansions in canine hepatocytes in the absence of tumor formation warrant additional studies in the field more generally; however they may reflect natural clonal dynamics in aging animals

Summary: ASGCT Recommendations

- **Methods for Assessment**
  - There is value today in performing integration-site analysis with existing methods on tumor tissue samples from patients, and WGS on tumor samples from non-clinical and clinical studies should they occur. ISA, WGS and gene expression analysis of any malignant tumors should be performed.
    - Limitations to these methods may be overcome by rapid and ongoing improvements in sequencing technologies, such as target enrichment and long-read sequencing methods. These methods may be used as a secondary screening method, and are likely to provide greater insight into concatemeric and highly rearranged integrated vector genomes.
  - ASGCT proposes an expert group be convened to make recommendations regarding the shortcomings of these existing methods used to assess oncogenic events when detected, and assess methods that would overcome them, to enable better understanding of the relationship between AAV integration and HCC in humans.
  - Cell-free DNA (cfDNA) should be further researched and validated in the field as a potential safety biomarker.
- **Clinical Assessment**
  - Biopsies of neoplastic nodules and surrounding non-tumor tissue should be conducted to further investigate any positive signal picked up in non-invasive methods.
  - Duration of long-term follow-up monitoring should be risk-based and depend on the product-specific risk factors (e.g., promoter, route of administration, dose) and patient population’s background risk, including age and presence of chronic liver regeneration.

Acknowledgements

Thanks to the co-chairs (Kevin Eggan and Doug McCarty) speakers, panel members, and participants in the August 18, 2021 ASGCT AAV Integration Roundtable for comprehensive background information, lively discussion, and thoughtful comments and questions.

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Special thanks to the ASGCT cross-stakeholder working group that compiled the Society's recommendations:

- Markus Grompe, MD, Oregon Health & Science University
- Nimi Chhina, PhD, JD, BioMarin Pharmaceutical
- Kevin Eggan, PhD, BioMarin Pharmaceutical
- John Gray, PhD, Vertex Pharmaceuticals
- Tim MacLachlan, PhD, Novartis
- Doug McCarty, PhD, unaffiliated
- Snehal Naik, PhD, Spark Therapeutics
- Marjolaine Phan, PhD, Novartis
- Kristin Van Goor, PhD, Vertex Pharmaceuticals
- Adora Ndu, PharmD, JD, BioMarin Pharmaceutical (*FDA Liaison meeting Co-chair*)

Discussion
2:15 – 2:30 pm ET

Break

The meeting will resume at 2:40 pm ET

FDA / CBER
Office of Tissues and Advanced Therapies (OTAT) Update

American Society of Gene & Cell Therapy (ASGCT) Liaison Meeting

November 8, 2021

Wilson W. Bryan, MD

Center for Biologics Evaluation and Research (CBER)

Office of the Director

Peter Marks, MD, PhD, Director

Celia Witten, PhD, MD, Deputy Director

Office of Management

Office of Communication, Outreach, and Development

Office of Compliance and Biologics Quality

Office of Biostatistics and Epidemiology

Office of Vaccines Research and Review

Office of Blood Research and Review

Office of Tissues and Advanced Therapies

Diversity of OTAT-Regulated Products

- **Gene therapies (GT)**
  - Ex vivo genetically modified cells
  - Non-viral vectors (e.g., plasmids)
  - Replication-deficient viral vectors (e.g., adenovirus, adeno-associated virus, lentivirus)
  - Replication-competent viral vectors (e.g., measles, adenovirus, vaccinia)
  - Microbial vectors (e.g., Listeria, Salmonella)
- **Stem cells/stem cell-derived**
  - Adult (e.g., hematopoietic, neural, cardiac, adipose, mesenchymal)
  - Perinatal (e.g., placental, umbilical cord blood)
  - Fetal (e.g., neural)
  - Embryonic
  - Induced pluripotent stem cells (iPSCs)
- **Products for xenotransplantation**

