FREELINE

CLINICAL TRIAL PROTOCOL

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

Protocol Number:	FLT201-01
Investigational Medicinal Product:	FLT201
Indication:	Gaucher Disease (Type 1)
Development Phase:	Phase 1/2
Sponsor:	Freeline Therapeutics Ltd Sycamore House Gunnels Wood Road Stevenage Hertfordshire SG1 2BP United Kingdom
Regulatory Agency Identifier:	EudraCT Number: 2020-005032-30
Protocol Version:	v8.0
Protocol Date:	16 November 2023

1 Protocol Approval Page

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

Protocol Number: FLT201-01

This study will be conducted in compliance with the clinical study protocol, adhere to the principles of the International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor:

PPD

16-NOV-2023

Print Name Signature Date

2 Investigator's Signature Page

Protocol Title:	A Phase 1/2, Open-label, Safety, Tolerability, and Efficacy Study of FLT201 in Adult Patients with Gaucher Disease Type 1 (GALILEO-1)
Protocol Number:	FLT201-01

I have read protocol FLT201-01.

I have fully discussed the objective(s) of this study and the contents of this protocol with the Sponsor's representatives.

I understand that the information in this protocol is confidential and should not be disclosed without written authorisation from the Sponsor. It is, however, permissible to provide the information contained herein to those directly involved in the execution or the scientific/ethical review of the study, and/or to a patient in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines (GCP), and to conduct the study in accordance with applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

Investigator:

Print Name	Signature	Date

Address: _____

_____

_____

_____

3 Emergency Contact Information

In the event of a serious adverse event (SAE) or adverse event of special interest (AESI), the investigator must record, within 24 hours of the first awareness of the event, all relevant SAE or AESI information in the CRFs of the Medidata RAVE project database. If Medidata RAVE is not available, the investigator should utilize the back-up paper SAE/AESI report form to report the initial SAE/AESI or follow-up information (email/fax the form within 24 hours to the pharmacovigilance contract research organisation [PV CRO]) (SAE Hotline: +44 (0) 1223 374 240; SAE Faxline: +44 (0) 1223 374 102). The CRF of Medidata RAVE must be updated as soon as it is available. Applicable fax numbers and email addresses can also be found on the form.

For emergency protocol- or safety-related issues, the investigator must contact the CRO Medical Monitor in their region:

REGION	**24-HR SAFETY HOTLINE**
Europe Middle East Africa Asia Pacific	+44 122 337 4240
Latin America	+55 11 4504 4801

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4 List of Abbreviations

Abbreviation	Definition
AAV	Adeno-associated virus
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATIMP	Advance therapy investigational medicinal product
AUC	Area under curve
BPI-SF	Brief Pain Inventory – Short Form
CIMP	Clinical Immune Management Plan
CMV	Cytomegalovirus
CRO	Contract research organisation
DBS	Dried blood spot
DEXA	Dual energy x-ray absorptiometry
DLT	Dose-limiting toxicity
DMC	Data monitoring committee
DNA	Deoxyribonucleic acid
eCRF	Electronic case report form
ECG	Electrocardiogram
EOS	End of study
ERT	Enzyme replacement therapy
EU	European Union
FACIT-Fatigue	Functional Assessment of Chronic Illness Therapy Fatigue Scale
FIX	Factor IX
FEF25	Forced expiratory flow at 25% of vital capacity
FEF50	Forced expiratory flow at 50% of vital capacity
FEF75	Forced expiratory flow at 75% of vital capacity
FEF25–75	Forced expiratory flow over the middle half of the vital capacity
FEV1	Forced expiratory volume in one second
FRC	Functional residual capacity
FVC	Forced vital capacity
GBA1	Gene encoding for β-glucocerebrosidase
GCP	Good Clinical Practice
eGFR	Estimated glomerular filtration rate
GI	Gastrointestinal
GCase	Glucocerebrosidase
GD-DS3	Gaucher Disease Type 1 Severity Scoring System
αGLA	Alpha-galactosidase A
GL1	Glucosylceramide
GLP	Good Laboratory Practice
Hb	Haemoglobin
HBsAg	Hepatitis B surface antigen
HepC Ab	Hepatitis C antibody
HepB	Hepatits B
HepC	Hepatitis C
HIV	Human immunodeficiency virus
HRQoL	Health-related quality of life
hs	High sensitivity
IB	Investigator's Brochure

