rAAV9 Vector Biodistribution in Brain and Spinal Cord via Lumbar Intrathecal Infusion in Nonhuman Primates (NHP): Assessing the Administration Route Leveraged in TSHA-102 Rett Syndrome Clinical Trials

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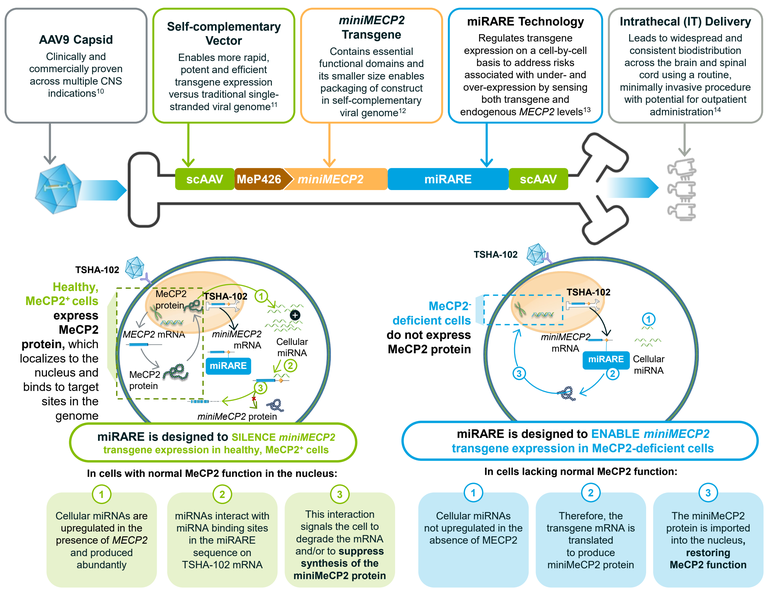
Aim

To assess biodistribution of rAAV9 vector products in NHP brain and spinal cord tissues following lumbar IT or ICM injection

Background

- Taysha is focused on advancing AAV-based gene therapies for severe monogenic CNS diseases. Its lead clinical program TSHA-102 is in development for Rett syndrome
- TSHA-102 is designed to deliver a functional form of the *MECP2* gene for the treatment of Rett syndrome, and TSHA-105 is designed to deliver the *SLC13A5* gene for the treatment of SLC13A5-related epilepsy
- TSHA-102 carries a 3' element, the miR-Responsive Auto-Regulatory Element (miRARE), designed to silence transgene expression in cells with normal MeCP2 function and permit expression in MeCP2-deficient cells[1] (**Figure 1**)
- Lumbar IT dosing of rAAV vectors is a well-studied ROA that has been explored in preclinical and clinical studies for targeted delivery of transgenes to the CNS[2,3]
- Like other CNS-targeted routes (e.g., ICM, ICV), lumbar IT dosing bypasses the blood-brain barrier, limits systemic exposure, and reduces the impact of pre-existing anti-AAV antibodies[3,4]
- Compared with other CNS-directed routes, including ICM administration, lumbar IT dosing has the advantage of being minimally invasive[5] (**Figure 2**)
- While there have been varied results regarding the biodistribution and transduction efficiencies of different ROA, evidence suggests that in NHPs, lumbar IT facilitates the biodistribution needed to target CNS disorders[6-9]

Figure 1: Key features of TSHA-102 construct with miRARE



Methods

- We examined biodistribution of TSHA-102 and TSHA-105 vectors from three studies using lumbar IT or ICM dosing
- TSHA-102 and TSHA-105 vectors were manufactured and tested for quality under similar conditions
- Juvenile cynomolgus monkeys (n=16) were dosed across three studies
- Stock vectors were diluted to dosing concentrations (**Table 1**) and 2.5 mL was administered to all NHPs at an approximate rate of 1 mL/min
- Biodistribution (vg/µg NHP DNA) was quantified at 90- and 180-days post-injection by qPCR of spinal cord and brain homogenates (**Figure 2**) following scheduled necropsy

