Preclinical Assessment of JAG201, a Clinical Stage Gene Therapy for Severe Neurodevelopmental Disorders Caused by Mutations or Deletions in *SHANK3* Including Phelan-McDermid Syndrome (PMS) and Autism Spectrum Disorder (ASD)

Michael P. Hughes[1], Janet Do[1], and Daniel D. McCoy[1]

[1]Jaguar Gene Therapy, LLC. Lake Forest, IL

Background

The SH3- and ankyrin repeat (SHANK) protein family are major scaffold proteins of the post-synaptic density of excitatory synapses. Loss of SHANK3 function leads to 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 designed to be a one-time treatment for *SHANK3* haploinsufficiency including Phelan-McDermid syndrome (PMS) and a genetic form of autism where a *SHANK3* mutation or deletion is present. 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 efficacy data following intracerebroventricular (ICV) administration of JAG201 in a mouse model of *Shank3* deficiency.

Objectives

To investigate the effect of ICV administered JAG201 in the *Shank3*$^{\Delta 4\text{-}22}$ mouse model on:

- Neurobehavioral phenotypes recapitulating deficits in the human *SHANK3* haploinsufficient patient population
- Restoration of SHANK3 protein and functional binding partner recruitment at the synaptic membrane within the brain
- Biodistribution of JAG201 vector DNA and transgene expression

Methods

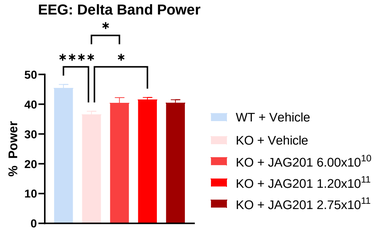
- On postnatal day 14 (±2 days), *Shank3*$^{\Delta 4\text{-}22}$ and wild-type (data not shown) mice received a single unilateral ICV administration of vehicle or JAG201 at a dose range of 2.40x10^{09} to 2.75x10^{11} vg/animal.
- Animals were followed for up to 10 months post-treatment, with assessments of established neurobehavioral phenotypes (EEG, rotarod, and open field) carried out at 3-4 months post-treatment.
- Post-life analyses included assessment of JAG201 protein expression and functional binding partners in synaptic membrane preparations (SPM), biodistribution (BD) of JAG201 vector DNA and mRNA via droplet digital PCR (ddPCR) and RNA fluorescence in situ hybridization (FISH).

Results

JAG201 Significantly Improves Neurobehavioral Deficits and SHANK3 Protein Function in *Shank3*$^{\Delta 4\text{-}22}$ *M*ouse Model

Abnormal sleep significantly improved in *Shank3*$^{\Delta 4\text{-}22}$ mice treated with JAG201

Effect on Sleep (EEG Test): Sleep disturbances are often reported among PMS patients; these disturbances can be assessed in humans utilizing EEG delta band power as a marker of restorative sleep[3]. In *Shank3*$^{\Delta 4\text{-}22}$ (KO) mice, the EEG delta band power was significantly lower than in wild-type (WT) mice, reflecting the sleep disturbances commonly seen in PMS patients. *Shank3*$^{\Delta 4\text{-}22}$ mice treated with JAG201 at dose levels of 6.00x10^{10} and 1.20x10^{11} vg/animal demonstrated improvements in restorative sleep, with a significant increase in EEG delta band power, compared to *Shank3*$^{\Delta 4\text{-}22}$ (KO) vehicle-treated control mice (**Figure 1**).

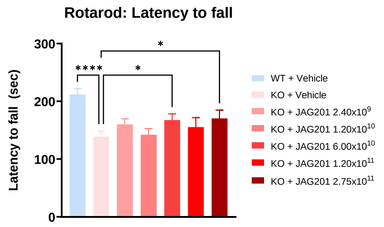


Treatment	Animal Number	Probability
WT – Vehicle	**22**	**<0.0001**
KO – Vehicle	20	-
KO – 6.00x10^{10} vg	**17**	**0.0272**
KO – 1.20x10^{11} vg	**9**	**0.0183**
KO – 2.75x10^{11} vg	9	0.0648

Figure 1. Treatment with JAG201 significantly improved sleep deficit in *Shank3*$^{\Delta 4\text{-}22}$ mice, as measured by EEG delta band power as a marker of restorative sleep. One-way ANOVA probability denotes statistical comparison relative to the vehicle-treated KO group. *, P ≤ 0.05; ****, P < 0.0001.

