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Gene Therapy Utilizing Adeno-Associated Viral (AAV) Vectors: Historical Data Review to Characterize Common Challenges and Identify Opportunities for Refinement

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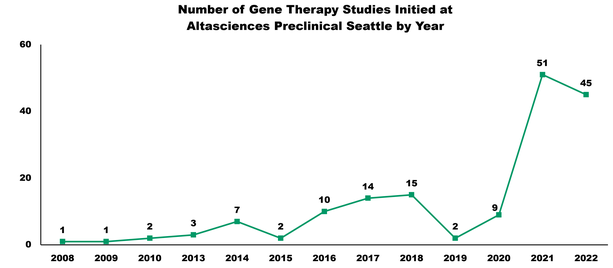
ABSTRACT

Recently, there have been significant advances in gene therapy utilizing viral and non-viral vectors at both the preclinical and clinical stages of drug development. In the preclinical space, safety assessment of adeno-associated viral (AAV) vector-based therapeutics requires the use of the nonhuman primate (NHP) as the main species; the presence of naturally occurring neutralizing antibodies (nAb) in the primate population necessitates screening of a large number of animals to obtain an adequate negative titer cohort for the study. Additionally, pre-treatment with corticosteroids prior to AAV vector administration is often required to counter any adverse reactions. Given the unique challenges of working with AAV vector-based test articles, we reviewed data collected across 22 toxicology studies conducted in the past few years with the aims of (1) establishing ranges for the number of animals to screen for nAb; (2) establishing the dosage range for commonly used corticosteroid given prior to AAV vector administration; and (3) describing the most common in-life findings. Approximately 38% (430/1119) of animals screened for nAb against AAV8 were suitable for study assignment based on established criteria for negative or low viral titers by AAV neutralizing antibody assay (≤ 5 nAb50 in HEK293 cells). Pretreatment with 2 mg/kg of dexamethasone at approximately 1-2 hours prior to AAV administration was adequate to mediate immune-related responses. There was no discernable effect of AAV administration on body weight, and most abnormal post-dose clinical signs were minor and not directly attributable to the AAV vector. In conclusion, this historical data set serves as a guide for more informed study designs for AAV vector-based therapeutics and allows for potential reduction and refinement of animal use in their safety testing.

INTRODUCTION



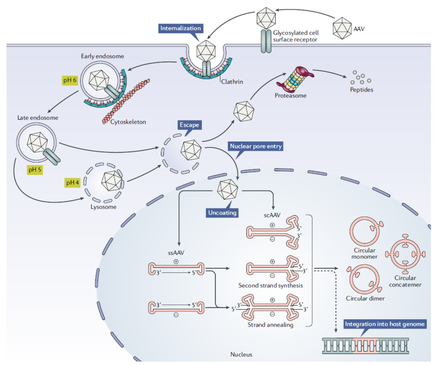


Figure 1. Diagram of rAAV Transduction Pathway

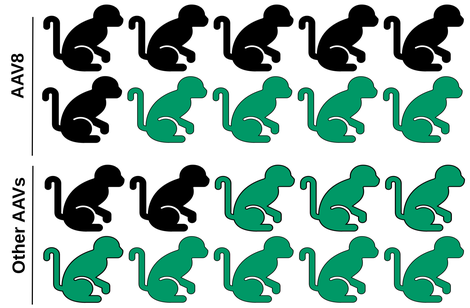
Wang, D., Tai, P.W.L. and Gao, G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov* **18**, 358–378 (2019). https://doi.org/10.1038/s41573-019-0012-9

- AAV is a non-enveloped virus that can be engineered to deliver DNA to target cells.
- Eleven serotypes of AAV have been identified that differ in the types of cells they infect, making AAV a very useful system for preferentially transducing specific cell types.
- AAVs are the leading platform for gene delivery for the treatment of a variety of human diseases and are often utilized for the delivery of CRISPR/Cas9 components for genome editing.

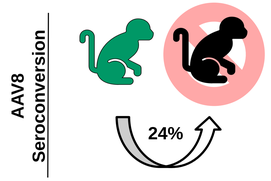
Neutralizing Antibodies (nAb) Prescreening and Seroconversion Rate:

Approximately 38% (430/1119) of NHPs screened for nAb against AAV8 were suitable for study assignment based on established criteria for negative or low viral titers by AAV neutralizing antibody assay (≤ 5 nAb50 in HEK293 cells).