- **Functionally mature/differentiated cells**
  (e.g., retinal pigment epithelial cells, pancreatic islets, chondrocytes, keratinocytes)
- **Therapeutic vaccines and cellular immunotherapies** including antigen-specific active immunotherapies
- **Blood- and Plasma-derived products**
  - Coagulation factors
  - Fibrin sealants
  - Fibrinogen
  - Thrombin
  - Plasminogen
  - Immune globulins
  - Anti-toxins
  - Venom antisera for snakes, scorpions, and spiders
- **Combination products**
  - Engineered tissues/organs
- **Devices**
- **Tissues**

Approved Gene Therapies

- KYMRIAH (tisagenlecleucel)
- YESCARTA (axicabtagene ciloleucel)
- TECARTUS (brexucabtagene autoleucel)
- **BREYANZI** (lisocabtagene maraleucel)
- **ABECMA** (idecabtagene vicleucel)
- LUXTURNA (voretigene neparvovec-rzyl)
- ZOLGENSMA (onasemnogene abeparvovec-xioi)

Approved Cellular Therapy Products

- PROVENGE (sipuleucel-T)
- Hematopoietic Progenitor Cells, Cord Blood
- LAVIV (azficel-T)
- GINTUIT (allogeneic Cultured Keratinocytes and Fibroblasts in bovine collagen)
- MACI (autologous Cultured Chondrocytes on porcine collagen membrane)
- **STRATAGRAFT** (allogeneic cultured keratinocytes and dermal fibroblasts in murine collagen-dsat)
- **RETHYMIC** (allogeneic processed thymus tissue–agdc)

All OTAT INDS
(i.e., Research and Expanded Access (EA))
1963 – 2020

666

223

700
600
500
400
300
200
100
0

Expanded Access INDs
INDs with product development programs

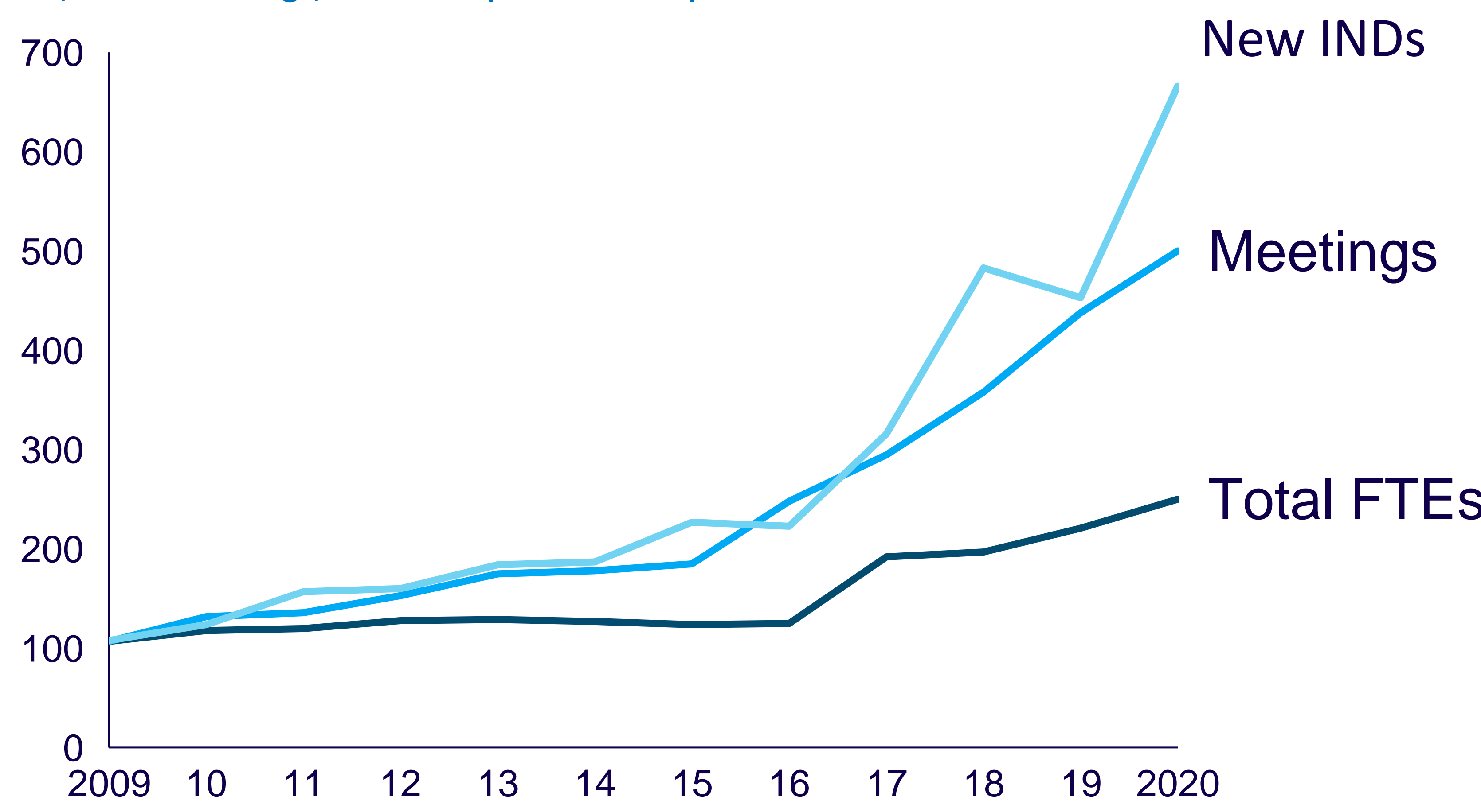
1963 1976 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020

