Optimization of intrathecal delivery of an infused AAV9 vector for delivery of a gene-therapy candidate for adrenomyeloneuropathy in nonhuman primates

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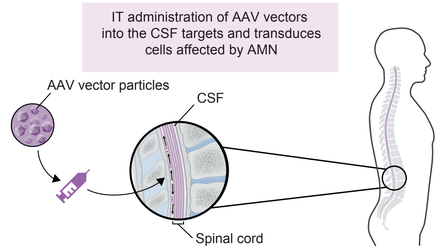
INTRODUCTION

- Adrenomyeloneuropathy (AMN) is caused by mutations in the gene encoding the hABCD1 protein, which transports very long-chain fatty acids from the peroxisome, and is characterized by a dying-back axonopathy affecting spinal cord tracts that ultimately leads to loss of mobility.
- We are developing SBT101, an adeno-associated virus serotype 9 (AAV9)-based gene therapy encoding functional hABCD1, as a candidate treatment for patients with AMN.
- Prior research in mice indicated that 24-hour intrathecal (IT) lumbar administration delivered the transgene to the entire spinal cord while minimizing biodistribution to the periphery as compared with other routes of administration.[1]
- We have assessed multiple infusion parameters to enhance widespread distribution to the spinal cord and dorsal root ganglia (DRG) following IT delivery of a vector (Figure 1).

Figure 1: IT administration of AAV vector gene therapy in the spinal cord



AAV, adeno-associated virus; AMN, adrenomyeloneuropathy; CSF, cerebrospinal fluid; IT, intrathecal

Objective

- To identify delivery parameters of IT administration of AAV9 that produce widespread gene transfer/biodistribution to the spinal cord and DRG.

Methods

Preclinical analysis of AAV9 delivery and biodistribution

- An AAV9–green fluorescent protein (GFP) reporter vector was used to investigate infusion parameters and their effect on biodistribution to the spinal cord and DRG in nonhuman primates (NHPs) (Table 1).
  - Animals received either a bolus (20 minutes) or an extended infusion (6 hours or 24 hours) of AAV9–GFP (cervical or lumbar).
  - Vector biodistribution throughout multiple tissues was analyzed using two independent approaches: immunohistochemistry and quantitation of vector genome distribution by Droplet Digital PCR (ddPCR).

Table 1: Study design – infusion parameters

Model	Number of animals	Test article	ROA	Dose (vg/an)	Volume (mL)	Infusion duration
IT lumbar infusion vs IT cervical infusion						
Male cynomolgus monkeys	n = 3 per group	AAV9–GFP	IT-L	1.06E13	2.5	20 minutes
				1.26E13	10.0	24 hours
				3.38E13	10.0	24 hours
			IT-C	1.20E13	10.0	24 hours
IT lumbar infusion, volume and time						
Male cynomolgus monkeys	n = 4 per group	AAV9–GFP	IT-L	1.55E13	2.5	6 hours
				1.39E13	5.0	6 hours
				1.46E13	5.0	24 hours
				1.23E13	10.0	24 hours

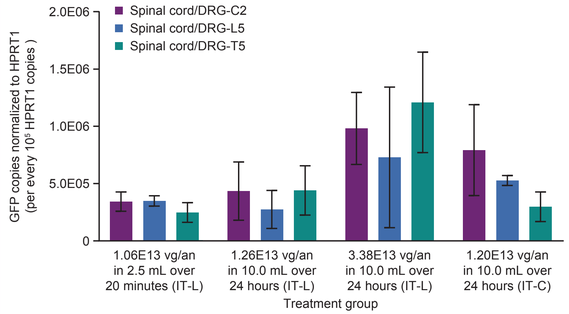
AAV9, adeno-associated virus serotype 9; an, animal; GFP, green fluorescent protein; IT, intrathecal; IT-C, intrathecal cervical; IT-L, intrathecal lumbar; ROA, route of administration; vg, vector genomes

Results

AAV9 biodistribution after IT lumbar and cervical infusions

- IT administration over 20 minutes through 24 hours resulted in a widespread AAV9–GFP biodistribution to the entire spinal cord and DRG at 2 weeks after infusion (Figure 2).
- Lumbar infusion over 24 hours delivered widespread biodistribution to the entire spinal cord and DRG compared with both cervical and bolus delivery (2.5 mL over 20 minutes) (Table 2).

