#566

Development of AAV-GBA1 Gene Replacement Therapy for IV Delivery via Blood Brain Barrier Penetrant AAV Capsid

Charlotte Chung, Giridhar Murlidharan, Smita Jagtap, Adewale Adeluyi, Elisabeth Knoll, Abigail Ecker, Brian Ezell, Tatiana Athanasopoulos, Brett Hoffman, Tatiana Knox, Jenna Tocci, Emalee Peterson, Ambreen Sayed-Zahid, Maneesha Paranjpe, Kyle Grant, Kelly Bales, and Todd Carter

Voyager Therapeutics Inc., 64 Sidney Street, Cambridge, MA 02139, USA

SUMMARY

- AAV vectorized optimized GBA achieved widespread CNS distribution and GCase activity after intravenous administration in GBA patient cells and Gaucher mouse model
- *In vitro* screening of enhanced GBA transgene
  - Increased GCase activity in patient fibroblasts
  - Increased substrate reduction
- *In vivo* target engagement:
  - Strategy to achieve superior CNS distribution with non-invasive one-time IV AAV-GBA gene therapy
  - Consistent VG distribution and GCase activity observed across the CNS following IV delivery
  - GCase activity of 30-50% increase is anticipated to be clinically impactful, our data suggest the potential of dose reduction
- Safety: Parallel build of tissue-detargeting approach
  - GBA transgene level DRG tissue-detargeting approach validated in mice

INTRODUCTION

- Adeno-associated viral vector-based gene-replacement therapies can be used to achieve sustained correction of lysosomal storage disorders affecting the central nervous system. *GBA1* gene encodes the lysosomal enzyme β-glucocerebrosidase [*GCase*] and *GBA1* loss of function (LOF) results in *GCase* deficit and cellular build-up of glycosphingolipid-substrate. Homozygous *GBA1* LOF mutations manifest as Gaucher disease, the most common lysosomal storage disorder. Heterozygous *GBA1* LOF mutations and reduced GCase activity are strong genetic risk factors for Parkinson disease and Lewy Body dementia. Enzyme replacement therapy can have clinical impact in the periphery but fails to adequately cross the blood-brain barrier. Here, we report development of an AAV-based *GBA1*-gene replacement therapy that delivers GCase to a widespread CNS-footprint and periphery following intravenous (IV) dosing.
- Several classes of enhanced *GBA1* transgenes with favorable attributes, including promoter and gene optimization; cell and lysosomal targeting; tissue-detargeting were designed. Following expression validation, *GBA1* transgenes were packaged into AAV for testing on patient-derived cells for *in vitro* target engagement. Multiple AAV-*GBA1* constructs demonstrated dose-dependent GCase activity increases, GBA protein normalization, and correction of glycosphingolipid levels to match healthy human comparator cells.
- Single IV-dosing of our optimized AAV-*GBA1* vector in WT mice resulted in widespread vector genome CNS distribution and GCase activity increases in CSF and multiple relevant brain regions [up to ~5x over baseline]. We are currently evaluating optimized AAV-*GBA1* vectors in a relevant rodent model of GBA1 LOF.
- In summary, we present our strategy to address gaps and accelerate development of an IV-delivered, CNS-directed AAV-*GBA1* gene replacement therapy.

GBA Disease

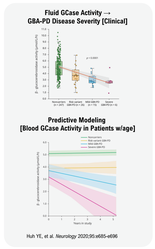
GENETICS & EPIDEMIOLOGY

- *GBA-1* mutations- strong genetic risk factor for PD, also associated with earlier age of onset
- 5-10% of PD patients have *GBA-1* mutations (~90000 PD patients in US)
- *GBA1* LOF mutations and reduced GCase activity [BM] are associated with GBA-PD; Gaucher disease and other Parkinsonism such as DLB

GCase activity ↓ [30-50%]	Validated Fluid BMs
GBA substrate accumulation	Strong disease association w/GBA-LOF mutations
α-Syn/Lewy body pathology	GD-II patient cell lines and transgenic mouse model

TARGET BIOLOGY

- *GBA-1* encodes lysosomal enzyme glucocerebrosidase (GCase) which degrades glycosphingolipids [e.g. GlcCer]
- GBA is partially secreted into fluid compartment/CSF [valid biomarker for disease / target engagement]
- GCase interaction with α-Syn thought to promote degradation via lysosome-mediated autophagy
- *GBA-1* mutations lead to decrease protein and decrease GCase catalytic activity resulting in glycosphingolipid accumulations
  - Associated with downstream feedback e.g. α-Syn aggregation
  - Severity of LOF mutation strongly correlates w/loss of GCase activity and worse clinical outcome



