# Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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# FDA Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70

**September 2-3, 2021**

# Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy (GT)

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AC Planning Working Group
Office of Tissues and Advanced Therapies (OTAT)
CBER, FDA

# Outline

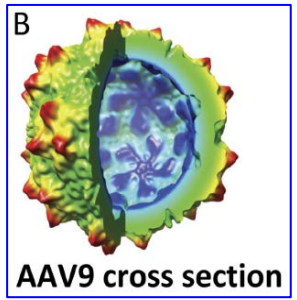
- Introduction
- Hepatotoxicity
- Thrombotic microangiopathy (TMA)
- Neurotoxicity
  - Dorsal root ganglia (DRG) toxicity
  - Brain magnetic resonance imaging (MRI) findings
- Oncogenicity
- Summary

# INTRODUCTION

# Adeno-associated Virus (AAV) and AAV Vectors

- AAV is a small DNA virus (20 - 25 nm diameter, ~ 5 kb genome)
  - Widespread in humans and animals
  - More than 100 serotypes, with tropisms for different cell types
  - Only replicates in the presence of a helper virus (e.g., adenovirus, HSV-1, HHV-6, EBV)
  - No known disease association

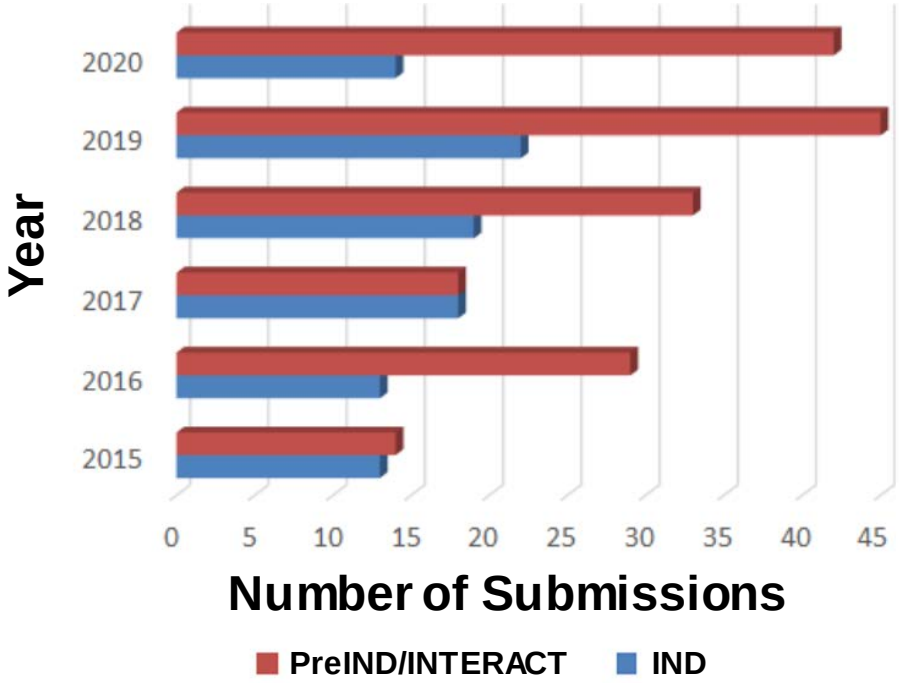
- AAV vectors
  - Do not replicate
  - Are engineered to express a therapeutic gene




*DiMattia et al. (2012) J. Virol. 86:6947*

# AAV Vector-based Gene Therapy (GT) Products

## AAV Vector-based GT Submissions to OTAT





Voretigene neparvovec-rzyl
Luxturna
[2017]


Onasemnogene abeparvovec-xioi
Zolgensma
[2019]

