



**Freeline Therapeutics Ltd**

**FLT201-01**

**A Phase 1/2, Open-label, Safety, Tolerability and Efficacy Study of FLT201  
in Adult Patients with Gaucher Disease Type 1 (GALILEO-1)**

**16JUN2025**

Statistical Analysis Plan

**Version 4.0**

Prepared by:

**PPD on Behalf of  
Freeline Therapeutics Ltd**

Sycamore House  
Gunnels Wood Road  
Stevenage  
Hertfordshire SG1 2BP  
United Kingdom

## Document History

Version	Date	Changes
1.0	08MAY2024	Addressed sponsor comments and finalized
Draft 2.0	03JUL2024	<p>Removed interim analysis section as per sponsor request.</p> <p>Updated section 8.4 to indicate the details of BPI-SF score analysis.</p> <p>Updated section 13 header title to reflect the protocol and cover for both total antibodies and neutralizing antibodies analyses planned for the study.</p> <p>Removed adverse events leading to study discontinuation section as required information is not collected in the eCRF.</p> <p>Added new section for non-serious adverse events as required as part of EU CTR clinical trial results summary.</p> <p>Added extra description to relevant sections detailing EU CTR requirements of clinical trial results and lay person summary as part of the EU CTR reporting managed by the sponsor.</p>
2.0	08AUG2024	<p>Updated with sponsor comments on SF36 scoring and pain scores.</p> <p>SF-36 scoring now refers to the QualityMetric User's Manual for SF-36v2 Health Survey Third Edition.</p> <p>Addressed sponsor comment on section 5.2 and updated terminology used for protocol deviations significance categorization.</p>
3.0	23JAN2025	<p>Updated List of Abbreviations.</p> <p>Added handling of BLQ, LOD, LOQ and ULOQ values to Section 4.</p>

		<p>Updated section 7 to summarize medications/therapies by the closest ATC class level if ATC class level 4 is not available.</p> <p>Added mention of chitotriosidase activity levels plot to Section 8.4.</p> <p>Simplified the plots details described in sections 8.3, 9.6.1 and 9.6.2.</p> <p>Updated Section 9.2.2 to replace CO<sub>2</sub> by bicarbonate.</p>
4.0	16JUN2025	<p>Femoral neck replaced with Hips in Screening bone mineral density T-score and Z -score in Section 6.1.</p> <p>“Z-score &amp; T-score at lumbar spine (L1-L4); hip (femoral neck)” changed to “Z-score &amp; T-score at lumbar spine; hip (left and right)” in Table 2-3.</p> <p>“Z-score and T-score at lumbar spine (L1-4) and hip (femoral neck)” changed to “Z-score and T-score at lumbar spine and hip (left and right)” in Section 8.4.</p>

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## List of Abbreviations

AAVS3	Adeno-associated viral vector serotype S3
AE	Adverse Event
AESI	Adverse Events of Special Interest
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AR	Adverse reaction
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under curve
BLQ	Below the Limit of Quantification
BPI-SF	Brief Pain Inventory- Short Form
bsALP	Bone specific Alkaline Phosphatase
CHIT1	Gene encoding for Chitotriosidase
CMV	Cytomegalovirus
DEXA	Dual energy x-ray absorptiometry
DLT	Dose-limiting toxicity
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EOS	End of study
ERT	Enzyme replacement therapy
EU CTR	European Union Clinical Trial Regulation
FACIT-Fatigue	Functional assessment of chronic illness therapy fatigue scale
GBA1	Gene encoding for $\beta$ -glucocerebrosidase
GCase	Glucocerebrosidase
GD-DS3	Gaucher Disease severity score
HRQoL	Health-related quality of life
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
INR	International normalized ratio
IV	Intravenous(ly)
LFT	Liver function test
LOD	Limit of Detection
LOQ	Limit of Quantification
Lyso-Gb1	Glucosylsphingosine
MCS	Mental Component Summary Score
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NIMP	Non-investigational medicinal product
PCR	Polymerase chain reaction
PCS	Physical Component Summary Score
PD	Pharmacodynamic
PK	Pharmacokinetic
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SF-36	Short Form Health Survey
SOC	System organ class
SRT	Substrate Reduction Therapy
SUSAR	Suspected, unexpected, serious adverse reaction
TEAE	Treatment emergent adverse event
ULOQ	Upper Limit of Quantification
vg	Vector genomes

WHO

World Health Organization

## 1. Introduction

FLT201 is an investigational AAV-gene therapy administered as a single dose, slow intravenous (IV) infusion, that is being developed as a treatment to improve the clinical phenotype in patients with Gaucher disease type 1.

The aim of this first-in-human, Phase 1/2, open-label study is to evaluate the safety, tolerability, efficacy, and pharmacokinetics of FLT201, and to investigate the relationship of FLT201 dose to augmentation of residual glucocerebrosidase (GCase) expression (activity and concentration), and its potential to improve the clinical phenotype by reduction of cellular accumulation of GCase substrate.

The purpose of this statistical analysis plan (SAP) is to define the planned statistical methods consistent with the study objectives. This plan should be read in conjunction with the study protocols (Global (ex-US/ex-Brazil), US only and Brazil only) and is prepared in compliance with International Conference on Harmonisation (ICH). All analyses will be conducted using SAS® Version 9.4 or higher (SAS Institute Inc., Cary, North Carolina).

