Clinical Trial Protocol

Future Rare Diseases

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GALILEO-1: a Phase I/II safety and efficacy study of FLT201 gene therapy for Gaucher disease type 1

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Gaucher disease type 1 (GD1), caused by mutations in the *GBA1* gene, results in β-glucocerebrosidase (GCase) deficiency. Gene therapy is under investigation as a potential treatment option for patients with GD1. The investigational gene therapy FLT201 consists of an adeno-associated virus (AAVS3) encoding a novel GCase variant (GCase-85). Preclinical characterization of FLT201 showed promising results, with GCase-85 being more stable at physiological pH than wild-type GCase and delivered effectively to target tissues. Here, we describe the design of GALILEO-1, a first-in-human Phase I/II safety, tolerability and efficacy study of FLT201 gene therapy in adult patients with GD1. The study results will inform the decision to start a Phase III study of FLT201 in patients with GD1.

Clinical Trial Registration: NCT05324943 (ClinicalTrials.gov)

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Gaucher disease is an autosomal recessive disease caused by mutations in the *GBA1* gene, resulting in deficient activity of the enzyme β-glucocerebrosidase (GCase) [1,2]. Gaucher disease is rare, with an estimated prevalence of 0.70–1.75/100,000 individuals in the general population; the prevalence is higher in the Ashkenazi Jewish population [3]. There are three main forms of Gaucher disease, defined by the absence (type 1) or presence (type 2 and type 3) of overt involvement of the central nervous system. Gaucher disease type 1 (GD1) is the most common form of the disease in Europe and the USA, accounting for over 90% of all patients in these regions [1].

Insufficient GCase activity in the cells of patients with Gaucher disease leads to accumulation of the lipid substrate glucocerebroside. Macrophages become engorged with the accumulated glucocerebroside, forming what are known as Gaucher cells [1,4]. The clinical manifestations of Gaucher disease reflect long-term progressive infiltration of tissues by macrophages and Gaucher-cell deposition in the spleen, liver, bone marrow and other tissues. This can result in splenomegaly, hepatomegaly and the signs and symptoms associated with thrombocytopenia, anemia and bone involvement [1,4]. Bone involvement can include acute and chronic pain, as well as osteonecrosis, osteopenia and osteoarthritis.

Current treatments for GD1

The current standard-of-care treatments for patients with GD1 comprise enzyme replacement therapy (ERT) and substrate reduction therapy (SRT). ERT consists of delivering exogenous, functional GCase enzyme to patients at regular intervals (every 2 weeks) by intravenous infusion [4,5]. Currently available recombinant GCase ERT formulations include the following: imiglucerase (Cerezyme™, Sanofi/Genzyme), which is derived from Chinese-hamster-ovary cells; velaglucerase alfa (VPRIV™, Shire), which is derived from human fibroblasts; and taliglucerase alfa (Elelyso™, Protalix/Pfizer), which is derived from carrot plant root cells. Imiglucerase and velaglucerase alfa are available in Europe, the UK and the USA, and taliglucerase alfa is available in the USA. SRTs are

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oral treatments that decrease production of the GCase substrate glucocerebroside [1,4,5]. Currently there are two available SRT formulations, miglustat (N-butyldeoxynojirimycin; Zavesca™, Actelion) and eliglustat (Cerdelga™, Sanofi/Genzyme).

The introduction of ERT for the treatment of Gaucher disease in 1991 led to dramatic improvements in clinical outcomes, reversing the key features of the disease (anemia, thrombocytopenia, hepatomegaly, splenomegaly and bone disease) [6,7]. ERT has the most potential for effect outside of the hematological system when initiated before the development of irreversible changes including fractures or osteonecrosis of bones, severe pulmonary infiltration or cirrhotic liver changes, which underscores the importance of early diagnosis of Gaucher disease [8]. However, ERT does not cross the blood–brain barrier and is not effective for neurologic symptoms. Depending on severity and starting point of treatment, responses can be variable and incomplete [9–11], with some patients continuing to experience substantial life-limiting symptoms such as bone events [9–13].

The costs of therapy are high, and the lifelong need for treatment with ERT and SRT results in high cumulative healthcare costs and substantial patient burden [5,12,13]. Gene therapy is a new therapeutic approach that is being investigated as a potential treatment option for patients with GD1.

