

Adeno-Associated Virus-Related Toxicities in Nonhuman Primates

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Cellular, Tissue and Gene Therapies Advisory Committee

Sept 2, 2021

Disclosure Statement

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

Acknowledgements



Juliette Hordeaux
DVM, PhD



Liz Buza
DVM, Diplomate ACVP



Cecilia de Souza Dyer
DVM, MS, DACLAM



Christian Hinderer
MD, PhD

Sponsors

Biogen
Passage
Amicus
FA
NHLBI

Gene Therapy Program

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

Spectrum of Toxicities Associated With Systemic AAV

XLMTM

*Cholestatic
liver failure*

DMD

*Hemolytic Uremic
Syndrome (TMA)*

Initial insult

↓ platelets

+/- ↑ LFTs

SMA1

*Hepatocellular
liver failure*

Hemophilia

*Loss of transgene
expression*

Potential Mechanisms Leading to Organ Damage

Disseminated Intravascular Coagulation (DIC)

Tissue factor
Inflammation

Activation and consumption
of coagulation factors

Thrombotic Microangiopathy (TMA)

Activation and consumption
of platelets

Endothelial damage

**Microvascular
Thrombosis**

Secondary activation
of fibrinolysis

Organ damage

Symptoms	Severe DIC	Severe TMA
Organ Failure	Often (Multi organ)	Usually (Kidney, CNS)
Bleeding	Frequent	Frequent
BP	Low	High
Anemia	Often	Usually (hemolytic)

Lab data	Severe DIC	Severe TMA
Platelets	Low	Low
Hemoglobin	Often low	Low
Fibrin products	Markedly high	Slightly high
PT	Often prolonged	Normal
Creatinine	Often high	High

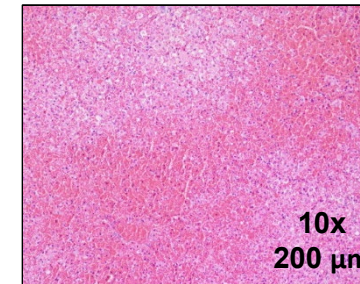
Wada et al Thrombosis Journal 2018

Case Report #1 of Fatal Systemic Toxicities in NHPs

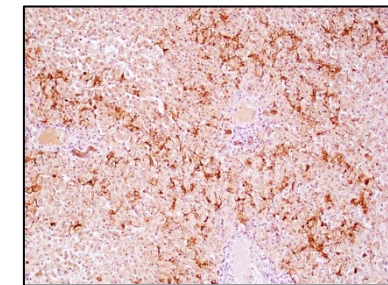
Case #1: AAVhu68.CB7.hSMN1 2e14 GC/kg IV in juvenile rhesus macaque

- 2.2 kg 1-year-old female rhesus macaque
- Shock/disseminated intravascular coagulation syndrome day 4 post-AAV dosing
- 2 other animals dosed in the same group were bled day 4/5: asymptomatic thrombocytopenia with elevated ALT

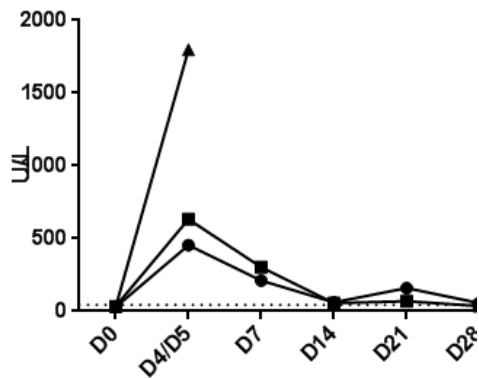
Severe (grade 5) acute liver necrosis (H&E)



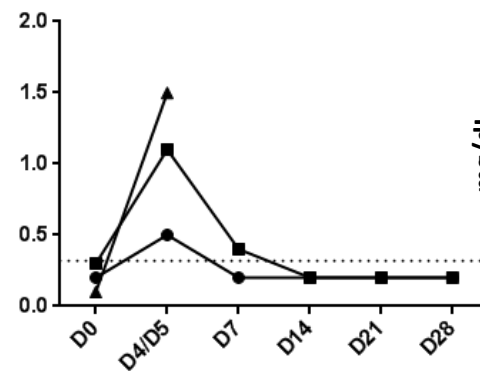
Severe liver (grade 5) intravascular coagulation (Fibrinogen IHC)