Table 1: Dosing and sample information of TSHA-102 and TSHA-105

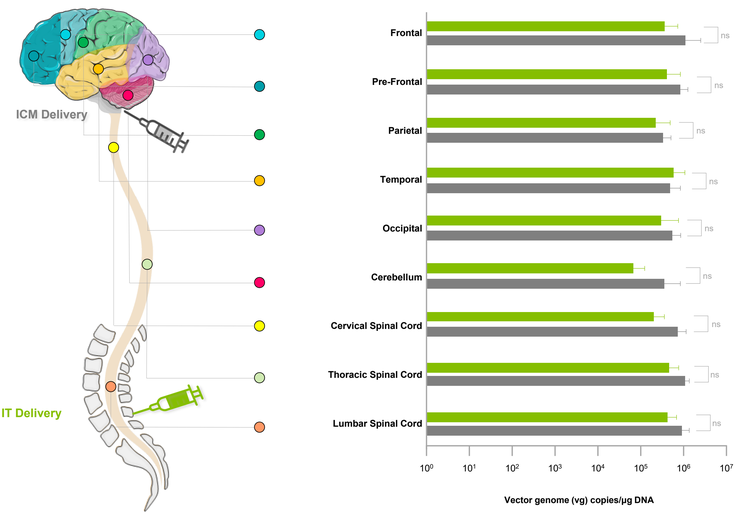
	Route	Dose per animal (vg)	HED (vg)*	Time to necropsy (days post-dosing)	
				90	180
TSHA-102†	Lumbar IT	$5.8x10^{13}$	$5.0x10^{14}$	3F	3F
TSHA-102	ICM	$2.3x10^{14}$	$2.0x10^{15}$	3F	–
TSHA-105†	Lumbar IT	$2.3x10^{14}$	$2.0x10^{15}$	2M, 2F	2M, 1F

Tissues from animals indicated were examined by qPCR to quantify CNS distribution of the rAAV9 vector. Vehicle-treated control animals are not shown.

*Estimated based on the ratio of CSF volumes of NHPs versus humans.[15]

†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.

Figure 2: Lumbar IT administration is potentially an effective, safe and minimally invasive delivery approach for broad targeting of the CNS



TSHA-102 (ICM): $2x10^{15}$ total vg HED, TSHA-105 (Lumbar IT): $2x10^{15}$ total vg HED

Results

- At 90 days and 180 days, TSHA-102 (lumbar IT) DNA was detected in all tissues analyzed post-lumbar IT infusion and biodistribution was consistent across all tissues (**Figure 3**)
- At 90 days, lumbar IT (TSHA-105) and ICM (TSHA-102) biodistribution both demonstrated widespread and consistent biodistribution across the brain and spinal cord at comparable mean levels of 10^5–10^6 vg/µg NHP DNA (**Figure 4**)
- Following lumbar IT delivery, vector DNA levels were dose-dependent; the 4-fold dose increase was associated with a 1.5–2.5-fold vector copy number increase in brain tissues (**Figure 5**)
- No or minimal safety issues were observed with any dose or ROA

Figure 3: Broad biodistribution of TSHA-102 following lumbar IT administration of $5.8x10^{13}$ vg/animal

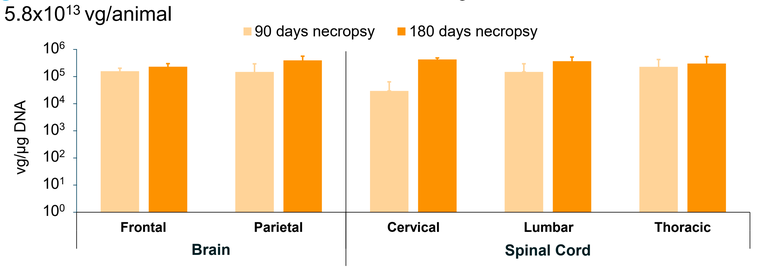


Figure 4: Comparison of vector DNA biodistribution of rAAV9 following equivalent dosing ($2.3x10^{14}$ vg/animal) by lumbar IT (TSHA-105) and ICM (TSHA-102) administration at 90 days post-dosing

