Biodistribution Assessment in Non-Human Primates of JAG201, a *SHANK3* AAV9 Vector Delivered via ICV Injection for ASD, Phelan-McDermid Syndrome, and Other *SHANK3* Mutation or Deletion Related Conditions

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Background

The SH3- and ankyrin repeat (SHANK) protein family are major scaffolds of the post-synaptic density of excitatory synapses, where loss of SHANK3 function provokes synaptic dysfunction in the brain. Patients with SHANK3 haploinsufficiency present with a debilitating neurodevelopmental disorder characterized by severe intellectual disabilities, impaired or absent speech and language, deficits in motor skills, behavioral challenges, and autism and/or autistic features[1,2].

JAG201 is as an investigational AAV9-based gene therapy in preclinical development intended to deliver a functional version of SHANK3 to treat autism spectrum disorder (ASD), Phelan-McDermid syndrome (PMS), and other neurodevelopmental disorders that result from a genetically confirmed SHANK3 haploinsufficiency. Genetic sequencing studies indicate that SHANK3 mutations or deletions may be present in ~1% of patients with ASD, equating to about 30,000 patients in the U.S.

Here, we present data from a non-GLP study in NHPs evaluating the biodistribution of vector genome DNA and RNA transgene expression of JAG201 in the CNS and peripheral organs following unilateral and bilateral intracerebroventricular (ICV) administration.

Objectives

To investigate ICV delivery of JAG201 at two dose levels in NHPs on:

- CNS transduction and transgene expression following JAG201 ICV administration
- JAG201 transduction efficiency following unilateral vs bilateral ICV administration
- Off-target transgene expression in peripheral tissues

Methods

24 NHPs (12 males and 12 females) aged approximately 2 years received either an ICV administration of vehicle or JAG201 via unilateral or bilateral injection at a dose of $1x10^{13}$ or $1x10^{14}$ vg/animal in a total volume of 2.0 mL given via bolus injection.
Injection coordinates were determined via MRI followed by stereotaxic administration targeting the lateral ventricles.

Animals were followed for a 90-day in-life period, and biodistribution of JAG201 vector DNA and RNA in CNS and peripheral tissues were analyzed via droplet digital PCR (ddPCR) and supported via RNA fluorescence in situ hybridization (FISH).

T0 ICV Administration
- Vehicle vs JAG201
- Unilateral vs Bilateral

T90 Euthanasia
- JAG201 DNA and mRNA biodistribution
- JAG201 mRNA FISH

Results

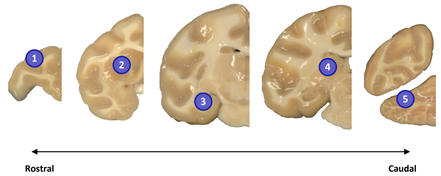
Study Design and JAG201 Brain Biodistribution

NHPs received a unilateral or bilateral ICV infusion using stereotaxic techniques to target the lateral ventricle(s), according to the following study design:

Group	JAG201 Dose (Total vg)	Unilateral ICV	Bilateral ICV	Total
Vehicle	-	4 (2M/2F)	4 (2M/2F)	8
JAG201	$1x10^{13}$	4 (2M/2F)	4 (2M/2F)	8
JAG201	$1x10^{14}$	4 (2M/2F)	4 (2M/2F)	8

Table 1. Experimental ICV study design. When administered bilaterally, half the total dose was administered to each ventricle. M = male, F = female, vg = vector genomes.

The unilateral and bilateral ICV administration procedures and doses of JAG201 were generally well tolerated through the 90-day in-life period. To evaluate JAG201 delivery to the CNS, key brain regions were selected for biodistribution and transgene expression analysis:



Sample	Brain Region
1	Frontal Cortex
2	Striatum
3	Hippocampus
4	Thalamus
5	Cerebellum

JAG201 vector genome (DNA) and transgene (RNA) biodistribution were examined in five key brain target regions 90 days after unilateral or bilateral ICV administration of JAG201 at a dose of $1x10^{13}$ vg or $1x10^{14}$ vg (**Figure 1**). JAG201 administered animals demonstrated dose-dependent widespread brain transduction and subsequent transgene expression of JAG201 following both unilateral and bilateral ICV administration.

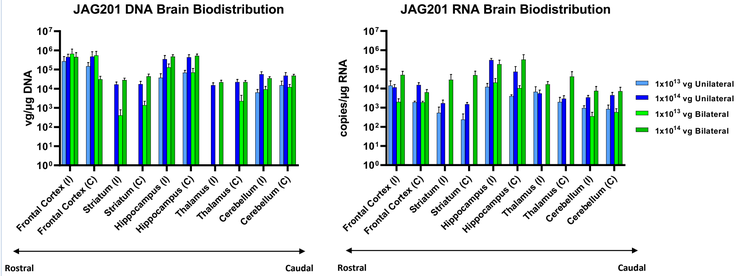


Figure 1. Brain JAG201 DNA and RNA expression biodistribution via ddPCR analysis, 90 days after unilateral or bilateral ICV administration. I – Ipsilateral hemisphere; C – Contralateral hemisphere (relative to the unilaterally administered hemisphere).