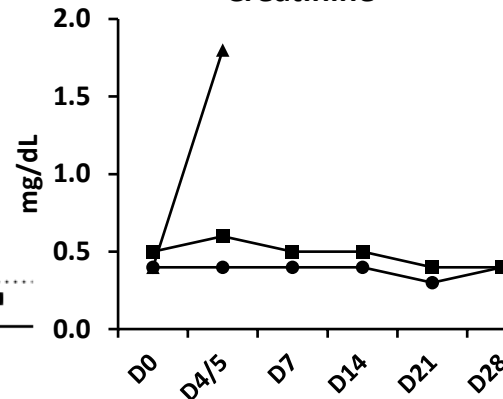
ALT



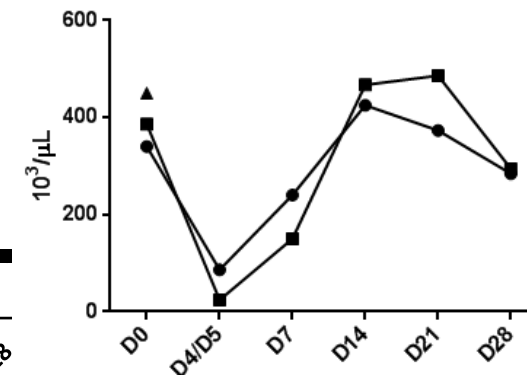
Total bilirubin



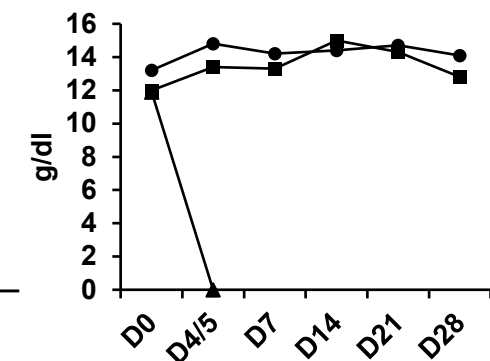
Creatinine



Platelets



Hemoglobin

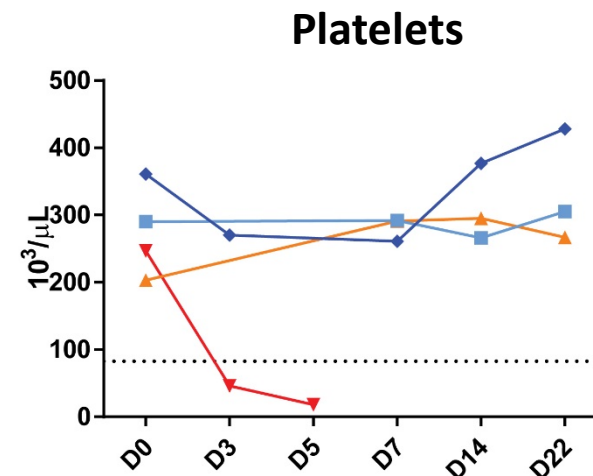
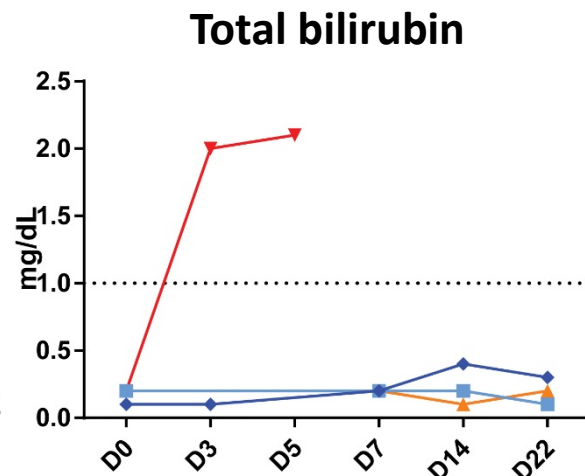
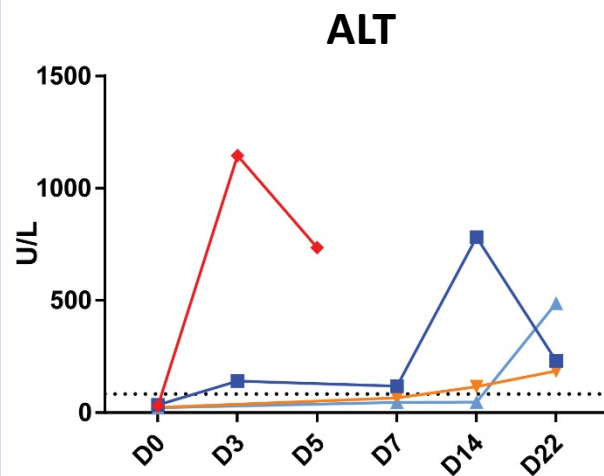


Case Report #2 of Fatal Systemic Toxicities in NHPs

Case #2: AAV.PHP.B.GFP 7.5e13 GC/kg IV in adult rhesus macaque

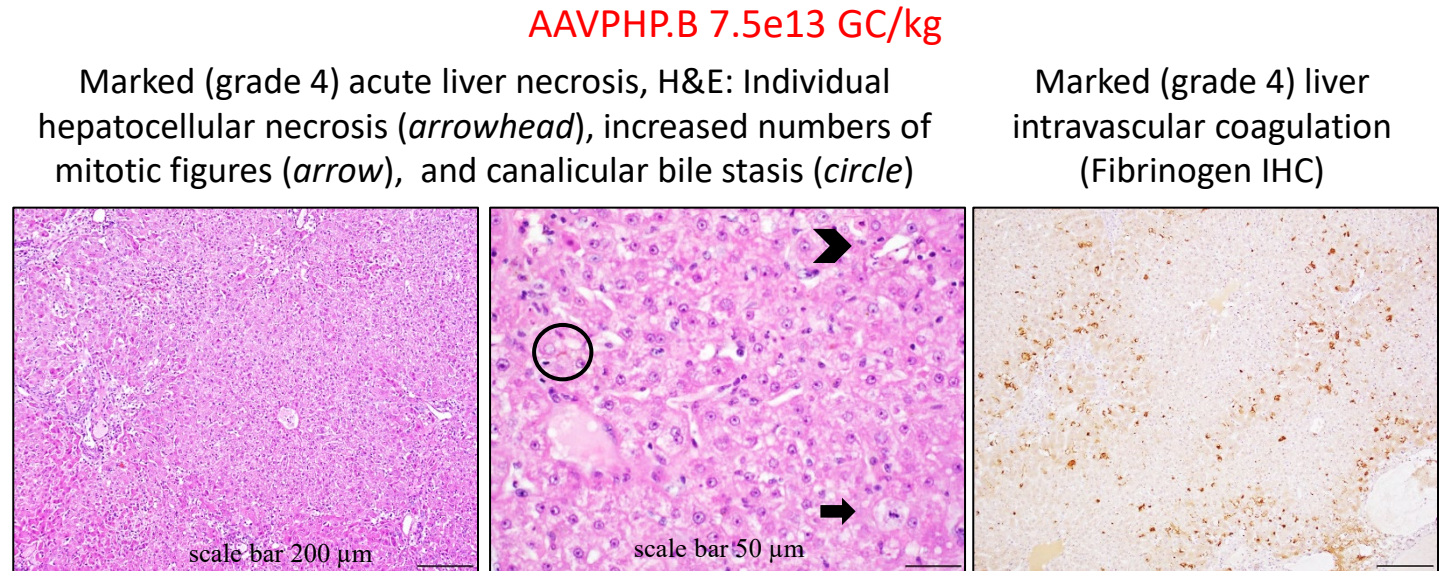
- 5.9 kg 4-year-old female rhesus macaque
- AAV9 and AAV.PHP.B.GFP evaluated at both 2 and 7.5e13 GC/kg IV
- Thrombocytopenia and liver enzyme elevations Day 3 – progressed to severe cutaneous hemorrhages and anemia Day 5 at high dose AAV.PHP.B.GFP
- Other groups well tolerated

AAV9 2e13 GC/kg AAVPHP.B 2e13 GC/kg
AAV9 7.5e13 GC/kg AAVPHP.B 7.5e13 GC/kg



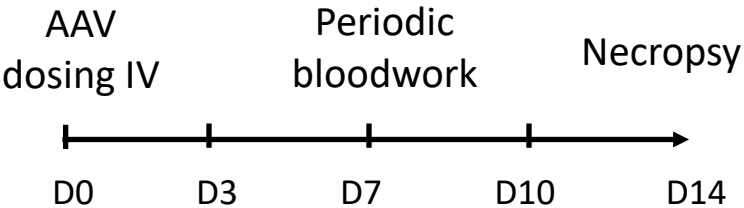
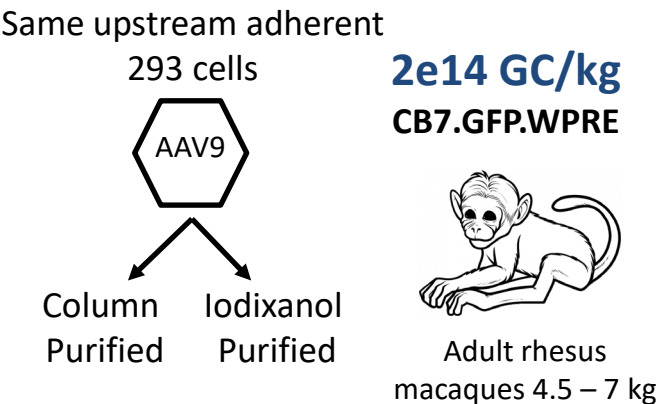
AAVPHP.B 7.5e13 GC/kg

	Baseline	Day 3	Day 5
PT (sec)	9.5	11.9	12
PTT (sec)	25.4	31.5	29.8
Fibro (mg/ml)	136	134	79
D dimer (ng/ml)	42	147	20