Gene therapy as a potential treatment approach for GD1

Gene therapy has the potential to provide a one-time, long-lasting treatment for patients with monogenic disorders such as GD1 [14,15]. Although the duration of therapeutic benefits with adeno-associated virus (AAV) gene therapy is unknown, the first study of AAV gene therapy in patients with severe hemophilia B has shown stable therapeutic expression of factor IX protein over a period of up to 8 years post treatment [16].

For patients with GD1, the aim of gene therapy is to deliver a functional copy of the *GBA1* gene to enable the body to produce active GCase and prevent substrate accumulation [14,15]. There are currently no gene therapies available for patients with GD1. Gene therapy by transplanting gene-corrected cells (*ex vivo* gene therapy) has been investigated in patients with Gaucher disease [17]. One study used retroviral gene transfer of *GBA1*; however, GCase activity did not increase in any patient [18]. Another study, which is currently ongoing, is using lentiviral gene therapy [19]. In contrast to these *ex vivo* gene therapy approaches, the aim of *in vivo* gene therapy is to administer genes directly into somatic cells. AAV vectors are widely used for *in vivo* gene therapy [20] and comprise the capsid and DNA vector genome [21]. AAVs are not known to cause disease in humans [22], and the host genes can be replaced with an expression cassette containing a gene of interest between the AAV inverted terminal repeat elements [23]. AAV gene therapy is currently the only approved *in vivo* gene therapy [22].

FLT201

FLT201 is an AAV gene therapy candidate consisting of an AAV capsid (AAVS3) carrying a codon-optimized *GBA1* transgene that encodes a novel, engineered GCase variant (GCase-85) protein, expressed via a liver-specific promotor (Figure 1) [24,25]. FLT201 has the potential to produce high levels of active GCase in target tissues using relatively low viral doses based on three main features: the use of a novel, engineered AAV capsid with high potency (AASV3); codon optimization of the transgene and the use of a liver-specific promoter to support high levels of protein production from the liver; and the use of a transgene encoding an engineered GCase variant (GCase-85) with a longer half-life than wild-type GCase.

The AAVS3 capsid has been constructed from domains of natural serotypes [26]. AAVS3 targets the human liver, allowing for functional transduction and subsequent protein production at therapeutic levels [26], and transduces substantially more liver cells *in vitro* than other currently used, natural serotypes (AAV5, AAV8) [27]. The high potency of AAVS3 gene therapy provides the opportunity to deliver lower doses than other natural serotypes while still reaching required levels of protein expression [26].

The same AAVS3 capsid has been administered to humans in ongoing clinical development programs for hemophilia B (FLT180a encoding factor IX) [26] and Fabry disease (FLT190 encoding α-galactosidase) [28]. As recently published, results from the FLT180a Phase I/II clinical trial in patients with hemophilia B showed sustained expression of factor IX transgene in 9 of 10 patients with relatively low doses of FLT180a (3.84×10^{11} to 1.28×10^{12} vector genomes per kilogram of body weight [vg/kg]). FLT180a was generally well tolerated. There were no deaths and no infusion reactions. Of the 12 serious adverse events that were thought to be associated with FLT180a, 9 were transient increases in liver aminotransferase levels [26].

Another important feature of FLT201 is that it encodes an engineered variant of GCase, GCase-85; this was developed to provide increased stability compared with wild-type GCase, which has a short half-life at physiological

