FLT201: AN AAV-MEDIATED GENE THERAPY FOR TYPE 1 GAUCHER DISEASE DESIGNED TO TARGET DIFFICULT TO REACH TISSUES

Poster #44

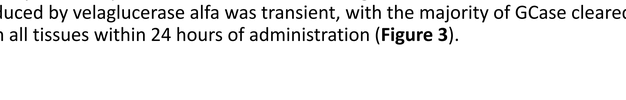
Romuald Corbau[1], Carlos J Miranda[1]; Fabrizio Comper[1], Petya Kalcheva[1], Elisa Chisari[1], Clement Cocita[1], Samantha Correia[1], Jalpa Pandya[1], Benjamin Liou[2], Natalie Northcott[1], Emmaline Stotter[1], Erald Shehu[1], I-Mei Yu[1], Jaminder Khinder[1], Rose Sheridan[1], Ying Sun[2], Amit C Nathwani[3]

[1] Freeline, Stevenage Bioscience Catalyst, Stevenage, United Kingdom; [2] Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; [3]Department of Haematology, University College London Cancer Institute, London, United Kingdom

INTRODUCTION

- Gaucher disease (GD) is one of the most common lysosomal storage disorders. Mutations in the *GBA1* gene attenuate or abrogate the activity of the lysosomal acting enzyme glucocerebrosidase (GCase).[1]
- Enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) are currently standard of care for the treatment of type 1 GD (GD1) patients.[2] However, significant unmet needs remain:
  - Intravenous infusion every 2 weeks is required because of the short half-life of ERTs, which produces a high burden of treatment and consumes significant healthcare resources.[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]
- Oral SRTs are primarily limited to patients for whom ERT is not suitable or who are not CYP2D6 ultra-extensive metabolisers.[2]
- A single infusion with adeno-associated virus (AAV) gene therapy has the potential to provide continuous endogenous levels of enzyme to achieve the ultimate treatment goal of a functional cure for GD1 patients.
- Our proprietary AAVS3 has shown significantly higher transduction efficiency and protein expression in human liver cells compared with wild-type (WT) AAV serotypes used in many other gene therapy programmes.
- FLT201, an investigational gene therapy for the treatment of GD1, is a novel, potent, engineered AAVS3 containing an expression cassette that encodes for a GCase variant 85 ($GCase_{var85}$) (**Figure 1**).
- This paper describes the *in vitro* and *in vivo* characterisation of FLT201.

Figure 1. FLT201: investigational AAVS3 gene therapy for GD1.



METHODS

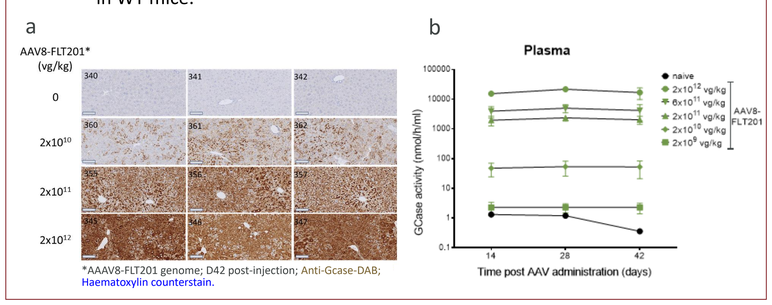
- AAV constructs were optimised to express full-length $GCase_{var85}$. Constructs were packaged as AAV8 particles for *in vivo* (mouse studies), or as AAVS3 (Freeline proprietary capsid optimised for human liver transduction) particles for non-human primate (NHP) studies.
- FLT201 was administered as a single injection at doses indicated for each experiment. Velaglucerase alpha (ERT) 60 U/kg was administered either once (PK studies in WT mice) or every other week ($Gba^{9V/null}$ mice).
- GCase activity in plasma and tissues was determined fluorometrically with 4-methylumbelliferyl-β-D-glucopyranoside based on a 4-methylumbelliferone standard curves. Immunohistochemistry was performed on paraffin sections following standard protocols.
- Data are shown as mean ± SD and statistical analysis performed using one-way ANOVA.

RESULTS

AAV8-FLT201 increases GCase expression in WT mice

- AAV8-FLT201 produced robust GCase expression in murine liver and showed a dose-dependent increase in immunoreactivity to human GCase (**Figure 2a**).
- $GCase_{var85}$ expression in the liver resulted in a sustained and linear dose-dependent increase in secretion of active GCase into the bloodstream (**Figure 2b**).

Figure 2. GCase expression in the liver (**a**) and GCase secretion into the bloodstream (**b**) in WT mice.