Cell and Gene Therapies: Research INDs 2002 – 2020

Breakthrough (BT) and RMAT Designation Requests

All Meeting Types (A, B, C, and Other)

OTAT workload outpaces FTE increases



FDA/CBER/OTAT

GROWTH PROGRAM

OTAT Growth Program

Primary Goals

- Expedite advances in cell and gene therapy
- Improve staff satisfaction and sustainability

Program Phases

- Generate ideas
- Prioritize objectives and pilot solutions
- Refine and implement solutions

OTAT Growth Program: Generate Ideas

- Interviews and focus groups with CBER and OTAT staff
  - Analysis of workload data
- Interviews and listening sessions with sponsors and industry trade groups
  - 25 sponsor interviews
  - Listening sessions with four trade organizations, including ASGCT
- Data analysis to characterize and quantify opportunities
- Prioritize ideas

What we heard from OTAT Staff

Challenges in interactions with sponsors

1) More staff is needed to meet increasing workload
2) Difficult to meet expectations for the degree of engagement
3) Submissions may arrive missing key documents or information
4) Sponsors do not necessarily communicate changes in a way that facilitates efficient review
5) Limited precedents for some products, questions have become more complex and time-intensive to address

What we heard from sponsors

Strengths in interactions with OTAT

1) High quality scientific advice
2) Strong working relationships with OTAT staff (e.g., responsive/engaged interactions with project managers)
3) Digital innovations implemented as a result of the COVID-19 pandemic (e.g., digital submissions)
4) PDUFA timelines are being met
5) OTAT staff clear commitment to the patient mission

What we heard from sponsors

Challenges in interactions with OTAT

1) Response quality and consistency varies, especially for more nascent technologies
2) Clarity and specificity of OTAT responses is mixed (e.g., in written responses)
3) Limited opportunities for informal interactions and follow-ups to answer clarifying questions (e.g., after meetings)
4) Unclear expectations on several topics

OTAT Growth Program: Idea Summary

- Rapid growth in the development of cell and gene therapies has created new challenges for OTAT.