Motor coordination significantly improved in *Shank3*$^{\Delta 4\text{-}22}$ mice treated with JAG201

Effect on Balance and Motor Learning (Rotarod Test): Motor deficits are frequently observed in PMS patients[4], which was reflected in the reduced rotarod test performance by *Shank3*$^{\Delta 4\text{-}22}$ mice. KO mice treated with JAG201 at dose levels of 6.00x10^{10} and 2.75x10^{11} vg/animal demonstrated improvements in motor function, with a significant increase in average latency to fall, compared to KO vehicle-treated control mice (**Figure 2**).

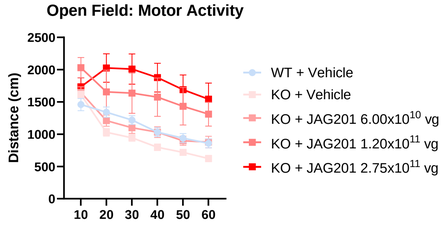


Treatment	Animal Number	Probability
WT – Vehicle	**35**	**<0.0001**
KO – Vehicle	34	-
KO – 2.40x10^{9} vg	25	0.1685
KO – 1.20x10^{10} vg	23	0.8464
KO – 6.00x10^{10} vg	**30**	**0.0467**
KO – 1.20x10^{11} vg	9	0.4471
KO – 2.75x10^{11} vg	**22**	**0.0452**

Figure 2. Treatment with JAG201 significantly improved motor deficit in *Shank3*$^{\Delta 4\text{-}22}$ mice, as measured by average latency to fall in the rotarod test. One-way ANOVA probability denotes statistical comparison relative to the vehicle-treated KO group. *, P ≤ 0.05; ****, P < 0.0001.

Motor activity significantly improved in *Shank3*$^{\Delta 4\text{-}22}$ mice treated with JAG201

Effect on Locomotor Activity (Open Field Test): Abnormal gait and motor deficits are frequently observed in PMS patients[4], which was reflected by reduced motor activity of *Shank3*$^{\Delta 4\text{-}22}$ mice in the open field test. *Shank3*$^{\Delta 4\text{-}22}$ mice treated with JAG201 at dose levels of ≥6.00x10^{10} vg/animal demonstrated increased motor activity, compared to KO vehicle-treated control mice (**Figure 3**).



Treatment	Animal Number	Probability
WT – Vehicle	**35**	**0.0365**
KO – Vehicle	34	-
KO – 6.00x10^{10} vg	**31**	**0.0253**
KO – 1.20x10^{11} vg	**9**	**<0.0001**
KO – 2.75x10^{11} vg	**22**	**0.0001**

Figure 3. Treatment with JAG201 significantly improved motor activity in *Shank3*$^{\Delta 4\text{-}22}$ mice, as measured by distance travelled in the open field test. Mixed model for repeated measures probability denotes statistical comparison relative to the vehicle-treated KO group.

Restoration of SHANK3 Protein Function at the Synaptic Membrane

SPM miniSHANK3 and Homer 1 protein levels: A dose-dependent increase in miniSHANK3 protein levels from brain synaptic membrane preparations was observed in KO animals following JAG201 treatment compared to vehicle-treated animals (**Figure 4A**). These dose-dependent increases in miniSHANK3 protein levels equated to intermediate and supraphysiological levels of endogenous SHANK3 expression, when compared to vehicle treated WT animals.

miniSHANK3 synaptic function was assessed via quantification of Homer1 recruitment at the synaptic membrane, which when bound with SHANK3, acts as a scaffold to stabilize metabotropic glutamate receptors at the PSD[4]. KO animals treated with a JAG201 dose level of 2.75x10^{11} vg/animal demonstrated a significant increase in Homer1 recruitment to the synaptic membrane, when compared to vehicle treated KO animals (**Figure 4B**).