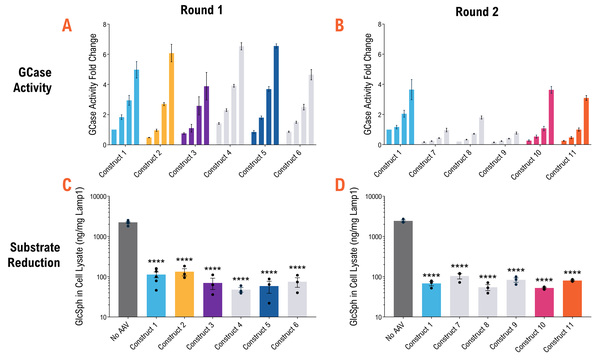
GBA BACKGROUND

- Reduced levels of lysosomal enzyme beta-glucocerebrosidase/GCase cause increased accumulations of glycosphingolipid glucosylceramide/GluCer
- Correlated with multiple disease manifestations in the CNS and periphery [GBA-PD; Neuronopathic Gaucher; Dementia with Lewy body disease]
- High patient-prevalence: 7-10% of PD patients have GBA1 mutations (~90k PD-GBA patients in the US); Sporadic PD patients also known to have reduced GCase activity. Gaucher disease is one of most prevalent LSD (1:30-100k worldwide)

APPROACH

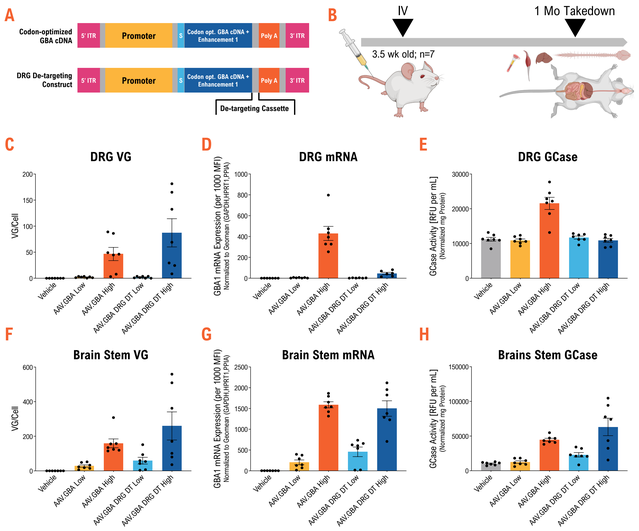
- Increasing GCase enzyme activity with AAV gene transfer of optimized GBA1 transgene cassette in a widespread CNS footprint successfully shown to decrease substrate glycosphingolipid glucosylceramide levels; and slowdown pathogenesis in GBA-PD
- With modifications in delivery approach, strategy could be applied for patients with other manifestations of GBA dysfunction such as Gaucher disease and Dementia with Lewy body disease

Figure 1. *In vitro* Screening of AAV2.GBA1 VYGR Constructs in Patient Fibroblasts



Enhancements of GBA1 transgene were made, including promoter, cell- and lysosomal-targeting, tissue-targeting. Eleven VYGR optimized AAV2.GBA1 constructs were tested in our *in vitro* screening in patient fibroblasts. (A,B) Glucocerebrosidase (GCase) activity was measured by SensoLyte® Blue Glucocerebrosidase activity assay in patient fibroblasts 7 days post treatment with construct 1-11 at 4 increasing doses. All constructs demonstrate dose dependent increase in GCase activity. (C,D) Substrate reduction in glucosylsphingosine (GlcSph) levels were detected were detected by LC/MS-MS 7 days post treatment at the highest dose with VYGR constructs 1-11. Five constructs were chosen for *in vivo* target engagement study in GBA LOF mouse model. Mean +/-SEM.

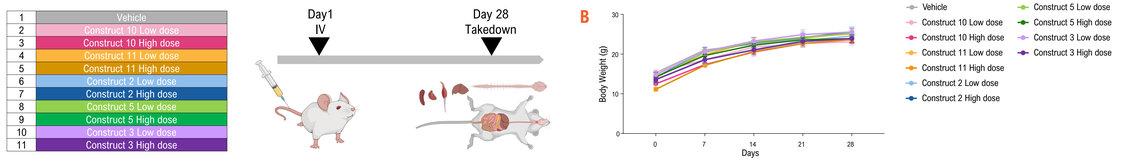
Figure 2. Optimized GBA Construct Demonstrates Successful Reduction of PHP.eB.GBA1 GCase Transduction with the Incorporation of DRG-detargeting Cassette



VYGR optimized PHP.eB.GBA1 with or without DRG detargeting cassette (A) were delivered to wildtype mice via tail vein injection and dorsal root ganglia (DRG) and brain stem were collected 1-month post-injection (B). VG Biodistribution was measured by ddPCR, FAM-RbGpA and VIC-Ms TFRC probes accounting for diploid animal cells. (C,F), GBA mRNA expression was evaluated by branched DNA (bDNA) technology. (D,G) GCase activity was measured by SensoLyte® Blue Glucocerebrosdase activity assay. (E,H). Mean +/-SEM. Mouse graphics generated in BioRender.

