Generation of β-Glucocerebrosidase Variants with Increased Half-life in Human Plasma for Liver Directed AAV Gene Therapy for the Treatment of Type 1 Gaucher Disease

Poster #41

Fabrizio Comper[1], I-Mei Yu[1], Petya Kalcheva[1], Carlos J Miranda[1], Elisa Chisari[1], Clement Cocita[1], Samantha Correia[1], Jalpa Pandya[1], Natalie Northcott[1], Emmaline Stotter[1], Erald Shehu[1], Rose Sheridan[1], Amit C Nathwani[2], Romuald Corbau[1]

[1]Freeline, Stevenage, United Kingdom; [2]University College London Cancer Institute, London, United Kingdom

INTRODUCTION

- Mutations in the *GBA1* gene result in deficiency of β-Glucocerebrosidase (GCase) and cause Gaucher disease (GD), the most common lysosomal storage disorder.[1]
- Enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) are currently standard of care for the treatment of non-neuronopathic GD patients.[2] However, significant unmet needs remain:
  - The short duration of activity of ERTs requires IV administration every 2 weeks, which translates into high treatment burden and cost.[2]
  - Response to treatments can be variable and incomplete.[3,4]
  - Patients continue to experience significant life-limiting symptoms and resultant poor quality of life.[3-6]
- Systemic liver-directed AAV gene therapy may offer superior therapeutic benefit for patients with GD, but the short half-life of GCase in blood poses substantial challenges in delivering active enzyme to target tissues.
- Here, we present the design, screening, and preliminary *in vitro* and *in vivo* characterisation of GCase variants aimed at increasing enzymatic stability.

METHODS

- Recombinant proteins were expressed *in vitro* using the Expi293™ expression system (Invitrogen) or in a human hepatocellular carcinoma (Huh7) cell line, and *in vivo* in wild-type C57Bl/6 mice.
- Tissues were processed using standard techniques, in turn homogenised by a Precellys Evolution Tissue Homogenizer to measure the GCase activity content or collected for immunohistochemical (IHC) analysis using the automated Ventana Discovery XT system (Roche Diagnostics) and the Ventana DAB Map detection Kit. Anti-GBA antibody (Abcam, Cat. #: ab125065) was used.
- GCase activity was determined using a fluorometric assay with the fluorescent substrate 4-Methylumbelliferyl-β-D-glucopyranoside (4MUG) in GCase assay buffer (AB buffer). Stability was measured as % of residual activity at different time points following incubation in the chosen buffer.

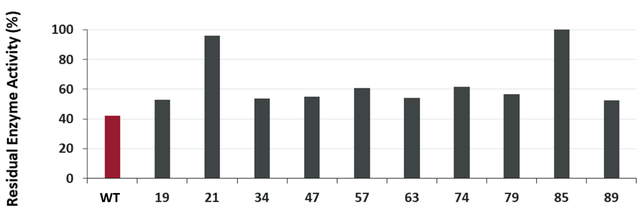
RESULTS

In Vitro Stability

- We designed and engineered 86 GCase variants and ten of these variants showed improved stability compared with wild-type (WT) Gcase after 2 hours of incubation at neutral pH (**Figure 1**).
- The two variants with the highest stability (21 and 85), and their combination (21-85), were further characterised.

Figure 1. Relative *in vitro* stability of GCase variants (shown in black) compared with WT GCase (red).

Ten GCase variants showed higher stability *in vitro* compared with WT GCase

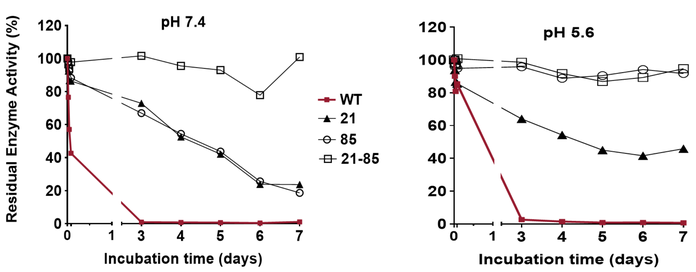


Cell conditioned media were used. WT = wild type.