Purification Method Impact on Systemic Toxicity After HD AAV in NHPs

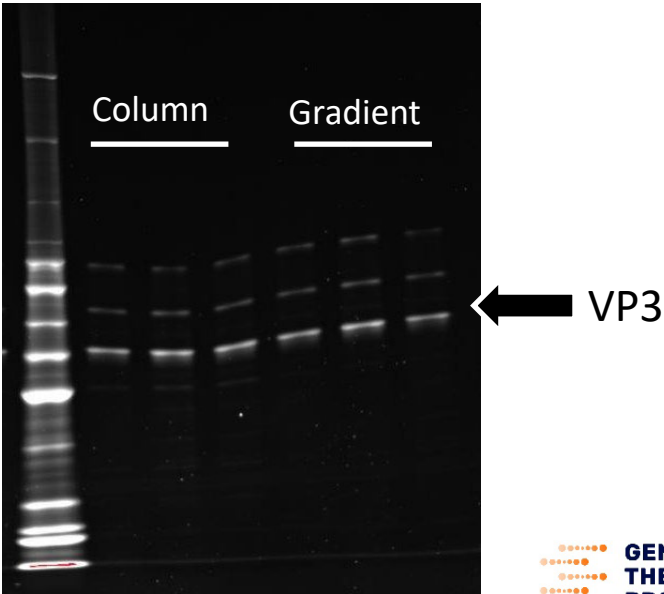
Study : **Purification
method effect**



All animals baseline NAb titer <1:5

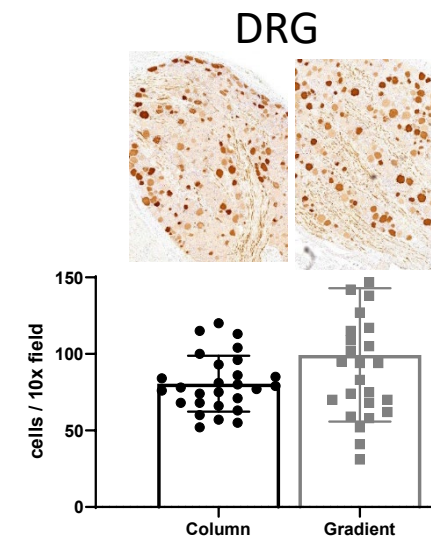
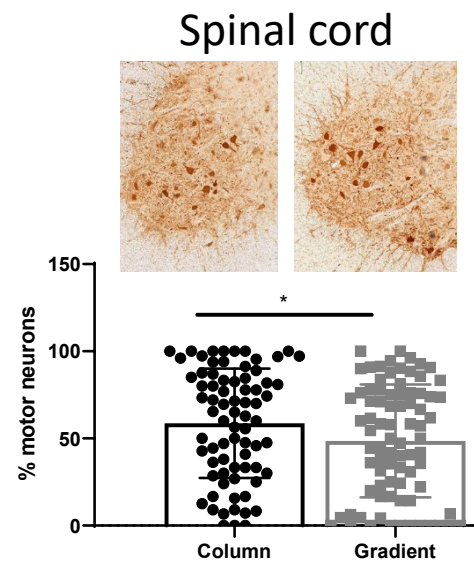
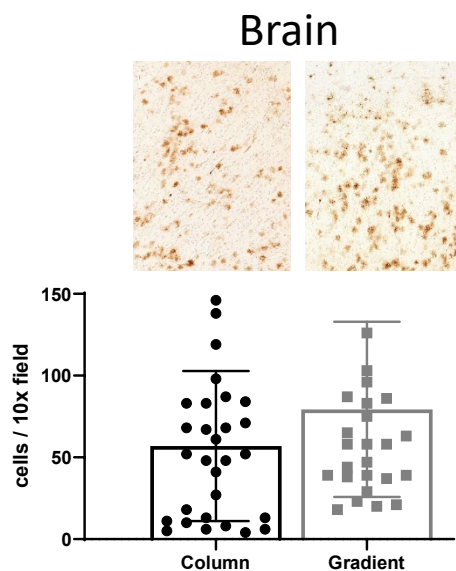
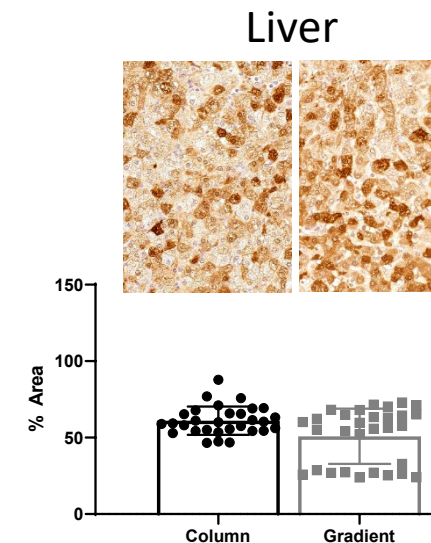
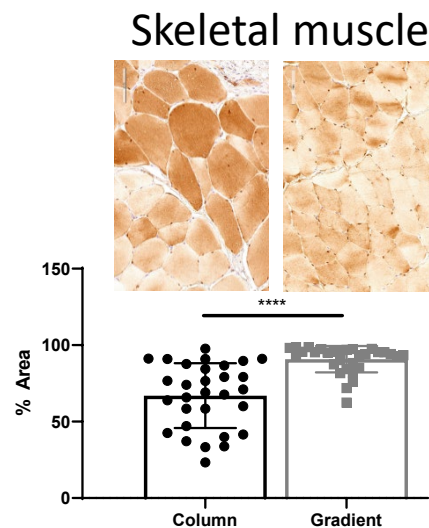
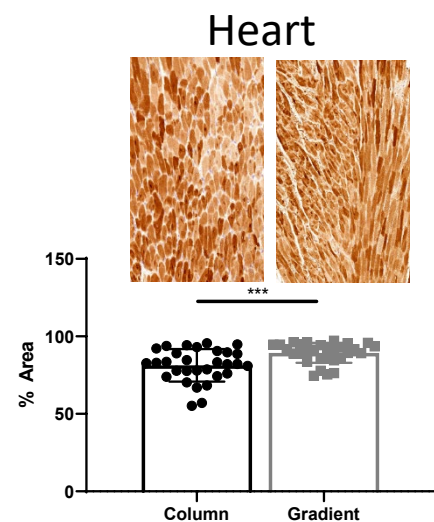
	Column Purified	Iodixanol Purified
Titer dd PCR (GC/ml)	5.87 E13	8.79 E13
Full:empty ratio (%)	89 : 11	71 : 29
Potency % Reference lot	40	68
Endotoxin (EU/ml)	<0.500	<0.500

