Broad CNS Biodistribution of AAV9-based Gene Therapies Delivered by Intrathecal Lumbar Puncture in Non-Human Primates

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Introduction

Intrathecal (IT) dosing of recombinant adeno-associated viral (rAAV) vectors is a well-studied route of administration (ROA) that has been explored in preclinical and clinical studies for delivering therapeutic transgenes directly to the central nervous system (CNS). As with other CNS-directed ROAs (e.g., intra-cisterna magna (ICM) or intracerebroventricular (ICV) infusion), IT dosing:

- Bypasses the blood-brain barrier
- Reduces vector load in systemic circulation, and
- Minimizes risk of interference of pre-existing anti-AAV antibodies.

Compared to other CNS-directed routes, IT delivery by lumbar puncture may be preferred as a minimally invasive approach for clinical use (**Figure 1**).

- There is debate in the scientific community about the transduction efficiency of AAV9 in non-human primate (NHP) brains, using different ROAs[1-4]
- A survey of ongoing human gene therapy trials in ClinicalTrials.gov shows that IT continues to be the most common route of administration for centrally targeted AAV9 vectors.[5]

Since 2020, Taysha Gene Therapies has developed four rAAV9 candidate gene therapy vectors for rare and ultrarare neurogenetic disorders that advanced to toxicology/ biodistribution studies in NHPs (**Table 1**). **Here, we examine biodistribution of these four vectors, from five studies using lumbar IT or ICM dosing.**

Methods

Across five studies, 67 cynomolgus macaques were studied:

- 47 animals treated with TSHA-101, -102, -105 or -120 at varying doses, of which 28 animals were analysed for vector biodistribution
- 20 control animals treated with vehicle alone

Table 1: Taysha gene therapy vectors studied in NHPs.

Construct	Vector structure*	Neurodevelopmental /neurodegenerative disorder	Therapeutic transgene	ROA
TSHA-101	ssAAV9	GM2 gangliosidosis	β-hexosaminidase A	Lumbar IT
TSHA-102	scAAV9	Rett syndrome	MeCP2	Lumbar IT
				ICM
TSHA-105	scAAV9	SLC13A5-related epilepsy	SLC13A5	Lumbar IT
TSHA-120†	scAAV9	Giant axonal neuropathy	Gigaxonin	Lumbar IT

*Single-stranded (ss) or self-complementary (sc). †TSHA-120 is also called scAAV9/JeT-GAN.

ROAs, dose per animal, and time to necropsy were as shown in **Table 2**.

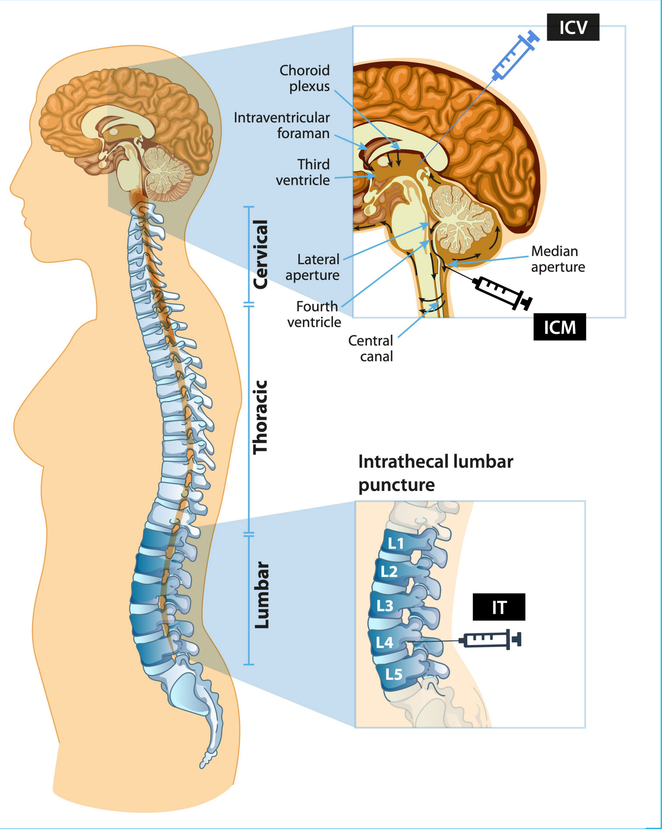
- Overall, five rAAV9 doses were tested, ranging from $3.0x10^{12}$ to $2.3x10^{14}$ vector genomes (vg) per animal.
  - Range corresponds to human equivalent doses of $2.6x10^{13}$ to $2.0x10^{15}$ vg
- CNS biodistribution was assessed following scheduled necropsy.

qPCR was used to quantify rAAV9 DNA in spinal cord (SC) or brain homogenates prepared from prespecified tissue samples. Levels are reported as vector genomes per microgram (vg/µg) NHP genomic DNA.