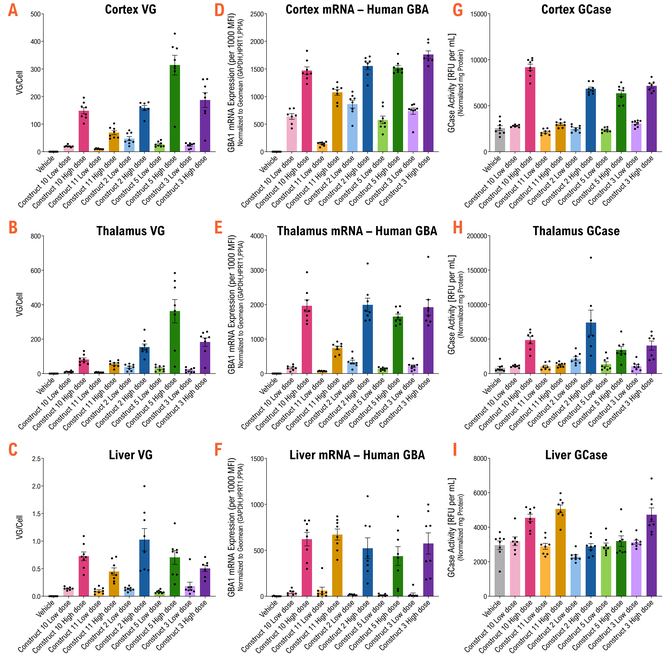
In vivo Target Engagement of VYGR Optimized PHP.eB.GBA1 in a GBA LOF Mouse Model:

Figure 3. Study Design and Cage Side Observation



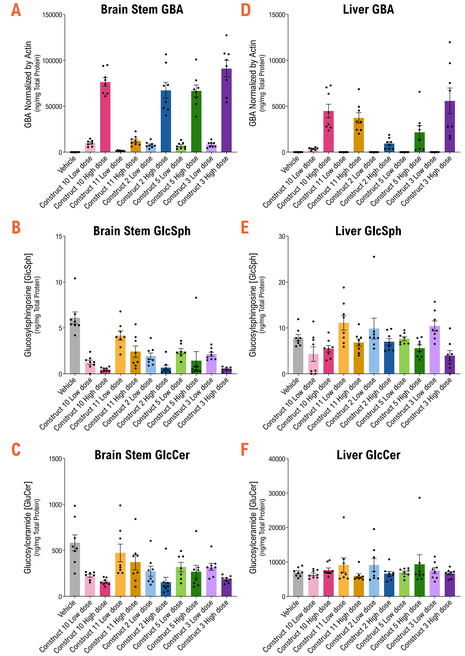
A) Five VYGR optimized PHP.eB.GBA1 vectors at 2 doses were injected in a GBA loss of function (LOF) mouse model and tissues were collected 28 days post-injection. B) VYGR optimized PHP.eB.GBA1 vectors was well tolerated with no-cage-side observations or body weight safety findings post-injection at any dosage. Mean +/-SEM. Mouse graphics generated in BioRender.

Figure 4. VG Biodistribution, GBA mRNA Expression, and GCase Activity



A-B) VG biodistribution was measured via ddPCR, FAM-RbGpA and VIC-MsTFRC probes accounting for diploid animal cells. Successful gene transfer across fore- and mid-brain regions was demonstrated in cortex (CTX) and thalamus (TH). Minimal gene transfer (<1VG/cell) was observed in liver (C). D-F) GBA1 mRNA expression was evaluated by branched DNA (bDNA) technology. The 7-plex probe-set used for this assay is custom designed to differentiate between transgene-specific GBA mRNA and mouse endogenous GBA mRNA. mRNA expression value is reported as mean fluorescence intensity (MFI). Human GBA1 mRNA is shown in figures D-F. Successful transcription across brain regions was demonstrated in cortex and thalamus. Reduced expression was observed in liver. G-I) GCase activity from cortex, thalamus, and liver was measured using SensoLyte® Blue Glucocerebrosidase activity assay. High dose of PHP.eB.GBA1 with enhancement 10, 2, 5, and 3 showed significant increase in GCase activities in CNS tissues. Mean +/-SEM.

Figure 5. Quantitative Analysis of GBA and Substrate Reduction



A-F) Brain stem and liver were submitted for LC-MS/MS analysis to quantify glucocerebrosidase (GBA) protein level and substrate reduction (glucosylsphingosine and glucocylceramide). PHP.eB.GBA1 with enhancement 10, 2, 5, and 3 showed significant increase in GBA protein expression (A). Substrate reduction in brain stem was observed in all constructs at both doses compared to vehicle treated mice. GBA expression level was increased in liver but did not reduce substrates compare to vehicle treated animals. Mean +/-SEM.

CONCLUSION

These results demonstrate that IV dosing of GBA1 transgenes using a blood brain barrier penetrant AAV capsid can effectively deliver therapeutically relevant levels of GBA1 protein to multiple brain regions in mouse models following a single dose.

