Poster #379

Lyso-Gb1 dynamics as a surrogate biomarker in type 1 Gaucher disease treated with FLT201 AAV gene therapy

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Background

Gaucher disease is an inherited autosomal recessive disease caused by deficient activity of the lysosomal enzyme, glucocerebrosidase (GCase), and the resultant accumulation of its undegraded substrate, glucosylceramide (Gb1), and its deacylated derivative, glucosylsphingosine (lyso-Gb1)[1]. Lyso-Gb1 directly reflects lysosomal substrate accumulation and Gaucher cell infiltration across multiple tissues, including those not readily accessible for biopsy or imaging. Lyso-Gb1 lies directly downstream of the genetic and biochemical defect and plays a dual role: as a sensitive and specific marker of substrate burden and as a bioactive, cytotoxic molecule that contributes directly to disease pathophysiology.

Lyso-Gb1 toxicity has been shown to promote chronic immune activation, neuronal toxicity, and bone disease[2]. Nonclinical studies demonstrate that elevated lyso-Gb1 impairs osteoblast function, which may contribute to osteopenia[3,4], and clinical imaging studies show strong correlations between lyso-Gb1 and bone marrow infiltration measured by quantitative chemical shift imaging (QCSI)[5]. Lyso-Gb1 has also been implicated in the development of gammopathy and increased risk of multiple myeloma through chronic B-cell stimulation[6,7,8].

Impact of FLT201 on lyso-Gb1

In the GALILEO-1 Phase 1/2 study, FLT201 was administered at a dose of 4.5 x 10^{11} vg/kg to six adult participants with Gaucher disease type 1 who were on stable enzyme replacement therapy (ERT) or substrate reduction therapy (SRT) for at least 2 years. Background ERT/SRT was discontinued at the discretion of the investigator once elevations in leukocyte GCase levels from baseline were seen. All patients rolled in to the long-term follow up GALILEO-2 trial.

Of the six dosed patients, 5 of 6 had DBS lyso-Gb1 above pathologic range (normal range: ≤ 6.8 ng/mL; pathologic: > 12 ng/mL) (mean: 146.36, range: 10.29-383.28) at baseline despite 4-22 years of prior ERT/SRT. All patients presented with residual disease (**Table 1**).

Table 1: Patient demographics at Baseline

Pt.	Age/ gender	Prior ERT/SRT (duration)	DBS lyso (ng/mL)	Residual disease
1	35/M	Velaglucerase* (4.25 yr)	102.85	Moderate bone marrow infiltration, moderate bone/joint pain, fatigue, low mental QoL (SF-36)
2	25/M	Eliglustat * (22 yr)	10.29	Moderate bone marrow infiltration, low BMD (≤ -2 lumbar DXA Z score)
3	24/M	Velaglucerase (4 yr) Eliglustat* (5.5 yr)	383.28	Marked/severe bone marrow infiltration, low BMD (≤ -1 lumbar DXA Z score), thrombocytopenia
4	30/F	Imiglucerase* (14.5 yr)	72.16	Marked/severe bone marrow infiltration
5	24/M	Eliglustat * (4 yr)	257.0	Marked/severe bone marrow infiltration, splenomegaly (> 5 MN)
6	58/F	Imiglucerase (12.25 yr) Miglustat* (11.5 yr)	52.6	Moderate bone marrow infiltration, mild bone/joint pain, low BMD (≤ -1 lumbar DXA Z score), thrombocytopenia, fatigue, low physical QoL (SF36)

* Therapy at study entry

Patients 1-4 discontinued ERT/SRT by Week 11 and have remained off through Year 2. Patients 5 and 6 either did not express GCase at all (low but detectable anti-AAVS3 NAbs at baseline) or lost expression and impact on lyso-Gb1. Of the 4 patients with meaningful data, mean baseline DBS lyso-Gb1 was 142.15 ng/L (range: 10.29-383.28). At Week 38/Month 9, mean DBS lyso-Gb1 was 19.3 (range: 12.0-30.2), an 86% reduction. This reduction was maintained through Year 2 (**Table 2**).