SDS Gel Purity



Study presented 05/11/2021, abstract #46 ASGCT 2021

Method of Purification Had Little Effect on Transduction Profiles

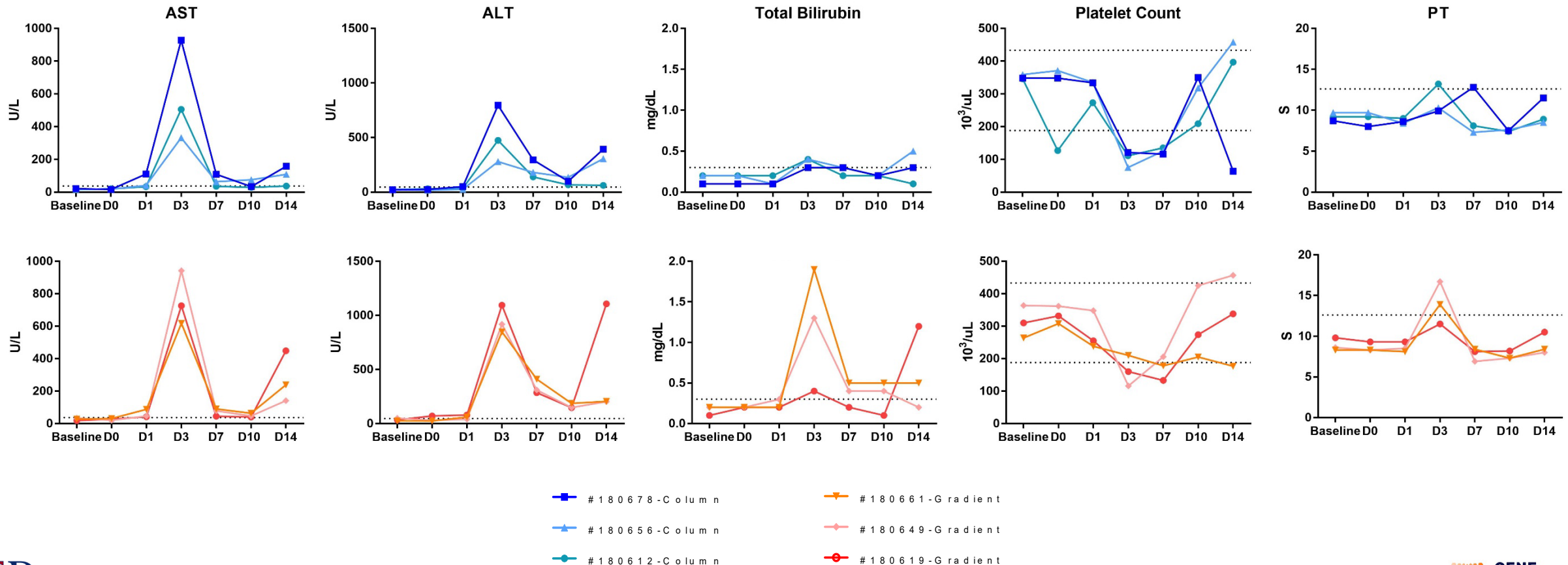


Transient Acute Toxicity in Both Groups

- Transient acute toxicity manifested on day 3 post-vector administration; all animals recovered
- Thrombocytopenia in 5/6 animals, marked liver enzymes elevation in 6/6, bilirubin increase in 2/6, increased coagulation times in 3/6
- Haptoglobin (not shown) unchanged or increased at day 3, normal RBC: no hemolysis; renal function unchanged

Column

Gradient

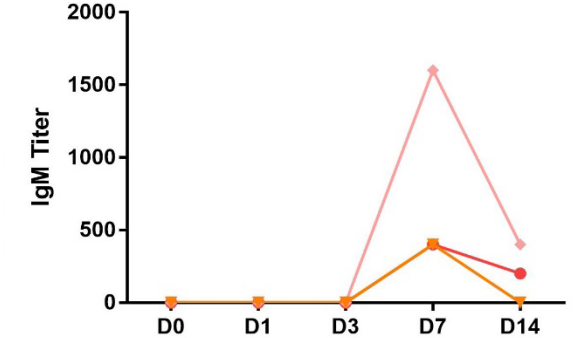
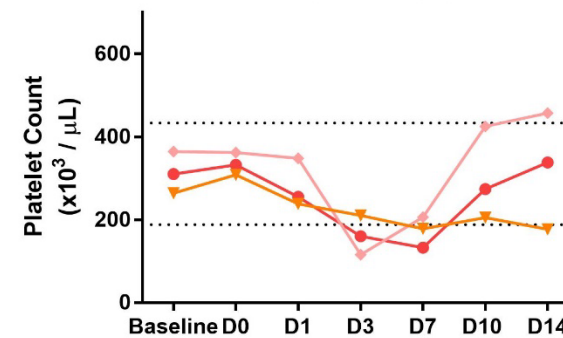
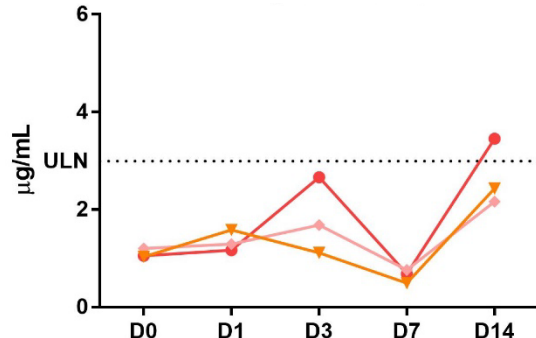
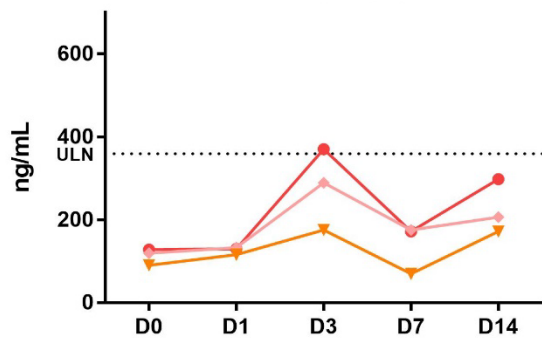
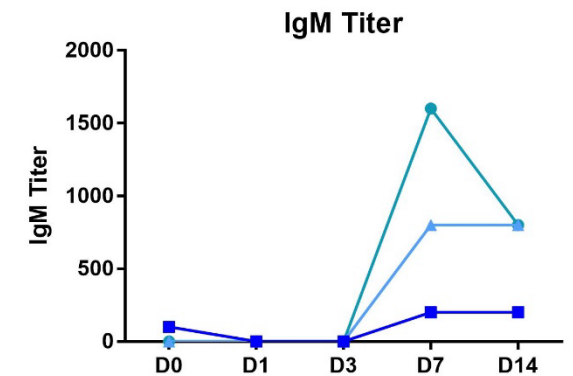
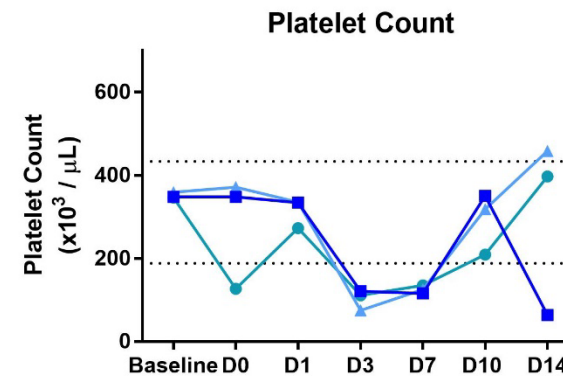
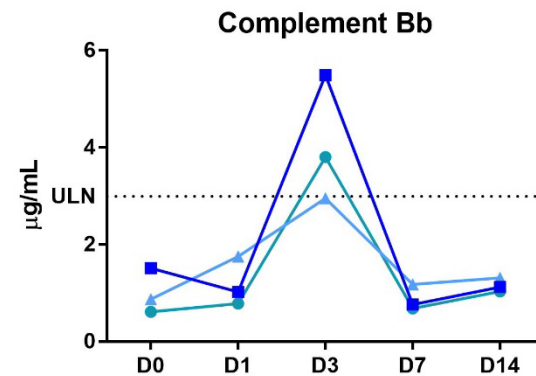
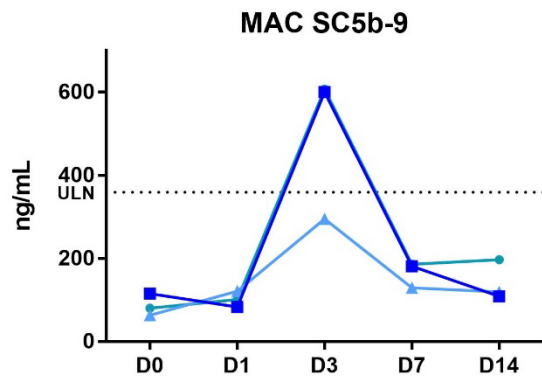