AAV Vector for Low-dose One-time IV GBA Gene Therapy

INVESTORS & MEDIA

CAREERS AT VOYAGER

Efficacy of a Novel Vectorized Antibody Targeting the C-Terminal Domain of Tau, Antibody 1, Using Systemic Dosing of a Blood Brain Barrier Penetrant AAV Capsid in Mouse Models of Tauopathies

Wencheng Liu, Maneesha Paranjpe, Jerrah Holth, Blaise Clarke, Jeffrey Thomson, Joe Clement, Elisabeth Knoll, Charlotte Chung, Adewale Adeluyi, Alex Powers, Vinodh Kurella, Dillon Kavanagh, Brian Ezell, Timothy Fiore, Kyle Grant, Jay Hou, Kelly Bales, Steve Paul, and Todd Carter

Voyager Therapeutics Inc., 64 Sidney Street, Cambridge, MA 02139, USA

INVESTORS & MEDIA #1031

CAREERS AT VOYAGER

INTRODUCTION

Anti-tau immunotherapy is being pursued as a promising therapy for tauopathies, including Alzheimer's disease (AD), frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP). The success of such an approach relies, in part, on the identification of efficacious antibodies and their delivery to affected or vulnerable brain regions. We have previously demonstrated broad distribution and expression of vectorized anti-tau antibodies in the mouse brain using a blood brain barrier penetrant capsid administered intravenously (IV). Here we describe the characterization and vectorization of a novel anti-tau antibody, antibody 1 (Ab01), and efficacy studies in two tauopathy mouse models.

We have carried out immunization campaigns with AD patient-derived PHF-tau (paired helical filamentous tau) as an antigen in mice. One of the antibodies discovered, Ab01, exhibits strong selectivity for PHF-tau, and potent functional inhibition of PHF seeding and propagation *in vitro* and *in vivo*. This antibody contains a novel sequence, recognizes a phospho-specific epitope in the C-terminal region of tau, and shows significant reduction of tau pathology in an AD-PHF induced P301S hippocampal seeding and propagation model. Furthermore, we have vectorized Ab01 into an AAV expression vector and evaluated it in two independent mouse models of tauopathy.

This gene therapy-based approach has potential advantages over the traditional passive immunization, including 1) continuous expression of antibody in the central nervous system (CNS) after a single gene therapy administration compared to repetitive administrations of high dose of antibody by passive immunotherapy; 2) increased CNS exposure of tau antibody relative to passive immunotherapy; and 3) the potential to target intracellular tau aggregates which are less effectively accessed by passively delivered antibody; all of which could enhance targeting of pathological tau species in the CNS and resulting efficacy against tau pathology. These results add to accumulating evidence that systemic dosing of a vectorized anti-tau antibody using a BBB-penetrant AAV capsid results in reduced tau pathology and may represent a new single-dose therapeutic strategy for treating various tauopathies.

METHODS

1. Paired Helical Filamentous Tau (sarkosyl insoluble fraction enriched for PHF, abbreviated as ePHF) Preparation: ePHF was isolated from cortices of Braak VI AD cases based on the protocol described by Liu et al., *J Neuroscience* 36, 12425, 2016.

2. Vector Genome Measurement: AAV vector genome levels were quantified via ddPCR and shown as per diploid genome number using the endogenous mouse transferrin receptor C gene (TFRC) for normalization.

3. Anti-tau Antibody (Ab) Measurement: Anti-tau Ab expression within the CNS was evaluated using a sandwich ELISA in which ePHF was used as a capture antigen and an anti-IgG antibody for detection.

4. Affinity Measurement: Binding to iPHF and WT Tau was measured using Surface Plasmon Resonance (SPR) on Biacore 8K instrument. Briefly, iPHF and WT Tau were directly immobilized on CM5 sensor chip by amine coupling at 225 RU and 921 RU level respectively. Antibody was injected using Single Cycle Kinetics (SCK) mode with association and dissociation time of 5 and 10 min respectively. Concentration range of 0.078 to 2.5 nM for high binders or 0.78 to 25 nM for weak to no binders was used. The sensorgrams were fitted to 1:1 binding model in the Biacore Evaluation software to determine kinetic rate constants and affinity value.

5. *In vitro* Seeding Assay: 5X dilution of antibodies were coated on Dynabeads and incubated with sonicated ePHF. Immunodepleted supernatant were then incubated with Lipofectamine 2000 and added to Tau RD P301S FRET Biosensor cells (Holmes et al 2014). Cells were fixed 2 days. post-treatment and aggregated tau was measured by FRET-signal and normalized to nucleus count.

6. Detection of Tau Pathology: AT8 ELISA was used to detect tau pathology in the CNS of tauopathy models as described by Liu et al., *J Neuroscience* 36, 12425, 2016.

7. Statistics: Statistics were performed using a one-way ANOVA-Tukey's multiple comparison test for all graphs except Ab01/passive graph in Figure 4C which was done by student T test. Data is expressed as mean ± SEM. (*,**,*** and **** indicate statistical significance (p<0.05; 0.005, 0.0005 and 0.0001., respectively).