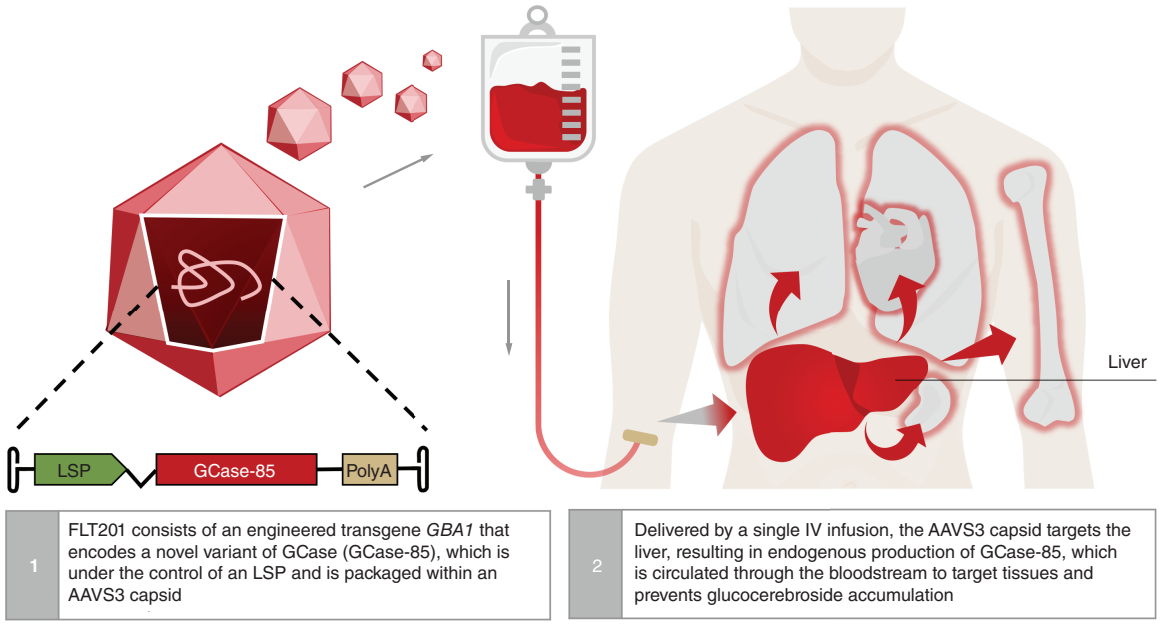

Figure 1. Schematic representation of FLT201 gene therapy.
AAV: Adeno-associated virus; GCase-85: GCase variant 85; IV: Intravenous; LSP: Liver-specific promoter; PolyA: Polyadenylation signal sequence.

pH [25]. GCase-85 contains two novel amino acid substitutions, which result in a sixfold increase in half-life in human serum and a 20-fold increase in GCase half-life at lysosomal pH conditions compared with wild-type GCase; however, the catalytic parameters (Michaelis constant [K_M]) of GCase-85 remain similar to those of wild-type GCase [25]. In mouse models, GCase-85 was found to be taken up by cells in bone marrow and lung tissue, which were not accessed by the wild-type protein when delivered either by ERT or gene therapy [24].

Codon optimization and the use of a liver-specific promoter, apolipoprotein E locus control region, human α1-antitrypsin (FRE76), are also designed to ensure efficient expression of the transgene in hepatocytes, using host-cell transcription machinery, and subsequent production of functional GCase, secretion into the blood and uptake into target cells [24,25]. GCase uptake is primarily mediated by endocytosis through the macrophage mannose receptor (CD206) [29,30]. Preclinical studies of FLT201 have shown that uptake of GCase-85 into Gaucher disease induced pluripotent stem cell-derived macrophages is also mediated through mannose receptors to a similar extent as ERT, as determined by blocking uptake with exogenous mannose addition (unpublished data).

On the basis of these features (capsid potency, optimized transgene expression in the liver and engineered GCase variant), it is hypothesized that FLT201 will provide a continuous level of endogenously expressed, more stable GCase-85 in hepatocytes, resulting in steady plasma activity levels. Compared with ERT, it has the potential to provide continuous uptake of the active enzyme by macrophages and better penetration into the target tissues of patients with GD1.

This paper describes the design of GALILEO-1, which will evaluate the safety and tolerability of FLT201, investigate the relationship between FLT201 dose and production of endogenous GCase and consider the potential of FLT201 to improve the clinical phenotype of GD1 by reducing cellular accumulation of the GCase substrate glucocerebroside.

GALILEO-1 clinical trial

GALILEO-1 is a first-in-human, Phase I/II, open-label, safety, tolerability and efficacy study of FLT201 gene therapy in adult patients with GD1 (EudraCT number: 2020-005032-30; ClinicalTrials.gov number: NCT05324943). GALILEO-1 is sponsored by Freeline Therapeutics. This manuscript reflects GALILEO-1 Study Protocol version 3.0 (October 2021), and elements of the study design may change with future protocol updates or vary by geographic region.