## 2. Objectives and Endpoints

Table 2-1 presents the primary objectives and endpoints.

**Table 2-1 Primary Objective and Endpoints - Safety**

Primary Objective	Primary Endpoints
To assess the safety and tolerability of a single IV administration of FLT201 in adults with Gaucher disease type 1 (previously treated and previously untreated).	<p><b>Primary safety variable:</b> incidence of TEAEs (including DLTs) from Day 1 to the last follow-up visit.</p> <p><b>Other safety endpoints:</b> incidence of AEs, SAEs, and changes from baseline in vital signs, 12-lead ECG, physical examination, and laboratory assessments from Day 1 to the last follow-up visit.</p>
<p><b>Abbreviations:</b> AE=adverse event; DLT=dose-limiting toxicity; ECG=electrocardiogram; IV=intravenous; SAE=serious adverse event; TEAE=treatment emergent adverse event.</p>	

Table 2-2 presents the secondary objectives and endpoints.

**Table 2-2 Secondary Objectives and Endpoints**

<b>Secondary Objectives</b>	<b>Secondary Endpoints</b>
<b>PK:</b> To investigate the relationship of FLT201 dose to endogenous production of GCCase.	Change from baseline to each assessment point in plasma and leukocyte GCCase activity level.
<b>Efficacy:</b> To assess the impact of FLT201 on: <ul style="list-style-type: none"> <li>• clearance of lyso-Gb1</li> <li>• spleen size</li> <li>• liver size</li> <li>• hemoglobin</li> <li>• platelet count</li> </ul>	Change from baseline to each assessment point in: <ul style="list-style-type: none"> <li>• lyso-Gb1 in plasma</li> <li>• spleen volume measured by MRI</li> <li>• liver volume measured by MRI</li> <li>• hemoglobin</li> <li>• platelet count</li> </ul>
<b>Immunologic:</b> To describe the immune response to GCCase transgene product.	Change from baseline to each assessment point in total anti-GCCase antibody titer and neutralizing antibody titer.
<b>Shedding:</b> To assess viral shedding after systemic administration of FLT201.	Clearance of vector genomes (vg) in plasma, urine, saliva, stool, and semen measured by PCR test.
<b>Abbreviations:</b> GCCase=glucocerebrosidase; lyso-Gb1=glucosylsphingosine; MRI=magnetic resonance imaging; PK=pharmacokinetic; PCR=polymerase chain reaction, vg=vector genomes.	

Table 2-3 presents the exploratory objectives and endpoints.

**Table 2-3 Exploratory Objectives and Endpoints**

Exploratory Objectives	Exploratory Endpoints
<b>Other efficacy/biomarkers:</b> To assess the impact of FLT201 on: <ul style="list-style-type: none"> <li>Gaucher disease severity and progression</li> <li>bone disease</li> <li>fatigue</li> <li>pain</li> <li>biomarkers</li> <li>lung disease</li> </ul>	Change from baseline to each assessment point in: <ul style="list-style-type: none"> <li>Gaucher disease severity measured by the Gaucher disease severity score (GD-DS3)</li> <li>bone marrow burden score measured by MRI</li> <li>bone mineral density measured by dual energy x-ray absorptiometry (DEXA) <ul style="list-style-type: none"> <li>Z-score &amp; T-score at lumbar spine; hip (left and right)</li> </ul> </li> <li>fatigue measured by FACIT-Fatigue</li> <li>pain measured by Brief Pain Inventory- Short Form (BPI-SF)</li> <li>disease activity biomarkers: chitotriosidase, CCL18</li> <li>bone biomarkers: bsALP, osteocalcin</li> <li>chest x-ray and pulmonary function tests</li> </ul>
<b>Pharmacokinetic:</b> To characterize the pharmacokinetics of FLT201.	<ul style="list-style-type: none"> <li>AUC, peak, and steady state GCase activity levels in plasma and leukocytes (baseline to Week 38)</li> <li>Change from baseline to each assessment point in GCase concentration (antigen levels)</li> <li>Dose response relationship</li> </ul>
<b>Immunologic:</b> To describe the immune response to AAVS3 capsid proteins.	<ul style="list-style-type: none"> <li>Immune response to AAVS3 capsid (AAVS3 antibody titer and T-cell response)</li> <li>Change from baseline to each assessment point in immune response biomarkers</li> </ul>
<b>HRQoL:</b> To assess the impact of FLT201 on health-related quality of life (HRQoL).	Change from baseline to each assessment point in HRQoL measured by SF-36.
<b>Abbreviations:</b> AAVS3=adeno-associated viral vector serotype S3; AUC=area under curve; BPI-SF=Brief Pain Inventory – Short Form; bsALP=bone-specific alkaline phosphatase; DEXA=dual energy x-ray absorptiometry; FACIT-Fatigue=Functional Assessment of Chronic Illness Therapy - Fatigue scale; GCase=glucocerebrosidase; HRQoL=health-related quality of life; MRI=magnetic resonance imaging.	

### 3. Investigational Plan

#### 3.1. Overall Study Design and Plan

FLT201-01 is a first-in-human, Phase 1/2, open label, safety, tolerability, and efficacy study in adult patients with Gaucher disease type 1. The study has two parts. Part 1 is a dose escalation scheme in previously treated patients. When the last participant in Part 1 has completed at least 12 weeks' follow-up post-FLT201 administration, a dose will be selected for Part 2 based on safety/tolerability (including DLT), PK, PD, and clinical data from participants in Part 1. Part 2 will not occur in the US.