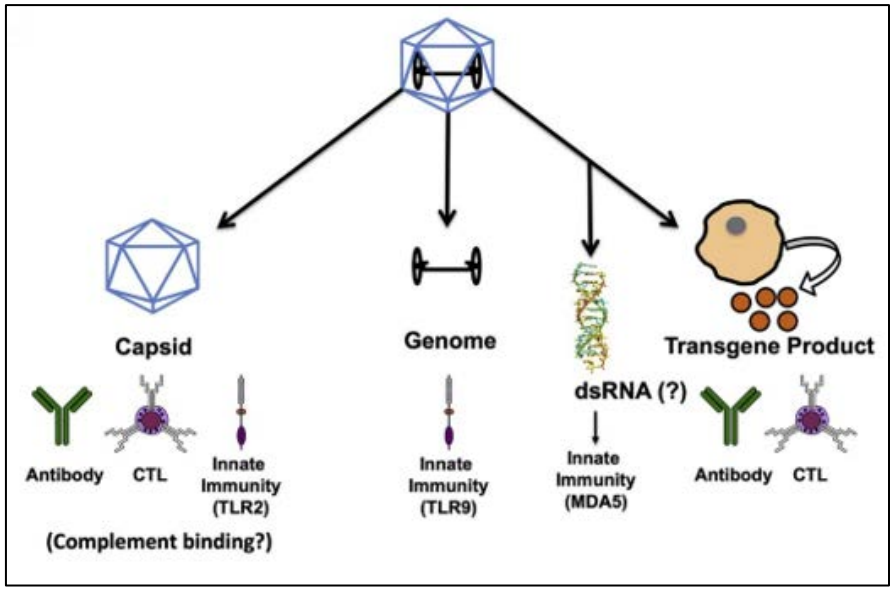
# Severe Adverse Events in AAV Vector Clinical Trials

| Toxicity | Serious Adverse Event | Vector Serotype | Indication | Route of Administration |
|---|---|---|---|---|
| **Hepatotoxicity** | Elevated liver enzymes, serious liver injury | AAV9 | SMA | Intravenous |
| | Elevated liver enzymes | AAV5 | Hemophilia | Intravenous |
| | Liver failure | AAV8 | XLMTM | Intravenous |
| **TMA** | Thrombocytopenia, hemolytic anemia, acute kidney injury | AAV9 | SMA, DMD | Intravenous |
| **Neurotoxicity (DRG Histopathology)** | DRG neuronal loss | AAV9 | GAN | Intrathecal |
| **Neurotoxicity (DRG Histopathology)** | DRG neuronal loss | AAVrh10 | ALS due to mutation in *SOD1* | Intrathecal |
| **Neurotoxicity (Brain MRI)** | Abnormal T2 hyperintensities | AAVrh10 | Late infantile Batten disease | Intraparenchymal |



TMA, thrombotic microangiopathy; DRG, dorsal root ganglion; SMA, spinal muscular atrophy; XLMTM, X-linked myotubular myopathy; DMD, Duchenne muscular dystrophy; GAN, giant axonal neuropathy; ALS, amyotrophic lateral sclerosis; *SOD1*, superoxide dismutase 1 gene