Acute Toxicity Associated With Activation of Alternative Complement Pathway

- Total SC5b-9 elevation showed transient complement activation on day 3
- Increase in Bb suggests activation of alternative complement pathway
- Parameters specific for classical pathway were not elevated (C4, C4a)
- Animals were Ab negative to AAV at baseline with increases in IgM detectable at day 7 but absent at day 3
- No evidence that immune complexes are the inciting event for acute toxicity

Column

Gradient

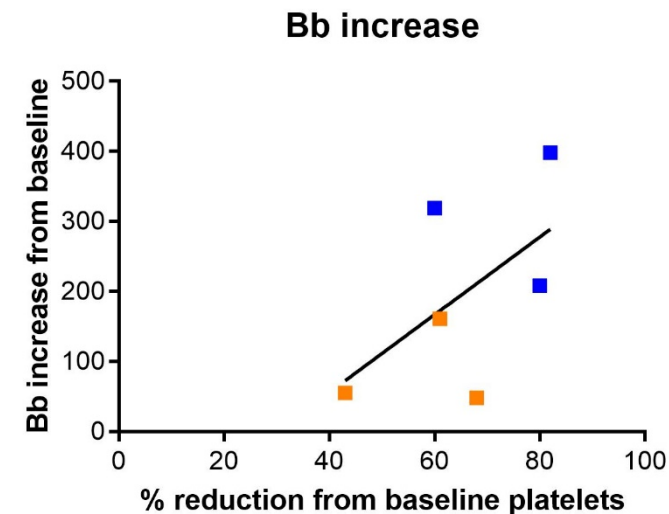
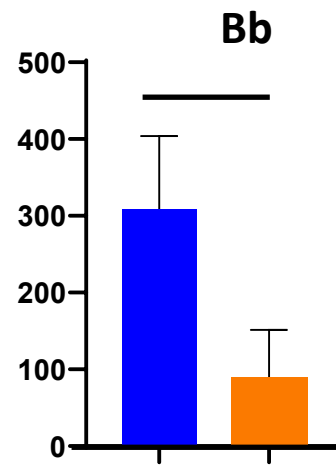
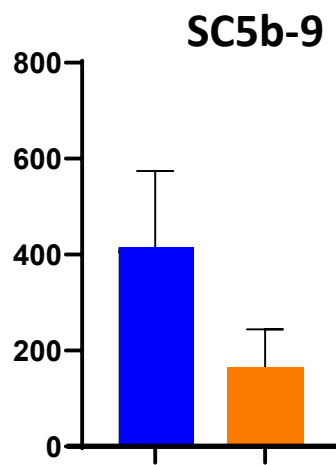
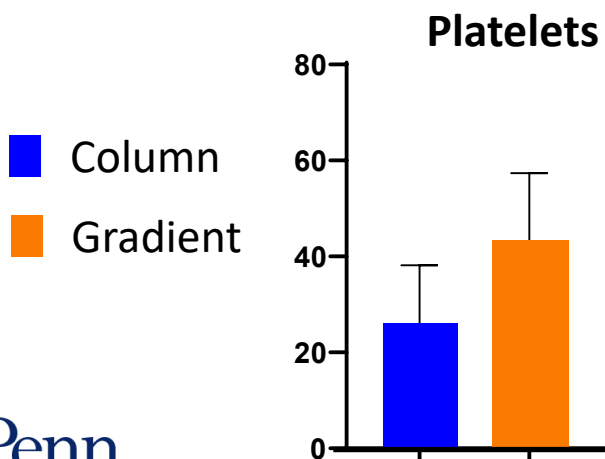
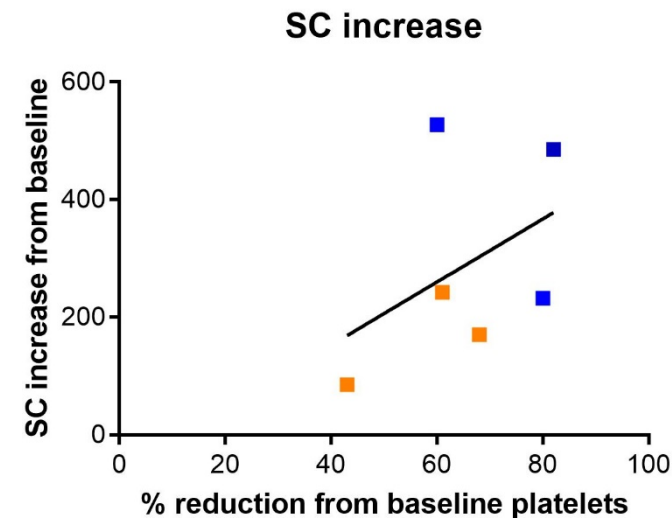
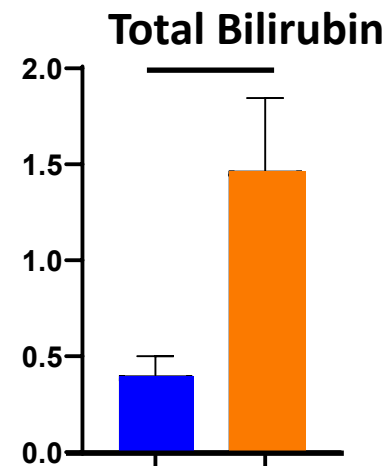
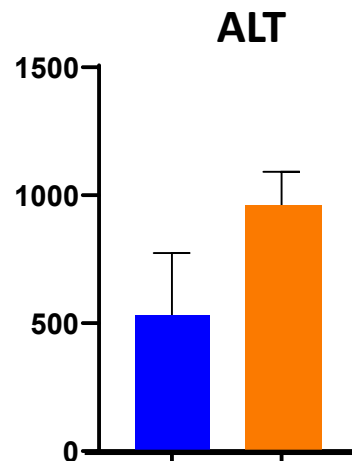