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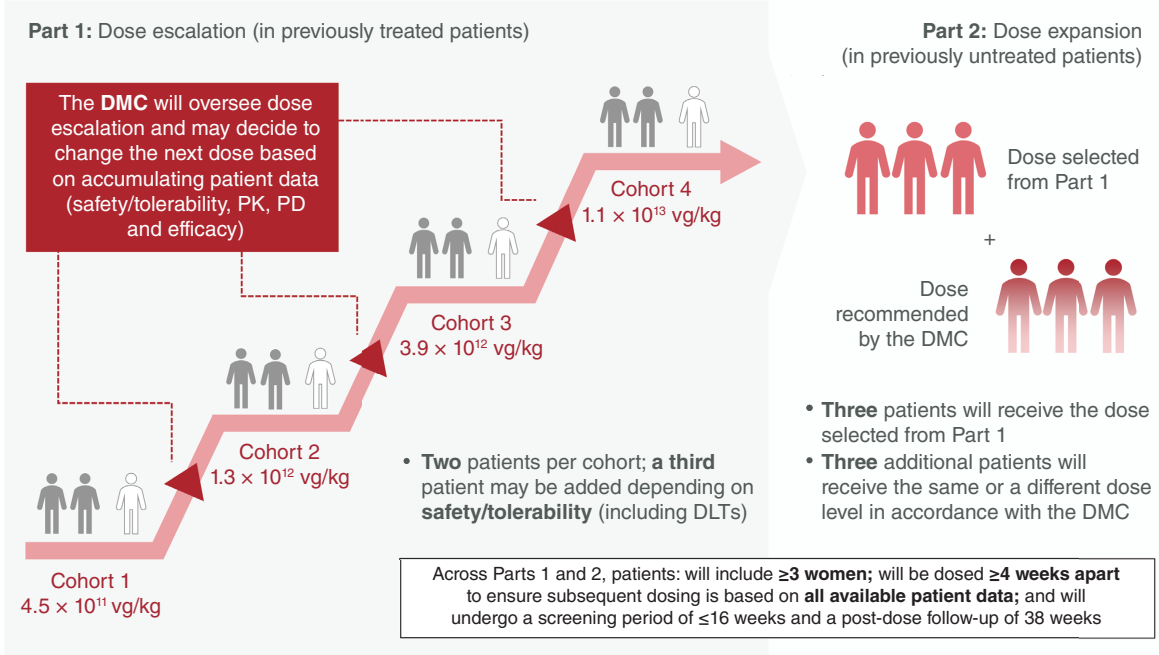

Figure 2. Dose-escalation and dose-expansion phases of GALILEO-1.
DLT: Dose-limiting toxicity; DMC: Data Monitoring Committee; PD: Pharmacodynamics; PK: Pharmacokinetics.

The starting dose of FLT201 is based on the totality of nonclinical data for FLT201, which includes pharmacokinetic (PK) and pharmacodynamic (PD) data, efficacy and toxicology data in mouse models and safety data in nonhuman primates (NHPs) [24], as well as NHP and clinical data from other clinical programs utilizing the AAVS3 capsid [26,28]. The starting dose is anticipated to be efficacious, and the maximum potential dose is 1.7-fold below the no observed adverse effect level (NOAEL) and is also supported by capsid safety data from other Freeline clinical development programs [26,28]. The predicted long-term production of GCase activity that would result from a successful single treatment with FLT201 would remove the burden of long-term treatment administration for patients with GD1, providing sustained delivery of functional GCase to target organs and offering a treatment option that has the potential to be more effective than existing therapies.

Design

Study design

In addition to assessing the safety and tolerability of FLT201, the aim of the study is to investigate the relationship between FLT201 dose and increase in residual GCase expression, and the potential of FLT201 to improve the clinical phenotype by reducing and preventing cellular accumulation of the GCase substrate, glucocerebroside. The study consists of two parts: dose escalation (Part 1), followed by dose expansion (Part 2) (Figure 2).

Part 1: dose escalation

Approximately 12 patients who have been previously treated with ERT or SRT will be enrolled across approximately four dose cohorts. Each dose cohort is planned to include at least two patients; a third patient may be added, depending on the observed safety and tolerability (including dose-limiting toxicities). The following doses are planned: Cohort 1, 4.5×10^{11} vg/kg; Cohort 2, 1.3×10^{12} vg/kg; Cohort 3, 3.9×10^{12} vg/kg; Cohort 4, 1.1×10^{13} vg/kg. For patients weighing greater than 90 kg, the dose will be based on a body weight of 90 kg. After Cohort 1, the subsequent dose tested may be adapted according to emerging data but will not exceed 1.5×10^{13} vg/kg.