#### Part 1: Dose Escalation in Previously Treated Patients

Up to approximately 12 participants will be enrolled across approximately 4 dose cohorts in a dose escalation scheme in previously treated patients only.

Dose escalation will be overseen by an independent data monitoring committee (DMC). The planned dose escalation scheme is explained in the protocol.

## **Part 2 (excluding US participants): Dosing in Previously Untreated Patients**

Approximately 6 participants will be enrolled across up to 2 dose cohorts.

On completion of the study, all participants will be followed under a separate long-term follow-up protocol for at least 5 years after dosing with FLT201.

The Schedule of Assessments is described in the protocol.

### **3.2. Treatments**

FLT201 drug product is supplied as a sterile Solution for Infusion in 10 mL Crystal Zenith<sup>®</sup> vials, each vial containing 5 mL extractable volume. The vials are sealed with rubber stoppers and aluminium seals with plastic flip tops.

The appropriate number of vials required for dosing is determined based on the weight of each participant and the allocated dose level (Day -1 weight should be used to determine the final dose).

On Day 1, FLT201 will be administered as a single-dose, slow IV infusion into a peripheral vein.

### **3.3. Dose Adjustment/Modifications**

#### **3.3.1. Dose Escalation**

Dose escalation will be performed by dose cohort, and the details including the provisional dose levels can be found in the protocol.

Since individual participants will only receive a single infusion of FLT201 in this study, dose modifications are not applicable. Participants may have their infusion interrupted or stopped for safety reasons based on the discretion of the investigator. All dose interruptions or discontinuations must be recorded in the participant's eCRF.

#### **3.3.2. Temporary Halt**

Further enrolment into the study will be put on hold in the event of any of the defined temporary halt rules described in the protocol.

Following a safety review by the independent DMC, the Sponsor deems it appropriate to restart the study; this can only be done following approval by all relevant competent authorities, IECs/IRBs and any local approvals required.

## **4. General Statistical Considerations**

All study data collected will be presented in data listings, and all relevant data will be tabulated and summarized by study part, dose cohort, and overall, unless stated otherwise.

Continuous data will be described using descriptive statistics (i.e., n, mean, standard deviation, median, minimum, and maximum). Categorical data will be described using the count and percentage in each category. For the summary statistics of all numerical variables unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported. Mean and median will be displayed to one level of precision greater than the data collected. Standard deviation, standard error, and confidence intervals will be displayed to two levels of precision greater than the data collected.

Missing data will not be imputed. All analyses will be based on observed cases for the safety population unless specified otherwise.

Clinical laboratory and PK results that are below the lower limit of quantification (BLQ/LOQ), below the limit of detection (LOD), above the upper limit of quantification (ULOQ) or missing will be displayed as such in the data listings.

To calculate descriptive statistics and PK parameters, all BLQ, LOQ and LOD results will be treated as half of the BLQ/LOQ/LOD values whereas all ULOQ values will be treated as the ULOQ values as applicable.

The end of the study will be defined as the last visit by the last participant. All participants will be followed up for at least 5 years in a separate long-term follow-up study.

Unless specified otherwise, baseline will be defined as the last non-missing measurement prior to the infusion of IMP.

Change from baseline is defined as: post-baseline value – baseline value.

Study Day 1 is the calendar day of the IMP infusion. Subsequent study days are calculated as the date of assessment/event – date of infusion + 1. Pre-infusion study days are calculated as date of assessment/event – date of infusion. There is no Study Day 0.

All data are summarized based on the visit name collected on the eCRF page.

#### **4.1. Date Imputations**

For the purpose of inclusion in prior and/or concomitant medication/procedure and AE tables, incomplete medications/procedures and AEs start, and end dates will be imputed as follows:

Missing start dates (where UK, UKN and UNKN indicate unknown or missing day, month, and year respectively):

- UK-MMM-YYYY: If the month and year are different from the month and year of the IMP infusion, assume 01-MMM-YYYY. If the month and year are the same as the IMP infusion month and year and the end date (after any imputation) is on or after the IMP infusion, then assume the date of the IMP infusion. If the month and year are the same as the IMP infusion month and year and the end date (after any imputation) is prior to IMP infusion, then assume the end date for the start date.
- DD-UKN-YYYY/UK-UKN-YYYY: If the year is different from the year of the IMP infusion, assume 01-JAN-YYYY of the collected year. If the year is the same as the IMP infusion year and the end date (after any imputation) is on or after IMP infusion, then assume the date of the IMP infusion. If the year is the same as the IMP infusion and the end date (after any imputation) is prior to the IMP infusion, then assume the end date for the start date.

Missing end dates (where UK, UKN and UNKN indicate unknown or missing day, month and year respectively):

- UK-MMM-YYYY: Assume the last day of the month.
- DD-UKN-YYYY/UK-UKN-YYYY: Assume 31-DEC-YYYY.

For prior/concomitant medication, if start date is completely missing and end date is not prior to the IMP infusion, then the medication will be classified as both prior and concomitant. If the start date is completely missing and the end date is within 30 days prior to the IMP infusion, then the medication will be classified as prior. If the end date is missing, then the medication will be classified as ongoing. Medications for which the start and end dates are missing will be classified as prior and concomitant.