■ #180678-C
▲ #180656-C
● #180612-C

▲ #180661-G
● #180649-G
● #180619-G

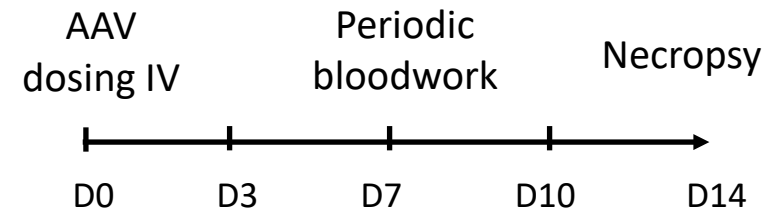
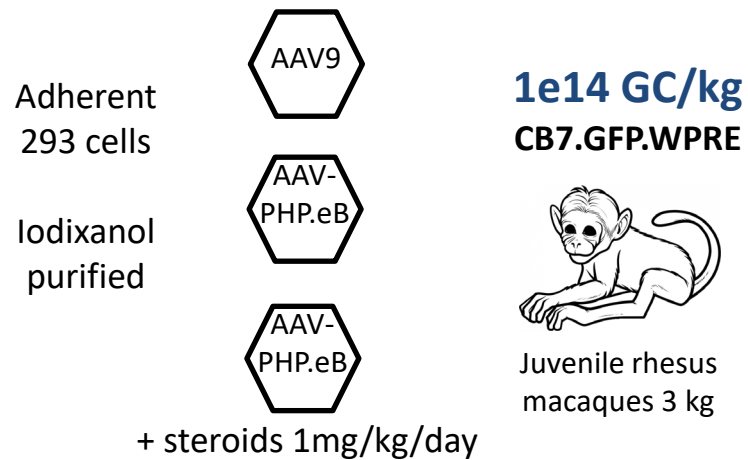
Impact of Downstream Process on Vector Toxicity

- Column material results in greater activation of complement and a greater reduction in platelets
- There is a correlation between complement activation and platelet reduction across all groups
- Gradient material results in more liver toxicity based on ALT, TB and pathology (data not shown)
- These are trends with statistical significance achieved only with TB and Bb ($p < 0.05$) by unpaired *t* test.



Studying the Impact of Capsid on Acute Toxicity and the Potential Impact of Steroids

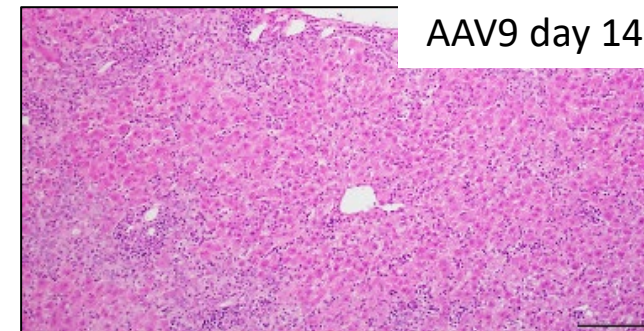
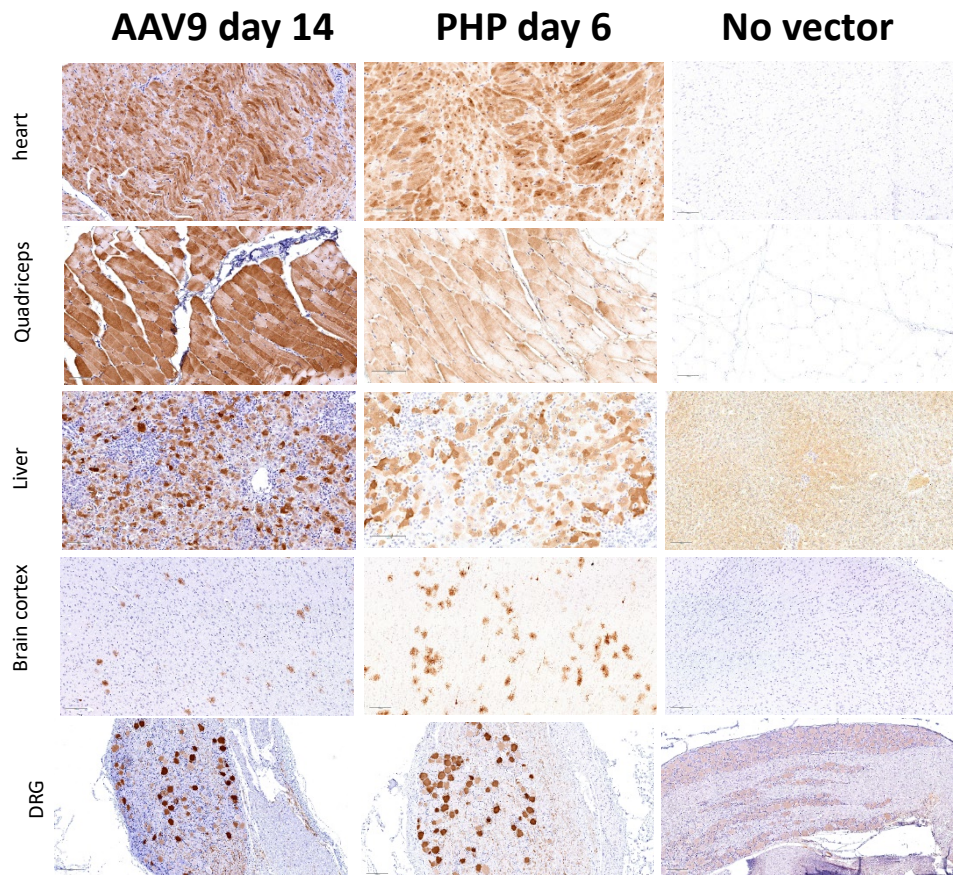
Study : Capsid and steroid effect



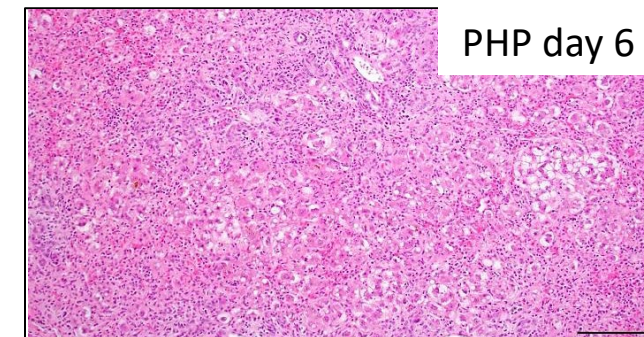
All animals baseline NAb titer <1:5

AAV9 and PHP Demonstrate Broad Distribution of Transduction Following IV 1e14/kg in Rhesus Macaques

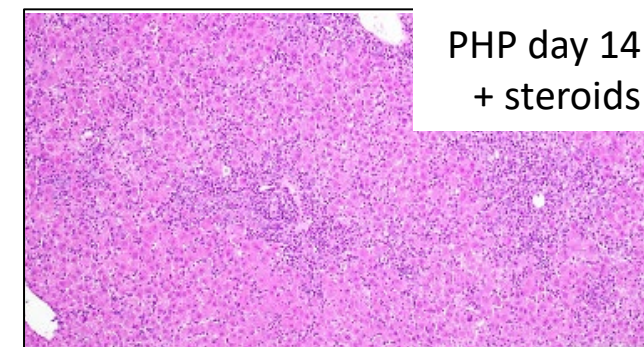
- Widely disseminated transduction with both AAV9 and PHP; no differences
- Steroids reduce liver toxicity as evaluated by histopathology