Figure 2: AAV vector biodistribution in the spinal cord and DRG, by delivery method



Plotted values represent mean ± SD; n = 3 NHPs per treatment group
AAV, adeno-associated virus; an, animal; C2, cervical section 2; DRG, dorsal root ganglia; GFP, green fluorescent protein; HPRT1, hypoxanthine phosphoribosyltransferase 1; IT-C, intrathecal cervical; IT-L, intrathecal lumbar; L5, lumbar section 5; NHP, nonhuman primate; SD, standard deviation; T5, thoracic section 5; vg, vector genomes

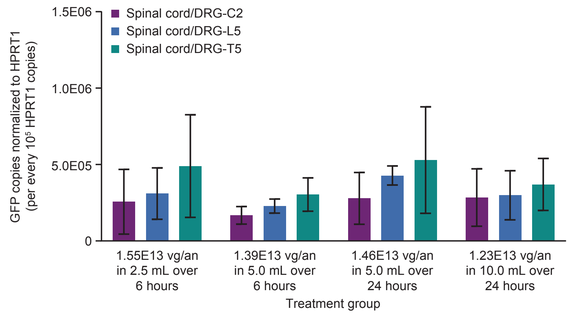
Table 2: Heat map of transgene expression in the spinal cord and DRG, by delivery method

Tissue	Bolus			24-hour IT-L infusion			Cervical		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
Spinal cord									
Cervical					2	2			
Thoracic	2		1	2	2	2	2		1
Thoracolumbar			3	1	3	2	1		
Lumbar	3	3	3	3	5	4	5	4	5
Sacral			3						
DRG									
Cervical		3		2	4	1	1	1	2
Thoracic	3		2	3	4	3		2	3
Thoracolumbar			1		4		1	1	4
Lumbar		1	1	2	5	2	1	2	4
Sacral	4	1	1	4	4		2	3	4

Neuron positivity (%): 5 4 3 2 1

A1 to A3 represent individual NHPs
Percentage neuron positivity is presented as grades 1–5: grade 1, ≤ 1%; grade 2, 1–25%; grade 3, 26–50%; grade 4, 51–75%; grade 5, 76–100%; blanks, unremarkable
DRG, dorsal root ganglia; IT-L, intrathecal lumbar; NHP, nonhuman primate

Figure 3: AAV vector biodistribution in the spinal cord and DRG, by IT-L infusion time



Plotted values represent mean ± SD; n = 4 NHPs per treatment group
AAV, adeno-associated virus; an, animal; C2, cervical section 2; DRG, dorsal root ganglia; GFP, green fluorescent protein; HPRT1, hypoxanthine phosphoribosyltransferase 1; L5, lumbar section 5; NHP, non-human primate; SD, standard deviation; T5, thoracic section 5; vg, vector genomes

Table 3: Heat map of transgene expression in the spinal cord and DRG, by infusion time

Tissue	6-hour IT-L infusion				24-hour IT-L infusion			
	A1	A2	A3	A4	A1	A2	A3	A4
Spinal cord								
Thoracic (cranial)	1			1				
Thoracic (caudal)	1				1	1		
Lumbar	2		2	1	2	3	2	
DRG								
Cervical	2			1	2		1	
Thoracic (cranial)	1		1	1	2		1	
Thoracic (caudal)	1		1	2	2	1	1	
Lumbar	3		2	2	2	2	2	

Neuron positivity (%): 5 4 3 2 1

A1 to A4 represent individual NHPs
Percentage neuron positivity is presented as a grades 1–5: grade 1, ≤ 1%; grade 2, 1–25%; grade 3, 26–50%; grade 4, 51–75%; grade 5, 76–100%; blanks, unremarkable
DRG, dorsal root ganglia; IT-L, intrathecal lumbar; NHP, nonhuman primate

AAV9 biodistribution at 6 hours and 24 hours after IT lumbar infusion

- IT lumbar infusion over 6 hours was equivalent to that over 24 hours for AAV9 biodistribution to the spinal cord and DRG, while total volume delivered did not substantially affect biodistribution (Figure 3 and Table 3).

CONCLUSIONS

- This study evaluated AAV9 biodistribution within multiple tissues through the detection of the transgene and through immunohistochemical protein detection, which is considered more informative.
- Extended IT infusion time (24 hours) was found to provide broader distribution to the target tissue (spinal cord and DRG) than bolus administration (20 minutes).
- Further refinement demonstrated that the time of vector delivery could be shortened from 24 hours to 6 hours to improve the patient experience, a critical element in the clinical setting.
- Even though there was a notable animal-to-animal variation observed in all cohorts, at doses predicted to be clinically relevant, a 6-hour IT lumbar infusion of an AAV9 vector could deliver widespread biodistribution to the spinal cord and DRG.
- Our results further established the optimal route of administration, volume and duration of AAV9 leading to a biodistribution best suited for the treatment of AMN.

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Disclosures: VV, SWC, DWA and KK are employees of SwanBio and may hold stock or stock options

Reference: 1. Gong Y *et al. Hum Gene Ther* 2019;30:544–55

Abbreviations: AAV, adeno-associated virus; AAV, adeno-associated virus serotype 9; AMN, adrenomyeloneuropathy; an, animal; ATP, adenosine triphosphate; C2, cervical section 2; CSF, cerebrospinal fluid; ddPCR, Droplet Digital PCR; DRG, dorsal root ganglia; GFP, green fluorescent protein; hABCD1, human ATP-Binding Cassette sub-family D Member 1; HPRT1, hypoxanthine phosphoribosyltransferase 1; IT, intrathecal; IT-C; intrathecal cervical; IT-L, intrathecal lumbar; L5, lumbar section 5; NHP, nonhuman primate; ROA, route of administration; SD, standard deviation; T5, thoracic section 5; vg, vector genomes

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