- Variants 21, 85 and 21-85 showed sustained activity over 7 days both at neutral and acidic pH (**Figure 2**).

Figure 2. Relative *in vitro* stability of GCase variants compared with WT GCase (shown in red) incubated at acidic (AB) and neutral (PBS) pH over 7 days.

Two GCase variants and their combination showed enhanced stability over 7 days



Cell-conditioned media were used and time points were taken at 0.5, 1, 2 hours, than at day 3, 4, 5, 6, 7 post incubation. WT = wild type.

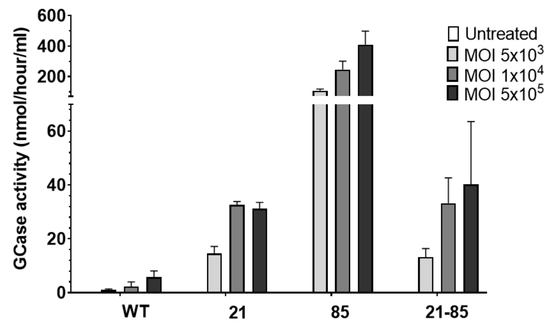
RESULTS (continued)

In Vitro Activity and Stability of GCase Variant 85

- The three GCase variants (21, 85 and 21-85) and the WT GCase were transduced using our proprietary AAV capsid (AAVS3) in the Huh7 cell line at different titres (Multiplicity Of Infection).
- GCase variant 85 showed the highest level of GCase activity (ranging from >100-fold increase at the lowest titre and >80-fold increase at the highest titre tested) compared with the WT GCase (**Figure 3**).
- Variant 85 was chosen as the lead construct and further characterised.

Figure 3. GCase activity of the three variants (21, 85 and 21-85) and WT transduced using AAV-S3 in Huh7 cell line.

Variant 85 expressed the highest activity when transduced in the Huh7 cell line



Conditioned media were used for the activity assay. MOI = multiplicity of infection. WT = wild type.

- The thermostability, the Michaelis-Menten enzymatic constant (K_M) and the *in vitro* stability of purified variant 85 and WT GCase in different biologic media were determined.
- Variant 85 showed improved stability in all media tested, ranging from a 6 to >21 times longer half-life compared with WT GCase, with a 3°C increase in thermostability, without a difference in K_M (**Table 1**).

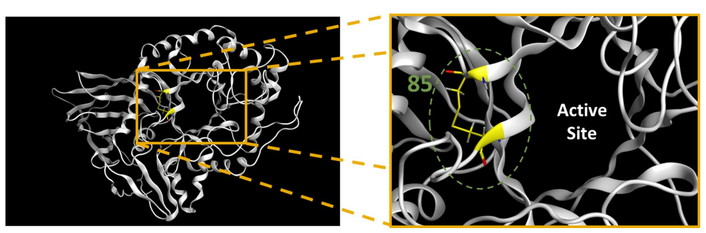
Table 1. Biophysical properties of GCase variant 85 and WT (velaglucerase alfa)

	Lysosomal pH	Physiological pH	Mouse serum	Mouse plasma	Human serum	Tm (°C), pH7	K_M (mM)
	Half-life (minutes)						
WT	388	70	7.4	77	24	51.5	2.3
Variant 85	>8639	141	74	508	143	54.5	2.4
Improvement	>21X	2X	10X	6.6X	6X	3°C	na

WT = wild type.

- Based on *in vitro* activity and biophysical properties, GCase variant 85 was selected as our candidate for testing in *in vivo* models.
- The structure of variant 85 is shown in **Figure 4**.

