SPR301 dose-dependent reduction of inflammation and substrates in a surrogate model for GBA1-linked neuroinflammation

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Introduction

Parkinson's disease (PD)

- PD is one of the most common neurodegenerative diseases, affecting nearly two million people across US and Europe.
- An association between Gaucher disease (GD), *GBA1* mutations which attenuate or abrogate the activity of the lysosomal enzyme glucocerebrosidase (GCase), and PD has been established, with approximately 5-15% of PD patients carrying a pathogenic or non-pathogenic *GBA1* mutation.[1]
- There is also evidence for reduced GCase activity in PD patients without a known *GBA1* mutation (idiopathic Parkinson's, iPD), with factors impacting GCase stability, activity, lipid clearance and α-synuclein aggregation.
- Although most *GBA1*^{mut} patients do not develop PD, *GBA1* mutation status is still the most predictive genetic factor for the development of PD and is associated with early onset PD and rapid progression.

SPR301

SPR301 is an AAV9 based gene therapy in development for *GBA1*-linked PD, containing an expression cassette optimised for CNS expression, encoding a novel engineered GCase variant 85 (GCase85).

- GCase85 with two amino acid substitutions to the wild type GCase (GCase WT), has been established as a more stable enzyme while retaining the same specific activity as wild type, resulting in significantly increased GCase exposure.[2]
- In the context of GD, the liver optimised FLT201 which delivers GCase85, has shown durability of clinical benefit.[3,4]

SPR301 delivering *GBA1-85*, the gene encoding GCase85, offers the potential for improved GCase delivery and distribution in the brain for GBA1-linked PD. In this study, initial proof of principle was established in an SH-SY5Y *in vitro* model of dopaminergic neurons (Fig.1 and 2):

- α-Synuclein aggregate seeded cells, pre-treated with SPR301, demonstrated a superior GCase activity in cell supernatants and a more efficient intracellular α-synuclein reduction when compared with AAV9-GBA1 WT pre-treated cells.

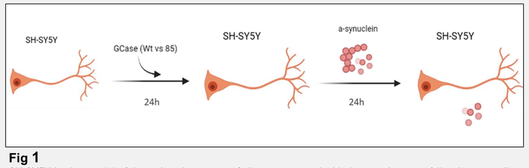


Fig 1
SH-SY5Y in vitro model of dopaminergic neurons. Cell are pre-treated with therapeutic vector 24h prior to seeding with α-synuclein aggregates (4 µg/mL). Supernatant and cell lysate are analysed after 24h further incubation.

In vivo proof of principle was established in a surrogate murine model for GBA1-linked neuroinflammation treated with conduritol-β-epoxide (CBE), a brain penetrant irreversible inhibitor of GCase (Figs 3-5).

- A dose response was observed for SPR301 for GCase activity and substrate reduction. SPR301 at low to mid dose, ≥ 25-fold lower dose than that required for AAV9-GBA1 WT, reduced CBE-induced inflammation to near naïve levels in the thalamus, hypothalamus and substantia nigra.

Background

SPR301 delivers higher measured GCase activity and demonstrates more efficient α-synuclein reduction in SH-SY5Y cells, a surrogate *in vitro* model of dopaminergic neurons.

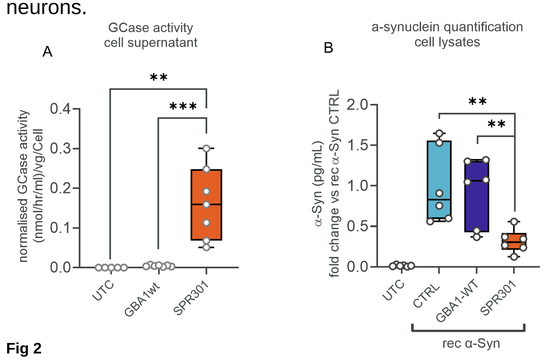
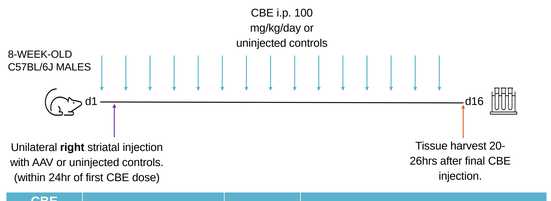


Fig 2

(A) GCase activity in supernatant of AAV9-GBA-WT or SPR301 treated SH-SY5Y cells or untreated control (UTC) and (B) α-synuclein reduction by ELISA in cell lysates of SH-SY5Y cells pre-treated with AAV9-GBA-WT or SPR301 in comparison to untreated control (UTC) and recombinant α-synuclein aggregate (rec α-syn) control. SH-SY5Y cells transduced at MOI of 1E6; N=2, data denoted as mean ± SEM; t-test shown.

Methods

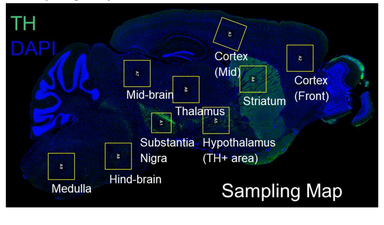
SPR301 in a murine CBE induced surrogate model for GBA1-linked neuroinflammation



CBE Treatment	Test Vector	Dose (vg)	Analysis Presented
Naïve	naive	-	GCase activity of the right hemibrain Glucosylsphingosine, glucosylceramide of the right hemibrain IHC Sagittal brain sections stained for human GBA1, microglia (Iba-1), nuclei (DAPI)
CBE	naive	-	
CBE	AAV9-GBA1-WT	1.32E+10	
CBE	AAV9-Negative Control	1.32E+10	
CBE	SPR301	5.20E+08	
CBE	SPR301	2.60E+09	
CBE	SPR301	1.32E+10	

Study performed at Scantox Neuro GmbH

Sampling Map for IHC



IHC analysis samples were sectioned and stained with antibodies against GCase, IBA-1 and TH. TH images established a Sampling Map which was used for quantification, blinded to experimental group, using QuPath software and normalised by sample area (µm^2 of positive staining per µm^2 of sampled area) for graphical presentation.