## **4.2. Sample Size**

Sample size is based on feasibility and not formal hypothesis testing. All statistical analysis will be descriptive.

It is planned to include approximately up to 12 participants into Part 1 and approximately 6 participants in Part 2 (excluding US participants). The actual number of participants will depend on emerging data.

The dose escalation scheme as described is intended to minimize the number of participants that may be dosed at suboptimal levels, whilst allowing for safety evaluation and option to expand each cohort where DLT is observed.

## **4.3. Randomization, Stratification, and Blinding**

This is an open-label study; therefore, participants will not be randomized, stratified or blinded.

## **4.4. Analysis Set**

### **4.4.1. Screened Set**

The Screened Set will include all participants with recorded data in the database, including screen failures.

### **4.4.2. Safety Set**

The Safety Set will include all participants who received any dose of FLT201. This population will be used to report both Parts 1 and 2. All data summaries will use the Safety Set, unless otherwise specified.

### **4.4.3. Pharmacokinetic (PK) Set**

The Pharmacokinetic (PK) Set will include all participants who received any dose of FLT201 and had had at least one measurable GCase activity level result for leukocyte, plasma or DBS data.

Where participants experience issues which may affect GCase activity level (e.g., dosing errors), data will be reviewed by the study pharmacokineticist and evaluated for exclusion from the PK Set on a case-by-case basis. All participants excluded from the PK Set will be documented in the data listings.

## 5. Participant Disposition

### 5.1. Disposition

Participant disposition will be summarized for the Screened Set. The number and percentage of participants enrolled, dosed participants, participants who completed the study, and participants who prematurely discontinued study (i.e., withdrawal) will be presented. Primary reasons for discontinuation of individual participants may include any of the following:

- Adverse Event
- Death
- Lost to Follow-up
- Physician Decision
- Protocol Violation
- Trial Screen Failure
- Trial Site Terminated by Sponsor
- Study Terminated by Sponsor
- Withdrawal by Subject
- Other

Participant disposition data (including subject identifier, informed consent date, date of dose, date of study completion/termination, and, for those who discontinued study early, the specific reason(s) for discontinuation will be presented in a listing for the Screened Set.

A listing of all participants that failed screening will be presented for the Screened Set.

#### 5.1.1. Additional Summaries

Additional summaries will be provided to support the clinical trial results summary part of European Union Clinical Trial Regulation (EU CTR) reporting along with the disposition summary table described in [Section 5.1](#). These include participants enrollment breakdown by country, the overall participants enrollment breakdown by age group and the overall participants gender breakdown.

### 5.2. Protocol Deviations

The number and percentage of participants with significant protocol deviations will be summarized by deviation category, study part, dose cohort, and overall, for the Safety Set.

Participants will be counted once within each deviation category regardless of how many deviations they have in that category. A review of the protocol deviations will be performed prior to database lock.

All protocol deviations will also be presented in a data listing for the Screened Set.

## 6. Demography and Baseline Characteristics

### 6.1. Demography

Demography data will be summarized by study part, dose cohort, and overall. Individual participant listings will be provided to support the summary table.

Age (year), weight (kg), height (cm) and body mass index (BMI) will be summarized using descriptive statistics (i.e., n, mean, standard deviation, median, minimum, and maximum). Age

category (year), race, sex, ethnicity, and other categorical variables (see list below) will be summarized with frequency tabulations.

Other characteristics include:

- Age group (<65 years, ≥65 years);
- Region (Europe, North America, Latin America);
- Screening hemoglobin level;
- Screening platelet count;
- Screening GCase activity levels (Plasma and DBS);
- Screening GCase concentration;
- Screening chitotriosidase activity (DBS);
- Screening Lyso-Gb1 (Plasma and DBS);
- Screening liver volume (multiples of normal);
- Screening spleen volume (multiples of normal);
- Screening bone mineral density T-score (Lumbar spine and Hips);
- Screening bone mineral density Z -score (Lumbar spine and Hips);
- Screening pulse rate (beats per minute);
- Screening blood pressure (mmHg);
- Screening respiratory rate (breaths per minute);
- Screening body temperature (°C);

## **6.2. Baseline Characteristics**

Baseline Gaucher disease characteristics collected at screening, including age at diagnosis, genotyping (genes encoding for  $\beta$ -glucocerebrosidase (GBA1) and CHIT1) results, Gaucher disease severity (GD-DS3), and ERT/SRT history, will be summarized descriptively by study part, dose cohort, and overall. A listing of the baseline Gaucher disease characteristics including the date symptoms began will be presented.

Baseline characteristics summary table will also be used for clinical trial results summary part of the EU CTR reporting.

## **6.3. Medical History**

### **6.3.1. General Medical History**

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 26.1 or higher.

The number and percentage of participants with any general medical history not related to Gaucher disease will be summarized overall and for each system organ class (SOC) and preferred term for each study part, dose cohort, and overall. Percentages will be calculated based on number of participants in the Safety Set. All medical history data will be presented in a listing.

### **6.3.2. Gaucher Disease Medical History**

Medical history related to Gaucher disease will be coded using the MedDRA dictionary (Version 26.1 or higher).

Medical history related to Gaucher disease will be summarized by SOC and preferred term for each study part, dose cohort, and overall. Medical history data related to Gaucher disease will be presented in a listing.