Figure 1. Voyager Anti-Tau Antibody Discovery

GOAL: Identify anti-tau antibodies that block tau-seed mediated propagation of pathology

APPROACH:

Focus on antibodies that target pathological tau species

Generate a diverse starting pool of anti-tau Abs

Immunization Campaigns

- Host animals: WT mouse, TauKO
- Immunogens: Paired Helical Filamentous tau (ePHF or sarkosyl insoluble fraction) from AD brain

Screen and prioritize anti-tau Abs based on:

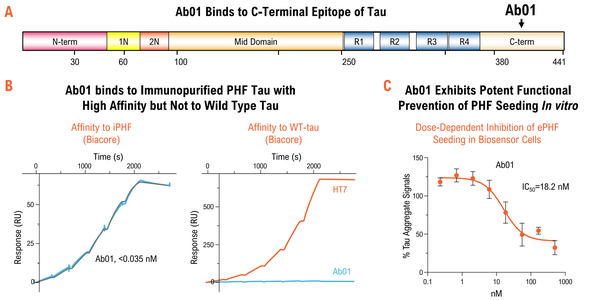
- Biochemical selectivity for pathological tau
- IHC selectivity for AD/PSP vs WT human brain
- Functional inhibition of PHF seeding *in vitro* and *in vivo*
- Developability based on low polyspecificity and lack of aggregation

Characterize additional attributes

- Sequence
- Epitope
- Western blot binding to AD, PSP vs WT human brain

Mouse immunization strategy and candidates' selection profiles for identifying efficacious anti-tau candidates.

Figure 2. Leading Candidate-Ab01: Biophysical Properties



Biophysical characteristics of leading candidate Ab01. A) Epitope position mapped against 2N4R-human tau isoform (441 residues). B) Antibody binding to immunopurified PHF (iPHF) or WT Tau was measured using Surface Plasmon Resonance (SPR) on Biacore 8K instrument. Ab01 demonstrated high affinity to iPHF but did not bind to wild type tau. HT7 is a Pan tau antibody and is used as a positive control for wild type tau. C) Ab01 demonstrated functional inhibition of ePHF-seeding activity *in vitro* with IC_{50} at 18.2 nM. D) Ab01 binds specifically to tau pathology on the cortical sections of AD, PSP or P301S but not non-AD, non-tauopathy or wild type/tau KO mouse cortical sections. Note that AD/non-AD or PSP/non-tauopathy cortical sections were purchased from Banner Sun Health Research Institute, Sun City, AZ.

Table 1. Biophysical Properties of Ab01

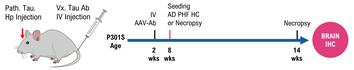
PROPERTY	CRITERIA*	Ab01
Approximate Epitope	-	C TERMINAL
Selectivity: iPHF:WT rec. Tau	> 100-fold	>838
Selectivity: ePHF:WT rec. Tau	≥ 100-fold	>222
IHC Fixed - Human AD Brain	Positive	POSITIVE
IHC Fixed - Human Ctl Brain	Negative	NEGATIVE
IHC Fixed - Mouse P301S	Positive	POSITIVE
IHC Fixed - Mouse Tau KO	-	POS/WEA
IHC Fixed - Mouse WT	-	WEAK
IHC Frozen - Human AD Brain	Positive	POSITIVE
IHC Frozen - Human Ctl Brain	Negative	NEGATIVE
IHC Frozen - Mouse P301S	Positive	POSITIVE
Inhibition of ePHF seeding in Biosensor Cells	≤ 20 nM IC_{50}	18.2
Low Polyspecificity (using BVP ELISA)	il range of comp. Abs	√
Solution and Colloidal Stability at >10 mg/mL	95% pure by SEC, no particulates	√

Summary of biophysical characteristics including selectivity, *in vitro* functional inhibition and developability of Ab01.

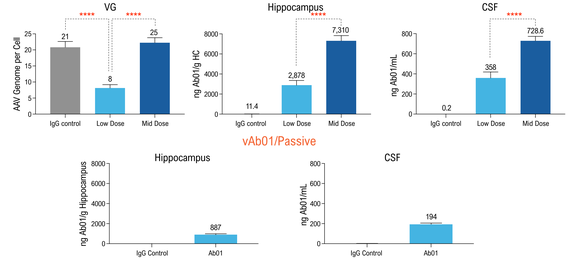
Figure 3. P301S Seeding Model Treated with Vectorized Ab01 Antibody

A Study Design

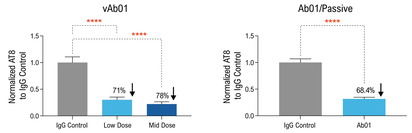
- Vector: PHPeB.CBA.Ab01
- ePHF is used to seed hippocampus
- Route: Vector IV dosed at age of 6 weeks
- Live phase: 8 weeks
- 2 doses: Low and Mid-dose
- Critical readout: AT8 ELISA
- Ab is present in hippocampus when seeded
- Ab was dosed one week prior to seeding in passive group



B AAV Biodistribution and Ab01 Expression in the CNS Regions at the Terminal Time



C Reduction of Tau Pathology in the Hippocampus



D vAb vs. Passive

GROUP	HC AB LEVEL (ng/g HIPPOCAMPUS)	CSF AB LEVELS (ng/g)	REDUCTION OF AT8 PATHOLOGY
vAb01, low dose	2,878	358	71%****
vAb01, mid dose	7,310	728	78%****
Passive, 40mg/kg	887	194	68.4%****