Protocols for harvesting brain and SC tissues varied across studies

- For TSHA-102 (IT dosing study), biodistribution was examined in two brain regions (right frontal and right parietal lobes) and three SC regions
- For TSHA-120, vector DNA was quantified in a total of 15 brain sections from a series of five coronal slabs along a rostro-caudal axis, including the brainstem in the caudal slab
- For other studies, vector DNA was quantified in six brain regions (frontal lobe, parietal lobe, occipital lobe, temporal lobe, pre-frontal lobe and cerebellum) and three SC regions (cervical, lumbar, and thoracic).

Figure 1: Direct dosing of gene therapy to the CNS by different routes of administration. Adapted from Ref. [5].



In this retrospective examination of five NHP studies quantifying the CNS biodistribution of Taysha vectors, we found that:

- **Lumbar IT dosing led to broad and consistent rAAV9 biodistribution of all payloads across the brain and spinal cord.**
- **Despite differences in proximity to the brain with IT versus ICM injection, biodistribution was comparable between the two routes.**

These findings provide strong support for IT dosing as a minimally invasive approach to CNS delivery of rAAV9 gene therapy vectors.

Table 2: Dosing of Taysha rAAV9 vectors to 47 NHPs across five safety / biodistribution studies.

	Route	Dose per animal (vg)	Human equivalent dose (vg)*	Time to necropsy (days post-dosing)			
				30	90	180	364
TSHA-101	IT	$5.8x10^{13}$	$5.0x10^{14}$	2M, 2F			
TSHA-102†	IT	$5.8x10^{13}$	$5.0x10^{14}$		3F	3F	
TSHA-102	ICM	$2.3x10^{14}$	$2.0x10^{15}$		3F		
TSHA-105†	IT	$2.3x10^{14}$	$2.0x10^{15}$		2M, 2F	2M, 1F	
TSHA-120	IT	$3.0x10^{12}$	$2.6x10^{13}$				2M, 2F
		$1.2x10^{13}$	$1.0x10^{14}$				2M, 2F

Tissues from animals indicated were examined by qPCR to quantify CNS distribution of the rAAV9 vector. Vehicle-treated control animals in each study are not shown.
*Estimated based on the ratio of CSF volumes of NHPs versus humans, as reported by Emami et al. (2018).[6]
†Per study protocol, of three doses of TSHA-102 tested in this study ($2.9x10^{13}$, $5.8x10^{13}$, and $2.3x10^{14}$ vg/animal; n=6 for each dose), only the intermediate dose was examined to quantify vector biodistribution. Similarly, one of two doses of TSHA-105 (N=7 for each dose) was examined for biodistribution.

Results

- No significant toxicity was observed in any of the five NHP studies.
- For IT-dosed TSHA-101, -102, and -105 (**Figure 2**), vector biodistribution was consistent across brain and SC tissues at:
  - 30 days post-dosing (TSHA-101)
  - 90 and 180 days (TSHA-102 and -105)
- TSHA-101 study (Day 30 necropsy) showed relatively high vector DNA in SC samples, a pattern not seen in the other studies and time points.
- Based on cross-study results at 90 and 180 days:
  - With $5.8x10^{13}$ vg IT dosing, vector DNA in brain was consistently ~$1.5 – 3.0x10^{5}$ vg/µg (~1 to 2 copies/diploid genome (DG))
  - With a 4-fold higher dosing of TSHA-105, vector copy number in brain tissue samples was ~1.5 – 2.5-fold greater.
- At 90 days post-dosing, biodistribution of TSHA-102 vector (**Figure 3**) was generally consistent across brain and SC tissues for both IT and ICM routes of administration:
  - Vector levels were ~$5x10^{5}$ vg/µg (3.25 copies/DG) with IT dosing, across tissues
  - Higher values observed for ICM dosing were likely due to 4-fold higher dose of TSHA-102 in the ICM study ($5.8x10^{13}$ versus $2.3x10^{14}$ vg/animal)
  - Note that this difference is seen in SC as well as brain tissue samples, despite direct suboccipital instillation of the vector in the ICM study.
- One year after IT dosing, TSHA-120 showed consistent brain biodistribution across 15 samples from a five-slice rostro-caudal series
  - Tissue vector DNA levels were typically 10^{4} vg/ µg (**Figure 4**).
- Pooled data from the 90- and 180-day necropsy time-points confirm that all brain and SC tissues showed numerically greater mean vector DNA levels with $2.3x10^{14}$ versus $5.8x10^{13}$ vg IT dosing (**Figure 5**).