Figure 4. Model of GCase variant 85

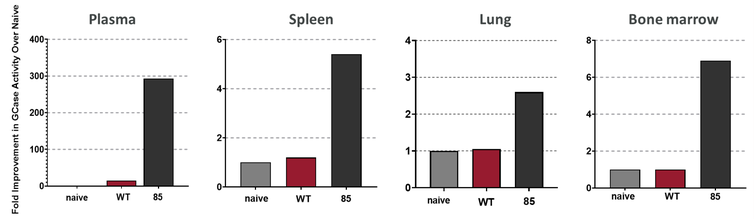


In Vivo Activity of Variant 85

- Variant 85 and WT GCase vectors pseudotyped with AAV8 capsid were transduced in C57bl/6 mice at a titre of $6x10^{10}$ vg/kg, and GCase activity was analysed in plasma, spleen, lung and bone marrow.
- At the low vector dose of $6x10^{10}$ vg/kg, mice exposed to the WT construct had a 15-fold increase in GCase activity in plasma compared with untreated mice, but substantial accumulation of GCase in target organs was not observed (**Figure 5**).
- In contrast, GCase protein variant 85 at the same dose showed almost 300-fold higher GCase activity in plasma, and more than 5, 2, and 6 times in spleen, lung and bone marrow, respectively (**Figure 5**).

Figure 5. Fold increase in GCase activity over naive in plasma and various tissues in mice transduced with variant 85 and WT vectors pseudotyped with AAV8 capsid ($6x10^{10}$ vg/kg)

Variant 85 showed improved GCase activity *in vivo*

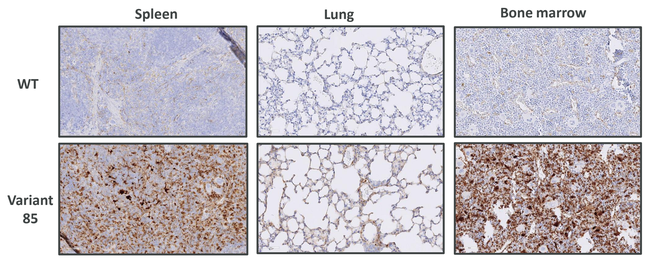


Naïve = mice not transduced. Samples taken 28 days post injection. WT = wild type.

- Immunohistochemistry (IHC) showed substantial accumulation of the variant 85 GCase protein in target organs, with WT being only faintly detectable (**Figure 6**).
- Importantly, variant 85 was also observed in lung and bone marrow, which are difficult-to-reach organs (**Figure 6**).

Figure 6. Immunohistochemical analysis of GCase proteins in target organs.

IHC analysis confirmed accumulation of variant 85 GCase in difficult to reach targets organs



Samples were taken 28 days post infection at a titre of $6x10^{10}$ vg/kg

CONCLUSIONS

- Treatment of patients with GD may be improved through a gene therapy approach that provides sustained, endogenous production of GCase following a single IV infusion.
- We have designed a GCase protein variant that shows increased stability in different physiological media compared with ERT, without differing in its fundamental enzymatic parameter K_M.
- This higher stability translated into a substantially higher level of GCase activity in plasma and target organs of mice (including difficult to treat organs such as lung and bone marrow) transduced with a very low dose AAV vector ($6x10^{10}$ vg/kg).
- GCase variant 85 (FLT201) is part of the Freeline GD product development programme and further studies are ongoing to evaluate its therapeutic potential; a first-in-human clinical trial is targeted for later this year.

References

1. Stirnemann J, Belmatoug N, Camou F, et al. *Int J Mol Sci.* 2017;18(2):441. **2.** Gary SE, Ryan E, Steward AM, Sidransky E. *Expert Rev Endocrinol Metab*. 2018;13:107–118. **3.** Shayman JA. Advances in Gaucher disease: basic and clinical perspectives. Future Medicine Ltd, Grabowski: London; 2013; 240–256. **4**. Weinreb NJ, et al. *J Inherit Metab Dis.* 2013;36: 543–553. **5.** Wyatt K, et al. *Health Technol Assess.* 2012;16:1-543. **6.** Revel-Vilk S, et al. *Br J Haematol.* 2018;182:467-480.

E-Poster Presented at the WORLD*Symposium* 2021 Virtual Scientific Meeting, 11 February 2021.