Results

SPR301 achieves equivalent GCase activity at a dose ≥ 25-fold lower than AAV9-GBA1 WT

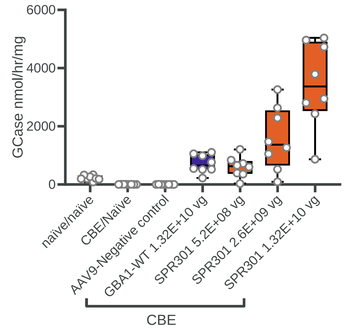


Fig 3

GCase activity in the right hemibrain (N=8) of CBE treated mice injected with SPR301 compared to AAV9-GBA1-WT.

SPR301 shows toxic substrate reduction at a dose ≥ 25-fold lower than that required for AAV9-GBA1 WT

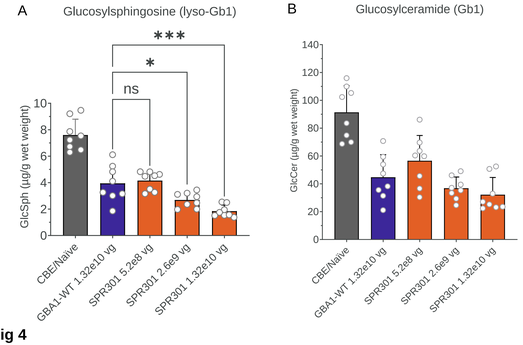


Fig 4

(A) Glucosylsphingosine and (B) Glucosylceremide levels in the right hemibrain (N=8) of CBE treated mice injected with SPR301 compared to AAV9-GBA1-WT.

SPR301 shows a dose dependent GBA distribution

CBE treatment +/- the negative AAV9 control stimulated equivalent inflammation (microglial IBA-1 marker) in all areas assessed, except for the striatum which did not appear to be affected by CBE. No GBA staining was detected in the contralateral untreated hemispheres (data not shown).

- Dose dependent GBA distribution was observed (Fig. 5A) for the mid cortex, hypothalamus and substantia nigra, with SPR301 dosed at 2.6 x 10^9 vg equal or greater than that observed for AAV9-GBA1 WT at 1.32 x 10^{10} vg.
- High dose cohorts of both SPR301 and AAV9-GBA1 WT appeared to induce further inflammation, but mid and low dose appeared to reduce inflammation (Fig 5B).
- SPR301 at low to mid dose successfully reduced CBE induced inflammation to naïve levels in the thalamus, hypothalamus and substantia nigra and did not stimulate inflammation at the site of injection (Fig 5B & 5C).

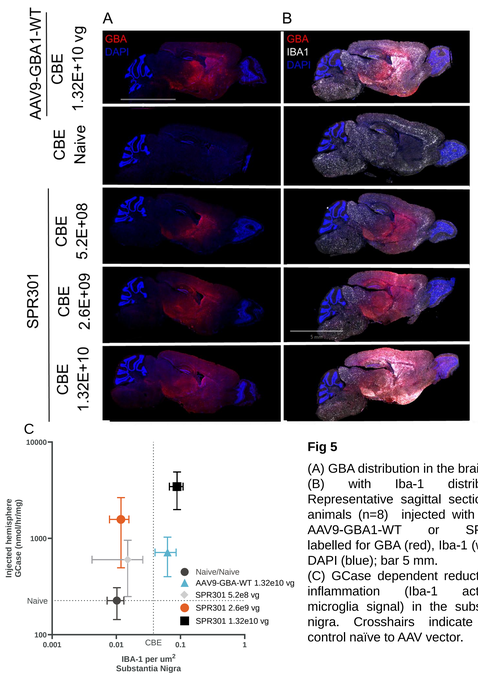


Fig 5

(A) GBA distribution in the brain and (B) with Iba-1 distribution. Representative sagittal sections of animals (n=8) injected with either AAV9-GBA1-WT or SPR301 labelled for GBA (red), Iba-1 (white), DAPI (blue); bar 5 mm.
(C) GCase dependent reduction of inflammation (Iba-1 activated microglia signal) in the substantia nigra. Crosshairs indicate CBE control naïve to AAV vector.

Conclusions

SPR301 shows potential for *GBA1*-linked Parkinson's disease with a favourable therapeutic window

1. SPR301 demonstrates efficient intracellular α-synuclein reduction vs AAV9-GBA1 WT in α-synuclein seeded SH-SY5Y cells.
2. A dose response was observed for SPR301 for GCase activity and substrate reduction in a murine CBE model for GBA1 deficiency.
3. SPR301 at low dose reduced CBE induced Gb1, lyso-Gb1 & inflammation to near naïve levels in the thalamus, hypothalamus & substantia nigra.

References

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3. Khinder, J., *et al.* Durability of FLT201: an Investigational Gene Therapy for Gaucher Disease Type 1 Encoding an Engineered Variant of the GCase Enzyme. ASGCT Abstract 174, May 15, 2025. Time: 8:00 AM - 9:45 AM , ASGCT 28th Annual Meeting
4. GALILEO-1 clinical trial NCT05324943

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