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The Nonhuman Primate Model of CNS Therapies and Utility of Adeno-Associated Viral (AAV) Vectors in Gene Therapy: From Discovery to IND-Enabling Studies

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ABSTRACT

Making the transition from exploratory discovery proof of concept studies to IND-enabling studies requires a coordinated effort between discovery researchers and toxicologists. Discovery studies require specialized delivery techniques and fewer animals, while IND-enabling studies transition to more standard routes of administration. For example, MRI-guided technologies allow delivery into specific areas of the brain, while intrathecal dosing allows delivery to reach the cerebrospinal fluid via injection into the spinal cord or into the subarachnoid space. The overall goal of these approaches is to circumvent the blood-brain barrier. In this presentation, we will provide examples of the specialized delivery methods in the nonhuman primate gene and cell therapy model. A discussion will include historical information, and data gathered in recent years relating to gene therapy. Here, we reviewed data collected in a large number of studies conducted in the past few years with the aims of (1) establishing ranges for the number of animals to screen for neutralizing antibody (nAb), (2) establishing the dosage range for commonly used corticosteroid given before AAV vector administration. Approximately 37% (182/491) 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 before AAV administration was adequate to mediate immune-related responses.

INTRODUCTION

Animal use procedures approved via protocol by the IACUC
Gene therapy and stem cell transplantation:

- MRI-guided intraparenchymal delivery into brain regions (cortical and subcortical)—Discovery
- AAV vector delivery using intrathecal (IT), intravenous (IV), and other routes of administration (ROA)—Discovery and IND-enabling general and ocular toxicology studies
- Samples collected during in-life—liver (up to 150 mg), muscle, skin, blood

Table 1. Enhanced Delivery—Discovery at Altasciences Sacramento

Organ System	Method	Number of Studies Completed
Brain—putamen, thalmus, cerebellum, intracerebroventricular	MRI-guided	51
	Stereotaxic (non-MRI-guided)	2
Spinal cord	Intrathecal	14
	Cisterna magna	3
	Chronic CSF port	49
Ocular	Intravitreal	25

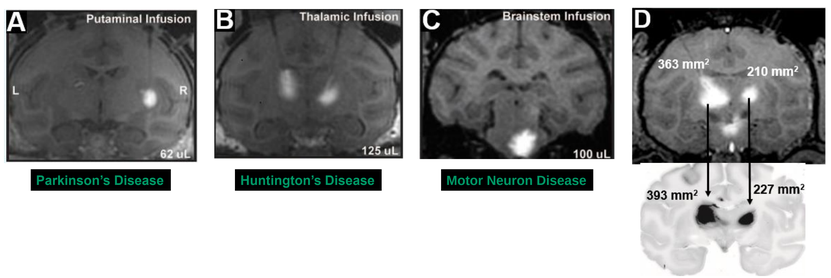

Figure 1. MRI Images Showing Real-Time Monitoring of Infusate Into Various Target Regions of Interest

IND-ENABLING STUDIES—GENE THERAPY

- + Biodistribution
- + Screening animals for anti-AAV antibodies
- + De-risk germline editing/inadvertent germline integration of gene therapy (GT) vectors

Table 2. Naïve Screening Initial Pre-study Positivity Rate—Neutralizing Antibodies

AAV Serotype	Number of Animals Screened	% Positive	% Negative
AAV1	152	19	81
AAV2	30	23	77
AAV3	30	27	73
AAV5	30	0	100
AAV6	30	0	100
AAV7	30	33	67
AAV8	1119	62	38
AAV9	120	32	68
AAV10	30	20	80

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.

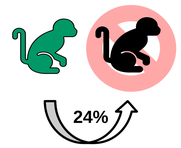

Figure 2. AAV8 Seroconversion

IMMUNOSUPPRESSION PRIOR TO DOSING

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

Table 2. Immune Suppression Prior to Dosing GT Products

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 infusion at durations of up to 1 hour.

Table 3. Viral Vector Dose Range and Duration of Dosing

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

MOLECULAR BIOLOGY LAB SUPPORT

Ligand Binding, PCR

- Biodistribution
- RNA quantification
- Relative gene expression
- Vector shedding
- Germline editing/integration: oocytes/sperm

IMMUNOGENICITY EVALUATIONS ASSAYS

Table 4. Examples of Common Assays

Assay	Platform	Characteristics
Anti-AAV capsid tAbs	Immunoassay	Tiered approach (screen, confirm, titer)
Anti-transgene protein tAbs	Immunoassay	Tiered approach
Anti-AAV capsid nAb	Cell-based assay	Inhibition of transduction
Anti-transgene protein nAb	Cell-based assay	Inhibition of activity
Anti-AAV capsid of anti-transgene protein T cell response	ELISPOT Flow cytometry	Cytokine responses Immunophenotyping
Anti-transgene antibodies	Immunoassay	Tiered approach

IN-LIFE STUDY CONDUCT RESULTS

- Incidence clinical observations on day 1

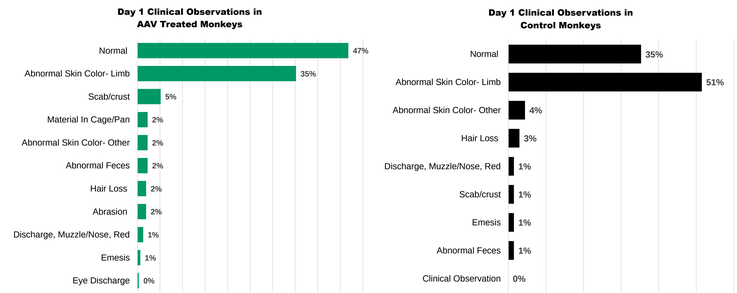

Figure 3. Clinical Observations in AAV-treated and Control Monkeys

- Body weight changes in AAV vs. control animals

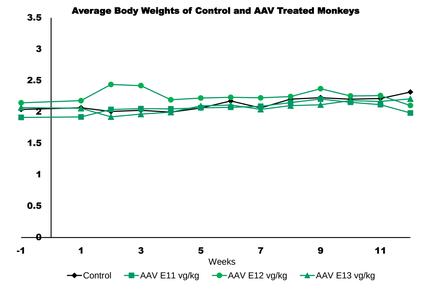

Figure 4. Body Weight in AAV-treated and Control Monkeys