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

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Disclosures

- **V Vasireddy** – employee, stock/shareholder: SwanBio Therapeutics
- **SW Clark** – employee, stock/shareholder: SwanBio Therapeutics
- **DW Anderson** – employee, stock/shareholder: SwanBio Therapeutics
- **K Kozarsky** – employee, stock/shareholder: SwanBio Therapeutics

Introduction

- AMN is caused by mutations in the gene encoding the hABCD1 protein, and is characterized by a dying-back axonopathy affecting spinal cord tracts that ultimately leads to loss of mobility
- We are developing SBT101, an AAV9-based gene therapy encoding functional hABCD1, as a candidate treatment for patients with AMN
- We have assessed multiple infusion parameters to enhance widespread spinal cord/DRG distribution following intrathecal delivery of a vector

Objective: to evaluate delivery parameters of intrathecally administered AAV9 that produce widespread gene transfer/biodistribution within the spinal cord and DRG as a candidate treatment for AMN

AAV, adeno-associated virus; AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate; DRG, dorsal root ganglia; hABCD1, human ATP-Binding Cassette sub-family D Member 1

Preclinical Analysis of AAV9 Delivery and Biodistribution

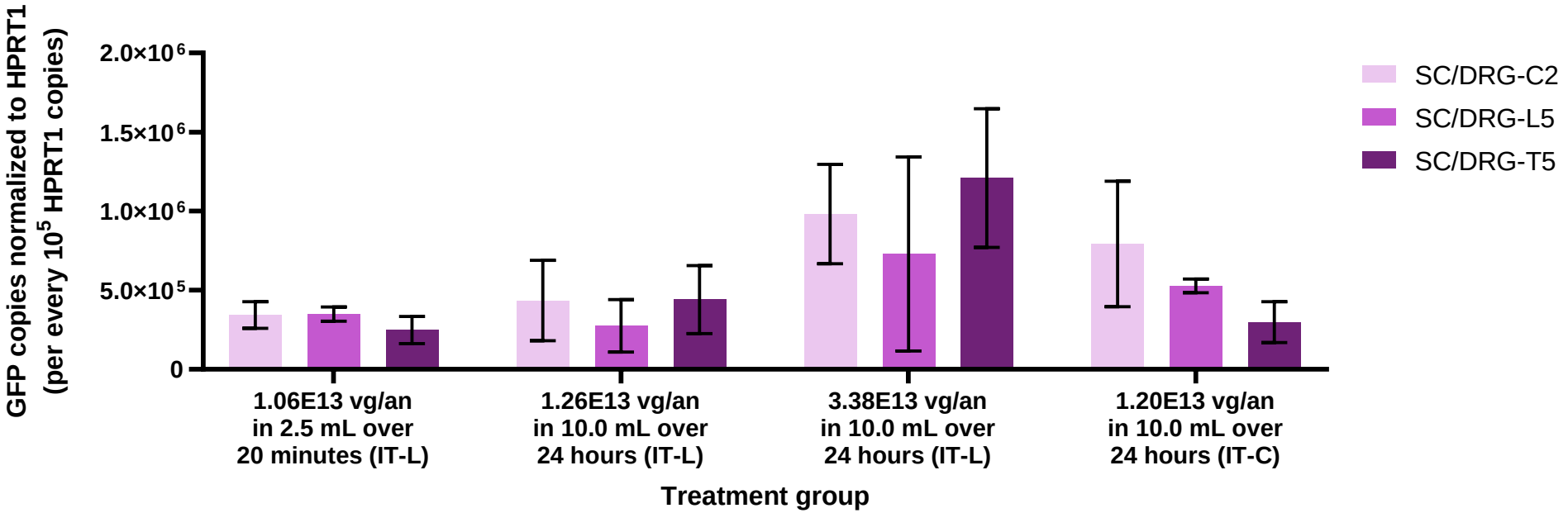
- An AAV9-GFP reporter vector was used to investigate infusion parameters and their effect on biodistribution throughout the spinal cord/DRG in NHPs
  - Animals received either a bolus (20 minutes) or extended (6- or 24-hour) infusion of AAV9-GFP (cervical or lumbar)
  - Outcomes included 14-day survival and immunohistochemistry analysis of GFP throughout multiple tissues

Model	Number of animals	Test article	ROA	Dose (vg/an)	Volume (mL)	Infusion duration
IT lumbar versus 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

AAV, adeno-associated virus; an, animal; DRG, dorsal root ganglia; GFP, green fluorescent protein; IT, intrathecal; IT-C; intrathecal cervical; IT-L, intrathecal lumbar; NHP, non-human primates; ROA, route of administration; vg, vector genome

AAV9 Biodistribution After IT Lumbar and Cervical Infusion

- Widespread AAV9 biodistribution to the entire spinal cord and DRG was observed



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

Slower IT Infusion of AAV9 Shows an Increase in Transduction of Neurons in Spinal Cord and DRG

- Lumbar infusion over 24 hours delivered widespread biodistribution to the entire spinal cord and DRG compared to either cervical or bolus delivery
- Evaluation of transgene expression by immunohistochemistry is more informative than the vector genome distribution
- Can the time of vector delivery be shortened to improve patient experience?