Figure 2: Broad biodistribution of TSHA-101, -102 and -105 following lumbar IT dosing at $5.8x10^{13}$ vg/animal (TSHA-101 and TSHA-102) or $2.3x10^{14}$ vg/animal (TSHA-105).

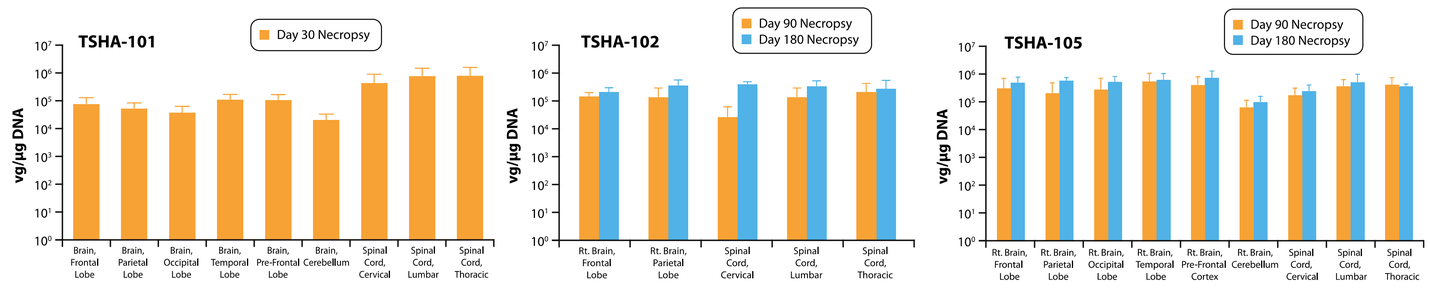


Figure 3: Similar biodistribution of TSHA-102 following IT (5.8x1013 vg) or ICM (2.3x1014 vg) dosing.

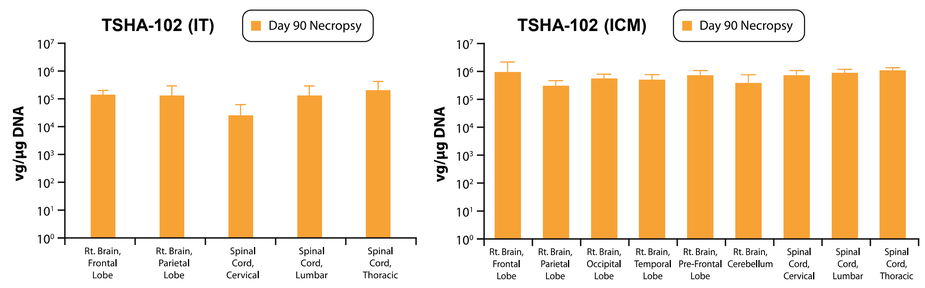
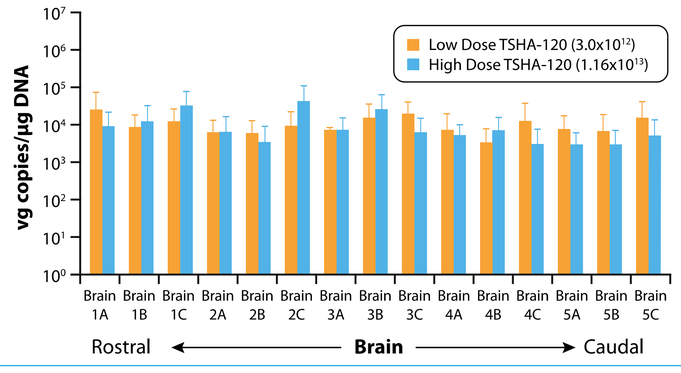
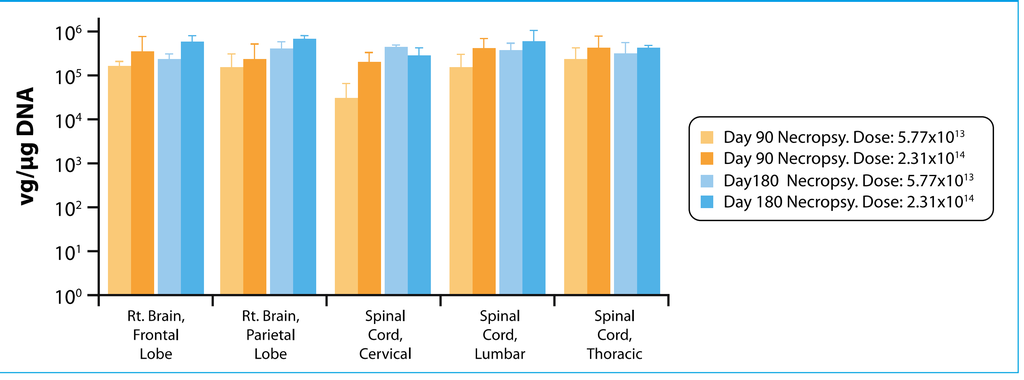