# Human Immune System and AAV Vectors

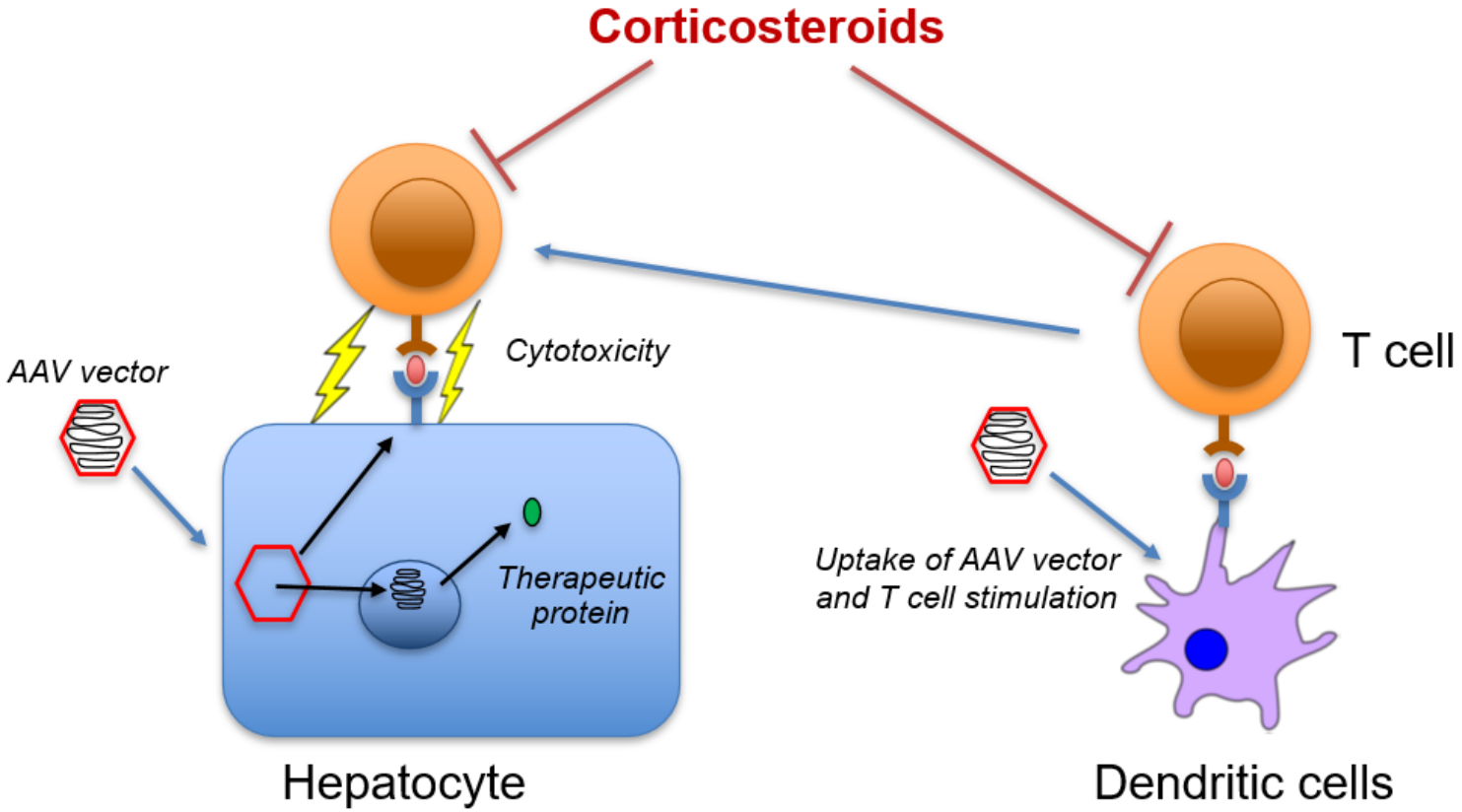



dsRNA, double-stranded RNA; CTL, cytotoxic T lymphocyte; MDA5, melanoma differentiation-associated protein 5; TLR, Toll-like receptor

Source: Shirley et al., 2020. Immune Responses to Viral Gene Therapy Vectors. Molecular Therapy 28: 709-722 (16)

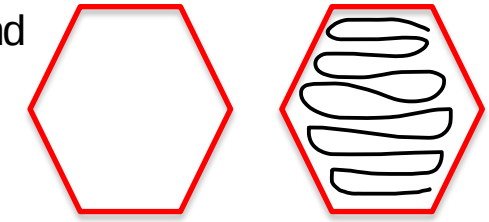
- Older children and adults often have anti-AAV antibodies.
  - Can block vector activity
  - Many clinical trials exclude subjects with pre-existing antibodies
- Innate and adaptive immune systems
  - Activation of complement pathways
  - Anti-AAV T cells mediate hepatotoxicity
- Role for immunosuppressive drugs

# Anti-AAV T Cells Can Mediate Hepatotoxicity



# Other Potential Causes of AAV Vector-related Toxicities

- Transgene expression levels
  - Neuronal death in dorsal root ganglia (DRG)
- Vector DNA integration into host genome
  - Hepatocellular carcinoma (HCC)
- Product impurities
  - Empty capsids can be targets for antibodies, complement, and T cells
  - AAV capsids can package non-vector DNA during manufacturing
  - Safe levels often unknown