ICH	International Council for Harmonisation
ICF	Informed consent form
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IMP	Investigational medicinal product
INR	International normalised ratio
IRB	Institutional Review Board
IV	Intravenous(ly)
KCO	Carbon monoxide transfer coefficient corrected for lung volume and haemoglobin
LFT(s)	Liver function test(s)
Lyso-Gb1	Glucosylsphingosine
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NHP	Non-human primate
NIMP	Non-investigational medicinal product
NOAEL	No observed adverse effect level
PEFR	Peak expiratory flow rate
PCR	Polymerase chain reaction
PTT	Partial thromboplastin time
RV	Residual volume
rcAAV	Replication-competent AAV
SAE	Serious adverse event
SmPC	Summary of Product Characteristics
SRT	Substrate reduction therapy
ss	Single stranded
SUSAR	Suspected, unexpected, serious adverse reaction
TEAE	Treatment emergent adverse events
TLC	Total lung capacity
vg	Vector genomes

5 Protocol Synopsis

Protocol Number: FLT201-01	**Drug:** FLT201
Study Title: A Phase 1/2, open-label, safety, tolerability, and efficacy study of FLT201 in adult patients with Gaucher disease type 1	
Number of Participants: Approximately 18 participants will be enrolled across Part 1 and 2 of the study; the actual number of participants will depend on emerging data. Part 1: Approximately 12 participants will be enrolled across approximately 4 dose cohorts. Part 2: Approximately 6 participants will be enrolled across up to 2 dose cohorts.	
Sites and Regions: This is a global multicentre study.	
Overview: This study is a first-in-human, phase 1/2, open-label, safety, tolerability, and efficacy study in adult patients with Gaucher disease type 1. The aims are to investigate the safety/tolerability and efficacy 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 and prevention of cellular accumulation of GCase substrate.	
Inclusion Criteria: 1. Adult ≥ 18 years of age. 2. Diagnosis of Gaucher disease type 1 with deficient GCase enzyme activity ≤30% of normal in leukocytes at diagnosis. 3. All female participants of childbearing potential must not be lactating and must have a negative serum pregnancy test at screening and confirmed negative by urine testing prior to dosing on Day 1. 4. Female participants of childbearing potential and male participants must be willing to follow protocol guidelines for barrier protection/contraception. 5. Able to give full informed consent for the trial. **Part 1 only** (previously treated patients)**:** 6. Treatment status at screening (screening period is 16 weeks): Treated with either enzyme replacement therapy (ERT) or substrate reduction therapy (SRT) and started this treatment at least 2 years prior to dosing with no change in regimen for the prior 3 months. ERT dose ≥15 U/kg and ≤60 U/kg every other week. **Part 2 only** (previously untreated [naïve i.e., never received ERT/SRT] patients)**:** 7. Haemoglobin (Hb) level ≥1 g/dL below the lower limit of normal adjusted for age and sex, and at least one of the following at screening: a. Platelet count <120,000/mm^3. b. Hepatomegaly on abdominal magnetic resonance imaging (MRI). c. Splenomegaly on abdominal MRI.	
Exclusion Criteria: 1. Diagnosed or suspected type 2 or type 3 Gaucher disease (including any participant with eye movement abnormality on clinical examination). 2. Positive for neutralising antibodies to AAVS3 at screening. 3. Evidence of significant and persistent liver dysfunction at screening defined as >1.5 x upper limit of normal (ULN) in alanine aminotransferase (ALT), aspartate aminotransferase (AST) or total bilirubin.	