Reductions in DBS lyso-Gb1 from baseline were seen as early as Week 4 (first post-dosing assessment timepoint measured) in 2 patients with additional reductions seen thereafter. One patient entered the trial with low lyso-Gb1, which remained low through the 2-year follow up.

Table 2: DBS lyso-Gb1 from baseline over time (ng/mL)

Patient	Baseline	Week 4	Week 8	Week 12	Week 24	Week 38/Month 9 (% change)	Year 1 (% change)	Year 2 (% change)
1	102.85	115.39	97.8	59.9	25.8	30.2 (-71%)	25.9 (-75%)	48.8 (-53%)
2	10.29	12.56	37.43	26.3	11.0	12.0 (+17%)	6.1 (- 41%)	10.8 (+5%)
3	383.28	153.2	15.3	12.6	12.2	12.0 (-97%)	19.5 (-95%)	21.3 (-94%)
4	72.16	20.7	17.7	16.9	28.3	23.0 (-68%)	22.0 (-72%)	13.1 (-82%)
Cohort: Mean	142.15	75.46	42.06	28.93	19.3	19.3 (-86%)	18.4 (-87%)	23.5 (-83%)

Lyso-Gb1 as a potential new standard for regulatory approval pathways

Lyso-Gb1 provides a proximal, mechanistically relevant, and quantifiable measure of disease burden and therapeutic response in GD1. It complements, and extends beyond, traditional clinical endpoints by providing a quantitative linkage to the primary pathophysiology of GD1, enhanced sensitivity to detect subclinical or residual disease activity, and an early indication of biochemical response or relapse. Given its strong biological rationale, analytical robustness, and consistent correlation with clinical outcomes, lyso-Gb1 is considered scientifically and clinically justified for use as a surrogate endpoint to assess efficacy in GD1 therapeutic trials.

Please see **Poster #315** for clinical outcomes after FLT201 dosing

References

1. Hughes D et al. *J Bone Miner Res*. 2019;34(6):996-1013. **2.** Murugesan V et al. *Am J Hematol*. 2016;91(11):1082–9. **3.** Mistry P et al. *Proc. Natl. Acad. Sci. USA* 2010, 107,19473–19478. **4.** Revel-Vilk S et al. *Int. J. Mol. Sci*. 2020, 21, 7159. **5.** Dekker N et al. *Blood* 118:e118–e127. **6.** Liu J et al. *Proc. Natl. Acad. Sci. USA* 2012, 109,10018–10023. **7.** Rosenbloom B et al. *Am J Hematol.* 2022 Oct;97(10):1337-1347. **8.** El Khoury C et al. *Hematol Transfus Cell Ther.* 2022 Jan-Mar;44(1):136-137. **9.** Dinur T et al. *Int. J. Mol. Sci.* 2021; 22, 7699. **10.** Zimran A et al. *Orphanet J Rare Dis*. 2025 Jan 29;20(1):43. **11.** Kleytman N et al. *Mol Genet Metab Rep*. 2021 Aug 28:29:100798. **12.** Basiri M et al. *eLife* 2023;12:e87537. **13.** Mistry P et al. *Am J Hematol.* 2021;96(9):1156–65. **14.** Cox T et al. *Genet Med*. 2023; 25,100329. **15.** Elstein D et al. *Orphanet J Rare Dis.* 2022 Jan 6;17(1):9. **16.** Comper F. et al. *Mol Ther*. 2025; 33(8):3789-3807.

Abbreviations: AAV: adeno-associated virus; BMD: bone mineral density; DBS: dried blood spot; DXA: Dual-Energy X-ray Absorptiometry scan; ERT: enzyme replacement therapy; GCase: Glucocerebrosidase; GD1: Gaucher disease type 1 ; Lyso-Gb1: glucosylsphingosine; MN: Multiples of normal; NAbs: neutralizing antibodies; QoL: quality of life; SF36: 36-Item Short Form Health Survey; SRT: substrate reduction therapy; vg: vector genome.

