Assessment of Intracerebroventricular AAV9-hTPP1co Efficacy in Batten Mice Using a Digital Vivarium (Vium, Inc.)

Juliette Hordeaux, Aldo Pourchet, Laura K. Richman, Yan Che, Brianne Jeffrey, Elizabeth L. Buza, Peter Bell, and James M. Wilson

Gene Therapy Program, Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

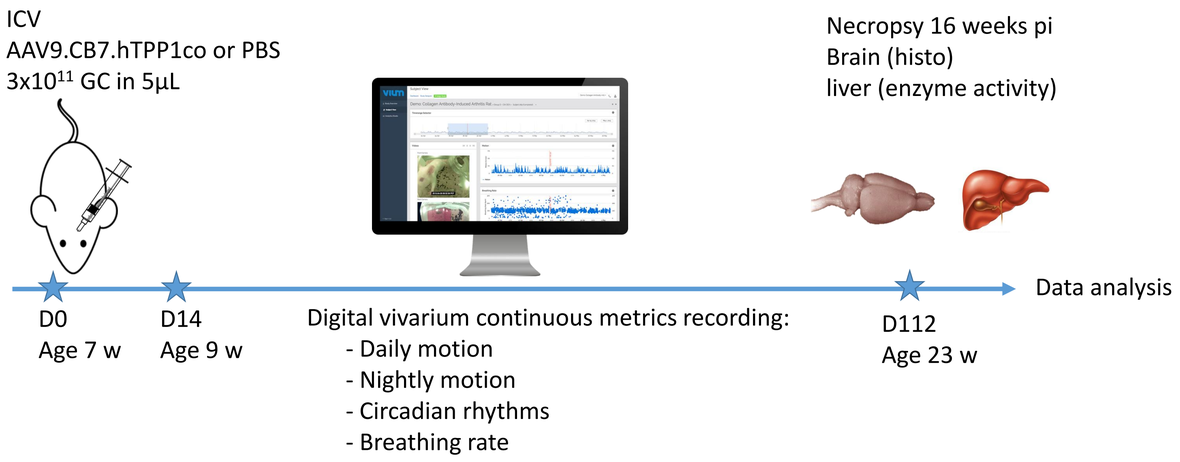
Introduction

CLN2 disease, a form of Batten disease, is a neurodegenerative lysosomal storage disorder caused by mutations in the gene encoding the soluble enzyme tripeptidyl-peptidase-1 (TPP1). The disease is characterized by early onset at 2-4 years of age with seizures and ataxia before death by mid-childhood. To minimize the burden associated with repeated intrathecal administration of recombinant enzyme (Brineura®), we are investigating gene therapy via intracerebroventricular (ICV) AAV9 encoding human TPP1 in the TPP1^{m1J} mouse (JAX stock #012876). This model recapitulates most features of the human disease such as shortened lifespan, seizures, or abnormal gait. Monitoring of the neurobehavioral function in this model is challenging, however, as they are prone to noise- or stress-induced fatal seizures when handled.

Aim

To assess the impact of ICV AAV9-mediated gene therapy on the neurologic function of the TPP1^{m1J} mouse, using an unbiased non-invasive full time continuous monitoring system in a digital vivarium (Vium, Inc.).

Experimental design



Gene therapy ameliorates clinical condition and survival

- AAV9.CB7.hTPP1co vector was well tolerated via ICV administration at the dose of 3x10^{11} GC
- 61.5% of treated KO mice were bright, alert, responsive, and hydrated throughout study versus 0% of vehicle treated KO mice (Table 1)
- The vector delayed and decreased the incidence of clinical events (Table 1)
- The lifespan of vector-treated mice was significantly increased (Fig. 1)

Table 1. **Clinical events (incidence)** of TPP1^{m1J} and WT littermates treated with intracebroventricular injection of vehicle or of 3x10^{11} GC AAV9.CB7.hTPP1co

Clinical event	KO PBS n=10	KO AAV9 n=13	WT PBS n=13
BARH throughout study	0%	61.5%	100%
Tremors	60%	38.5%	0%
Seizure	40%	7.7%	0%
Moribund	60%	15.4%	0%
Found dead	40%	23.1%	0%
1st clinical event	14.9 to17.6 weeks	17 to >23 weeks	/

PBS: Phosphate buffered saline (vehicle control)
BARH: bright, alert, responsive, and hydrated