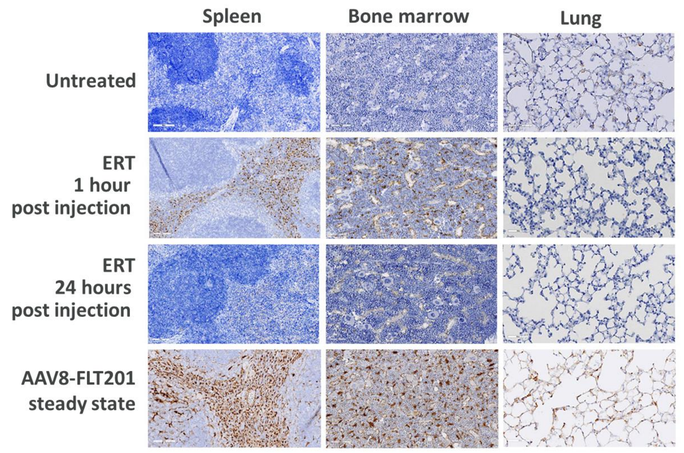
*AAV8-FLT201 genome; D42 post-injection; Anti-Gcase-DAB; Haematoxylin counterstain.

AAV8-FLT201 produces enhanced and sustained uptake of GCase in key tissues in WT mice

- Administration of AAV8-FLT201 to WT mice produced a steady-state level of GCase uptake into all tissues analysed (liver, spleen, bone marrow and lung) (**Figure 3**).
- In contrast, administration of velaglucerase alfa produced uptake of GCase in spleen, but only limited uptake in bone marrow and lung tissue. The level of Gcase produced by velaglucerase alfa was transient, with the majority of GCase cleared from all tissues within 24 hours of administration (**Figure 3**).

RESULTS (continued)

Figure 3: GCase uptake in key organs following a single injection of AAV8-FLT201 or ERT

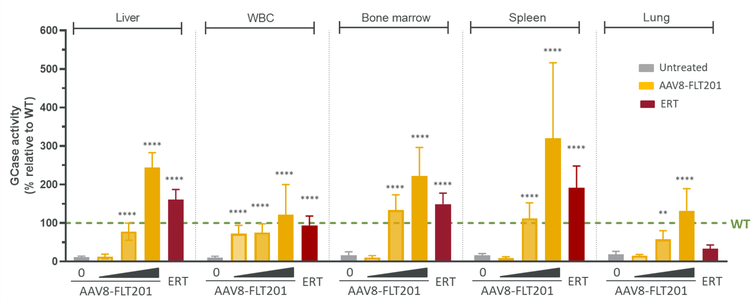


WT mice, n=3; GCase; Haematoxylin; ERT = velaglucerase alfa at 60 U/kg; AAV8-FLT201 at $6x10^{10}$ vg/kg.

AAV8-FLT201 restores GCase activity in *GCase*-deficient mice

- A single injection of AAV8-FLT201 to $Gba^{9V/null}$ mice resulted in a dose-dependent increase in GCase activity in the different tissues analysed (**Figure 4**).
- Restoration of GCase activity was observed at the dose of $2x10^{11}$ vg/kg in bone marrow and spleen, and at the dose of $2x10^{12}$ vg/kg dose in white blood cells (WBC) and lung (**Figure 4**).

Figure 4: GCase activity in tissues following administration of FLT201 or ERT in *GCase*-deficient mice

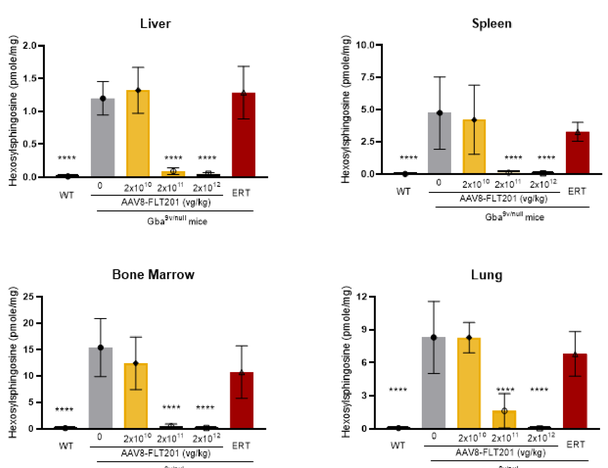


AAV8-FLT201 = AAV8 pseudo-typed FLT201 genome; $2x10^{10}$, $2x10^{11}$ and $2x10^{12}$ vg/kg; ERT = velaglucerase alfa 60 U/kg biweekly; WT = wild type mice; WBC = White blood cells; **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle control.