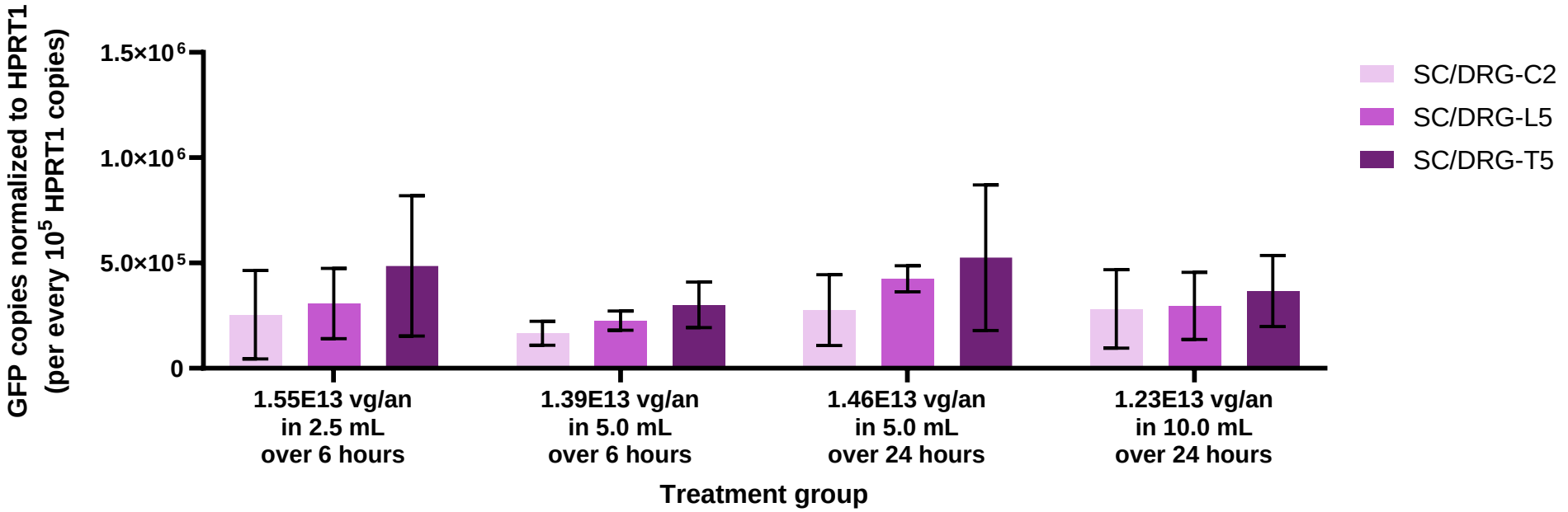
Tissue		Bolus			24-hour IT-L infusion			Cervical		
		A1	A2	A3	A1	A2	A3	A1	A2	A3
Spinal Cord	Cervical					2	2			
	Thoracic	2		1	2	2	2	2		1
	Thoracolumbar			3	1	3	2	1		
	Lumbar	3	3	3	3	5	4	5	4	5
	Sacral			3						
DRG	Cervical		3		2	4	1	1	1	2
	Thoracic	3		2	3	4	3		2	3
	Thoracolumbar			1		4		1	1	4
	Lumbar		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 animals
AAV, adeno-associated virus; DRG, dorsal root ganglia; IT, intrathecal

AAV9 Biodistribution at 6 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/DRG, while total volume delivered did not significantly impact biodistribution



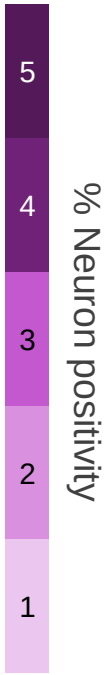
Plotted values represent mean ± standard deviation; n = 4 NHPs per treatment group

AAV, adeno-associated virus; C2, cervical section 2; DRG, dorsal root ganglia; GFP, green fluorescent protein; HPRT1, hypoxanthine phosphoribosyltransferase 1; IT, intrathecal; L5, lumbar section 5; NHP, non-human primate; SC, spinal cord, T5, thoracic section 5; vg, vector genome

Lumbar Infusion Over 6 Hours was Equivalent to that Over 24 Hours for Biodistribution to the Spinal Cord and DRG

- Time of vector delivery could be shortened from 24 to 6 hours to improve patient experience (critical element for clinic)

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	



A1 to A4 represent individual animals
AAV, adeno-associated virus; DRG, dorsal root ganglia; IT-L, intrathecal lumbar

Conclusions

- Extended times of IT infusion provides superior biodistribution over bolus administration
- At doses predicted to be clinically relevant, a 6-hour IT lumbar infusion of an AAV9 vector can deliver widespread biodistribution to the spinal cord/DRG

AAV, adeno-associated virus; DRG, dorsal root ganglia; IT, intrathecal

Acknowledgements

- The authors thank Dr David Gothard of Oxford PharmaGenesis, Oxford, UK, for providing medical writing support, which was sponsored by SwanBio in accordance with Good Publication Practice guidelines

Funding

- This study was funded by SwanBio Therapeutics Ltd