Patients will be dosed at least 4 weeks apart to ensure that all patient data contributes to the emerging totality of safety and tolerability (including dose-limiting toxicities), PK, PD and clinical data, which can be used to inform dosing of subsequent patients. Dose escalation will be overseen by a Data Monitoring Committee (DMC). Patients will continue to receive their current therapy until at least two consecutive assessments at least 4 weeks after dosing;

when leukocyte GCase activity level is higher than their pre-dose trough GCase activity level, the ERT or SRT may be stopped.

Part 2: dose expansion

When the final patient in Part 1 has completed at least 12 weeks of follow-up, a dose will be selected for Part 2 according to data collected during Part 1. Approximately six treatment-naive patients will be enrolled across up to two dose cohorts in Part 2. As in Part 1, patients will be dosed at least 4 weeks apart to ensure that all patient data contributes to the emerging totality of data, which can be used to inform subsequent dosing. If a second dose cohort is selected in Part 2 on the basis of recommendations from the DMC, any dose increase would not be greater than threefold that of the first cohort in Part 2.

Eligibility criteria

The study will include adults (aged ≥18 years) with a diagnosis of GD1 and GCase enzyme activity in leukocytes at no more than 30% of normal at diagnosis. The threshold of leukocyte GCase activity was selected as part of the screening process to further ensure that patients in the trial were those with homozygous Gaucher disease. There is wide variability in leukocyte GCase activity in patients with heterozygous Gaucher disease, but it would not be expected to be ≤30% of normal levels [31]. The study population has been selected to optimize the likelihood of patients benefiting from FLT201 treatment and to manage important risks; for example, patients with pre-existing neutralizing antibodies to AASV3 are excluded because this would limit transduction efficiency, thereby limiting the potential for these patients to benefit from FLT201 treatment. Patients who were stable on ERT/SRT and minimally symptomatic having received treatment for 2 years were included in the study because these patients have demonstrated good hematologic responses to therapy and are therefore unlikely to be at clinical risk during this early phase study. The goal of this study is to establish the dose response and show that FLT201 expression can maintain the patients' response to ERT/SRT. It is important to establish a response to FLT201 in patients before it is tested in more symptomatic patients in whom there would be greater risk for exacerbating disease. Full inclusion and exclusion criteria are shown in Box 1.

Planned sample size

The sample size is based on feasibility and not formal hypothesis testing. The planned sample size is approximately 18 patients.

Planned study period

Figure 3 shows the planned study periods. Screening will be carried out up to 16 weeks from initial consent. Infusion will be carried out on day 1, with FLT201 being administered as a single dose via slow intravenous infusion into a peripheral vein. Follow-up visits and laboratory monitoring will be carried out from week 1 to week 38 after the infusion. Finally, on completion of the study, patients will be followed up under a separate long-term follow-up protocol for at least 5 years after dosing.

Outcome measures/end points

All end points will be assessed for baseline values during screening prior to FLT201 dosing and at various times post treatment. The primary end point is the incidence of treatment-emergent adverse events (including dose-limiting toxicities) from day 1 to the last follow-up visit. Other safety end points include the incidence of adverse events, the incidence of serious adverse events and the changes from baseline in vital signs, 12-lead electrocardiogram and physical examination.

Secondary end points include PK, efficacy (Lyso-Gb1 in plasma, spleen and liver volume as measured by magnetic resonance imaging, hemoglobin level and platelet count), immunological and shedding evaluations (Table 1). Additional efficacy, PK and immunological evaluations, as well as assessments of biomarkers and health-related quality of life, were included as exploratory end points (Table 1).

Study procedures

On the day of infusion (day 1), FLT201 will be infused through a catheter in a suitable peripheral vein (e.g., the median cubital vein) over no more than 2 h. Patients will then remain at the infusion center for at least 8 h after the infusion to observe any immediate toxicity of the procedure. In the event of early withdrawal or discontinuation

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Box 1. Inclusion and exclusion criteria.