A

Treatment	Animal Number	Total miniSHANK3 and SHANK3 Protein Levels Relative to WT Endogenous SHANK3 (% ± S.E.M.)
WT – Vehicle	12	97.06 ± 9.85
KO – Vehicle	11	0.48 ± 0.06
KO – 2.40x10^{9} vg	6	2.65 ± 1.56
KO – 1.20x10^{10} vg	6	21.65 ± 5.70
KO – 6.00x10^{10} vg	10	43.62 ± 12.09
KO – 1.20x10^{11} vg	5	74.90 ± 46.04
KO – 2.75x10^{11} vg	5	589.20 ± 97.72

B

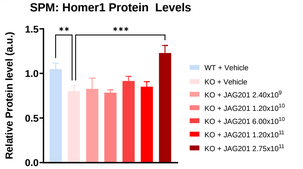


Figure 4. A) Total miniSHANK3 and SHANK3 protein levels relative to WT endogenous SHANK3 levels in brain synaptic membrane preparations at 3 months post-dosing; B) Relative protein levels of SHANK3 binding partner Homer1 in brain synaptic membrane preparations at 3 months post-dosing. One-way ANOVA probability denotes statistical comparison relative to the vehicle-treated KO group. **, P ≤ 0.01; ***, P ≤ 0.001.

JAG201 Biodistribution and Persistent Transgene Expression

JAG201-treated animals demonstrated dose-dependent brain transduction as measured by vector genome DNA levels (**Figure 5A**) and transgene expression as measured by JAG201 transgene mRNA (**Figure 5B**), throughout all evaluated brain regions.

Generally comparable levels of JAG201 transduction and transgene expression were observed in evaluated regions for both hemispheres of the brain at both evaluated timepoints, demonstrating widespread brain transduction and persistent transgene expression following a single unilateral ICV administration of JAG201. Supporting JAG201 mRNA FISH analysis demonstrated widespread transgene expression throughout the brain of JAG201-treated animals within cells with neuronal morphology (**Figure 5C**).

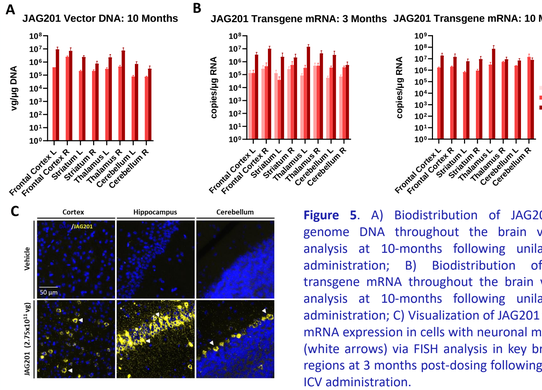


Figure 5. A) Biodistribution of JAG201 vector genome DNA throughout the brain via ddPCR analysis at 10-months following unilateral ICV administration; B) Biodistribution of JAG201 transgene mRNA throughout the brain via ddPCR analysis at 10-months following unilateral ICV administration; C) Visualization of JAG201 transgene mRNA expression in cells with neuronal morphology (white arrows) via FISH analysis in key brain target regions at 3 months post-dosing following unilateral ICV administration.

Summary and Conclusions

These data demonstrate the therapeutic benefit of ICV administered JAG201 in a model of SHANK3 deficiency, as shown by:

- Significant improvement in three key neurobehavioral deficits in *Shank3*$^{\Delta 4\text{-}22}$ mice
- Dose-responsive restoration of SHANK3 protein and significant increase in functional binding partner Homer1 recruitment at the synaptic membrane within the brain
- Widespread biodistribution and persistent transgene expression throughout the CNS following a single unilateral ICV administration

A first-in-human clinical study with JAG201, an AAV9-based gene therapy to treat *SHANK3* haploinsufficiency, is slated to begin this year.

References and Acknowledgements

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We thank Laurent Bogdanik, Bob Schneider, and technologists at JAX Preclinical Services for leading *in vivo* work and preparing study reports.

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Contact: medinfo@jaguargenetherapy.com | Website: Jaguargenetherapy.com