- OTAT is undertaking a variety of initiatives to meet these challenges, including:
  - improving communications with stakeholders and
  - increasing capacity and efficiency in OTAT operations

OTAT Growth Program: Prioritize Objectives

Four priority objectives to support initiatives and achieve primary goals:

1) Clarify expectations and create tools to help sponsors engage OTAT productively
2) Re-design core operational practices to drive efficiency, transparency, and collaboration
3) Increase frequency of scientific exchange externally and internally
4) Create more staff and management capacity and sustainability

Objective 1 - Pilot Solutions

1) Clarify expectations and create tools to help sponsors engage OTAT productively

   - Revise website, with initial focus on meetings with OTAT

   - Consolidate resources related to cell and gene therapies on CBER’s website (e.g., OTAT Learn recordings, guidance documents)

Objective 2 - Pilot Solutions

2) Re-design core operational practices to drive efficiency, transparency, and collaboration

- Standardize practices for clarifications after meetings, particularly after "Written Responses Only"
- Investigate opportunities for increased communication regarding status of submissions, including both original INDs and IND amendments

3) Increase frequency of scientific exchange externally and internally

- Collaborate with trade and scientific organizations (e.g., ASGCT) to facilitate mutual learning
  - Identify priority topics
  - Webinars
  - Workshops
  - White Papers

Pending 2021 OTAT Guidances

FINAL GUIDANCES

- Regulation of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps): Small Entity Compliance Guide
- Interpreting Sameness of Gene Therapy Products Under the Orphan Drug Regulations

DRAFT GUIDANCE

- Considerations for the Development of Human Gene Therapy Products Incorporating Human Genome Editing
- Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products
- Studying Multiple Versions of a Cellular or Gene Therapy Product in an Early-Phase Clinical Trial

4) Create more staff and management capacity and sustainability

- PDUFA VII
- Reconsider OTAT structure

- There is a commitment to patients and high-quality scientific exchange in the development of cell and gene therapies.
- Rapid growth in the development of cell and gene therapies has created new challenges for OTAT
- Ideas from OTAT staff and sponsors spurred initiatives to sustain strengths and meet challenges
- OTAT is piloting solutions to
  - improve communications with stakeholders and
  - increase capacity and efficiency in OTAT operations

FDA

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- Xiaofei Wang, PhD

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FDA Headquarters

OTAT Learn Webinar Series:

http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm

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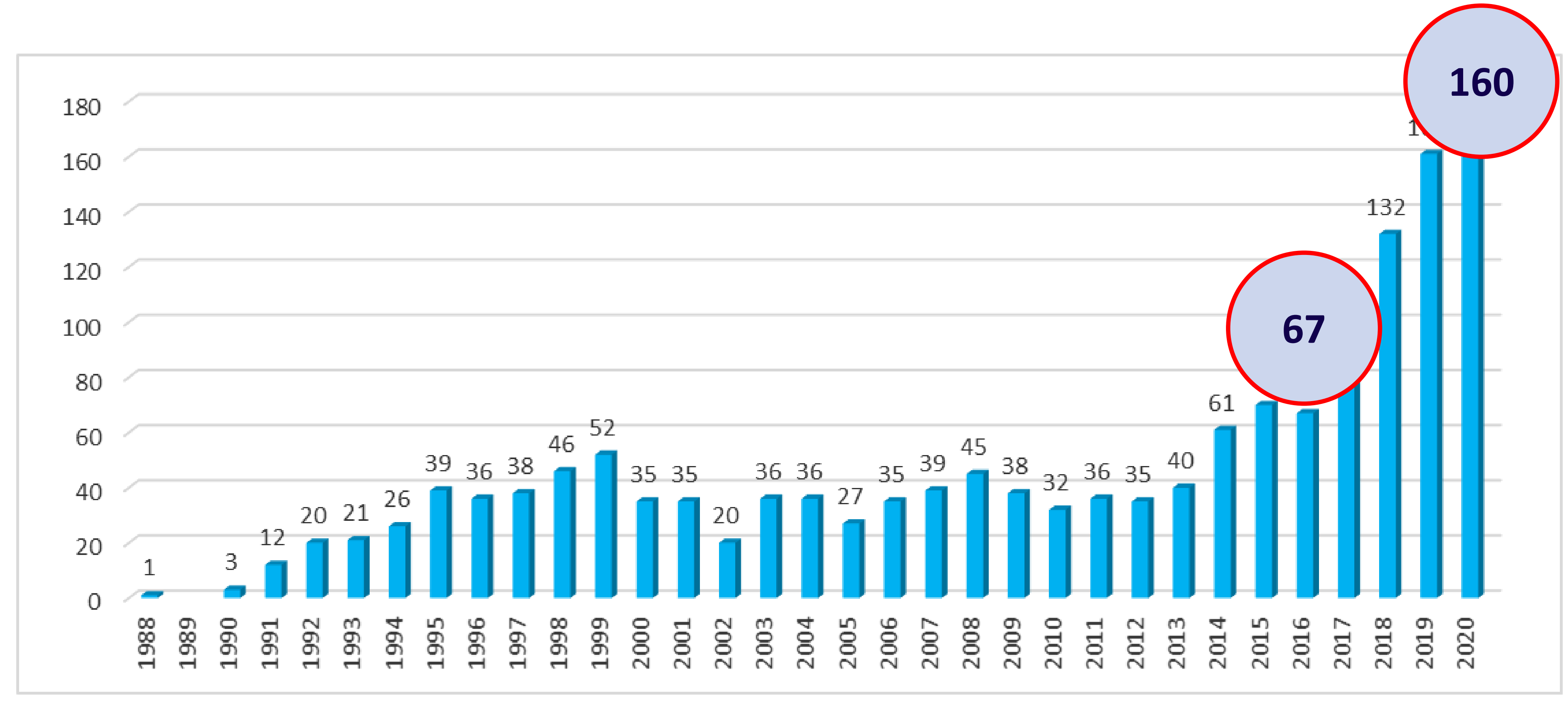
FDA / CBER
Office of Tissues and Advanced Therapies (OTAT) Update