Inclusion criteria:

- ≥18 years of age
- Diagnosis of GD1 with deficient GCase enzyme activity (≤30% of normal in leukocytes) at diagnosis
- Female patients of childbearing potential must not be lactating and must have negative pregnancy tests at screening (serum) and on day 1 (urine)
- Female patients of childbearing potential and male patients must be willing to follow protocol guidelines for barrier protection/contraception
- Patients must be able to give full informed consent

Part 1 only: previously treated patients

- Treatment status at screening[†]
  - Treated with either ERT[‡] or SRT and started this treatment ≥2 years prior to dosing with no change in regimen for the prior 3 months

Part 2 only: previously untreated patients[§]

- Hb level ≥1 g/dL below the LLN, adjusted for age and sex, and ≥1 of the following at screening
  - Platelet count <120,000/mm^3
  - Hepatomegaly on abdominal MRI
  - Splenomegaly on abdominal MRI

Exclusion criteria:

- Diagnosis of, or suspected, Gaucher disease type 2 or type 3[¶]
- Positive for neutralizing antibodies to AAVS3 at screening
- Evidence of significant and persistent liver dysfunction at screening, defined as >1.5 × ULN in ALT, AST or total bilirubin
- Evidence of any of the following at screening
  - Hb <8 g/dl
  - Platelets <45,000/mm^3
  - Pulmonary hypertension
  - New osteonecrosis within the past 12 months
  - Fragility fracture or bone crisis within the past 12 months
  - Positive for hepatitis B surface antigens
  - Positive for hepatitis C antibodies and subsequent positive PCR for hepatitis C RNA[#]
  - Positive PCR for CMV IgG and CMV DNA
  - Positive for HIV-1 or -2 antibodies
- Received live attenuated vaccination within the past 16 weeks or intends to receive such vaccination during the study
- History of clinically advanced liver disease, e.g., cirrhosis, portal hypertension
- History of bone marrow transplantation
- History of splenectomy (partial or total)
- History of splenic infarct within the past 12 months
- History of receiving any gene transfer medicinal product
- History of receiving any investigational therapy for Gaucher disease within 60 days of screening
- Participation in any other clinical study of an IMP, and/or receiving any other IMP during the study
- History of idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, thrombocytopenia, anemia, hepatomegaly, splenomegaly and/or osteoporosis, unrelated to Gaucher disease
- History of neoplastic disease within the past 5 years, or currently active neoplastic disease[††]
- History of uncontrolled cardiac failure, unstable angina or myocardial infarction, or other acute cardiac conditions requiring clinical management in the past 6 months
- History of acute myocarditis or presence of acute myocarditis during screening
- History of substance abuse, including alcohol abuse or dependence
- Known or suspected intolerance, hypersensitivity or contraindication to the IMP (FLT201) and non-IMPs (prednisolone/prednisone and/or tacrolimus) or their excipients
- History of anaphylaxis or infusion-related reactions to ERT
- Contraindication(s) to MRI[‡‡]
- Any clinical condition (medical or psychiatric) that, in the opinion of the investigator, could jeopardize safety or compromise the ability of the patient to participate in this study

[†]Screening period is 16 weeks.
[‡]ERT dose of at least 15 U/kg and no more than 60 U/kg every other week.
[§]Naive (i.e., never received ERT/SRT).
[¶]Including any patient with eye-movement abnormality on clinical examination.
[#]As follow-up test if positive for hepatitis C antibodies.
[††]Except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* that has been definitively treated.
[‡‡]For example, ferromagnetic metallic implants, some types of pacing and defibrillator devices, and nerve stimulators.
AAV: Adeno-associated virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CMV: Cytomegalovirus; ERT: Enzyme replacement therapy; GCase: β-Glucocerebrosidase; GD1: Gaucher disease type 1; Hb: Hemoglobin; HIV: Human immunodeficiency virus; IgG: Immunoglobulin G; IMP: Investigational medicinal product; LLN: Lower limit of normal; MRI: Magnetic resonance imaging; PCR: Polymerase chain reaction; SRT: Substrate reduction therapy; U: Units; ULN: Upper limit of normal.

Table 1. Study end points.

Primary end points	
Safety	Incidence of TEAE (including DLTs) from day 1 to last follow-up visit Other safety end points: incidence of AEs and SAEs, and changes from baseline in vital signs, 12-lead ECG, physical examination and laboratory assessments from day 1 to last follow-up visit
Secondary end points	
PK	Change from baseline† in plasma and leukocyte GCase activity level
Efficacy	Change from baseline† in the following • Lyso-Gb1 in plasma • Spleen and liver volume, measured by MRI • Hb level • Platelet count
Immunological	Change from baseline† in total anti-GCase antibody titer and neutralizing antibody titer
Shedding	Clearance of vg in plasma, urine, saliva, stool and semen, measured by PCR
Exploratory end points	
Efficacy and biomarkers	Change from baseline† in the following • Gaucher disease severity, measured by GD-DS3 • Bone marrow burden score, measured by MRI • Bone mineral density, measured by DEXA (Z-score and T-score at lumbar spine [L1-4]; hip [femoral neck]) • Fatigue, measured by FACIT-Fatigue • Pain, measured by BPI-SF • Disease activity biomarkers: chitotriosidase, CCL18 • Bone biomarkers: bsALP, osteocalcin • Chest x-ray and pulmonary function tests
PK	Assessment of AUC, peak and steady-state GCase activity levels in plasma and leukocytes from baseline to week 38 Change from baseline† in GCase concentration (antigen levels) Dose response relationship
Immunological	Assessment of AAVS3 antibody titer and T-cell response Change from baseline† in immune response biomarkers
HRQoL	Change from baseline† in HRQoL, measured by SF-36