## **6.4. Inclusion and Exclusion Criteria**

The inclusion and exclusion criteria are listed in the protocol. All inclusion/exclusion information will be included in a by-participant listing for the Screened Set. For participants who did not satisfy these criteria, the criteria numbers will be listed with the deviation.

## **7. Treatments and Medications**

### **7.1. Gaucher Disease Therapy**

ERT/SRT use will be summarized using Anatomical Therapeutic Chemical (ATC) Level 4 (if this is not available, the closest ATC level will be used) by preferred term, study part, dose cohort, and overall. Rules on imputation of incomplete dates are found in [Section 4.1](#). All ERT/SRT data will be presented in a listing.

### **7.2. Prior and Concomitant Medications**

All medications will be coded according to the World Health Organization (WHO Drug dated September 2023 or later) Drug Dictionary and summarized using the ATC class Level 4 (if this is not available, the closest ATC level will be used) and preferred term. All prior and concomitant medications information will be presented in a listing.

#### **7.2.1. Prior Medications**

Prior treatment includes all non-Gaucher disease therapy received from 30 days prior to the initial informed consent up until the time of IMP infusion.

Prior medications will be summarized using ATC Level 4 (if this is not available, the closest ATC level will be used) by preferred term, study part, dose cohort, and overall. Rules on imputation of incomplete dates are found in [Section 4.1](#).

#### **7.2.2. Concomitant Medications**

Concomitant treatment refers to all treatment taken between the date of IMP infusion and the end of study visit (Week 38 for all participants excluding US and Month 24 for participants in the US only) inclusive.

Concomitant medication includes prescription and non-prescription medication and herbal treatments. Concomitant medication also includes immunosuppressants used as part of an immune management and monitoring regimen. If immunosuppressant drugs are used, tacrolimus levels will be monitored, and data will be presented (see [Section 9.6.2](#)).

Concomitant medications will be summarized using ATC Level 4 (if this is not available, the closest ATC level will be used) by preferred term, study part, dose cohort, and overall. Rules on imputation of incomplete dates are found in [Section 4.1](#).

In addition, participants immunosuppressant drug dose levels will be plotted over time for all participants who received at least one dose of any immunosuppressant drugs described in the protocol.

#### **7.2.3. Procedures**

Data from prior and concomitant procedures form (procedure name, date, reason or indication, and associated AEs or medical history) will be presented in a listing.

### **7.3. Study Treatments**

#### **7.3.1. IMP Infusion**

IMP infusion data (i.e., total amount of IMP received, if infusion is interrupted, any related reactions) will be summarized by study part, dose cohort, and overall. IMP infusion data will be presented in a listing.

#### **7.3.2. Treatment Compliance and Modifications**

A single IMP infusion will be administered to the participant at the study site by trained study staff. All IMP will be tracked and accounted for at the investigational site following receipt, dispensing and administration to participant, and ultimately including destruction or return to the Sponsor. Therefore, no analysis of compliance is planned.

### **8. Efficacy Analysis**

Summaries and analysis of efficacy endpoints will be performed using the Safety Set unless otherwise specified.

Primary endpoints and secondary efficacy endpoints descriptive summaries will also be used to support the clinical trial results summary part of the EU CTR reporting.

#### **8.1. Derivations of Efficacy Endpoints**

Participant data will be entered into Rave (Medidata's electronic data capture system), but the following endpoints require additional programming to be calculated from the entered data.

##### **8.1.1. Health-related Quality of Life (SF-36 Score)**

The eight SF-36 health domain scores (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role emotional, mental health) and two component summary scores (physical and mental) will be derived according to the QualityMetric SF-36v2 Health Survey Third Edition User's Manual. Absolute values and changes from baseline in the health domain scores and component summary scores will be summarized.

#### **8.2. Primary Endpoint**

The primary endpoint for this study is to assess the safety and tolerability of FLT201, and the analysis is summarized in [Section 9](#).

#### **8.3. Secondary Efficacy Endpoint**

Summary tables presenting observed values and changes from baseline for each scheduled post-baseline visit will be presented for the following secondary efficacy endpoints:

- lyso-Gb1 in plasma and DBS
- Spleen volume measured by MRI
- Liver volume measured by MRI
- Hemoglobin level
- Platelet count

The above summaries will be presented by study part, dose cohort, and overall. GCase activity levels in plasma and leukocytes (as well as DBS) are discussed in [Section 10](#).

Additionally, GCase concentrations and the clearance of lyso-Gb1 (plasma and DBS), spleen volume and liver volume will be plotted by-participant over time.

#### **8.4. Exploratory Efficacy Endpoint**

Observed values and changes from baseline for each scheduled post-baseline visit will be summarized descriptively for the following endpoints:

- Gaucher disease severity score (GD-DS3)
- Bone marrow burden score measured by MRI
- Bone mineral density measured by DEXA:
  - Z-score and T-score at lumbar spine and hip (left and right)
- Fatigue measured by Functional Assessment of Chronic Illness Therapy - Fatigue score (FACIT-Fatigue)
- Gaucher disease activity biomarkers: chitotriosidase
- Bone biomarkers: bsALP, osteocalcin
- Lung disease measured by chest x-ray and pulmonary function tests
- GCase concentration
- HRQoL measured by SF-36 domains

The above summaries will be by study part, dose cohort, and overall.

Additionally, chitotriosidase activity levels will be plotted by-participant over time.