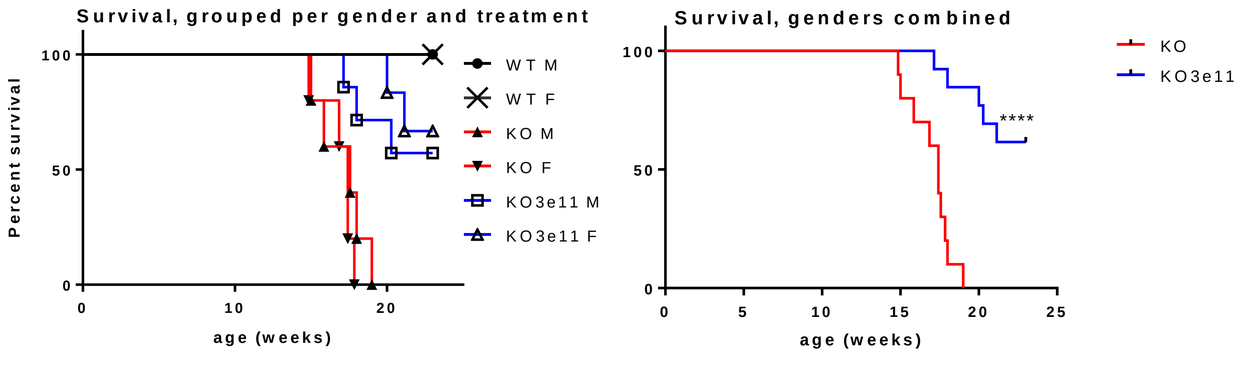


Figure 1. Increased survival of KO mice treated with intracebroventricular injection of 3x10^{11} GC AAV9.CB7.hTPP1co.
All vehicle treated KO animals were found dead or humanely euthanized before the age of 19 weeks whereas 67% of vector treated females (KO 3e11 F) and 57% of vector treated males (KO 3e11 M) were alive at the scheduled 23 weeks endpoint. **** Log-rank Mantel-Kox test, p<0.0001

Conflict of Interest Statement
This work was supported by REGENXBIO. J.M. Wilson is an advisor to, holds equity in, and has a sponsored research agreement with REGENXBIO and Scout Bio; he also has a sponsored research agreement with Ultragenyx, Biogen, and Janssen, who are licensees of Penn technology. In addition, he has sponsored research agreements with Precision Biosciences and Moderna Therapeutics. JMW holds equity in Solid Bio and he is an inventor on patents that have been licensed to various biopharmaceutical companies.

Gene therapy prevents neurobehavioral impairment

- The disease-related weight loss, more pronounced in females, was prevented by AAV9.CB7.hTPP1co vector (Fig. 2)
- Treatment preserved the biphasic circadian motion profile (Fig. 3), and weekly average night time motion (Fig. 4)
- Continuous recording of motion using the smart cages allowed to detect neurobehavioral impairment in vehicle treated TPP1^{m1J} mice from 14.7 weeks of age onward, in otherwise asymptomatic mice (Fig. 4)
- Breathing rate was comparable in all groups (not shown)

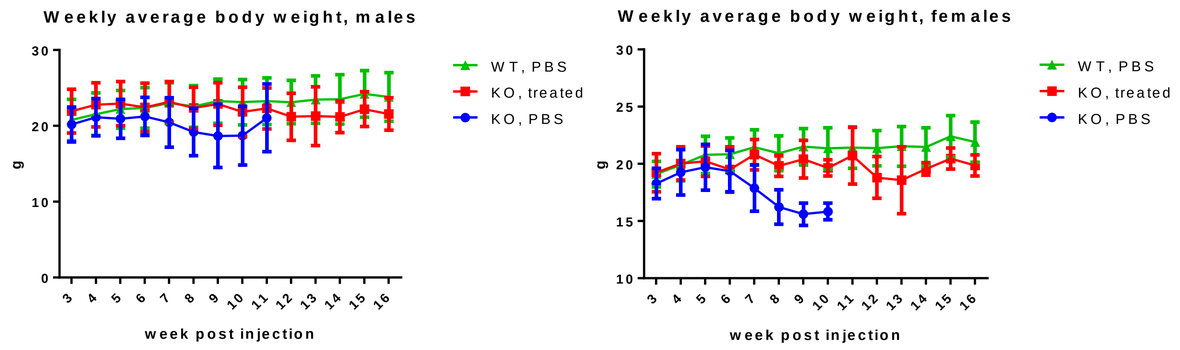


Figure 2. Weekly average body weight. Vehicle-treated KO mice started losing weight 6 weeks pi. Vector-treated animals maintained their body weight until sacrifice.

