Altasciences, 6605 Merrill Creek Pkwy, Everett, WA 98203, United States
Altasciences.com | contact@altasciences.com

CNS-Targeted Therapies Delivery Strategies and Sampling in Non-rodent Preclinical Species: Considerations During Early Phase Discovery to IND-Enabling Regulatory Studies

Norbert Makori
Altasciences, Seattle, WA, United States

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ABSTRACT

Continuous refinement of delivery techniques and sampling methods in large animals used in preclinical studies is among several critical steps in developing safe and effective therapies, such as anti-sense oligonucleotides and gene and cell therapies. In addition, collecting tissue samples for analysis, either during in-life or at necropsy, requires a clear understanding of all steps to ensure sample quality and correctly process the tissues for analysis. These requirements apply to exploratory early discovery research and toxicology studies, thus requiring a coordinated effort between research scientists and toxicologists. Generally, discovery phase research requires specialized delivery techniques and fewer animals, while IND-enabling toxicology studies transition, in most part, to more standard routes of administration. To investigate biodistribution, the analysis of tissue may require collection both during in-life and necropsy. MRI-guided technologies in nonhuman primates allow delivery into specific areas of the brain. In contrast, intrathecal dosing into the spinal cord or the subarachnoid space allows a therapy to reach the cerebrospinal fluid. The overall goal of these delivery approaches is to circumvent the blood-brain barrier. In this presentation, we will provide examples of specialized delivery methods and sampling (in-life and necropsy) in the nonhuman primate model of gene and cell therapy. A discussion will include historical background information, and data gathered in recent years relating to gene therapy. Data from many studies conducted in the past few years was also reviewed with the aims of (1) establishing ranges for the number of animals to screen for neutralizing antibodies (nAb), (2) establishing the dosage range for commonly used immunosuppressive regimens given before adeno-associated virus (AAV) administration. Approximately 36% (438/1219) of animals screened for nAb against AAV8 were negative and therefore suitable for study assignment based on established criteria for negative or low viral titers by AAV neutralizing antibody assay (≤ 5 nAb50 in HEK293 cells), while 61% (97/160) screened for AAV9 were negative. Generally, pretreatment with 2 mg/kg of dexamethasone at approximately 1-2 hours before AAV administration was adequate to mediate immune-related responses.

MATERIALS AND METHODS

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

RESULTS—EARLY DISCOVERY: NONHUMAN PRIMATES MODEL

Figure 1. MRI images showing real-time monitoring of infusate into various target regions of interest—volume to be distributed based on pre-scanning data performed one week before scheduled dosing.

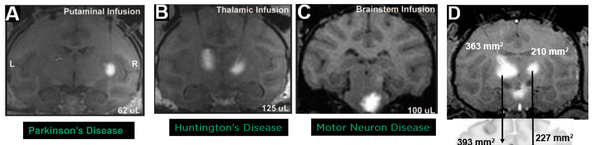
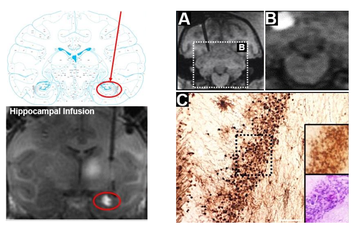


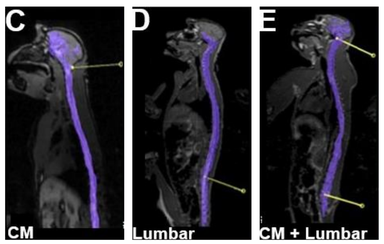
Figure 2. Infusing into “deeper” regions of interest

Hippocampal delivery Cerebellum delivery



Hematoxylin and eosin staining and immunostaining against green fluorescent protein staining were performed six weeks post-infusion.

Figure 3. Delivery into cisterna magna (CM) or lumbar intrathecal space



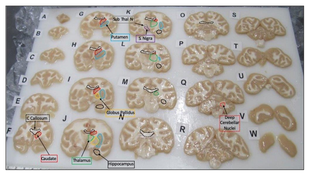
Chronic CM catheter

- Vascular access ports
- CSF collection on chair-trained NHPs

Assessment of distribution

- Cervical via cisterna magna puncture
- Lumbar puncture

Figure 4. Brain Regions of Interest For Assessment of Delivered Test Material



Acknowledgments

Figures1-4 Images credit: Valley Biosystems (2018)

IND-ENABLING STUDIES

A: Biodistribution

- High-throughput isolation preparation
  - QIAcube HT; KingFisher
- DNA and RNA
  - Quantification and dilution
- Vector Shedding

B: Screening for Anti-AAV Antibodies in Nonhuman Primates

- Screen animals before study placement for nAb and/or Total Ab (TAb)

Table 1. AAV-Specific Sero-Negativity Rate/Origin—Altasciences

AAV Serotype/Origin	% Negative (n=Number of Animals Screened)		
	Cambodian	Mauritian	Philippine
AAV1	81 (n=30)		
AAV2	77 (n=30)		83 (n=24)
AAV3	73 (n=30)		
AAV5	100 (n=100)		97 (n=34)
AAV6	100 (n=30)		
AAV7	67 (n=30)		
AAV8	36 (n=1219)	44 (n=25)	
AAV9	61 (n=160)	40 (n=25)	79 (n=34)
AAV10	80 (n=30)		

C: De-risk Germline Editing/Inadvertent Germline Integration of Gene Therapy (GT) Vectors: Oocyte and Sperm PCR

Figure 5. Oocyte preparation for genomic analysis. Note the cumulus oophorous around the oocyte in the left image. The oocytes in the right image are clean, with no foreign cells, and are suitable for DNA/RNA isolation.