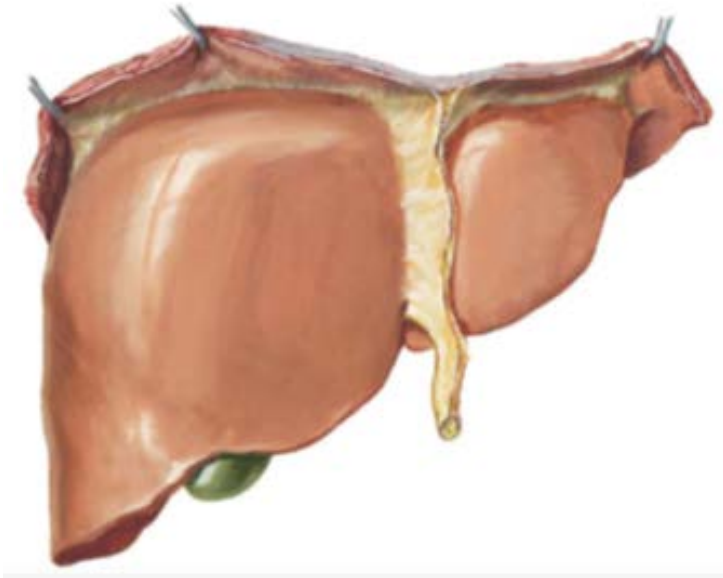
Empty capsid

Full capsid

# HEPATOTOXICITY

# Hepatotoxicity

- Most common adverse event associated with intravenous (systemic) administration of AAV vectors
- Prophylactic use of corticosteroids
- Presentations of hepatoxicity
  - Elevated liver enzymes (ALT, AST)
  - Drug-induced liver injury*
  - Hepatic failure
  - Death



[Image modified from *Netter Atlas of Human Anatomy, 6th edition*]

**As defined by Hy's Law*

# Clinical Experience: Spinal Muscular Atrophy (SMA)

- Onasemnogene abeparvovec (Zolgensma)
  - AAV9-based GT
  - FDA approved in 2019
  - Single intravenous (IV) infusion: $1.1 \times 10^{14}$ vg/kg of body weight
  - Children < 2 years of age with SMA with bi-allelic mutations in *SMN1*
  - Over 500 patients exposed

# Clinical Experience: SMA

- About one-third of clinical trial participants had at least one adverse event of hepatotoxicity
- Elevated aminotransferases > 20x ULN, treated with corticosteroids
- One case of acute serious liver injury / liver failure
  - Baseline aminotransferases ↑, unknown etiology
  - Jaundice, total bilirubin ↑, prothrombin time ↑ about 7 weeks after treatment
  - Biopsy: Massive hepatocytes degeneration and inflammatory infiltrates
  - Recovered with prednisolone
- Risk mitigation in the US Prescribing Information (PI)
  - Boxed warning
  - Systemic corticosteroids

# Clinical Experience: Hemophilia

- Elevated serum levels of aminotransferases
  - Soon after treatment
  - Transient
  - Asymptomatic

- Variability of AAV capsid-specific cell-mediated immune response
  - Many participants: Elevated aminotransferases and immune response
  - Some participants: Elevated aminotransferases, no immune response
  - Some participants: Immune response, no elevation of aminotransferases

- Loss of transgene expression in some subjects
  - Coincided with elevated aminotransferases
  - Subset of these participants had AAV capsid-specific cell-mediated immune response.

# Clinical Experience: X-Linked Myotubular Myopathy (XLMTM)

- ASPIRO Trial (NCT03199469)
  - Product: AAV8 vector expressing *MTM1*
  - Single intravenous administration
  - Low dose, $1 \times 10^{14}$ vg/kg: n=6, age range 0.8 - 4.1 years
  - High dose, $3 \times 10^{14}$ vg/kg: n=17, age range 0.6 - 6.8 years

- Prophylactic treatment with corticosteroid
  - Prednisolone (1 mg/kg/day) for 8 weeks, then tapered over next 8 weeks

ClinicalTrials.gov
https://clinicaltrials.gov/ct2/results?cond=NCT03199469&term=&cntry=&state=&city=&dist=

