Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.
Systemic AAV: Clinical Findings of Hepatotoxicity

CTGTAC: Toxicity Risks of AAV Vectors for Gene Therapy
September 2-3rd, 2021

Lindsey A. George, MD
Assistant Professor of Pediatrics, UPENN
Director of Clinical In Vivo Gene Therapy, CHOP
Attending Hematologist, CHOP
<table>
<thead>
<tr>
<th>CONFLICT</th>
<th>DISCLOSURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant / Research Support</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Director / Officer / Employee</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Shareholder</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Paid Instructor</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Speaker Bureau</td>
<td>No relevant conflicts of interest to declare</td>
</tr>
<tr>
<td>Consultant</td>
<td>Pfizer, Bayer, Biogen, Intellia</td>
</tr>
<tr>
<td>Other</td>
<td>Avrobio (DSMB), STRM.Bio (SAB)</td>
</tr>
</tbody>
</table>
Observed clinical hepatotoxicity following systemic AAV :

• Hemophilia A and B
• Spinal Muscular Atrophy
• X-linked myotubular myopathy
Systemic AAV Efficiently Targets the Liver

• Liver functions:
  • CHO, lipid, bilirubin metabolism
  • Protein synthesis: 60% of proteins, all plasma proteins (except γ-globulins)

• Hepatic Blood Flow
  • 1L/minute, 10-15% of total blood volume
  • Dual blood supply: hepatic portal v., hepatic a.

• Fenestrated endothelium in hepatic sinusoids

• Independent of therapeutic target, systemic AAV traffics the liver

• Hepatotoxicity is the most common AE in AAV clinical trials
Hemophilia A and B

<table>
<thead>
<tr>
<th></th>
<th>Hemophilia A</th>
<th>Hemophilia B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (males)</td>
<td>1 in 5,000</td>
<td>1 in 30,000</td>
</tr>
<tr>
<td>Deficient protein</td>
<td>FVIII</td>
<td>FIX</td>
</tr>
<tr>
<td>Endogenous synthesis</td>
<td>LSECs (predominantly)</td>
<td>hepatocytes</td>
</tr>
</tbody>
</table>

- All current hemophilia AAV trials target hepatocyte transgene expression
- Enrollment criteria: adults, with \( \leq \) stage 2 fibrosis

90% of severe hemophilia patients >40 years contracted HCV

Mazepa, Blood 2016
Hepatic Toxicity: Hemophilia A and B trials

AAV2-ssFIX
High Dose cohort: $2 \times 10^{12}$ vg/kg

Mannos CS et al., Nat Med 2006;12:342-347

Mingozzi F et al., Nat Med 2007
Mingozzi F and High KA, Blood 2013
Hepatic Toxicity: Cytotoxic Immune response

- **Presentation**: transient transaminase elevations (ALT most pronounced), decline in FVIII/FIX activity, IFN-γ ELISPOT to AAV capsid peptides; 1-3 months post vector

- **Management**: glucocorticoids or other immune-modulating agents (*e.g.* MMF, tacrolimus)

- **CTL response pattern observed in multiple HA and HB trials:**
  - Nathwani et al., NEJM 2011 (AAV8-scFIX, 2E12 vg/kg)
  - George et al., NEJM 2017 (AAV-SPK100-ssFIX-Padua, 5E11 vg/kg)
  - Chowdary et al., Res Pract Thromb Haemost 2020 (AAVS3-FIX-Padua, 7.5-9.5E11 vg/kg)
  - George et al., Res Pract Thromb Haemost 2020 (AAV-LK03-FVIII, 1.5-2E12 vg/kg)*
  - Konkle et al., Blood 2021 (AAV8-scFIX-Padua, 2E11-3E12 vg/kg)*
    * some participants lost all transgene expression

- **Higher vector CpG content**: CTL responses not abrogated by steroid intervention resulting in loss of expression
  - Konkle et al., Blood 2021 (AAV8-scFIX-Padua, 2E11-3E12 vg/kg)*
  - Faust SM et al., J Clin Invest 2013

- **2 trials observed recrudescence of a cellular immune response when weaning glucocorticoids**
  - AAVS3-FIX-Padua and AAV-LK03-FVIII; both AAV3-derived capsids

- **Thus far limited efficacy, but not raised major safety concerns** (Dose ranges: 2E11-6E13 vg/kg)
Hepatic Toxicity: Hemophilia A and B trials