Moderate-to-minimal degeneration, infiltrates and bile stasis



Severe hepatocellular loss, degeneration and individual cell necrosis with bridging fibrosis, bile duct hyperplasia, canalicular bile stasis, mononuclear cell infiltrate



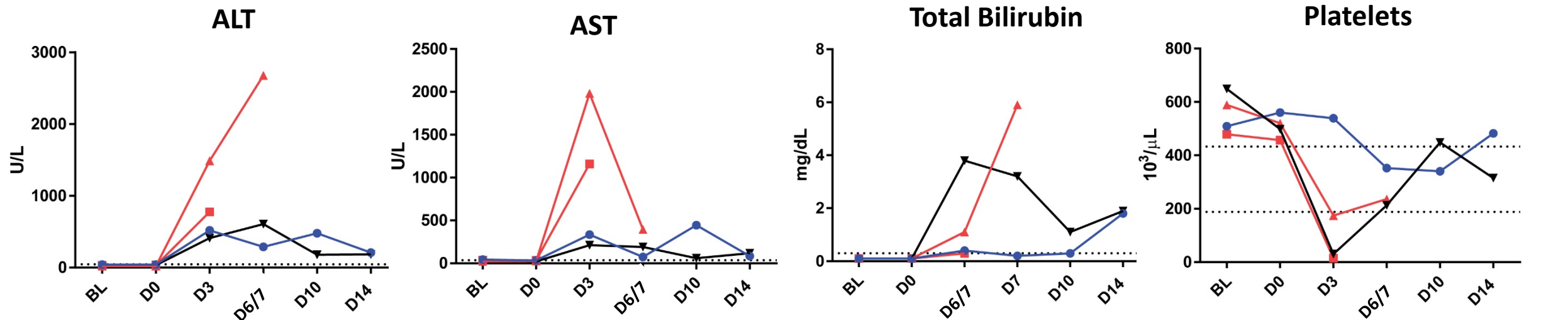
Moderate-to-minimal degeneration, infiltrates and bile stasis

Steroids Diminishes Liver Toxicity But Not Thrombocytopenia and Coagulopathy

- Acute liver toxicity, thrombocytopenia, and coagulopathy are much worse with PHP than AAV9
- Euthanasia required for PHP animals at days 3 and 6
- Steroids reduce liver toxicity of PHP but have no impact on thrombocytopenia and coagulopathy

- 17-029 AAV9
- ▲ 17-044 PHP.B
- 17-030 PHP.B
- ▼ 17C033 PHP.B + Steroids

	Baseline	Day 6 PHP	Day 3 PHP + steroids
PT (sec)	9	42	43
PTT (sec)	23	49	53
Fibro (mg/ml)	156	35	76



Non-linear Dose-Response of Transduction and Toxicity Following Systemic High Dose AAV9 and Related Variants

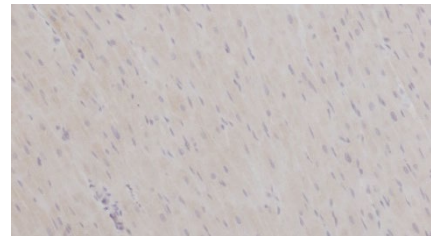
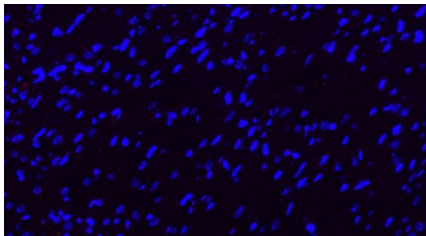
Unpublished data

Cardiac transduction 28 days following IV AAV expressing cardiac gene

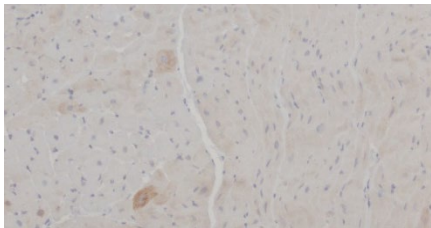
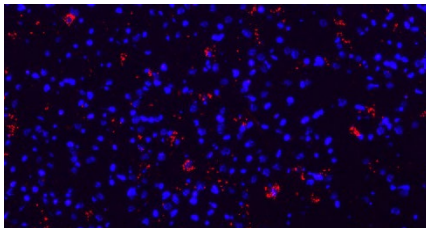
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IHC

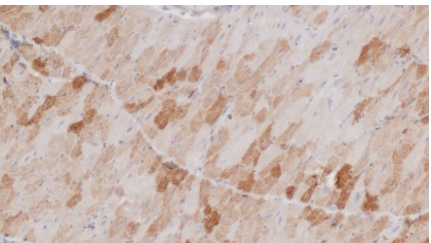
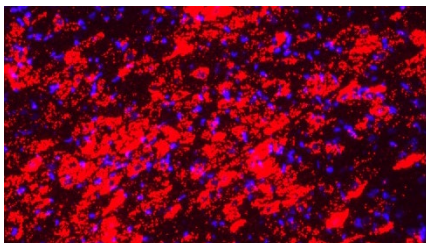
Untreated



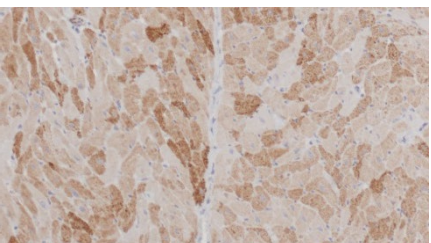
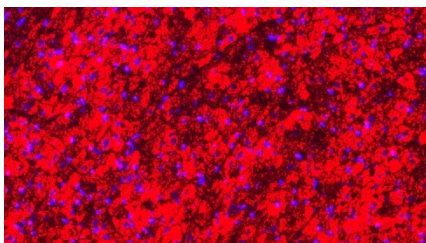
1×10^{13} GC/kg