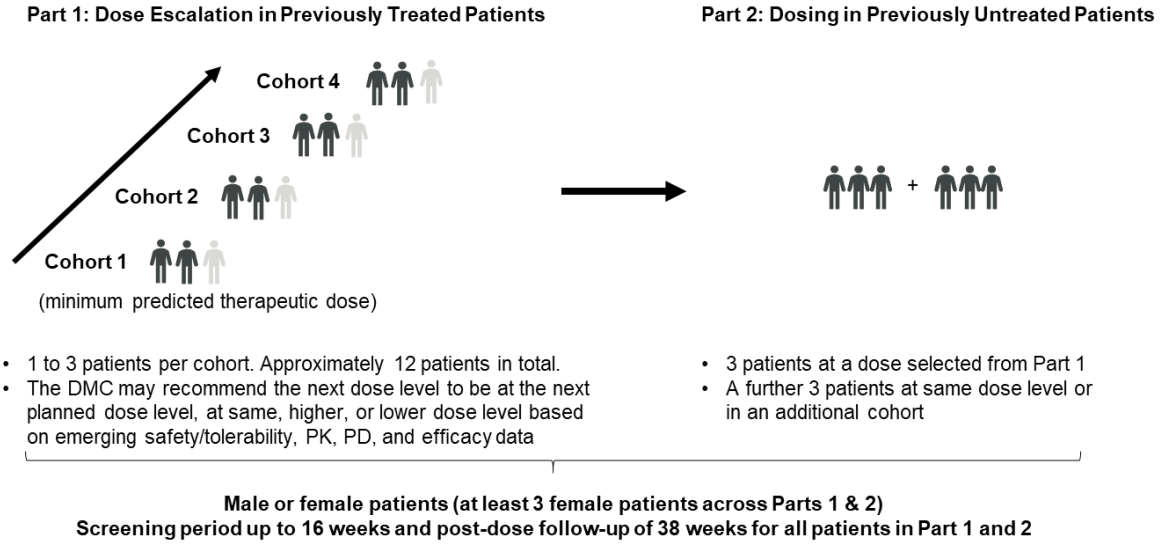
4. Evidence of any of the following at screening:
    a. Hb <8 g/dL.
    b. Platelets <45,000/mm^3.
    c. Pulmonary hypertension.
    d. New osteonecrosis within 12 months of screening.
    e. Fragility fracture or bone crisis within 12 months of screening.
5. Hepatitis B surface antigen (HBsAg) positive at screening.
6. Hepatitis C antibody (HepC Ab) positive and hepatitis C RNA polymerase chain reaction (PCR) (as follow up test if HepC Ab-positive) positive at screening.
7. Cytomegalovirus (CMV) immunoglobulin G (IgG) and CMV DNA PCR positive at screening.
8. Human immunodeficiency virus (HIV)-1 or -2 antibody positive at screening.
9. Receipt of live attenuated vaccination within 12 weeks prior to screening or intends to receive such vaccination during the study.
10. History of clinically-advanced liver disease e.g., cirrhosis, portal hypertension.
11. History of bone marrow transplant.
12. History of splenectomy (partial or total).
13. History of splenic infarct within 12 months of screening.
14. History of receiving any gene transfer medicinal product.
15. History of receiving any investigational therapy for Gaucher disease within 60 days of screening.
16. Participation in any other clinical study of an investigational medicinal product (IMP), and/or receiving any other IMP during the study.
17. History of idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, thrombocytopenia, anemia, hepatomegaly, splenomegaly, and/or osteoporosis, unrelated to Gaucher disease.
18. History of, or active neoplastic disease within 5 years of screening (except for basal or squamous cell carcinoma of the skin or carcinoma-in-situ which has been definitively treated).
19. History of uncontrolled cardiac failure, unstable angina, or myocardial infarction or other acute cardiac conditions requiring clinical management in the past 6 months.
20. History of acute myocarditis or presence of acute myocarditis during screening.
21. History of substance abuse, including alcohol abuse or alcohol dependence.
22. Known or suspected intolerance, hypersensitivity or contraindication to the investigational medicinal product (IMP) and non-investigational medicinal products (NIMPs) or their excipients.
23. History of anaphylaxis or infusion related reactions to ERT.
24. Contraindication(s) to MRI. (e.g., ferromagnetic metallic implants, some types of pacing and defibrillator devices, nerve stimulators).
25. Any clinical condition (medical or psychiatric) that, in the opinion of the investigator, could jeopardise safety or compromise ability of the participant to participate in this study.

Methodology:

This is a phase 1/2, open-label, dose escalation and expansion study of FLT201 in patients with Gaucher disease type 1. Participants who provide consent to participate in this study will be screened for eligibility over a 16-week period. On the day prior to infusion (Day -1), the participant

will attend the investigational site and final eligibility assessments will be conducted. On Day 1, FLT201 will be administered as a single dose, slow intravenous infusion into a peripheral vein, and the participant will be monitored closely for at least 8 hours following the infusion. Following satisfactory results from safety evaluations conducted on Day 1, the participant will be discharged from the investigational site. Post FLT201 dosing, all participants will enter a 38-week post-infusion follow-up period during which safety and efficacy assessments will be performed. On completion of the 38-week follow-up period, all participants will be followed under a separate long-term follow-up protocol that will assess safety and efficacy for at least a total of 5 years post-dosing.

Study Schema:



The study will be conducted in 2 parts:

Part 1: Dose Escalation in Previously Treated Patients

Approximately 4 dose cohorts (12 participants) are planned to be tested in a dose escalation scheme in previously treated patients. Each dose cohort is planned to include at least 2 participants (except if GCase activity in the first participant in the cohort is below the normal range [e.g., <1.5 µmol/L/h, though this value will vary depending on the specific laboratory] and no DLT observed; see below). A third participant may be added, depending on safety/tolerability (including DLT) observed following treatment. Safety, PK, PD and clinical data will be evaluated at pre-defined timepoints.