Animals were less likely to have nAb against other AAVs. Approximately 81% (123/152) of animals screened for AAV1 were suitable for use on study, 77% (23/30) for AAV2, 73% (22/30) for AAV3, 100% (30/30) for AAV5 and AAV6, 67% (20/30) for AAV7, 73% (44/60) for AAV9, and 80% (24/30) for AAV10.



For AAV8, rescreening of animals 5 to 7 months following initial viral titer assessment revealed 24% (32/135) were positive (≥10 nAb50 in HEK293 cells) for nAb when they previously had low to negative viral titers.



Predose Corticosteroid Administration Prior to Dosing:

Pretreatment with 2 mg/kg of dexamethasone at approximately 1-2 hours prior to AAV administration was adequate to mediate immune-related responses. Of the studies reviewed, 50% (11/22) utilized pretreatment.

Pretreatment	Dose Level (mg/kg)	Dose Route	Administration Timing
Dexamethasone	2	IV	30 minutes to 2 hours pre-dose

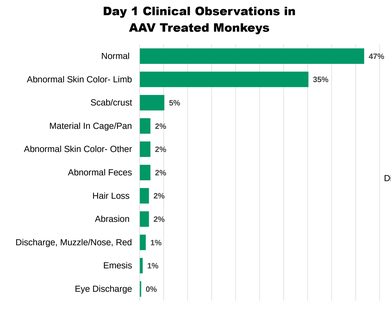
AAV Dose Administration:

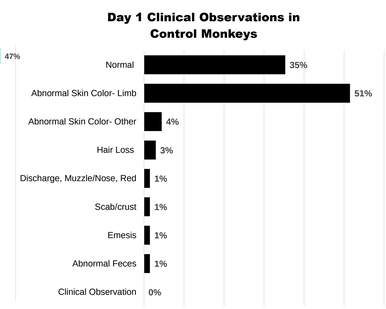
Common AAV study designs include the administration of AAVs alone or in combination with other test materials at viral concentrations of 1.5 x 10^{11} to 6 x 10^{13} vg/mL. Dose administration typically occurs via IV bolus injection or IV infusions at durations of up to 1 hour.

Dose Concentration (vg/mL)	Dose Volume (mg/kg)	Dose Route	Administration Duration
1.5 x 10^{11} to 6 x 10^{13}	1 to 10	IV bolus or IV infusion	Up to 1 hour

Common Clinical Observations in Animals Administered AAVs on Day 1:

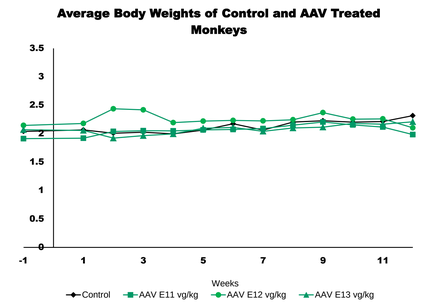
The most common clinical observations on Day 1 in animals administered AAVs were similar to those observed in control animals and typical of animals housed in similar laboratory conditions. Observations included bruising/abnormal skin color, abnormal feces, hair loss, abrasion, and, scab/crust. Compared to controls (1 observation), increased incidence of emesis or evidence of material in the cage/pan (11 observations) in animals administered AAV without pretreatment with corticosteroids may be indicative of immune activation.





Body Weight Changes in AAV vs. Control Animals:

Body weight changes assessed over 12 weeks post-AAV administration were comparable to concurrent control animals in both males and females.



CONCLUSIONS:

The number of safety assessment studies run at Altasciences utilizing AAV vectors for gene therapy applications has increased significantly in the last few years. Prior to the initiation of a program utilizing AAVs, it is important to understand the necessity and constraints of screening animals for nAb against the specific AAV types. Once animals are selected, the AAV is typically administered by IV-bolus injection or infusion, depending on the viral concentration and dose-volume constraints. Premedication with a corticosteroid may help to mediate immune-related responses to AAV administration. Clinical observations on Day 1 in AAV animals are similar to control animals and typical of animals housed under laboratory conditions. Observations indicative of emesis may be related to AAV administration in animals which were not pretreated with corticosteroid but are typically limited to Day 1. Over the course of the study, significant body weight changes are not observed following AAV administration.