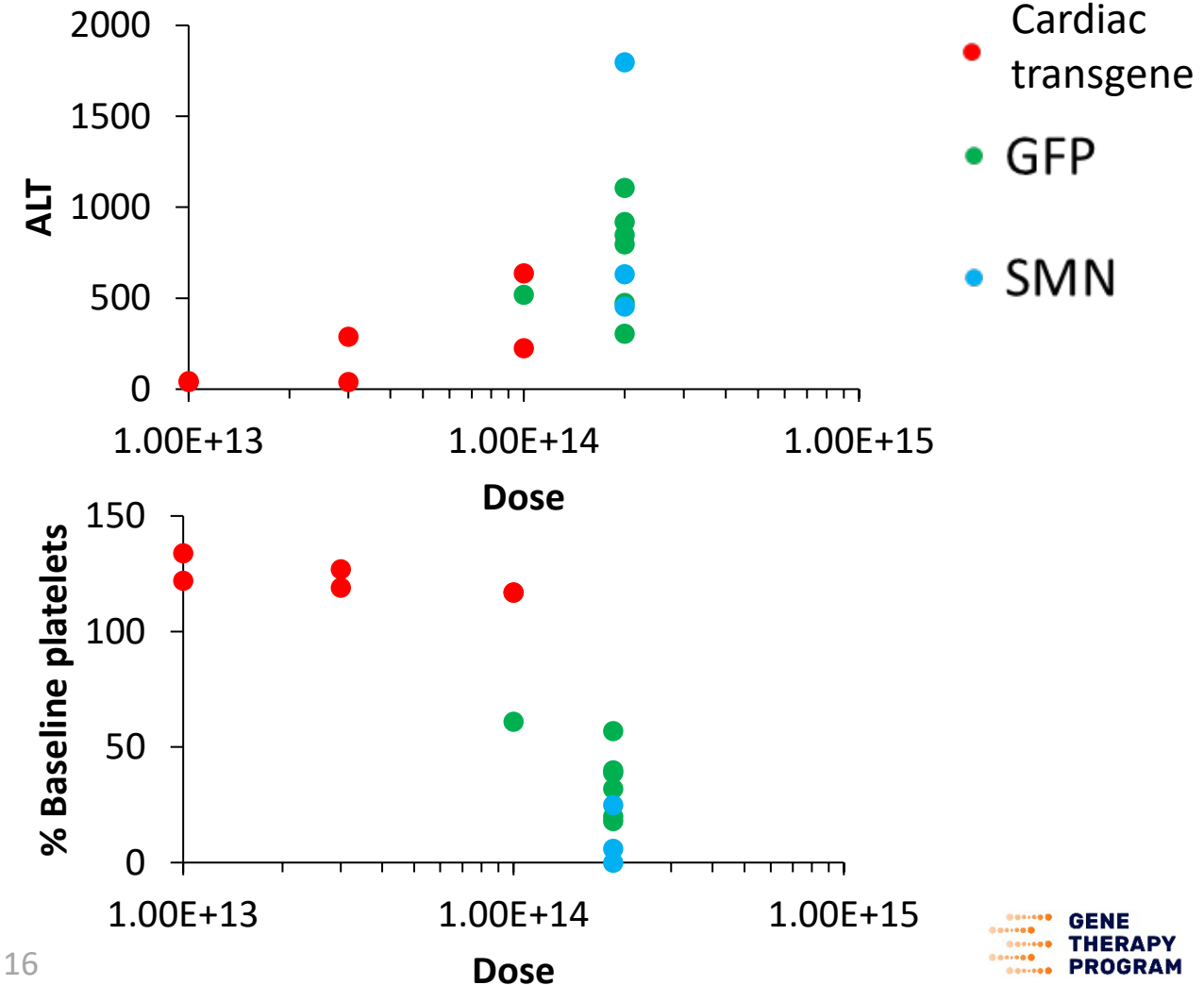
3×10^{13} GC/kg



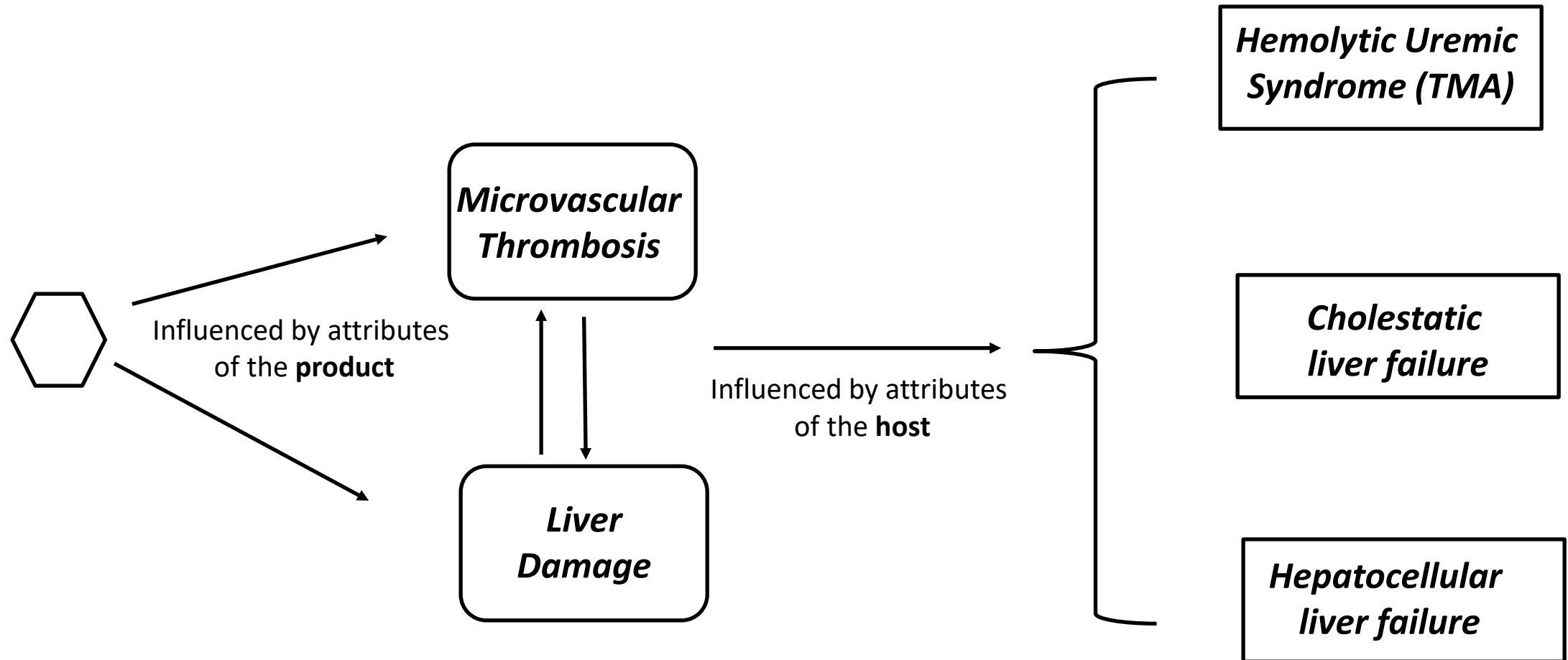
1×10^{14} GC/kg



Peak ALT and platelet reduction following IV AAV expressing various transgenes



Model for Toxicities Following High Dose IV AAV



Macaques as a Model for Toxicity of Systemic AAV

- Where is there **agreement** with human studies?
 - Acute host responses of thrombocytopenia +/- transaminase elevations
 - Pathology (NHPs) and laboratory (NHPs and humans) evidence for microvascular thrombosis
 - Development of hepatocellular liver failure
 - Non-linear (biphasic) dose response of efficacy and toxicity
 - Threshold for serious toxicity (2E14 GC/Kg)
 - Variation between research subjects
- Where are there **differences** with human studies?
 - Time course of severe liver toxicity that can be delayed in humans
 - No evidence of HUS in the setting of microvascular thrombosis
 - Evidence for bile stasis but not severe cholestatic liver failure
- Limitations of macaques
 - Can't provide supportive care (e.g., pressors, assisted ventilation etc.) to allow for an assessment of the evolution of toxicity syndromes
 - Hard to evaluate impact of disease-specific host factors