The planned dose escalation scheme is as follows (if subject weight is >90 kg, the dose will be calculated based on 90 kg weight):

- Cohort 1: 4.5×10^{11} vector genomes (vg)/kg of body weight
- Cohort 2: 1.3×10^{12} vg/kg
- Cohort 3: 3.9×10^{12} vg/kg
- Cohort 4: 1.1×10^{13} vg/kg

The dose level to be tested in each cohort after Cohort 1 may be an intermediate dose between the current dose and that of the next planned dose cohort based on emerging data but would not be greater than the next planned dose. In addition, based on emerging data, including safety/tolerability, PK, PD, and clinical data, all dose cohorts may not be tested, an additional dose cohort may be added and/or a dose cohort may be further expanded beyond 3 participants. If an additional dose cohort is added the dose will not exceed 1.5×10^{13} vg/kg.

Participants will be dosed at least 4 weeks apart to allow for every participant to contribute to the emerging totality of safety/tolerability (including DLT), PK, PD, and clinical data used to inform dosing of the subsequent participant(s).

Part 2: Dosing in Previously Untreated 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 be initiated after the approval of a protocol amendment providing rationale for the recruitment of previously untreated participants (i.e., naïve) and the dose(s) chosen for Part 2, based on available data from Part 1.

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

Participants in Part 2 will be dosed at least 4 weeks apart to allow for every participant to contribute to the emerging totality of safety/tolerability (including DLT), PK, PD, and clinical data used to inform dosing of the subsequent participant(s). If the first 3 participants in Part 2 have an acceptable post-dose safety profile, up to 3 further participants may be dosed at this dose level or a different dose level, following recommendations from the Data Monitoring Committee (DMC). If a second dose is selected and is a dose increase, the dose increase would not be greater than 3-fold that of the first cohort in Part 2.

Dose Escalation and Cohort Expansion Rules

A minimum of 4 weeks of safety/tolerability (including DLT), PK, PD, and clinical data will be assessed before dosing each participant (both within a dose cohort and before escalating to the next dose level). Four weeks post-dose approximates the time when leukocyte and plasma GCase activity levels are predicted to achieve steady state, giving an early indication of likely risk:benefit for participants at a given dose level. Any acute dose-limiting toxicities (DLT) may thus be adequately assessed in the 4 weeks post-dose period. A DLT is defined as any severe adverse event (AE) at least possibly related to FLT201 except for increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) that are not associated with increases in bilirubin.

Dose escalation will be overseen by a DMC in accordance with the following rules:

1. Safety Assessment Based on DLT
    - If 1 participant experiences a DLT, that cohort will be expanded to 3 participants.
    - If 2 out of 3 participants in the same cohort experiences a DLT, the DMC will be convened to consider a dose reduction. The DLT rate is expected to be ≤33%.
2. GCase Expression

If GCase activity is below the normal range (e.g., <1.5 µmol/L/h) in the first participant in the cohort and no DLT is observed, dose escalation to the next dose cohort will occur after this first participant to avoid treating the next participant with a dose predicted to be below the therapeutic range.

3. Safety Rules Based on NOAEL

The highest dose that may be tested will not exceed 1.5×10^{13} vg/kg, which is below the no observed adverse effect level (NOAEL) of the FLT201 Good Laboratory Practice (GLP) murine toxicology study (2.57×10^{13} vg/kg).

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)	Primary safety endpoint: • incidence of TEAEs (including DLTs) from Day 1 to the last follow-up visit Other safety endpoints: • 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
Secondary Objectives	**Secondary Endpoints**
To investigate the relationship of FLT201 dose to endogenous production of GCase	Change from baseline to each assessment point in plasma and leukocyte GCase activity level
To assess the impact of FLT201 on: • clearance of lyso-Gb1 • spleen size • liver size • haemoglobin • platelet count	Change from baseline to each assessment point in: • lyso-Gb1 in plasma • spleen volume measured by MRI • liver volume measured by MRI • haemoglobin • platelet count
To describe the immune response to GCase transgene product	Change from baseline to each assessment point in total anti-GCase antibody titre and neutralising antibody titre
To assess viral shedding after systemic administration of FLT201	Clearance of vg in plasma, urine, saliva, stool, and semen measured by PCR
Exploratory Objectives	**Exploratory Endpoints**
To assess the impact of FLT201 on: • Gaucher disease severity and progression • bone disease • fatigue • pain • biomarkers • lung disease	Change from baseline to each assessment point in: • Gaucher disease severity measured by the Gaucher Disease Severity Scoring System (GD-DS3) • bone marrow burden score measured by MRI • bone mineral density measured by dual energy x-ray absorptiometry (DEXA) o Z-score and T-score in the lumbar spine (L1-4) and hip (femoral neck) • fatigue measured by FACIT-Fatigue • pain measured by Brief Pain Inventory – Short Form (BPI-SF) • disease activity biomarkers: chitotriosidase, CCL18 • bone biomarkers: bsALP, osteocalcin • chest x-ray and pulmonary function tests
To characterize the pharmacokinetics of FLT201	• AUC, peak, and steady state GCase activity levels in plasma and leukocytes (baseline to Week 38) • Change from baseline to each assessment point in GCase concentration (antigen levels)