- Transaminase elevations without evidence of a cellular immune response
  - Rangarajan et al., NEJM 2017
  - Miesbach et al., Blood 2017

- Multi-month, unclear etiology, mild transaminase elevations (up to 2X ULN) without associated decline in FVIII activity or responsiveness to steroid intervention in some participants

AAV5-FVIII 6E13 vg/kg (Rangarajan et al., NEJM 2017)
Hepatic Toxicity: SMA

- AR disorder resulting in loss of LMN due to decreased SMN protein that is ubiquitously expressed; now part of NBS in most states

- 1 in 11,000; SMA I (45-65% of cases): mortality or require respiratory support by 2 years

- Natural history related to liver function:
  - Up to 1/3 of SMA I patients have some degree of underlying liver disease (steatosis on autopsy), possibly due to abnormal fatty acid metabolism or decreased glutathione stores

- Onasemnogene abeparvovec (Zolgensma®): AAV9-CMV/CAG-SMN1: 1.1E14 vg/kg
- N = 800 patients treated

**Boxed Safety Warning**

**WARNING: ACUTE SERIOUS LIVER INJURY**

See full prescribing information for complete boxed warning.

- Acute serious liver injury and elevated aminotransferases can occur with ZOLGENSMA. (5.1)
- Patients with pre-existing liver impairment may be at higher risk. (5.6)
- Prior to infusion, assess liver function of all patients by clinical examination and laboratory testing (e.g., hepatic aminotransferases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], total bilirubin, and prothrombin time). Administer systemic corticosteroid to all patients before and after ZOLGENSMA infusion. Continue to monitor liver function for at least 3 months after infusion (2.1) (2.3).
Hepatic Toxicity: SMA

- 61% of patients had transaminase elevations before vector, median age: 2.9 months

- N = 100 clinical trial patients, 90% had ALT or AST elevation; non-cholestatic pattern
  - Most <3X ULN
  - 9% mild (≥ 3X to <5X ULN)
  - 6% moderate (≥ 5 to <20X)
  - 5% severe (≥20X)

- Transaminase elevations: 1 week post vector with second peak at 1 month, concurrent with steroid wean or discontinuation

- Patients treated with prophylactic prednisone, mean 83 days (range: 33-229 days)

Chand et al., J of Hepatol 2021
SMA: case reports of acute liver failure

- **6mo term male with SMA1 presented 7 weeks post vector in acute liver failure with encephalopathic clinical features**
  - Note: elevation of transaminases ~3-4X ULN prior to vector
  - AST 4447 U/L, ALT 2014 U/L, GGT 45 U/L, DBili 3.9 mg/dL, GGT 273 U/L, INR 5.3 (factor studies c/w liver failure), LDH 3226 U/L; ammonia normal
  - Extensive work up (include whole genome sequencing): + only for norovirus; liver biopsy at presentation (below)
  - Managed with glucocorticoids weaned over a year; liver biopsy at 2 months, persistent fibrosis
  - 18 months later continues to make motor gains

- **20mo term female with SMA presented 8 weeks post vector in acute liver failure with stage 1-2 encephalopathy**
  - Received prednisone before infusion through day 14, where dose was increased due to increase in LFTs and mild hepatomegaly
  - AST 1950 U/L, ALT 1634 U/L, GGT 572 U/L, TBili 3.5 mg/dL, GGT 273 U/L, INR 1.5 (factor studies c/w liver failure), ammonia 103 mM
  - Work up negative: infectious, autoimmune
  - Liver biopsy: inflammatory infiltrates (mostly CD8+ and neutrophils)
  - Managed with glucocorticoids that were weaned over 3.5 months
  - 9 months later continues to make motor gains

- **Time course, responsiveness to glucocorticoids and hepatic biopsy findings favor an AAV vector immune response**
  - Federman AG et al., J Pediatrc 2020
Hepatic Toxicity: XLMTM

- Most common and severe form of centronuclear myopathy typically due to \textit{MTM1} mutations resulting in extreme skeletal muscle weakness

- 1 in 50,000 male births, disease classification based on ventilator support

- Heterogenous phenotype, 50% mortality by 18 months

- Liver disease reported of unclear etiology, modest natural history data available

- Treatment: none

- Multiple therapeutic strategies in clinical trial (including ERT)

Annoussamy M et al., Neurology 2019
Hepatic Toxicity XLMTM: AT132 (Audentes/Astellas)