##### **8.4.1. Pain measured by Brief Pain Inventory - Short Form (BPI-SF)**

The two pain domains (pain severity and pain interference) scores measured by BPI-SF questionnaire will be calculated as the average daily pain scores during the 7 days immediately following each screening visit (rounding to nearest whole number is not permitted).

Post-infusion two pain domains (pain severity and pain interference) values will be computed similarly, i.e., as an average of 7 daily values. The computation will be performed for Weeks 4, 12, 24 and 38/EOS.

The baseline will be defined as the observed BPI-SF pain domain score for pre-infusion (Day -1) visit. If pre-infusion (Day -1) visit score is missing, the weekly average of the latest screening visit pain domain score recorded during the 7 days following the screening visit (and prior to infusion) will be used as the baseline.

A summary table by study part, dose cohort, and overall will be provided for BPI-SF each pain score averages and changes from baseline for each scheduled post-baseline visit.

#### **9. Safety Analysis**

The safety profile is based on the incidence of Treatment Emergent Adverse Events (TEAEs) including DLTs and Serious Adverse Events (SAEs), as well as the change from baseline in standard clinical laboratory assessments, physical examination findings, vital signs, and 12-lead ECG recordings and will be summarized descriptively.

##### **9.1. Adverse Events**

Any AEs that occur from the time the initial Informed Consent Form (ICF) is signed by a participant until the end of study visit. This includes events occurring during the screening phase of the study and regardless of whether FLT201 is administered.

For a full definition of AEs for this study, see the protocol.

### **9.1.1. Treatment Emergent Adverse Events**

A TEAE is defined as an AE that meets any of the following conditions:

- begins during or after the infusion
- begins before the infusion and worsens in severity during or after the infusion
- is completely missing an onset date and end date
- is completely missing an onset date and the end date is on or after the date of infusion

All adverse events will be classified by SOC and preferred term according to MedDRA (Version 26.1 or higher).

An overview summary of the number and percentage of participants with any AE, any TEAE, serious TEAE, non-serious TEAE, IMP-related TEAE, IMP-related serious TEAE, TEAE leading to treatment discontinuation, and AE leading to death will be provided by study part, dose cohort, and overall. This summary will also be used for Lay Person summary part of the EU CTR reporting. A listing for all AEs will also be presented.

### **9.1.2. Relationship of Adverse Events to Investigational Medicinal Product**

The investigator will determine whether there is a reasonable causal relationship between the occurrence of the AE and exposure to IMP (FLT201) and non-investigational medicinal products (NIMP) (prednisolone/prednisone, methylprednisolone, and tacrolimus). If there is no valid reason for suggesting a relationship, then the AE should be classified as “Not Related.” Otherwise, if there is any valid reason for suspecting a possible cause-and-effect relationship between the IMP/NIMP and the occurrence of the AE, then the AE should be considered “Related.”

If the relationship to IMP/NIMP information is missing, then the corresponding AE is assumed to be related with reasonable possibility to the IMP/NIMP. If a participant experiences more than one occurrence of the same AE, the occurrence with the most-related relationship to IMP/NIMP will be used in summary tables.

The relationship of AEs to IMP/NIMP will be summarized by SOC, PT, study part, dose cohort, and overall.

### **9.1.3. Severity of Adverse Event**

A summary of TEAEs by severity will be presented in a table. The severity that will be presented represents the most extreme severity captured on the eCRF page. The possible severities are measured on the CTCAE scale which goes from 1 to 5 where 1=‘mild’, 2=‘moderate’, 3=‘severe’, 4=‘life-threatening’ and 5=‘fatal’.

The severity of the AEs will be summarized by SOC, preferred term, study part, dose cohort, and overall. SOC and preferred terms within SOC will be presented by descending frequency based on overall count. If a participant reports more than one AE within the same preferred term, the AE with the maximum severity will be presented. If a participant reports more than one AE within the same SOC, then the participant will be counted only once with the maximum severity at the SOC level. If severity is missing, then Grade 3 “Severe” is assumed.

#### 9.1.4. Dose Limiting Toxicities

All DLTs will be summarized by SOC, preferred term, study part, dose cohort, and overall. DLTs will be presented using CTCAE grades, AEs that are not graded using the CTCAE scale are still recorded in the eCRF using the same mapping (1=‘mild’, 2=‘moderate’, 3=‘severe’, 4=‘life-threatening’ and 5=‘fatal’).

DLTs definitions by protocol regions are as presented in [Table 9-1](#).

**Table 9-1 DLTs Definition by Protocol Versions**

<b>Global (ex-US/ex-Brazil), Brazil only</b>	<b>US only</b>
DLTs are defined as any severe AE at least possibly related to FLT201 except for increases in ALT or AST that are not associated with increases in bilirubin.	DLTs are defined as any Grade 3 or greater AE that is deemed by the DMC to be possibly, probably, or definitely related to FLT201. Toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

#### **9.1.5. Serious Adverse Events**

SAEs are defined in the protocol. SAEs will be summarized by SOC, preferred term, study part, dose cohort, and overall.

Important medical events as described in the protocol that may jeopardize the participant or may require an intervention to prevent one of the above characteristics/consequences may be considered SAEs when, based upon medical judgment.

SAE data will also be presented in a listing.