	• Dose response relationship
To describe the immune response to AAVS3 capsid proteins	• Immune response to AAVS3 capsid (AAVS3 antibody titre and T-cell response) • Change from baseline to each assessment point in immune response biomarkers
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
Investigational Product and Mode of Administration: FLT201 Solution for Infusion will be given as a single dose, slow IV infusion over up to 2 hours.	
Non-investigational Medicinal Product: The non-investigational medicinal products are prednisolone (prednisone), methylprednisolone, and tacrolimus.	
Maximum Duration of Participant Involvement in the Study: The planned total study duration for each participant is up to 56 weeks comprising: • Screening period of up to 16 weeks • Dosing day for FLT201 infusion • Post-dose follow-up period of 38 weeks (+2-week window) All participants will be subsequently followed up for at least a total of 5 years post-dosing under a separate long-term follow-up protocol.	
Statistical Methods: Sample size is based on feasibility and not formal hypothesis testing. All statistical analysis will be descriptive. 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.	
Protocol Version and Date: v8.0 (16 November 2023)	

Table 1: Schedule of Assessments (Screening and Weeks 1 to 38/EOS)

Procedure	Screen 1[A]	Screen 2[A]		Week 1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	28	32	38/ EOS[R]	Unscheduled Visit[S]
Visit Window	**-16 to -2 wk**	**-3 wk ± 1 wk**		**± 1 day**												**± 3 days**						**±2 wk**	
Informed consent	X		For Day -1 to Day 3 see Table 2																				
Demography & medical history	X																						
Prior & concomitant medications	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gaucher disease & ERT/SRT history	X																						
Gaucher disease severity (GD-DS3)		X													X				X			X	
Physical examination[C]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[D]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X			X			X								X				X			X	
MRI: liver & spleen size		X													X				X			X	
MRI: bone marrow burden		X													X				X			X	
DEXA (Z-score, T-score; lumbar spine, hip)		X																				X	
BPI-SF, diary completion[E]	X	X					X								X				X			X	
FACIT-Fatigue completion[F]	X	X					X								X				X			X	
HRQoL (SF-36)		X																				X	
Chest x-ray & pulmonary function tests		X													X				X			X	
Immunosuppressants[G]						→	→	→	→	→	→	→	→	→	→	→	→						
Local Laboratory Tests																							
Pregnancy test (urine)				X			X				X				X		X	X	X		X	X	X
Pregnancy test (serum)[H]	X	X		X			X				X				X		X	X	X		X	X	X
Liver function test (local)				X[I]	X[I]	X[I]	X[I]	X[I]	X[I]	X[I]	X[I]	X[I]	X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[J]	X[I]	X[I]	X[I]	X
Test for CMV[K]						X	X	X	X	X	X	X	X	X	X	X	X						
Test for tacrolimus level[L]						X	X	X	X	X	X	X	X	X	X	X	X						
Central Laboratory Tests																							
Genotyping (*GBA1*, *CHIT1*)	X																						
Haematology, chemistry incl. LFTs & hs troponin-T[N]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Procedure	Screen 1[A]	Screen 2[A]		Week																			Unscheduled Visit[S]
				1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	28	32	38/ EOS[R]	
Visit Window	**-16 to -2 wk**	**-3 wk ± 1 wk**		**± 1 day**												**± 3 days**						**±2 wk**	
HIV, hepB, hepC, CMV screen[O]	X																						
Biomarkers: chitotriosidase, CCL18, bsALP & osteocalcin	X	X					X								X				X			X	
GCase activity levels	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GCase immune response		X		X	X	X			X						X				X			X	X
GCase concentration		X		X	X	X			X						X				X			X	X
Lyso-Gb1		X					X				X				X				X			X	X
AAVS3 immune response		X		X	X				X						X				X			X	
Research plasma sample				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vector shedding: plasma, saliva, urine, stool, (& semen, male participants only)[Q]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Abbreviations: AAVS3=adeno-associated virus; BPI-SF=Brief Pain Inventory – Short Form; CMV=cytomegalovirus; DEXA=dual-energy x-ray absorptiometry; ECG=electrocardiogram; EOS=end of study; ERT=enzyme replacement therapy; FACIT-Fatigue=Functional Assessment of Chronic Illness Therapy Fatigue scale; FEV1=forced expiratory volume in one second; FVC=forced vital capacity; GCase=glucocerebrosidase; GD-DS3=Gaucher Disease Type 1 Severity Scoring System; HepB=hepatitis B; HepC=hepatitis C; HIV=human immunodeficiency virus; HRQoL=health-related quality of life; Ig=immunoglobulin; incl=including; LFTs=liver function tests; lyso-Gb1=glucosylsphingosine; MRI=magnetic resonance imaging; PCR=polymerase chain reaction; BPI-SF=Brief Pain Inventory-Short Form; SRT=substrate reduction therapy; vg=vector genomes; wk=week(s).