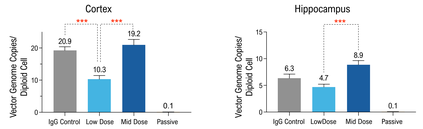
Treatment with vAb01 or with recombinant Ab01 administered passively demonstrated robust reduction of AT8 pathology in an ePHF seeding model. A) Ab01 was cloned as a full-length monoclonal antibody into a PHP-eB AAV vector using a ubiquitous promoter (CBA) and dosed intravenously (IV) two weeks prior to seeding. Two doses of vAb01 were used (low and mid-dose). In an independent experiment, recombinant Ab01 was administered Intraperitoneally (I.P.) twice in the week prior to seeding. 5 additional doses were administered by I.P. weekly after seeding in this experiment. For seeding, we injected 2 µl of ePHF (75 ng) into CA1 of hippocampus of P301S mice (Allen, et al., 2002 *J Neuroscience*), after which we allowed the pathology to develop for additional 6 weeks. Ipsilateral hippocampi were dissected, and their soluble and insoluble fraction were isolated for measuring antibody expression and tau pathology, respectively. B) AAV biodistribution and antibody expression of the vAb treatment groups are shown on the top. Ab expression from a passive group is used is shown on the bottom. Dose dependent increase of VG and Ab expression is observed in the CNS of animals treated with vAb01. C) Significant reduction of AT8 pathology is observed in all treatment groups compared to IgG control. D) Comparison of Ab expression and efficacy for different treatment groups.

Figure 4. P301S Intrinsic Model Treated with vAb01

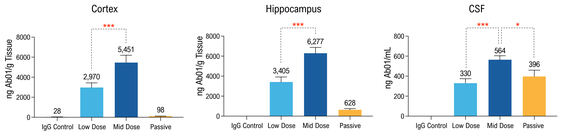
A Study Design

- Vector: PHPeB.CBA.Ab01
- Route: Vector IV dosed at age of 8 weeks
- Live phase: 13 weeks
- 2 doses: Low and Mid dose
- Critical readout: AT8 ELISA
- IgG control (Mid-dose)

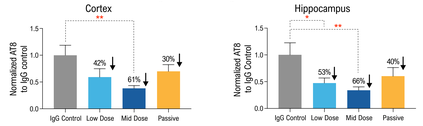
B AAV Biodistribution



C Ab01 Expression at the Terminal Time



D Efficacy Measured by AT8 ELISA



E vAb vs. Passive

GROUP	AB LEVEL (ng/g CORTEX)	AB LEVEL (ng/g HIPPOCAMPUS)	CSF AB LEVELS (ng/mL)	REDUCTION OF CORTICAL AT8 PATHOLOGY	REDUCTION OF HIPPO. AT8 PATHOLOGY
vAb01 (low dose)	2,970	3405	330	42%	53%*
vAb01 (mid dose)	5,451	6277	564	61%**	66%**
Passive, 40mg/kg	98	628	396	30%	40%

Treatment with vAb01 demonstrated significant reduction of AT8 pathology in the intrinsic model. A) Eight-week-old P301S mice (Allen. et al., *J Neuroscience*, 2002) were dosed with vAb01 by IV or recombinant Ab01 dosed by I.P. weekly for 13 weeks. Note that tau pathology is not developed yet in hippocampus and cortex at the age of 8 weeks in this model. Two doses (low or mid-dose) of vAb01 were used in the treatment, which lasted for 13 weeks. At the end of study, hippocampus and cortex were isolated for biochemical analyses as described in Figure 4. B) AAV biodistribution in the cortex and the hippocampus for all groups is shown. Dose dependent increase of VG is observed in the vAb treated groups. C) Antibody expression in the CNS regions and CSF for all groups is demonstrated. Dose dependent expression is observed for the groups with vAb01 treatment. D) Compared to IgG control, significant reduction is observed in the examined CNS regions with vAb01 treatment. A trend of reduction on AT8 pathology was observed in the passive arm. E) Comparison of Ab expression and efficacy for different treatment groups.

CONCLUSIONS

- We have identified a C-terminal antibody exhibiting strong selectivity for PHF-tau, and potent functional inhibition of PHF seeding and propagation *in vitro* and *in vivo*
- We have vectorized this antibody and examined its efficacy in two tauopathy models:
  - Vectorized Ab01 was well-tolerated at doses tested
  - We observed robust efficacy in all the treatment groups in the hippocampal seeding model
  - We observed significant efficacy in the P301S intrinsic model in vAb groups
  - A trend of reduction of AT8 pathology was observed in the P301S intrinsic model treated with Ab01 by passive administration at the dose tested

Intravenous Delivery of AAV Gene Therapy Provides Broad SOD1 Knockdown in the Spinal Cord and Robust Efficacy in a Mouse Model of SOD1-ALS

#1061

Michael D. Grannan, Ambreen Sayed-Zahid, Michael Hefferan, Mathieu Nonnenmacher, Shaoyong Li, Kyle Grant, Nilesh Pande, Katie Tyson, Jessenia Laguna-Torres, Anupriya Kulkarni, James Kaufman, Joe Clement, Jeffrey Thompson, Dinah W.Y. Sah, Holger Patzke