#### **9.1.6. Non-serious Adverse Events**

Non serious treatment emergent adverse events will be summarized by SOC, preferred term, study part, dose cohort, and overall. The non-serious TEAE summary is provided to support the clinical trial results summary part of the EU CTR reporting.

#### **9.1.7. Adverse Events of Special Interest (AESI)**

AESIs are defined in the protocol. AESIs will be summarized by SOC, preferred term, study part, dose cohort, and overall.

#### **9.1.8. Adverse Events Leading to Treatment Discontinuation**

An AE leading to treatment discontinuation is an AE where the answer to “Action Taken with FLT201” is “Infusion Stopped” in the Adverse Event eCRF page. Adverse events leading to treatment discontinuation will be presented in a table by SOC, and preferred term, study part, dose cohort, and overall. At each level of participant summarization, a participant is counted once if the participant reported one or more events.

#### **9.1.9. Death**

All participant deaths will be reported in a listing only. Data presented will comprise date of death, days on study, date of last visit, cause of death, autopsy performed (yes, no), and death certificate (yes, no).

### **9.2. Clinical Laboratory Evaluations**

Summary tables presenting observed values and changes from baseline will be presented for clinical laboratory tests with numeric values by study part, dose cohort, and overall, for participants in the Safety Set. Changes from baseline to each scheduled post-baseline visit will be presented.

Laboratory assessments will be performed by a central laboratory. All summaries will be based on the units provided by the laboratory; no conversion will be applied.

All relevant clinical laboratory test results will be classified as Low, Normal, and High, or Normal/Abnormal according to the normal ranges. These categorical data will be summarized in shift tables comparing the results at each scheduled post-baseline visit with those at the baseline visit.

When there are multiple values within a visit for a particular laboratory variable, the worst value will be taken (worst being the smallest value for criteria below a certain threshold or the largest value for criteria above a certain threshold). If a participant has a value below the threshold and above the threshold, the value furthest from the threshold will be chosen.

### **9.2.1. Hematology**

All hematology central laboratory tests will be summarized: complete blood count with differential, platelet count, prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT). All hematology data by participant will be presented in a listing. Note that in the occasions where central laboratory results are missing, local laboratory results will be summarized and listed.

Additionally, plots by-participant for hematology central laboratory tests over time will be produced for the following tests: hemoglobin, platelet count, APTT, and PT.

### **9.2.2. Serum Chemistry**

The following laboratory tests will be summarized: sodium, potassium, chloride, phosphate, bicarbonate, glucose, blood urea nitrogen, serum creatinine, C-reactive protein, hs troponin-T. All chemistry data by participant will be presented in a listing.

### **9.2.3. Pregnancy Testing**

A listing of pregnancy test results will be presented combining serum and urine tests.

## **9.3. Vital Signs**

Summary tables presenting observed values and changes from baseline will be presented for vital sign data, including systolic blood pressure (mmHg), diastolic blood pressure (mmHg), body temperature (°C), pulse rate (beats per minute), and respiration (breaths per minute) by study part, dose cohort, and overall. Changes from baseline to each scheduled post-baseline visit will be presented. All vital sign data will be presented in a listing.

## **9.4. Physical Examinations**

A table will summarize physical examination results by study part, dose cohort, and overall. Each visit captures the status of a body system and any finding associated with the body system as normal, abnormal, or not done. The summary will include the number and percentage of participants with each physical examination outcome for the following body systems: skin; head, neck, eyes, ears, nose, and throat (HEENT); respiratory; cardiovascular; gastrointestinal; endocrine/metabolic; genitourinary; neurological; blood/lymphatic; musculoskeletal; and other. Physical examination results for all participants will be presented in a listing.

## **9.5. Electrocardiogram**

Summary tables will be presented for 12-lead ECG data including ventricular rate (bpm), PR interval (msec), QRS duration (msec), QT interval (msec), and QTcF interval (msec), QTcB interval (msec), QTcLC interval (msec), QTcV interval (msec), by study part, dose cohort, and overall. Observed results at each visit will be presented.

## **9.6. Other Safety Data**

### **9.6.1. Liver Function Tests (LFTs)**

LFT data will be collected at each visit and summarized descriptively by laboratory (central, local), study part, dose cohort, and overall. The following laboratory tests will be collected: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, and AST. LFT data will also be presented in a listing. In addition, ALT, and AST levels and Total bilirubin will be plotted by-participant and laboratory (central, local) over time.

### **9.6.2. Tacrolimus Levels and CMV Testing**

A listing displaying cytomegalovirus (CMV) testing results throughout the study will be presented. In addition, a line plot by participant displaying tacrolimus levels over time will be presented for all participants who received at least one dose of tacrolimus.

### **9.6.3. Serology Screening**

The following serology tests will be listed: HBsAg, HepCAb, anti-HIV1/2 antibodies, CMV IgG antibodies, CMV PCR and SARS-CoV-2 (COVID-19).

## **10. Pharmacokinetics**

All PK listings and individual concentration-time profiles will be presented using the safety Set. PK tables, mean figures and all statistical analyses will be presented using the PK Set.

### **10.1. Data Handling**

Data rounding specifications for PK data are documented in the PK tables, listings, and figures shells.

Plasma, leukocyte, and dried blood spot (DBS) glucocerebrosidase (GCase) activity levels that are BLQ or missing will be displayed as such in the data listings.

For the calculating of descriptive statistics and PK parameters, all BLQ values will be treated as half of the lower limit of quantification.