†Change from baseline to be determined at each assessment point.
AAV: Adeno-associated virus; AE: Adverse event; AUC: Area under curve; BPI-SF: Brief Pain Inventory – Short Form; bsALP: Bone-specific alkaline phosphatase; CCL18: Chemokine (C-C motif) ligand 18; DEXA: Dual energy x-ray absorptiometry; DLT: Dose-limiting toxicity; ECG: Electrocardiogram; FACIT-Fatigue: Functional Assessment of Chronic Illness Therapy Fatigue Scale; GCase: β-Glucocerebrosidase; GD-DS3: Gaucher disease severity score; Hb: Hemoglobin; HRQoL: Health-related quality of life; L1-4: Lumbar vertebrae 1 to 4; lyso-Gb1: Glucosylsphingosine; MRI: Magnetic resonance imaging; PCR: Polymerase chain reaction; PK: Pharmacokinetic; SAE: Serious adverse event; SF-36: 36-Item Short-Form Health Survey; TEAE: Treatment-emergent adverse event; vg: Vector genome.

from the study, every effort will be made to collect data in line with the end-of-study assessments. Patients will attend on-site visits for assessments weekly up to week 12, every 2 weeks from week 12 to week 16 and every 4 weeks thereafter (Figure 3). Follow-up assessment and monitoring will continue for 38 weeks after infusion, with long-term follow-up occurring for at least 5 years after infusion under a separate protocol.

Immunosuppression management

To mitigate the risk of vector-associated transaminitis and efficacy loss due to subsequent loss of GCase expression, patients will receive prophylactic immunosuppression using oral steroid (prednisolone/prednisone) and/or oral

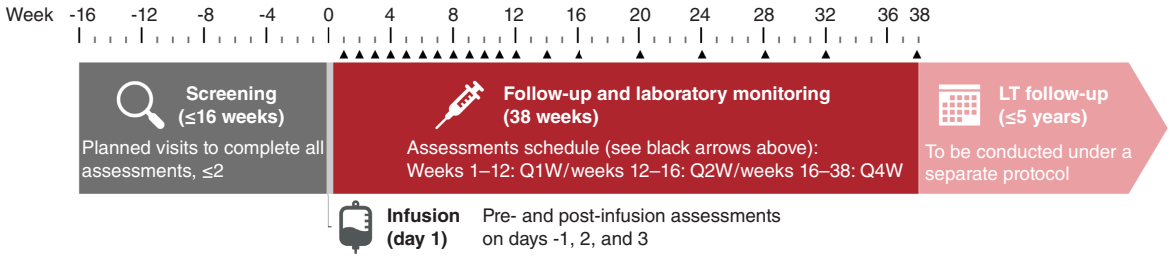

Figure 3. Study periods.
LT: Long-term; Q1W: Weekly; Q2W: Every 2 weeks; Q4W: Every 4 weeks.

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tacrolimus [26]. Prophylaxis will start at approximately 3 weeks after dosing with FLT201 and will continue for approximately 3 weeks, followed by a taper over 11–13 weeks. Prophylaxis is started at 3 weeks after dosing based on the results of a AAV2 gene therapy clinical trial in patients with hemophilia B, which showed that transgenic factor IX expression was lost approximately 8 weeks after dosing due to transaminitis [32]. Liver function will be monitored throughout the study to ensure timely identification and treatment of breakthrough liver transaminase elevations to preserve GCase expression. This immune management approach has been informed by data from the ongoing clinical development program for FLT180a in patients with hemophilia B [26]. It is important to note that in the Phase I/II clinical trial of FLT180a, factor IX expression declined in some patients upon removal of immunosuppression [26]. Hence, it will be important to closely monitor liver enzyme and GCase levels and intervene with additional immunosuppression treatment if transaminitis occurs.