# Clinical Experience: XLMTM

- Three deaths
  - All in the high-dose cohort ($3 \times 10^{14}$ vg/kg)
  - Occurred 20 - 40 weeks after product administration
  - Participants were older and heavier
  - Received higher total dose (range: 4.8 - 7.7 $\times 10^{15}$ vg)
  - Pre-existing intrahepatic cholestasis
  - Development of intrahepatic cholestatic liver failure
  - Treatment with ursodiol, augmented corticosteroids, immunosuppressants was ineffective
  - Immediate cause of death: Sepsis (2 cases), Gastrointestinal bleeding (1 case)

# Clinical Experience: XLMTM

- Role of disease-related factors in hepatotoxicity
  - XLMTM natural history study: Hepatobiliary disease common
  - More than 50% of participants in ASPIRO trial had some evidence of pre-existing hepatobiliary disease
  - Direct causal link between hepatotoxicity and the three deaths cannot be established
  - Dose-related hepatoxicity, in context of pre-existing hepatobiliary abnormalities in children with XLMTM

# Findings in Animal Studies

SMA

- Transient elevation in liver enzymes and minimal-to-moderate histopathological findings in the liver reported in neonatal FVB/NJ mice administered onasemnogene abeparvovec via the IV route (greater than $1.1 \times 10^{14}$ vg/kg)

- Acute liver failure, thrombocytopenia, and coagulopathy reported in healthy non-human primates (NHPs) administered an AAVhu68 vector via the IV route ($2 \times 10^{14}$ vg/kg)
    - Similar findings, along with transient complement activation, reported in healthy NHPs administered AAV9 or AAV-PHP.eB vectors via the IV route ($1 - 2 \times 10^{14}$ vg/kg)

# Findings in Animal Studies

Hemophilia

- Transient elevation in liver enzymes reported in hemophilia A dogs and in healthy NHPs administered different AAV vectors via the IV route (up to $4 \times 10^{13}$ vg/kg in dogs; up to $5 \times 10^{12}$ vg/kg in NHPs)

XLMTM

- No adverse liver findings reported in XLMTM dog and mouse models administered AAV8 vectors via the IV route (up to $5 \times 10^{14}$ vg/kg in dogs; $3 \times 10^{13}$ vg/kg in mice)

# Discussion Questions for the Committee: Hepatotoxicity

1. Please discuss the merits and limitations of animal studies to characterize the risk of hepatotoxicity and provide recommendations on preclinical study design elements, such as animal species / disease model and in-life and post-mortem assessments.

2. How should patients be screened and categorized based on their risk for developing liver injury, before AAV vector administration? Please discuss whether pre-existing hepatic conditions may predict the risk of serious liver injury.

3. What additional strategies could be implemented before or after AAV vector administration to prevent or mitigate the risk of liver injury?

# Discussion Questions for the Committee: Hepatotoxicity

4. What factors (e.g., level of disease severity) other than weight should be considered to determine the vector dose for systemic administration?

5. Considering the risk of toxicities observed in clinical trials with high doses of AAV vectors,

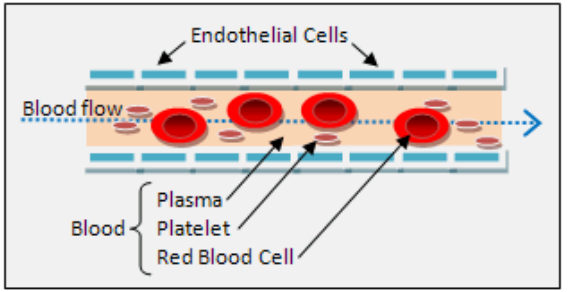
    a. Please discuss whether an upper limit should be set for the total vector genome dose per subject.

    b. Given that many AAV products contain significant amounts of empty capsids, please discuss whether an upper limit should be set on the total capsid dose.