Figure 4: Consistent distribution of TSHA-120 vector DNA via lumbar IT across brain sections.



The vector dose in the TSHA-120 study ($3.0x10^{12}$ – $1.16x10^{13}$ vg/animal) is lower than that used in the other Taysha rAAV9 studies ($5.8x10^{13}$ – $2.3x10^{14}$ vg/animal).

Figure 5: Dose-dependent biodistribution in two lumbar IT dosing studies.
Data from n=6 (TSHA-102) and n=7 (TSHA-105) animals treated at the doses indicated.



Discussion

The current retrospective analysis of all Taysha rAAV9 constructs studied in NHPs confirms that lumbar IT dosing led to widespread and generally uniform vector levels in the CNS.

- Within and across studies, vector DNA levels in brain and SC tissues were generally proportional to the rAAV9 vector dose
- Comparison of lumbar IT versus ICM dosing of TSHA-102 showed uniform biodistribution with both routes
  - Modestly (1.5 – 2.5-fold) higher vector levels observed in the ICM-dosed animals' tissues may be attributed to the 4-fold higher dose used in the ICM study versus the lumbar IT study.

These findings are consistent with a 2013 report by Gray et al.,[1] quantifying rAAV9 biodistribution and Green Fluorescent Protein (GFP) marker expression in NHPs after single-bolus lumbar IT dosing:

- The IT ROA, which bypasses the blood-brain barrier, was not affected by animals' pre-existing anti-AAV antibody titer
- Biodistribution of the vector was consistently ≥10^{3} vg/µg in brain tissues. This value is proportional to our observed vector DNA level (~10^{5} vg/µg, ~0.65 copies/DG) using the same route of administration for the Taysha vectors, but at doses ~100 times higher than used by Gray et al.[1]

Our findings also agree with those of Meseck et. al.[4] that, at doses of 1 – $3x10^{13}$ rAAV9 vg/animal, there was "no marked or consistent difference" in biodistribution between the lumbar IT and ICM ROAs.

- Biodistribution was consistent across brain and SC samples and was generally dose-dependent.
- Even at the lowest IT dose tested ($3x10^{12}$ vg/animal for TSHA-120), mean vector DNA levels remained ~10^{4} vg/µg in all brain tissues.
- These findings support the potential of lumbar IT administration as an effective, procedurally simple, minimally invasive approach to achieve widespread rAAV9 distribution across the CNS.
- The IT dose reported here for TSHA-102 corresponds to a human equivalent dose of $5x10^{14}$ vg, comparable to the lower of two doses currently being tested in the REVEAL clinical trials for adolescent/adult and paediatric patients with Rett syndrome.
- Hence, Taysha's overall IT biodistribution data support the clinical strategy currently studied in the REVEAL trials.

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