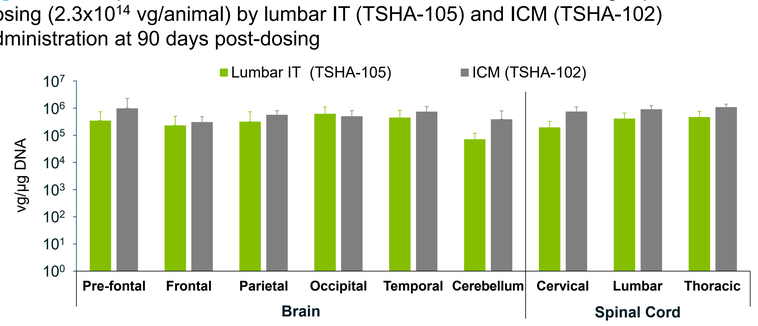
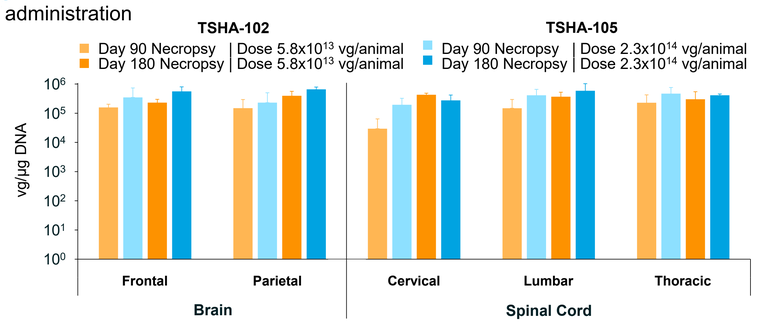


Figure 5: Dose-dependent biodistribution of TSHA-102 and TSHA-105 after lumbar IT administration



Conclusions

- These findings support lumbar IT administration as an effective and minimally invasive delivery route leading to widespread and consistent biodistribution across the brain and spinal cord comparable to ICM delivery
- Data supports TSHA-102 clinical development program approach, leveraging the minimally invasive lumbar IT administration for broad targeting of CNS regions impacted in Rett syndrome (**Table 2**)
- The CNS biodistribution profile reported here provides valuable context for interpreting clinical observations in the REVEAL first-in-human studies evaluating TSHA-102 administered via lumbar IT in Rett syndrome (NCT05606614, NCT06152237)[16]

Table 2: CNS regions with transduction based on TSHA-102 and biodistribution trends observed in rAAV9 studies[6] in NHPs

Clinical Efficacy Observed Post-TSHA-102	CNS Region Responsible for Function/Behavior	CNS Regions Targeted by TSHA-102 (Observed and Literature-Based[6])
Fine Motor Skills *Holds bottle unpropped, finger fed, reached for a toy, transferred an object from one hand to another* **Gross Motor Skills** *Walked with support, stood while holding on, pulled to standing, sat without support*	Motor cortex	✓
	Cerebellum	✓
	Brainstem	✓
	Basal ganglia	✓
Socialization/Communication *Spoke in phrases, used words with meaning, following a command with or without gesture, pointed to something they wanted, identified body parts*	Prefrontal cortex	✓
	Amygdala	✓

Key takeaway

Lumbar IT administration led to widespread and consistent biodistribution throughout the brain and spinal cord that was comparable to ICM, further supporting the clinical potential of lumbar IT administration as an effective, safe and minimally invasive delivery approach for broad targeting of the CNS

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Disclosures: Fred Porter is an employee of Taysha Gene Therapies and holds equity in the company.
Abbreviations: AAV9, adeno-associated virus serotype 9; CNS, central nervous system; CSF, cerebrospinal fluid; DNA, deoxyribonucleic acid; F, female; ICM, intra-cisterna magna; ICV, intracerebroventricular; IT, intrathecal; ITR, inverted terminal repeat; HED, human equivalent dose; M, male; miRNA, microRNA; NHP, non-human primates; ns, not significant; qPCR, qualitative polymerase chain reaction; ROA, routes of administration; rAAV, recombinant adeno-associated virus; sc, self-complementary; vg, vector genome.
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