- AT132 for XLMTM: AAV8-MTM1
- Excluded subjects with pre-existing clinically significant liver disease
- Protocol specified subjects given prednisone 1mg/kg/day weeks 0-8 and tapered weeks 9-16

<table>
<thead>
<tr>
<th>Cohort size</th>
<th>1.3E14 vg/kg</th>
<th>3.5E14 vg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (range)</td>
<td>1.7 yr (0.8-4.1)</td>
<td>2.6 yr (0.6-6.8)</td>
</tr>
<tr>
<td>AEs/SAEs</td>
<td></td>
<td>3 deaths</td>
</tr>
</tbody>
</table>

TABLE 2 Highest doses of AAV administered in gene therapy clinical trials

<table>
<thead>
<tr>
<th>Clinical trial ID</th>
<th>Capsid</th>
<th>Drug name</th>
<th>Condition</th>
<th>Dose (vg/kg)</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT03652259</td>
<td>AAV-7</td>
<td>AMT-061</td>
<td>Hemophilia B</td>
<td>2x10^13</td>
<td>uniQure</td>
</tr>
<tr>
<td>NCT03092974</td>
<td>AAV5</td>
<td>BMN-270</td>
<td>Hemophilia A</td>
<td>4x10^13</td>
<td>BioMarin</td>
</tr>
<tr>
<td>NCT03199469</td>
<td>AAV8</td>
<td>AT-132</td>
<td>XLMTM</td>
<td>1x10^14, 3x10^14</td>
<td>Audentes</td>
</tr>
<tr>
<td>NCT03306277</td>
<td>AAV9</td>
<td>Zolgensma</td>
<td>SMA</td>
<td>1.1x10^14</td>
<td>Novartis</td>
</tr>
<tr>
<td>NCT03368742</td>
<td>AAV9</td>
<td>SGT-001</td>
<td>DMD</td>
<td>5x10^13, 2x10^14</td>
<td>Solid Bio</td>
</tr>
<tr>
<td>NCT03362502</td>
<td>AAV9</td>
<td>PF-06930926</td>
<td>DMD</td>
<td>1x10^14, 3x10^14</td>
<td>Pfizer</td>
</tr>
</tbody>
</table>

Paulk, Biotechnology News 2020
AT132 3.5E14 vg/kg 3 deaths: what is known?

- **Baseline**: All subjects 5 years of age at death and had at least 1 elevated bilirubin prior to vector.

- **Total vector doses**: 4.8-7.7E15 vg

- **Presentation**: marked elevated bilirubin 35-50X ULN (peaking at 30-90X ULN) 4-6 weeks post vector followed by elevated transaminases.

- **Unresponsive to immune modulating therapy**

- **Liver at autopsy**:
  - No marked inflammatory infiltrate
  - intrahepatic and canicular cholestasis
  - Periportal and bile ductular reaction with secondary fibrosis

- **Death 20-40 weeks post vector due to sepsis (n = 2) and GI bleeding 9 (n = 1); >1 year ago**
  - May 6, 2020; June 23, 2020; August 21, 2020

Astellas press release Aug 2020
Shieh PB et al., Hum Gen Ther 2020
AT132 1.3 E14 vg/kg

- Clinical hold lifted by FDA in 12/2020, moved forward with 1.3E14 vg/kg dosing

- September 1, 2021 Astellas announced voluntary hold on screening and dosing

- 1 subject in the 1.3E14 vg/kg dose cohort developed abnormal liver function within a month after dosing

- Subject had a history of intermittent cholestasis, but normal liver US and LFTs prior to dosing

Summary/Conclusions: AAV hepatotoxicity

- **Clinical presentation:**
  - Hemophilia and SMA: non-cholestatic, hepatocellular pattern
  - XLMTM: progressive cholestatic hepatitis

- **Etiology:**
  - Hemophilia: CTL response likely accounts for most, but not all, hepatic toxicity
  - SMA: CTL/immune response
  - XLMTM: ?

- **Multiple variables:**
  - Patient population: Unclear natural history (XLMTM), underlying liver disease (hemophilia, 1/3 SMA patients)
  - Variable means of reported hepatotoxicity prohibiting comparison across trials and disease cohorts
  - Variety of AAV capsids, promoter/enhancer elements and transgenes, manufacturing
  - Vector doses: 2E11 – 3E14 vg/kg (1500X dose range)
  - Absence of publicly available data in some trials

- **Morbidity and mortality of hepatotoxicity only observed at AAV doses > 1E14 vg/kg in pediatric patients**