Voyager Therapeutics Inc., 64 Sidney Street, Cambridge, MA 02139, USA

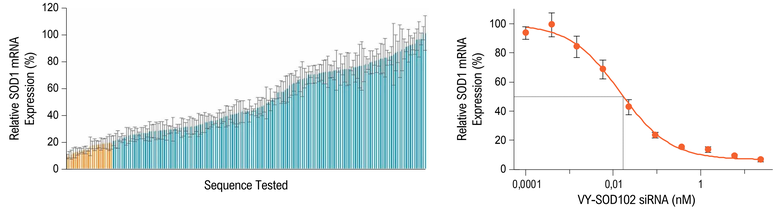
SUMMARY

- Mutations in SOD1 are responsible for a toxic gain of function and result in one of the most common forms of familial ALS
- Reduction of SOD1 in the spinal cord is thought to be a viable treatment approach, but conventional AAV therapies lack broad knockdown due to delivery limitations
- Using a capsid suitable for IV delivery in rodents, we have demonstrated robust knockdown of SOD1 in all levels of the spinal cord resulting in significant improvements in motor performance and survival in a mouse model of ALS
- Voyager's novel AAV capsids now allow for evaluation of this treatment approach in primates

INTRODUCTION

Mutations in superoxide dismutase 1 (SOD1) result in progressive motor neuron loss through toxic gain-of-function properties and are responsible for up to 20% of familial amyotrophic lateral sclerosis (ALS), or 2-4% of all ALS patients in the U.S. Studies using transgenic mice expressing SOD1 mutations have demonstrated reduced neuropathology, improved motor behavior, and extension of survival following several methods of SOD1 reduction. While these approaches have demonstrated varying degrees of efficacy, they often rely on direct spinal cord delivery and fail to achieve the broad SOD1 reduction throughout the CNS thought to be necessary for maximal clinical benefit. Voyager's novel AAV capsids now allow for intravenous delivery that is expected to provide appropriate biodistribution to motor neurons along the entire primate spinal cord, a key translational step for successful therapy. These novel AAV vectors carry transgenes encoding artificial pri-miRNAs using RNA interference (RNAi), a naturally occurring process that mediates gene silencing, to selectively reduce SOD1. Here, we report the results of an AAV gene therapy targeting SOD1 with RNAi, using IV delivery with a BBB-penetrant capsid, in studies in a G93A mouse model of ALS. These studies demonstrated robust SOD1 knockdown throughout the rostral-caudal extent of the spinal cord that correlated with vector genome levels and significant improvements in motor performance and survival extension, beyond what we have previously reported with intraparenchymal, intrathecal, or intracisternal delivery. These results suggest that the combination of potent and tolerable AAV-siRNA mediated knockdown with intravenous dosing of a BBB-penetrant capsid can demonstrate substantial phenotypic rescue in an SOD1-ALS mouse model and support its continued development and translation into the clinic.

Figure 1. Primary Screen of SOD1 siRNA Sequences in HeLa Cells



Primary screen of SOD1 siRNA sequences in HeLa cells. 169 sequences selectively targeting SOD1 were designed, synthesized and evaluated in HeLa cells. 24hr after transfection of 100pM SOD1 siRNA, cells were harvested, and SOD1 and GAPDH mRNA were quantified by RT-qPCR. SOD1 mRNA levels were normalized to GAPDH mRNA levels, and then expressed relative to a negative control. Data shown represent mean ± SD. 22 SOD1 siRNAs (yellow bars) resulted in >80% SOD1 mRNA knockdown.

G93A Efficacy Study

Mice: ~56-day old B6SJL-Tg(SOD1*G93A)1Gur/J (male and female)

Vector: PHP.eB.VY-SOD102 siRNA

Dose: 100µl, IV administration 2e12, 6.3e12, and 2e13 vg/kg

Endpoints: Motor performance, body weight, survival, IHC in spinal cord

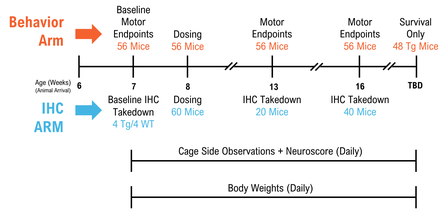
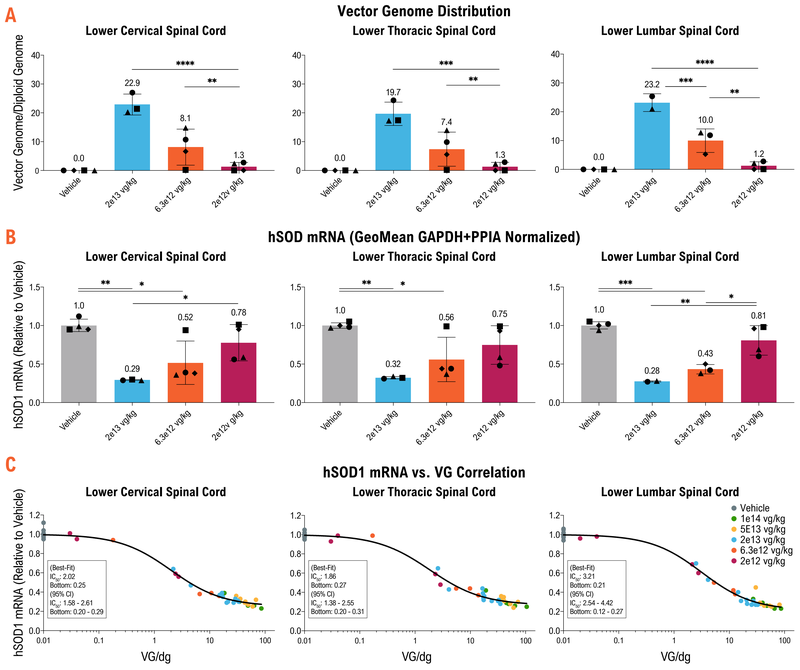
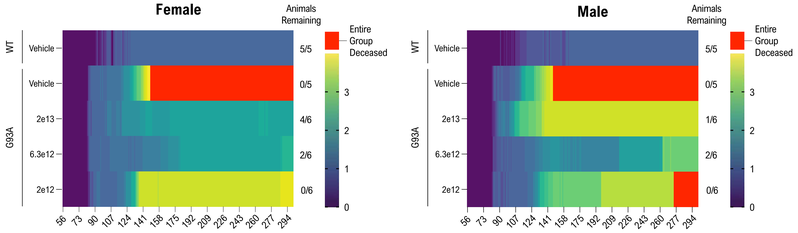