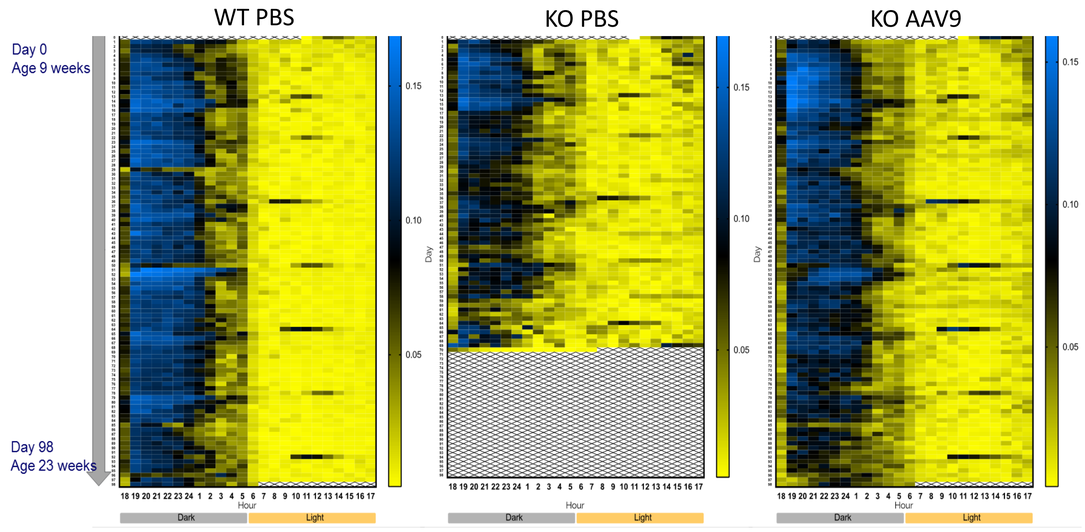


Figure 3. Circadian rhythm profile is maintained in treated KO mice.
The color scale represents speed of the mice (m/s) during the dark and the light phase. Scale is from slow motion in yellow to fast motion in blue. Both WT (left panels) and treated KO animals (right panels) have a clear biphasic circadian motion profile whereas vehicle treated mice (middle panels) started to lose the biphasic profile around 16 weeks of age.

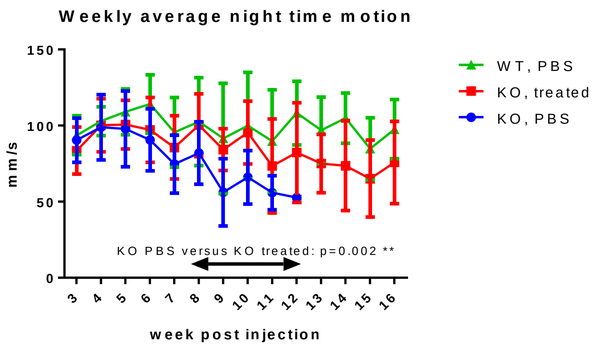


Figure 4. Weekly average night time motion speed monitoring shows therapeutic efficacy before the onset of clinical symptoms. Comparisons were carried out using linear mixed effect modeling within the R program. The analysis was not stratified by gender. Statistical Significance was assessed at the 0.05 level. Treatment effect in KO animals was significant from Vium Day 40 (7.7 weeks post-injection; age 14.7 weeks).

Gene therapy reduces brain astrocytosis

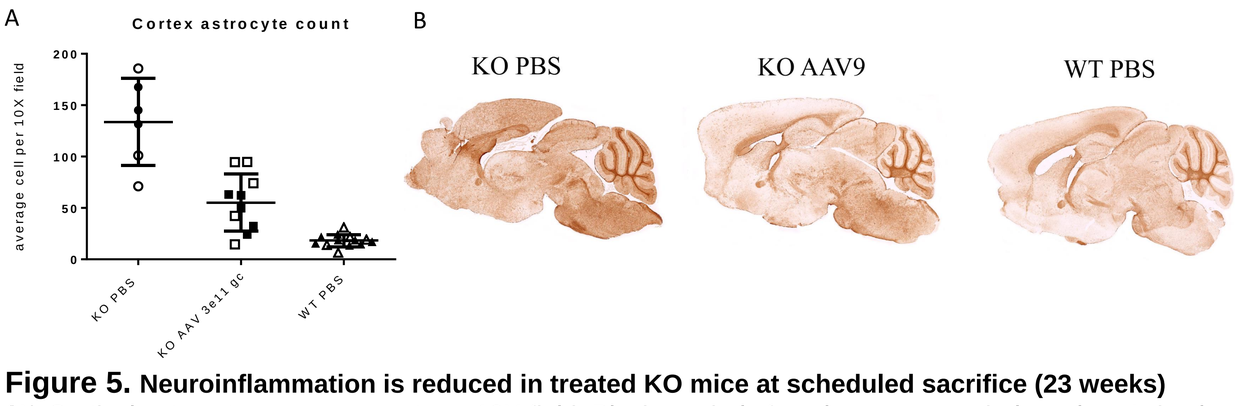


Figure 5. Neuroinflammation is reduced in treated KO mice at scheduled sacrifice (23 weeks)
(A) Cortical astrocyte average count per 10X field. Black symbols females, open symbols males. Animals found dead were excluded due to autolysis. (B) Representative immunostaining of astrocytes using an anti-GFAP antibody.

Conclusion

- ICV AAV9.CB7.hTPP1co increases survival, improves clinical condition, and reduces neurobehavioral manifestations and neuroinflammation in TPP1^{m1J}, a mouse model of CLN2 disease.
- Real time non-invasive continuous monitoring using smart cages (Vium, Inc.) allows sensitive and non-biased recording of disease phenotype and treatment-related rescue in a seizure-prone mouse model, while limiting handling-related deaths

Acknowledgements
We would like to thank Penn vector core, the GTP Program of Comparative Medicine and the staff of Vium, Inc. for invaluable assistance.