Data cut off as of November 20, 2025

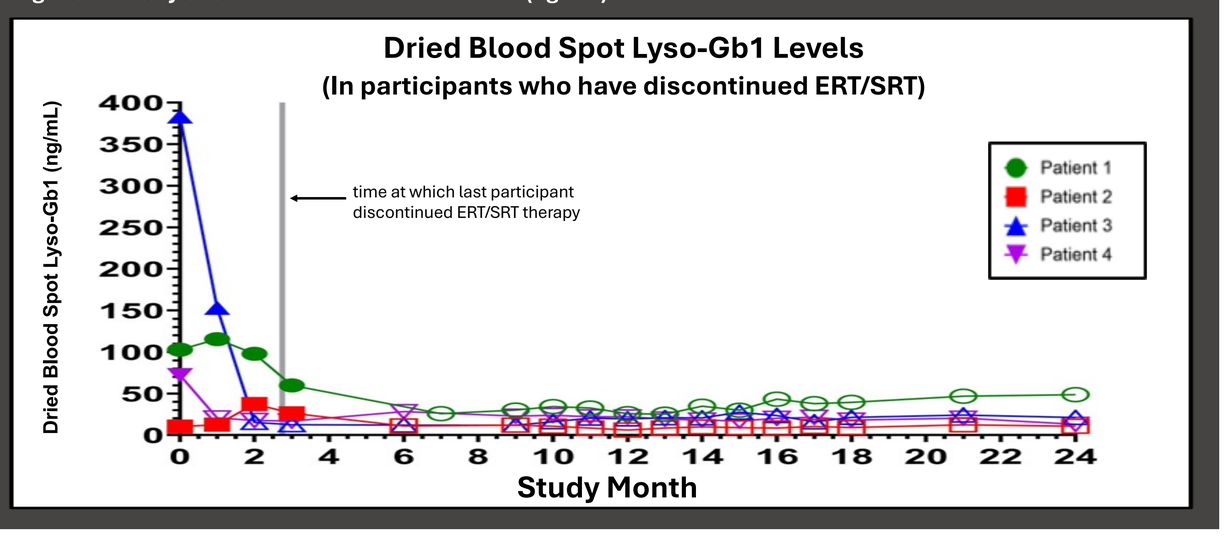
Clinical relevance of residual lyso-Gb1

While levels of DBS lyso-Gb1 improve with ERT and SRT, they often remain elevated. In large cohorts of adult patients with GD1[9,10], mean DBS lyso-Gb1 tend to range above 60 ng/mL despite years of treatment, reflecting continued toxic substrate accumulation.

Data demonstrates the continued presence of signs and symptoms of disease in patients with various ranges of lyso-Gb1 levels after years on ERT or SRT. Despite treatment, patients with mean DBS lyso-Gb1 levels >65 ng/mL continue to demonstrate splenomegaly[10,11,12], thrombocytopenia[11,12,13,14], significant bone disease with increased risk of AVN[12], moderate bone marrow infiltration and bone pain[13,14], chronic fatigue and reductions in QoL[15] emphasizing the complicated nature of GD pathology.

Measuring the impact of therapy beyond traditional hematologic and organ volume endpoints is challenging as whole-body manifestations of GD1 may occur slowly and rely on imaging, functional assessments, and patient reported outcomes that lack sufficient sensitivity to detect early or partial responses that are clinically meaningful. Therefore, lyso-Gb1 could serve as a dynamic and sensitive surrogate endpoint and biomarker for residual disease and response.