GCase activity levels will be baseline adjusted. Baseline is defined as any collection before the administration of the IMP. An average of the screening and pre-dose collection results will be taken to determine baseline for each of the matrices. Baseline adjustment leading to results <0 will be reported as 0.

### **10.2. Plasma, Leukocyte, Dried Blood Spot GCase Activity**

Sample collections that have an actual sampling time that deviates from the predefined collection time windows will be flagged in the data listings.

The following visit windows will be applied for the GCase sample collections:

Screening: +/-1 week

Weeks 1 to 12: +/-1 day

Weeks 14, 16, 20, 24, 28, 32, 38: +/-3 days

Week 38: +/-1 weeks

Individual GCase activity levels will be presented in data listings. A summary statistic for observed and changes from baseline values will be presented by study part, matrix, cohort, and overall.

Individual GCase raw activity levels will be plotted by-participant over time.

### **10.3. Plasma, Leukocyte and DBS Pharmacokinetic Parameters**

Plasma, leukocyte, and DBS GCase baseline adjusted activity level-time data will be analyzed by non-compartmental analysis using Phoenix<sup>®</sup> WinNonlin<sup>®</sup> Version 8.3 or higher (Certara USA, Inc., Princeton, NJ). The following PK parameters will be calculated for baseline adjusted GCase activity levels, where data permit:

$C_{\max}$	Maximum observed concentration.
$T_{\max}$	Time of maximum observed concentration.
$AUC_{\text{last}}$	AUC from time 0 to the last measurable observed concentration ( $C_{\text{last}}$ ), calculated using the linear trapezoidal rule.

Actual sampling times will be used for the estimation of all PK parameters, and all available GCaSe activity level data will be included in the analysis (including resulting from samples collected outside predefined collection windows).

Plasma, leukocyte, and DBS PK parameters will be presented in data listings and summarized separately using descriptive statistics (number of observations, arithmetic mean, SD, CV, geometric mean, geometric CV, median, minimum, and maximum) by study part and cohort.  $T_{\max}$  will be summarized using number of observations, median, minimum, and maximum only.

#### 10.4. Pharmacokinetic Statistical Analyses – Dose Proportionality (Power Model)

A non-linear Power Model will be used to assess dose-proportionality for baseline adjusted GCaSe Activity Levels in Part 1 of the study in all matrices (plasma, leukocytes, DBS). Actual dose received by each patient will be used. The proportional relationship between the expected value of each parameter  $E(y)$  and dose is written as a power function:

$$E(y) = \alpha * \text{dose}^{\beta} \text{ [Equation 1]}$$

where ' $\alpha$ ' is a constant, ' $\beta$ ' is the proportionality constant and ' $y$ ' is the parameter of interest. The exponent,  $\beta$ , will be estimated by performing a linear regression of  $\ln(\text{parameter})$  [ $C_{\max}$  and  $AUC_{\text{last}}$ ] versus  $\ln(\text{dose})$ . The exponent,  $\beta$ , is the estimated slope of the resulting regression line, since taking logs of Equation 1 gives the linear relationship:

$$\ln(y) = \alpha + \beta * \ln(\text{dose}) \text{ [Equation 2]}$$

The relationship is dose proportional when  $\beta = 1$ .

Linear regression plots of systemic exposure ( $C_{\max}$  and  $AUC_{\text{last}}$ ) versus dose will be presented using log-log scales with the power model overlaid.

#### 11. Shedding

Clearance of vg in plasma, saliva, urine, stool, and semen will be summarized by study part, dose cohort, and overall. Viral shedding data will be presented in a listing.

#### 12. GCaSe Immune Response

Immune response to the GCaSe transgene product measured as change from baseline to each assessment point in total anti-GCaSe antibody titer and neutralizing antibody titer will be summarized (the number of participants with non-missing data, median, min, max, geometric mean, geometric coefficient variation) separately by study part, dose cohort, and overall. Summary table will also include total and neutralizing titer assessments (screening cut point, screening response, specificity, titer), where applicable.

GCaSe immune response data will be presented in a data listing.

#### 13. AAVS3 Immune Response

Immune response to the AAVS3 capsid, measured as change from baseline to each assessment point in total AAVS3 antibody titer and total AAVS3 antibody assessments (screening cut point,

screening response, specificity, titer) will be summarized (the number of participants with non-missing data, median, min, max, geometric mean, cv% (geometric coefficient variation)) by study part, dose cohort, and overall.

In addition, screening neutralizing antibody titers will be summarized for screening and pre-infusion (Day -1) visits by study part, dose cohort, and overall.

AAVS3 immune response data will be presented in a listing.

#### **14. Changes in the Planned Analysis**

1. Section 8.4: Analysis of exploratory endpoint Gaucher disease activity biomarkers (CCL18) is omitted from this SAP as data is not collected.
2. Section 9.2.3: Analysis of safety endpoint, clinical laboratory evaluations urinalysis is omitted from this SAP as research urine samples are not available for analysis.
3. Section 9.1.8: Adverse Events Leading to Study Discontinuation is omitted from this SAP as data is not collected.
4. In the study protocol, an interim analysis is planned but will not be performed.

## **15. References**

Freeline Therapeutics LTD. FLT201-01 Protocol: A Phase I/2, open-label, safety, tolerability and efficacy study of FLT201 in adult patients with Gaucher disease type I (GALILEO-I)