JAG201 CNS Biodistribution

Comparison of JAG201 DNA biodistribution following unilateral and bilateral ICV administration in the contralateral hemisphere (relative to the unilaterally administered hemisphere) demonstrated comparable levels of JAG201 transduction at the $1x10^{14}$ vg dose level (**Figure 2**).

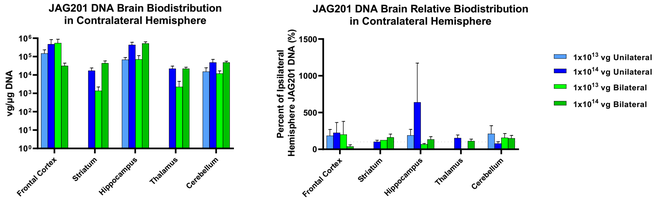


Figure 2. Brain contralateral hemisphere (relative to the unilaterally administered hemisphere) JAG201 vector biodistribution via ddPCR analysis at 90 days following unilateral or bilateral ICV administration.

JAG201 RNA FISH analysis supported further widespread brain biodistribution and transgene expression of JAG201 within cells with neuronal morphology in key brain target regions 90 days after unilateral ICV administration (**Figure 3**).

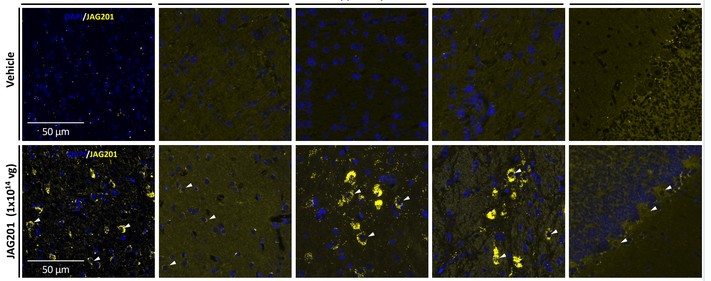


Figure 3. Visualization of JAG201 mRNA expression in cells with neuronal morphology (white arrows) via FISH analysis in five key brain target regions at 90 days following unilateral ICV administration.

JAG201 biodistribution analysis of spinal cord and dorsal root ganglia (DRG) further demonstrated widespread CNS transduction of JAG201 following unilateral ICV administration at both doses (**Figure 4**).

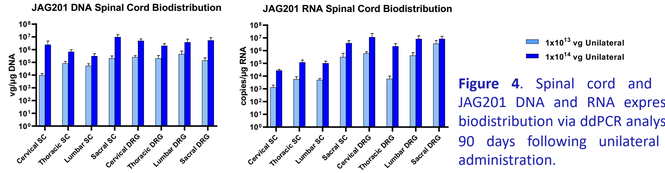


Figure 4. Spinal cord and DRG JAG201 DNA and RNA expression biodistribution via ddPCR analysis at 90 days following unilateral ICV administration.

JAG201 Peripheral Biodistribution

JAG201 biodistribution was examined in peripheral tissues 90 days after unilateral ICV administration of JAG201 (**Figure 5**). JAG201 administered animals demonstrated escape of the vector from the CNS, with JAG201 vector genome DNA detected in peripheral tissues. However, off-target JAG201 expression is limited in peripheral tissues as a result of JAG201 neuron selective promoter activity (**Figure 5 and 6**).

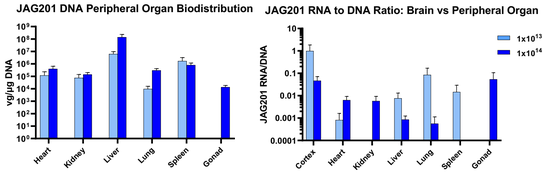


Figure 5. Peripheral organ JAG201 DNA and RNA expression biodistribution via ddPCR analysis at 90 days following unilateral ICV administration.

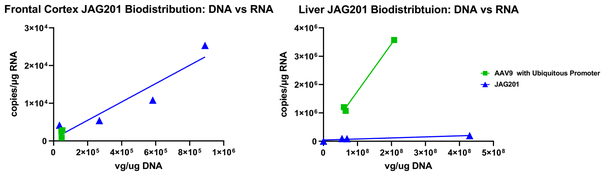


Figure 6. Comparison of vector genome levels (DNA) versus transgene expression (RNA) in frontal cortex and liver tissue from animals administered with JAG201 and comparable ubiquitous AAV9 vector.

Summary and Conclusions

The results of this JAG201 ICV NHP biodistribution study demonstrate:

- Single ICV administration of JAG201 results in widespread transduction and transgene expression throughout the CNS at both $1x10^{13}$ and $1x10^{14}$ vg dose levels.
- Unilateral and bilateral ICV administration of JAG201 were well tolerated and result in comparable CNS biodistribution.
- Neuron selective promoter activity of JAG201 reduces off-target expression in peripheral tissues.

In summary, these findings support the selection of the less invasive unilateral ICV administration for further evaluation of JAG201, a SHANK3 AAV9-based gene therapy targeting disorders resulting from genetically confirmed SHANK3 haploinsufficiency.

References and Acknowledgements

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We thank Mark Johnson and Northern Biomedical Research for their technical expertise in ICV administration to NHPs

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