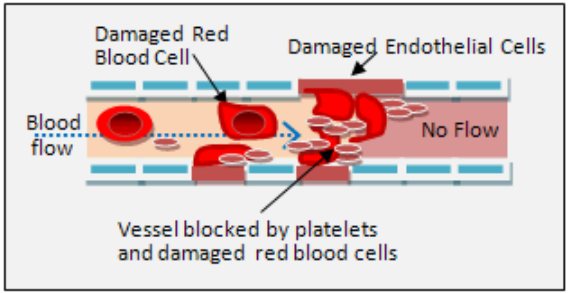
# THROMBOTIC MICROANGIOPATHY (TMA)

# Thrombotic Microangiopathy (TMA)

- Hematologic emergency
- Damage to arterioles and capillaries; microvascular thrombosis
- Syndrome of hemolytic anemia, thrombocytopenia, and acute kidney injury
- Two primary forms:
  - Thrombotic thrombocytopenic purpura
  - Hemolytic uremic syndrome (HUS)
- Typical vs. atypical HUS




**Normal capillary**




**Capillary damaged by TMA**

[Image modified from unckidneycenter.org/kidneyhealthlibrary/glomerular-disease/thrombotic-microangiopathy-tma/

# Clinical Experience: SMA

- At least 3 cases of TMA reported, about 1 week after receiving onasemnogene abeparvovec
- All received recommended dose of prednisolone
- Role of concomitant triggering events?
- Laboratory evidence of complement activation
- Outcomes:
  - Recovery from TMA within 2-4 weeks
  - One patient: Persistent hypertension
  - One patient: Hypertension and nephrotic syndrome; resolved 3 months later

# Clinical Experience: Duchenne Muscular Dystrophy (DMD)

- Several cases of aHUS in pediatric participants in DMC clinical trials

- Proposed risk mitigation plans
  - Prophylactic, off-label use of eculizumab and C1 esterase inhibitor
  - Increased dose of prednisone daily
  - Modification of manufacturing process to decrease percentage of empty vectors

# Findings in Animal Studies

- Acute thrombocytopenia, coagulopathy, transient complement activation and hepatotoxicity reported in healthy NHPs administered AAV vectors via IV route.

- No adverse kidney histopathology

# Discussion Questions for the Committee: TMA

1. Please discuss factors that may increase the risk of TMA following AAV vector administration.

2. Please provide recommendations on strategies that could be implemented before and after AAV vector administration to prevent or mitigate the risk of AAV vector-mediated TMA.

3. Considering the risk of toxicities observed in clinical trials with high doses of AAV vectors,

   a. Please discuss whether an upper limit should be set for the total vector dose.

   b. Given that many AAV products contain significant amounts of empty capsids, please discuss whether an upper limit should be set on the total capsid dose.

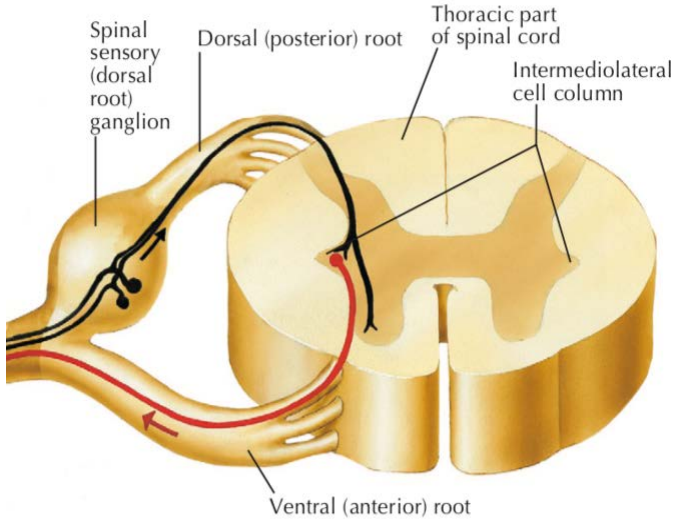
# NEUROTOXICITY:
# DORSAL ROOT GANGLION (DRG) TOXICITIES

# Adverse DRG Findings in Animal Studies

- Primary sensory neurons (PSN) in the DRG transmit sensory stimuli from the peripheral nerves to central terminals in the CNS.

- Degeneration of the PSNs of the DRG and axonopathy of the spinal cord reported in healthy NHPs administered AAV vector products via the intrathecal/cisterna magnus and IV routes
  - Minimal-to-moderate in severity with no associated clinical signs

- Similar AAV-mediated pathology have also been reported in Yucatan pigs and mice.