A For participant screening, at least two visits are expected but multiple visits may be required to perform and repeat assessments and prepare for enrolment. Screen 1 must be performed prior to Screen 2. Performance on the same day may be acceptable with Sponsor approval.

B If a site is not able to administer FLT201 the Sponsor will attempt to identify an alternative site for the infusion week only.

C Height (screening only) and weight will also be measured.

D Vital signs include pulse, blood pressure, respiratory rate, and temperature.

E Participants will complete BPI-SF questionnaire once a day covering the 7 days following each screening visit, and then for 7 consecutive days, on each occasion commencing at the start of Weeks 4, 12, 24 and 37 post-treatment (week prior to the 38-week visit). Study sites will contact participants at the beginning of Week 37 to remind them to complete the questionnaires during Week 38.

F Participants will complete the FACIT-Fatigue questionnaires at the end of each week during the screening period, and then at the end of Weeks 4, 12, 24, and 37 post treatment.

G A weight-based tapering immunosuppressive regimen will be initiated at the Week 3 visit. Cohort 1 may not require immunosuppression. For details see the pharmacy manual.

H Serum pregnancy test performed only at Week 1 to EOS and unscheduled visits if the urine pregnancy test is positive.

I Testing to be performed twice weekly (including the testing as part of the scheduled study visit), initially commencing at Week 1 and concluding at the end of Week 9 and later commencing at Week 28 and concluding at the end of Week 38/EOS. The tests should be as evenly spaced through the week as possible, for example: Monday and Thursday, or Tuesday and Friday.

J Testing to be performed three times weekly (including the testing as part of the scheduled study visit), commencing at Week 10 and concluding at the end of Week 27. The tests should be as evenly spaced through the week as possible, for example Monday, Wednesday, and Friday.

K Local testing. Weekly CMV PCR testing for the duration the participant is taking tacrolimus in the immunosuppressant regimen. Tests should be undertaken to coincide with use of tacrolimus if outside of the prophylactic regimen. CMV IgG testing followed by CMV PCR. CMV PCR only indicated for a positive CMV IgG.

L Local testing. Tacrolimus levels should be tested twice weekly until levels are in the therapeutic range and weekly thereafter for the duration the participant is taking tacrolimus in the immunosuppressant regimen. Tests should be undertaken to coincide with use of tacrolimus if outside of the prophylactic regimen.

M A negative assay outcome must be documented within 6 weeks of dosing. Should there be any problems with testing, it is acceptable to take repeat samples during the screening period as necessary.

N Vit D, Fe2+ and Vit B12 at screening only.

O Serology will be conducted by a central laboratory and will screen for; HepB (surface antigen), HepC (antibody and RNA) (HepC RNA PCR only indicated for a positive HepCAb screen), HIV 1 and 2 antibodies. CMV IgG testing followed by CMV DNA PCR (CMV PCR only indicated for a positive CMV IgG).

P In the event of an increase in ALT an additional plasma GCase sample should be taken.

Q Samples will be taken three times within 7-10 days after FLT201 infusion then weekly until the results from all matrices are negative at three consecutive visits. Collection of a particular matrix sample, e.g., plasma, can be stopped once negative results have been reported in that matrix at three consecutive visits at least 1 week apart after Week 1. Collection of the remaining matrices should continue until each delivers negative results at three consecutive visits at least 1 week apart after Week 1. Semen samples may be omitted at the discretion of the investigator for religious or other personal reasons expressed by the subject.

R In the event of participant discontinuation/withdrawal, every effort should be made to complete Week 38/EOS procedures.

S Minimum requirements for unscheduled visits are shown in the table. Additional assessments may be performed at the discretion of the investigator. A pregnancy test is only required if both (a) the unscheduled visit is 4 weeks or longer from previous pregnancy test and (b) viral shedding results are not negative at three consecutive visits taken at least 1 week apart after Week 1 in all matrices.