Figure 1: DBS lyso-Gb1 concentration over time (ng/mL)



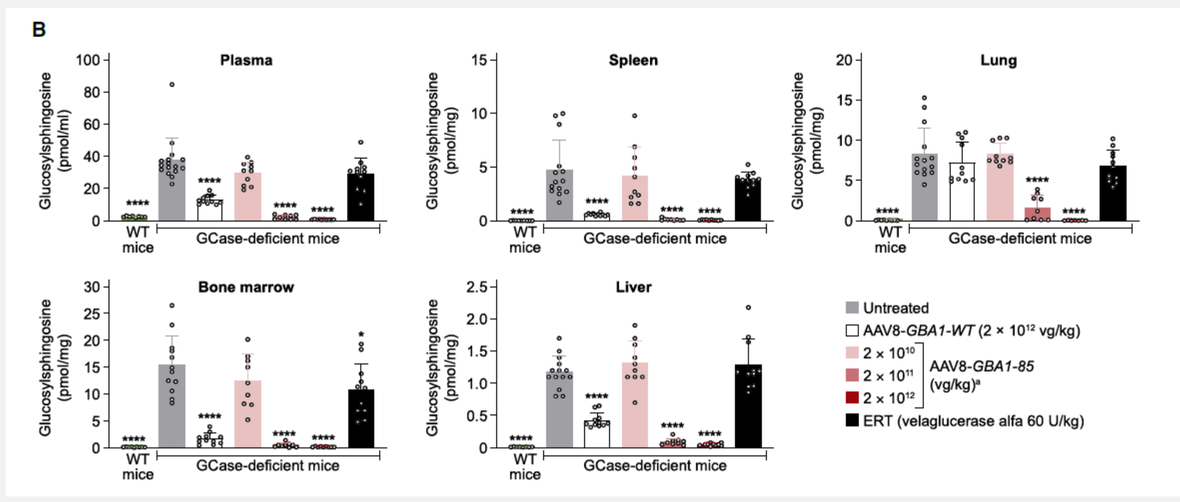
Impact of FLT-201

Reductions in lyso-Gb1 after receiving FLT201 were associated with clinical improvements. Fatigue, pain, and mental health (as reported by the SF36) were normalized in patient1; BMD was improved in patients 2 and 3; and bone marrow infiltration and thrombocytopenia were improved in patient 3.

Lyso-Gb1 builds up within cells, organs, and tissues in Gaucher disease, yet invasive biopsies are not appropriate in the population and current medical technology remains limited in its ability to fully assess the localized cellular toxicity. Lyso-Gb1 levels obtained via blood samples are a representation of the larger milieu and non-clinical data becomes essential to assess the link between lyso-Gb1 from blood samples and that occurring at the tissue and organ level.

FLT201's human hepatocyte selective capsid, AAVS3, demonstrated efficient transduction of human hepatocytes *in vitro* and *in vivo,* in the humanized liver mouse model (FRG) but not murine hepatocytes. Transduction of murine hepatocytes (but not human hepatocytes) by AAV2/8 (AAV8) is efficient; therefore, in murine models, AAV2/8 was used as a surrogate to deliver the FLT201 genome. The effect of FLT201 in the Gaucher mouse model on plasma lyso-Gb1 levels as well as that of diseased tissues was demonstrated (**Figure 2**)[16] with significant reduction of lyso-Gb1 across all tissue types (spleen, liver, bone marrow, lung), measured to near normal levels and to a greater extent than ERT (seven doses, every 2 weeks). Reductions in plasma levels corresponded strongly to reductions seen in tissues.

Figure 2: Impact of AAV8-GBA1-85 on glucosylceramide accumulation in visceral tissues in 9V/null mice



*$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$, ****$p \le 0.0001$ vs. untreated disease control, one-way ANOVA

Conclusions

- **Lyso-Gb1 is a mechanistically anchored, clinically validated, and treatment-responsive biomarker that reflects both disease burden and therapeutic effect.**
- **Lyso-Gb1 complements, and extends beyond, traditional clinical endpoints by providing a quantitative linkage to the primary pathophysiology of GD1, enhanced sensitivity to detect subclinical or residual disease activity, and an early indication of biochemical response.**
- **FLT201 demonstrates a significant and sustained reduction/maintenance in lyso-Gb1 in patients who had already been receiving long-term treatment with ERT/SRT, with subsequent clinical improvements.**
- **In the Gaucher mouse model lyso-Gb1 reduction in plasma correlates to organ and tissue improvements.**

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