American Society of Gene & Cell Therapy (ASGCT) Liaison Meeting

November 8, 2021

Wilson W. Bryan, MD

Research INDs: Gene Therapy



Research INDs: Cell Therapy

Year	INDs
1984	1
1985	1
1986	
1987	3
1988	3
1989	4
1990	3
1991	2
1992	6
1993	3
1994	16
1995	12
1996	23
1997	30
1998	25
1999	48
2000	46
2001	56
2002	47
2003	49
2004	56
2005	57
2006	56
2007	42
2008	52
2009	38
2010	48
2011	56
2012	58
2013	60
2014	65
2015	
2016	
2017	
2018	96
2019	91
2020	

y-axis: 0, 20, 40, 60, 80, 100, 120, 140, 160

Callouts: 63; 151

Discussion
3:05 – 3:20 pm ET

Recommendations on CMC Expectations for Gene and Cell Therapy Products

ASGCT-FDA Liaison Meeting
November 8, 2021

Jan Thirkettle, PhD, Chief Executive Officer, Transine Therapeutics

J. Fraser Wright, PhD, Professor of Pediatrics, Center for Definitive and Curative Medicine, Stanford University School of Medicine

Disclosures

- Jan Thirkettle is CEO of Transine Therapeutics, an oligonucleotide platform therapeutics company utilizing AAV as one of its delivery modalities
- J Fraser Wright is a co-founder of Spark Therapeutics, a scientific co-founder of Kriya Therapeutics, and an inventor on patents relating to methods to make viral vectors

Topics

- Introduction
- Product specifications
- *In vitro* potency assays
- Additional product characterization
- Manufacturing process comparability
- Concluding observations

Introduction

- Advances in manufacturing and analytical techniques have improved control and characterization of cell and gene therapy (CGT) products, but the link between product characteristics and clinical performance is still evolving.
- Small clinical trial populations that are characteristic of CGT product development make statistical analysis of CMC data from CGT batches challenging.
- The CGT category is wide, and the challenges presented by complex cell products (that may have multiple modes of action) and viral vector products may be different.

