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

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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

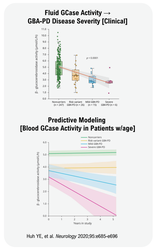
GENETICS & EPIDEMIOLOGY

- *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



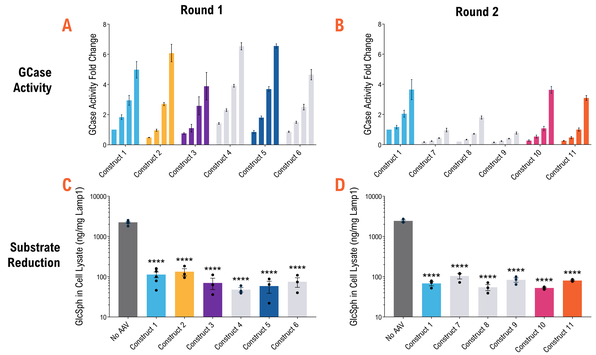
GBA BACKGROUND

- 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

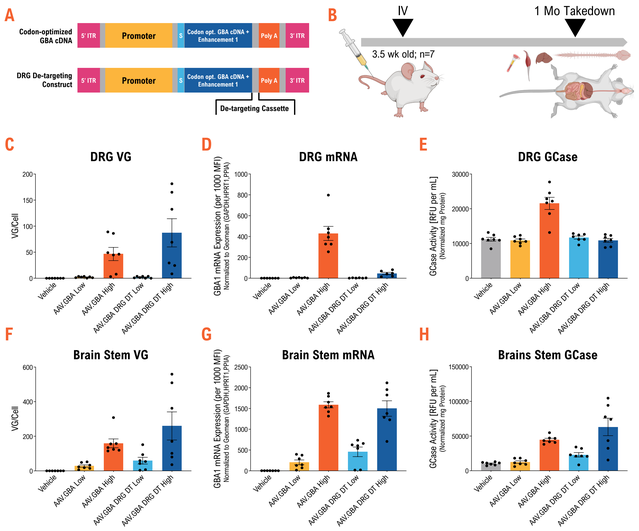
- Increasing GCase enzyme activity with AAV gene transfer of optimized GBA1 transgene cassette in a widespread CNS footprint successfully shown to decrease substrate glycosphingolipid glucosylceramide levels; and slowdown pathogenesis in GBA-PD
- With modifications in delivery approach, strategy could 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.

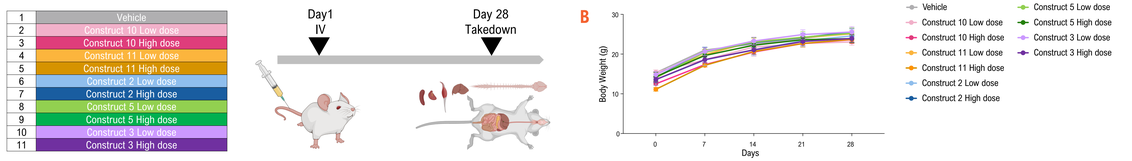
Figure 2. Optimized GBA Construct Demonstrates Successful Reduction of PHP.eB.GBA1 GCase Transduction with the Incorporation of DRG-detargeting Cassette



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.

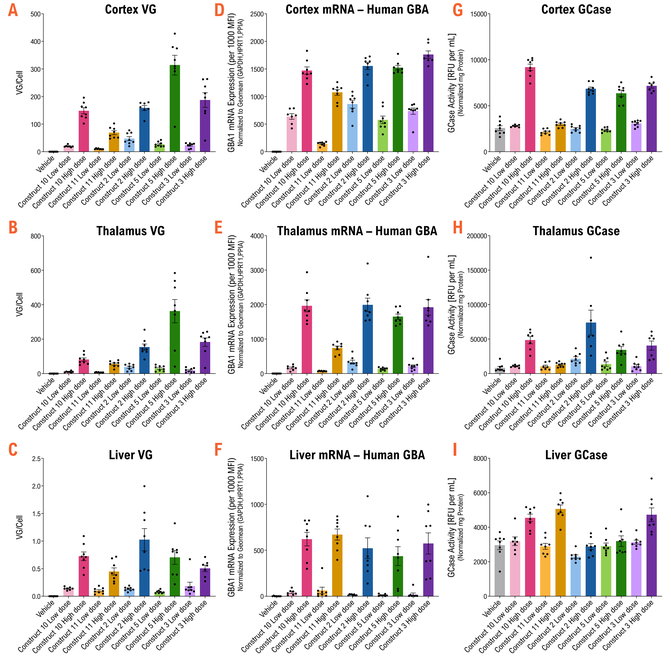
In vivo Target Engagement of VYGR Optimized PHP.eB.GBA1 in a GBA LOF Mouse Model:

Figure 3. Study Design and Cage Side Observation



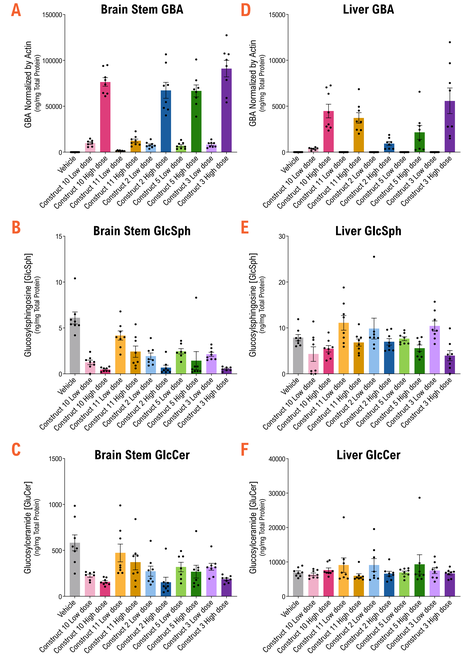
A) Five VYGR optimized PHP.eB.GBA1 vectors at 2 doses were injected in a GBA loss of function (LOF) mouse model and tissues were collected 28 days post-injection. B) VYGR optimized PHP.eB.GBA1 vectors was well tolerated with no-cage-side observations or body weight safety findings post-injection at any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

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