[Image modified from *Netter Atlas of Human Anatomy, 6th edition*]

# Adverse DRG Findings in Clinical Trials

- Two case reports of autopsy findings of DRG neuronal loss in participants of clinical trials
  - Giant axonal neuropathy (GAN) trial:
    - Severe DRG neuronal loss (autopsy at 8 months)
    - No clinical signs or symptoms of DRG toxicity
  - Amyotrophic lateral sclerosis (ALS) trial:
    - Tingling & pain in both hands & one foot, 3-4 weeks after AAV vector treatment
    - Sensory action potential: Normal at baseline, absent at 10 weeks
    - Neuronal loss (autopsy at 15.6 months)

# Discussion Questions for the Committee: DRG Toxicity

1. Based on the published data, please discuss the relevance of the NHP cases of DRG toxicity to human subjects.

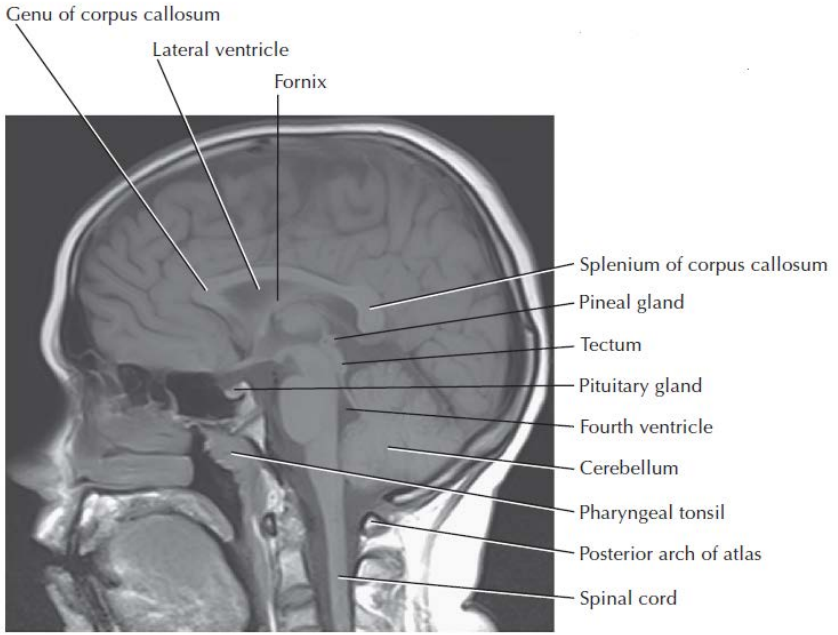
2. Please provide recommendations on preclinical study design elements, such as animal species / disease model, age, in-life and post-mortem assessments, and duration of follow-up, post-dose, that may contribute to further characterization of DRG toxicity.

3. In addition to periodic neurological examinations, please provide recommendations on other methods to mitigate the risk of DRG toxicity in clinical trials.

# NEUROTOXICITY:
# BRAIN MRI FINDINGS

# Clinical Experience

- Direct intraparenchymal delivery of AAV vectors to specific parts of brain may:
  - Improve targeting efficiency: Overcoming the need to administer high systemic doses of AAV vectors to cross blood-brain barrier
  - Decrease risk of systemic toxicity compared to high doses administered intravenously




[Image modified from Netter Atlas of Human Anatomy, 6th edition]

# Clinical Experience: Late Infantile Batten Disease

- Brain MRI T2 hyperintensities associated with intraparenchymal administration of AAVrh10 vector
  - Seen in all 13 participants 48 hours after vector administration
  - Localized to the sites of administration
  - Persistent in 7 participants on MRI after 18 months; resolved in 2 participants
  - Serious adverse events in 6 participants during acute period
  - Difficult to determine cause: AAV vector, delivery procedure, delivery devices

# Findings in Animal Studies

- Brain MRI and histopathological abnormalities reported in healthy NHPs following intraparenchymal administration of AAVrh10 vectors:
    - Immune cell infiltrates and gliosis at the injection tracks correlating with MRI abnormalities
    - Findings persistent up to 52 weeks
    - No neurobehavioral deficits observed
- Histopathological findings at the injection tracks also reported in healthy rats following intraparenchymal administration of AAVrh10 vector

# Discussion Questions for the Committee: Brain MRI Findings

1. Please provide recommendations for any preclinical in-life and post-mortem assessments (e.g., behavioral and neuropathological assessments) and duration of follow-up, post-dose, to identify and further characterize the risk of neurotoxicity following intraparenchymal administration of AAV vectors.