AAV8-FLT201 reduces levels of substrate in *GCase*-deficient mice

- A single injection of AAV8-FLT201 resulted in significant dose-dependent reductions in levels of hexosylsphingosine in tissues of *GCase*-deficient mice compared with untreated controls (**Figure 5**); these reductions were observed in plasma, liver, spleen and hard-to-reach tissues such bone marrow and lung.
- In contrast, ERT had little to no effect on levels of hexosylsphingosine compared with untreated controls.

Figure 5: AAV8-FLT201 dose-dependent reduction of the hexosylsphingosine observed in *GCase*-deficient mouse tissues

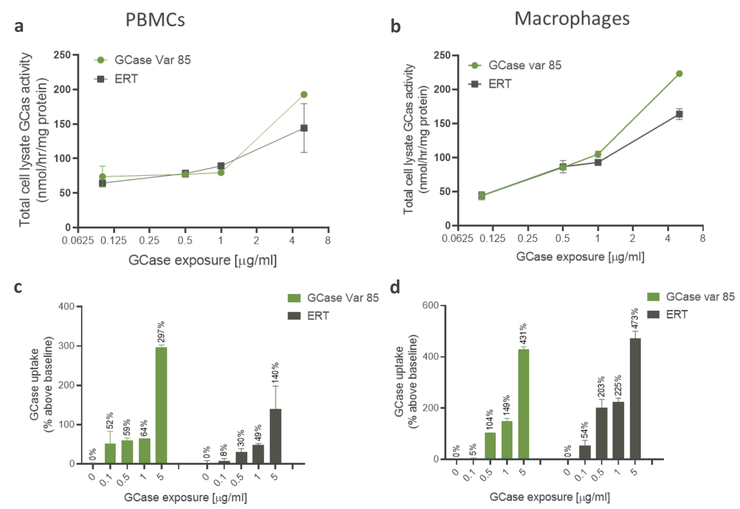


AAV8-FLT201 = AAV8 pseudo-typed FLT201 genome; ERT = velaglucerase alfa 60 U/kg biweekly; WT = wild type mice; ****p<0.0001 vs. vehicle control.

$GCase_{var85}$ can be taken up by human PBMCs and macrophages

- *In vitro* studies demonstrate that $GCase_{var85}$ can be taken up by human PBMCs (**Figure 6a and 6C**) and macrophages (**Figure 6b and 6d**) at similar levels to those observed with ERTs.

Figure 6: Uptake of $GCase_{var85}$ and ERT by human PBMCs (a,c) and macrophages (b, d)

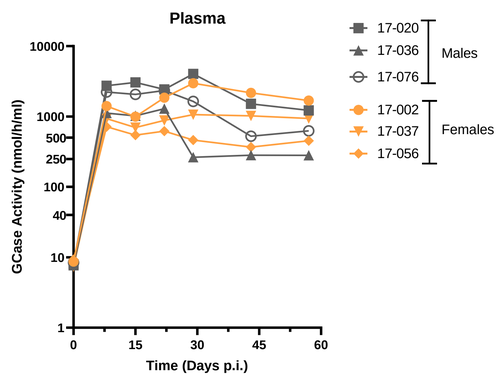


ERT=velaglucerase alfa; PBMCs = peripheral blood mononuclear cells.

FLT201 produces rapid and robust increases in GCase plasma levels in rhesus macaque

- A single injection of FLT201 to non-human primates (NHPs) was well tolerated and resulted in a rapid increase of GCase in plasma that was sustained for at least 2 months (**Figure 7**, study ongoing).
- In GLP toxicology studies, FLT201 demonstrated a good safety profile in mice with an NOAEL of $2.57 x10^{13}$ vg/kg (data not shown).

Figure 7: GCase plasma levels in NHPs following FLT201 treatment



Intravenous infusion over 30 minutes at the dose of $2x10^{12}$ vg/kg; n=6; p.i = post injection

CONCLUSIONS

- FLT201 is a potent, liver-directed AAVS3 gene therapy product that encodes a more stable GCase enzyme ($GCase_{var85}$) compared to WT enzyme.
- FLT201 can be administered by a single infusion.
- FLT201 in GD1 patients is anticipated to lead to continuous endogenous production of $GCase_{var85}$ in hepatocytes resulting in:
  - Steady presence of GCase in plasma and tissues
  - Improved GCase penetration in more target tissues of GD1 compared with ERT
  - Potentially improved clinical outcomes by producing GCase uptake in hard-to-reach tissues such as bone and lung
- First-in-human studies of FLT201 are planned to be initiated in 2021.

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.

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