Figure 2. Vector Genome Distribution in Mouse Spinal Cord Following IV Delivery



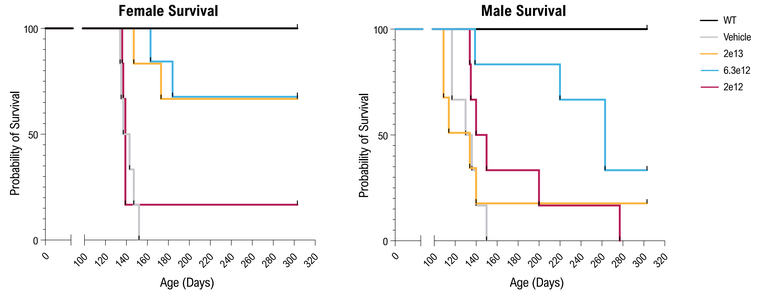
Vector genome distribution and huSOD1 knockdown in the cervical, thoracic, and lumbar spinal cord of G93A mice 32 days following AAV administration. A) Vector genome distribution was analyzed using a multiplex ddPCR assay against transgene and host targets in multiple regions of the spinal cord. B) huSOD1 expression measured using multiplexed RT-qPCR with huSOD1 expression level normalized to 2 host reference gene transcripts with vehicle control group as the comparator. C) Correlation of vector genome to huSOD1 knockdown in the mouse spinal cord. *p<0.05, **0.01, ***0.001, 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 3. Improvement in Neuroscore Composite Rating Following IV Delivery



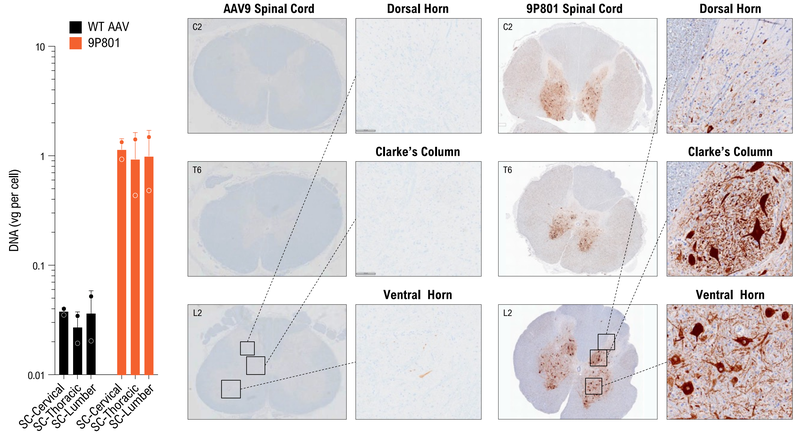
Neuroscore composite rating assessment in G93A mice. Mice were assessed for motor performance using the Neuroscore rating scale ~3x/week for the duration of the study. The scale ranges from 0-4, with 0 = no deficit, 1 = first symptoms, 2 = onset of paresis, 3 = paralysis, 4 = humane endpoint. Animals currently remaining are shown for each treatment group.

Figure 4. Increase in Survival in G93A Mice



Survival analysis of male and female G93A mice. Median survival could not be calculated for female 2e13 or 6.3e12 vg/kg dose groups due to over half of the cohort remaining.

Figure 5. Capsid 9P801 Mediates Strong Transduction in Macaque Spinal Cord



TRACER AAV9-derived capsid mediates widespread transgene expression in NHP brain. Previously disclosed data depicts enhanced spinal cord transduction compared to WT AAV9 when administered intravenously. Data shown was collected following an intravenous dose of 2e13 vg/kg.

CONCLUSIONS

- Robust knockdown of SOD1 in all levels of the spinal cord is observed following IV administration of and AAV gene therapy targeting SOD1 with RNAi
- Significant improvements in motor performance, body weight, and survival are observed in the G93A mouse model of ALS
- These data support the continued development of IV delivered RNAi using a novel BBB-penetrant capsid for use in primates

INVESTORS & MEDIA | CAREERS AT VOYAGER