2. Please discuss the clinical significance, if any, of brain MRI abnormalities observed in clinical trials of AAV GT. Please discuss whether the delivery procedure vs. AAV vector may have contributed to the abnormal brain MRI findings.

# Discussion Questions for the Committee: Brain MRI Findings

3. Please provide recommendations on strategies that could be implemented before and after vector administration to prevent or mitigate the risk of CNS injury.

4. Please recommend a duration of monitoring for subjects who have abnormal brain MRI findings, or factors to consider for the determination of an appropriate duration of monitoring.

# ONCOGENICITY

# AAV Vector Integration and Oncogenicity Risk

- AAV vector genomes can integrate into host genomic DNA
  - Tendency to integrate near active genes
  - Vector integration has been seen in both animals and humans.
- HCC observed in several mouse models
  - Vector genome integration into *Rian* locus that encodes numerous regulatory RNAs
- Hepatic clonal expansion in canine model of hemophilia A
  - Integration into genes associated with cell growth and / or transformation
  - No liver nodules or oncogenesis
- HCC incidence may be influenced by multiple factors such as vector components, animal species / disease model, timing of administration and duration of follow-up after vector administration.

# AAV Vector Integration and Oncogenicity Risk

- One case report of HCC in clinical trial
  - HOPE-B trial (NCT03569891): AAV5 vector for treatment of hemophilia B
  - Unlikely that AAV vector contributed to HCC

- Risk-based approach for determining duration of long-term follow-up protocol
  - FDA guidance document *Long Term Follow-up After Administration of Human Gene Therapy Products* (January 2020)
  - Recommended follow-up duration up to 5 years for AAV vectors
  - Up to 15 years recommended for retroviral vectors or vectors that carry genome editing components

# Discussion Questions for the Committee: Oncogenicity

1. Please discuss the merits and limitations of animal studies to characterize the risk of AAV vector-mediated oncogenicity, and provide recommendations on specific preclinical study design elements, to include:
    a. Animal species, healthy vs. disease models, and animal age
    b. In-life and post-mortem assessments, including methods for integration analysis
    c. Duration of follow-up, post-dose

2. Current literature suggests that various factors may affect AAV-mediated vector genome persistence, vector integration, and the risk of oncogenesis. Please discuss benefit-risk considerations for AAV vector-mediated oncogenesis, such as patient age at the time of treatment, pre-existing liver conditions (e.g., infection with hepatitis B or C virus), and high vector dose.

# Discussion Questions for the Committee: Oncogenicity

3. Considering the risk of oncogenesis,
   a. Please provide recommendations on safety monitoring measures that should be included in clinical trials.
   b. Please provide recommendations on duration, frequency, and method of long-term follow-up (LTFU) for recipients of AAV vectors.

4. Please discuss whether some vector designs may enhance the frequency of vector-mediated integration and the risk of oncogenesis. For example, how is the risk affected by promoter-enhancer elements, genome-targeted nucleases, or novel AAV vector designs for which there is limited clinical experience. Because AAV vectors can carry significant levels of co-packaged DNA impurities from the manufacturing process, is the risk of oncogenesis increased due to potential integration of non-vector DNA, and what types of studies should be performed to assess this risk?

# SUMMARY

# Summary

- Serious and life-threatening systemic toxicities have been reported as more patients receive AAV vectors, and as vector doses increase.

- There are many questions about these toxicities, including how to:
  - Understand mechanisms
  - Mitigate risks to participants in clinical trials

- FDA is seeking the committee's insights into AAV vector toxicities in the context of:
  - AAV vector design and quality
  - Preclinical and clinical study designs
  - Long-term monitoring of individuals receiving these products

# Acknowledgement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- Division of Cellular & Gene Therapies /OTAT
- Division of Clinical Evaluation & Pharmacology/Toxicology /OTAT

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