To minimize the impact of steroid-induced osteonecrosis, patients with new osteonecrosis and/or fragility fracture/bone crisis within 12 months of screening will be excluded from the trial (Box 1).

Conclusion

Gene therapy is a new therapeutic approach that is being investigated as a potential treatment option for patients with GD1. FLT201 is an investigational gene therapy that consists of a liver-directed AAV capsid (AAVS3) carrying a codon-optimized *GBA1* transgene that encodes a novel, engineered GCase variant protein with enhanced stability in plasma (GCase-85). The results from the GALILEO-1 study will inform the decision to start a Phase III study of FLT201 in patients with GD1.

Executive summary

Introduction

- Gaucher disease is caused by mutations in *GBA1*, resulting in deficient β-glucocerebrosidase (GCase) activity and cellular accumulation of its substrate glucocerebroside.
- There are three main forms of Gaucher disease, defined by the absence (type 1) or presence (types 2 and 3) of overt involvement of the central nervous system.
- Current standard of care for patients with Gaucher disease type 1 (GD1) is enzyme replacement therapy (ERT) and substrate reduction therapy (SRT).
- Gene therapy is a new therapeutic approach that is being investigated as a potential treatment option for patients with GD1.
- FLT201 is an investigational gene therapy that comprises a potent adeno-associated virus (AAV)S3 capsid carrying a codon-optimized *GBA1* transgene that encodes a novel engineered GCase variant (GCase-85) protein, expressed via a liver-specific promotor.
  - FLT201 has been designed to achieve high levels of functional GCase in target tissues using relatively low viral doses.
  - GCase-85 contains two amino acid substitutions that increase its half-life by up to 20-fold relative to wild-type GCase at physiological pH.
  - Studies in mouse models have demonstrated that GCase-85, delivered by gene therapy, is able to reach tissues that were not reached by wild-type GCase whether delivered by gene therapy or ERT.

GALILEO-1 study design

- Here, we describe the study design for GALILEO-1, a first-in-human, Phase I/II, open-label, safety, tolerability and efficacy study of FLT201 gene therapy in adult patients with GD1.
- The study includes dose escalation, followed by dose expansion (in ~18 patients).
  - The primary end point is the incidence of adverse events and serious adverse events, and changes from baseline in vital signs, physical examination and 12-lead electrocardiogram.
  - Secondary end points include pharmacokinetic, efficiency, immunological and shedding evaluations.
  - Exploratory end points include other efficacy assessments and biomarkers, further pharmacokinetic and immunological evaluations and health-related quality of life.
- The study period includes screening up to 16 weeks from initial consent, infusion on day 1 and follow-up visits from week 1 to week 38. Long-term follow-up will continue for at least 5 years after dosing, under a separate protocol.
- The Phase I/II study results will inform the decision to start a Phase III trial of FLT201 in patients with GD1.

Conclusion

- FLT201 has the potential to provide an effective treatment for patients with GD1. It is hypothesized that FLT201 will provide a continuous level of endogenously expressed, highly stable GCase, thereby preventing cellular accumulation of glucocerebroside and the associated clinical manifestations of the disease.

Author contributions

All named authors have made substantial contributions to the conception or design of the study, have contributed toward drafting this article or revising it critically for important intellectual content, have given their approval of this version for publication and have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Financial & competing interests disclosure

DA Hughes received honoraria for speaking and advisory boards administered through UCL consultants with benefit in part of research from Freeline, Takeda and Sanofi. F Ferrante is an employee of Freeline Therapeutics, the sponsor of the GALILEO-1 study. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Medical writing assistance and editorial support were provided by L Sloan and T Gristwood of Oxford PharmaGenesis, Oxford, UK, and were sponsored by Freeline in accordance with Good Publication Practice (GPP3) guidelines.

Ethical conduct of research

All procedures performed in the trial will be conducted in accordance with consensus ethical principles derived from international guidelines, including the Declaration of Helsinki, the Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council on Harmonisation Guidance on Good Clinical Practice (GCP) and the European Commission Guideline on GCP specific to Advanced Therapy Medicinal Products 2019. Informed consent will be obtained from the participants involved.

Data sharing statement

De-identified data relating to this study will be shared with qualified researchers upon request.

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Papers of special note have been highlighted as: • of interest

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