Rapid innovation in the CGT field warrants a CMC framework that remains flexible, risk-based, and correlated with the extent of clinical experience.

Challenges related to CGT product specifications

- Sponsors have experienced an expectation from FDA of setting specifications for complex assays for CGT products in very early-stage clinical trials even for parameters which are not known/confirmed CQAs.
- The overall objective of product developers is to improve product quality and clinical potency over the course of clinical development.
  - Specification assays critical in late-stage development/pivotal trials, such as potency assays, may not be possible early in clinical development due to:
    - Assay complexity/variability.
    - Small sample sizes (difficult to reach statistical significance).
    - Lack of mechanistic understanding.
  - CQA specifications should be added/tightened over time as more knowledge of the attributes and their impact on clinical data is gained.

ASGCT recommendations for specifications

- Take a pragmatic approach to the application of statistical analyses of specifications early in development. With limited data, these may not be as meaningful as robust qualitative analysis.
- Focus early-stage specifications on the limited number of well-defined CQAs at that stage.
- Allow characterization assays performed throughout development to inform the product specifications applied in late-stage development.
- Use available data and science-based risk assessment to guide the evolution of specifications at the appropriate point of development (including post-marketing if so justified).

Challenges related to *in vitro* potency assays

- ASGCT members who are sponsors agree with FDA that developing *in vitro* potency assays which mimic closely the mechanism of action (as far as it can be understood) of CGT products is an important, but very challenging goal.
  - Development of such assays suitable for a QC/GMP environment is a significant undertaking that may take many years.
    - These are highly complex assays with intrinsic limitations of precision and robustness and analytic methods.
    - These assays may need to be qualitative early in development until multi-batch experience is available to set specifications.
  - Challenges are heightened for cell therapy products with multiple modes of action e.g., immune cell products may secrete a wide range of immunomodulatory factors, or act through a range of synergistic mechanisms.
  - Full (quantitative) linkage between a potency assay and clinical effectiveness may require post licensure experience as clinical trial sizes are limited.
- ASGCT members who are sponsors have noted a trend through reviewer interactions towards requiring multiple potency assays on the product specification, for instance:
  - Infectivity assays for viral vectors in addition to a bioassay.
  - Requirements for potency assays for lentiviral vectors used to make gene modified cell therapies, which themselves have a potency assay.
- ASGCT members who are sponsors have noted a trend through reviewer interactions towards requiring extensive assay validation and statistically-driven specification setting approaches at the start of pivotal development even when there is insufficient data to support such approaches.

ASGCT recommendations for potency assays

- That FDA allow a risk-based approach in the context of the unmet medical need of the patient population in its requirements for potency assays of CGT products.
- The development of one strong potency assay addressing the main mechanism of action(s) of the final drug product should be sufficient for product release, obviating the need for other potency assays as product release tests.
  - For example, eliminate AAV infectivity or potency testing of rLV when these vectors are used to create a final genetically modified cell product which has its own potency assay.
- Additional measures of potency should be continued throughout development, but rather as general characterization assays with no acceptance criteria.
- Expectations for potency assay qualification and specification setting need to take into account the complexity of the assay, known correlation with clinical outcomes, and data availability.
- Allow continuous validation of potency assays during review and post-licensure to refine acceptance criteria for products where high replicate batch data is challenging (e.g., autologous products or those that have especially complex modes of action).

Challenges related to product characterization

- ASGCT members who are sponsors agree with FDA that maximizing use of available analytical tools to increase product understanding and quality is imperative; however, such methods are not always amenable to being deployed as specification assays.
    - Implementation of some complex characterization assays and validation in a GMP environment is not possible due to compliance challenges.
    - Biological assays possess inherent variability.
    - Information generated may be complex/not single-dimensional and so not amenable to setting specifications
    - The greatest value of characterization assays are not the specific results, but rather the role they play in increasing product understanding and risk assessment.
- ASGCT members who are sponsors are concerned that some reviewer requests increasingly drive towards moving assays from characterization to batch release assays (and therefore needing validation) even in cases where these challenges are manifest.