Table 2: Detailed Schedule of Assessment for Day -1 to Day 3 (Infusion Week)

Procedure[A]	Day -1[C]	Day 1 (Infusion Day)										Day 2	Day 3
		Pre-dose[B]	Timepoint (min) during infusion		Timepoint (hours) from end of infusion								
		-1h	0	Every 15 min	+1	+2	+3	+4	+5	+6	+8[J]		
Window	**-2 days**	**-**	**-**	**± 5 min**	**± 5 min**	**± 10 min**					**± 15 min**	**-**	**-**
Prior & concomitant medications	X	X	X	Every 15 min	X	X	X	X	X	X	X	X	X
Physical examination	X[D, E]											X	X
Vital signs[F]	X	X		Every 15 min	X	X	X	X	X	X	X	X	X
12-lead ECG	X											X	
FLT201 administration			X	----------▶									
Adverse events	X	X	X	Every 15 min	X	X	X	X	X	X	X	X	X
BPI-SF, FACIT-Fatigue, diary completion	X												
Local Laboratory Tests													
Pregnancy test (urine)	X												
Pregnancy test (serum)[G]	X												
SARS-CoV-2 (COVID-19)[H]	X												
LFTs[I]	X	X								X		X	X
Central Laboratory Tests													
Haematology, chemistry incl. LFTs & hs troponin-T	X											X	X
GCase activity levels		X											X
GCase immune response	X												
GCase concentration	X												
AAVS3 immune response	X												
Research plasma sample	X												
Vector shedding: plasma, saliva, urine, stool, (& semen, male participants only)[K]	X												X

Abbreviations: AAVS3=adeno-associated virus; BPI-SF=Brief Pain Inventory – Short Form; DBS=dried blood spot; ECG=electrocardiogram; FACIT-Fatigue=Functional Assessment of Chronic Illness Therapy Fatigue scale; GCase=glucocerebrosidase; LFTs=liver function tests; min=minutes; PCR=polymerase chain reaction; vg=vector genomes.

A If a site is not able to administer FLT201 the Sponsor will attempt to identify an alternative site for the infusion week only.
B Approximately 1 hour before infusion.
C The Day -1 assessments may be conducted as early as Day -3 for logistical reasons, if required.
D Including weight.
E Day -1 weight should be used in the final dose calculations for infusion of FLT201.
F Vital signs include pulse, blood pressure, respiration rate, and temperature.
G Serum pregnancy test performed only if the urine pregnancy test is positive.
H SARS-CoV-2 (COVID-19) testing should be conducted by local laboratories in line with current local practices within 72hrs prior to FLT201 dosing.
I LFTs (include albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, alanine aminotransferase, and aspartate aminotransferase.
J Following 8-hour post dosing monitoring, additional 2-hourly monitoring of vital signs can occur if deemed necessary by the investigator.
K Semen samples may be omitted at the discretion of the investigator for religious or other personal reasons expressed by the subject.

6 Introduction

6.1 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 (GL1), and other glycolipids [Pastores 2000]. The disease has a prevalence of 1:40,000 to 1:60,000 [Stirnemann 2017] and is classified by three main phenotypes delineated by the absence (type 1) or presence (type 2 and type 3) of primary CNS involvement [Pastores 2000]. Gaucher disease is caused by mutation in the β-glucosylceramidase gene (*GBA1)*, on chromosome 1q22 (GBA; 606463), encoding the enzyme GCase, resulting in deficiency in GCase activity and accumulation of GL1 in cells of the macrophage-monocyte system [Sidransky 2012].

The clinical manifestations of Gaucher disease reflect chronic, progressive macrophage tissue infiltration and Gaucher cell deposition in the spleen, liver, bone marrow, and other tissues resulting in splenomegaly, hepatomegaly, and the signs and symptoms associated with thrombocytopenia, anaemia, and bone involvement. Bone involvement can include acute and chronic pain, as well as osteonecrosis, osteopaenia, and osteoarthritis. Lung involvement and massive lymphadenopathy are less frequent.

The standard of care for Gaucher disease is long-term parenteral enzyme replacement therapy (ERT), which aims to increase the level of functional GCase enzyme, or oral substrate reduction therapy (SRT), which aims to reduce the amount of GL1 that is produced. Although many patients achieve clinically meaningful benefit within 2-5 years of treatment, response can be variable and incomplete. Platelet counts in some patients may be poorly responsive and, additionally, bone disease may sometimes progress despite otherwise successful treatment.

ERT consists of long-term, parenteral administration of recombinant GCase every 2 weeks. Current ERTs include Cerezyme® (imiglucerase), VPRIV® (velaglucerase), and Elelyso® (taliglucerase), with Elelyso not available in the European Union (EU).