ASGCT proposals related to product characterization

- FDA should develop broad, *high-level* guidance for the CQAs that should be the focus of characterization studies for each product class (i.e., rAAV, rLV, CAR-T cells, etc.).
    - This helps sponsors better understand the rationale and prioritization for characterization assays for each class of CGT product and develop appropriate methods to address them.
- FDA should provide feedback to sponsors in early-stage meetings regarding characterization expectations through the course of product development.
- Acceptance criteria should not be required for characterization assays (including when these support comparability assessments); rather, the results obtained should be used for science-based risk assessments.
- The focus of performing characterization assays should be to gain a deeper understanding of product CQAs during product development; while the focus of performing quality control assays is to ensure lot to lot consistency of *known* product CQAs within acceptable ranges.

Challenges related to manufacturing process comparability

- Multiple changes to the manufacturing process may be required during product development, often with limited batch datasets at each stage.
- ASGCT members who are sponsors believe the goal of studies to compare products before and after a process change is to prospectively ensure comparable safety and efficacy of an investigational product within bounds supported by risk assessments, rather than ensure identical performance on all measured characteristics.
- ASGCT members who are sponsors:
  - Have indicated that the agency has requested quantitative acceptance criteria and validation for characterization assays used for process comparability studies, which is sometimes not possible given the complexity of these assays.
  - Have interpreted agency data requests as indication that processes should be identical in comparability studies.
  - Feel agency expectations regarding similarity to the “pre-change” comparator(s) can be unclear or unrealistic, in effect requiring identicalness because of limited clinical trial sizes, in which there may be only one or few batches to compare.
  - Do not have clear guidance on what constitutes a manufacturing change requiring a comparability assessment.
- FDA moving toward requiring statistical analyses in setting comparability acceptance criteria is often not realistic given:
  - the lack of sufficient batch numbers that can realistically be generated during clinical development.
  - insufficient understanding of the clinical impact of biological variation.

ASGCT recommendations on comparability

- FDA should allow a more flexible and pragmatic approach to manufacturing process changes and comparability assessment, providing further guidance on principles for decision making.
- It is acceptable for characterization assays without acceptance criteria to provide relevant but difficult-to-quantify data to inform the overall assessment of comparability for attributes that are not expected to impact safety.
- Statistical analysis expectations should consider that low-replicate batches are an inherent feature of CGT investigational products. We propose greater weighting of science- and risk-based arguments and decision making that includes qualitative data.
- ‘Identicalness’ cannot be demonstrated in a comparability study given current assay limitations, natural biologic variation of complex (sometimes ‘living’) cell products, and poorly defined links to clinical benefit.
- Further guidance/clarity is required on the expectation for comparability testing of viral vectors used in genetically modified cell therapy products.
- Further guidance is needed on the parameters that FDA believes define a “change” in manufacturing that warrant comparability studies.
- The appropriate comparator for contemporaneous comparability testing following a process change should be the product manufactured using the preceding process, not all historical products and processes.

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Concluding observations

- **ASGCT appreciates the ongoing partnership and opportunity to engage in a scientific dialogue with FDA.**
- **ASGCT appreciates FDA's ongoing engagement with the community to share and discuss its thinking, such as the recent CTGT Advisory Committee meeting.**
- In addition to the specific regulatory recommendations presented, we suggest the following CMC policy approaches be considered:
  - Keeping CMC guidance consolidated to increase clarity in the Agency's views and avoid risks of divergence between therapeutic areas.
  - Greater coordination between OTAT and other offices with less experience in CGT to assist with product review consistency.
  - Continuing engagement with the scientific community at conferences and meetings, including sharing case studies – where possible, these should include blinded datasets representing broad areas/large samples to provide context and assist cross-field collaboration.
  - Continued engagement across HHS agencies, such as with the Bespoke Gene Therapy Consortium (BGTC).

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Discussion
3:45 – 4:00 pm ET

ASGCT and FDA Liaison Meeting

Concluding Remarks