SRT is a long-term oral medication but may be limited to specific populations; patients for whom ERT is not suitable Zavesca® (miglustat) or patients who are not CYP2D6 ultra-extensive metabolisers Cerdelga® (eliglustat).

There are currently no licensed advanced therapy investigational medicinal products (ATIMPs) available for the treatment of any form of Gaucher disease.

6.2 FLT201

FLT201 is an investigational ATIMP intended for the treatment of Gaucher disease. The FLT201 product is a single-stranded (ss) adeno-associated viral vector serotype S3 (AAVS3) and contains AAV and the human *GBA1* gene.

The FLT201 AAV construct has three major features:

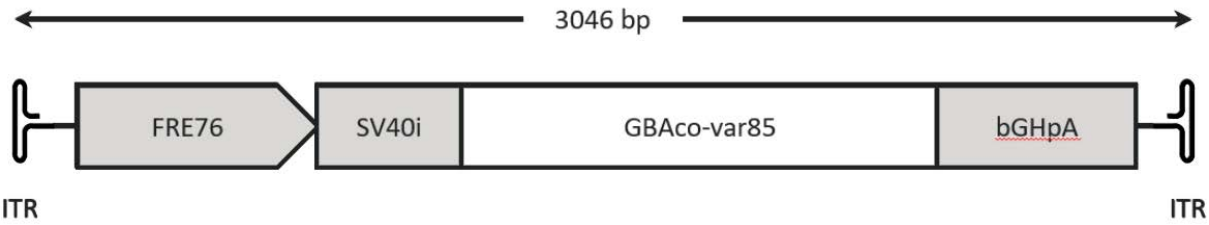
1. A novel engineered capsid (AAVS3) which has been selected based on its ability to transduce human hepatocytes with a high efficiency and more effectively than other capsids.
2. A codon-optimised transgene (*GBA1*) under the control of a liver-specific promoter (LSP), FRE76.

3. It encodes a protein engineered GCase (variant 85), which incorporates two amino acid (AA) substitutions that should result in a putative disulfide bond (S-S) in the GCase protein structure.

The AA substitutions are internal and involve no exposed residues. Changes are not predicted to affect the active site, the external structure, or the overall charge of GCase.

The construct for FLT201 is represented in Figure 1.

Figure 1: Schematic Representation of the FLT201 Construct for Gaucher Disease



Abbreviations: bGHpA=bovine growth hormone polyadenylation signal; bp=basepairs; FRE76=liver-specific promoter; GBAco-var85=codon-optimised β-glucocerebrosidase engineered for increased stability (variant 85); ITR=inverse terminal repeat; SV40i=SV40 intron; white box=protein coding sequence.

Freeline developed FLT201 encoding GCase (variant 85) since the wild type GCase displays a short half-life when exposed to physiological pH. GCase (variant 85) displays increased protein stability in various physiological matrices compared to the wild type GCase.

Following receptor-mediated uptake of AAV into the hepatocytes, transgenes are expressed by host cell transcription machinery [Mingozzi 2011]. Under the control of the LSP, the GBA1 gene will be expressed in host hepatocytes leading to the production and secretion of a functional GCase protein into the blood circulation. As GCase is produced in host hepatocytes, glycosylation will be host specific and thus be less likely than an exogenous protein to be recognised as foreign [Mingozzi 2003].

FLT201 is, therefore, anticipated to lead to continuous endogenous production of GCase (variant 85) in hepatocytes, resulting in steady plasma activity levels, and thus has the potential to provide enhanced uptake of the active enzyme by macrophages and better penetration into the target tissues of Gaucher disease.

6.3 Nonclinical Data

The overall nonclinical development programme consists of in vitro and in vivo pharmacodynamic studies, and in vivo proof of concept study in GBA-deficient mice and a single-dose GLP-compliant toxicology/biodistribution study in mice (C57BL/6) and a single-dose long-term investigational study in non-human primates (NHP) (rhesus macaques). Nonclinical studies showed the FRE76 promoter is liver specific and that the AAVS3 capsid is efficient at transducing human hepatocytes. The in vitro and humanised liver mouse model (FRG) studies demonstrated that transduction of murine hepatocytes (but not human hepatocytes) by AAV2/8 (AAV8) is efficient, whereas transduction of human hepatocytes by AAVS3 (but not murine hepatocytes) is very efficient. Studies in mice to test the therapeutic benefit of FLT201 genome were, therefore, performed with AAV8 capsid pseudotyped constructs. In GBA-deficient mice, following the administration of AAV2/8 pseudotyped FLT201 genome, plasma GCase activity level increased, resulting in normalisation of the biomarker glucosylsphingosine (Lyso-Gb1); the deacylated lysolipid of GL1 and the substrate for the GCase enzyme. In vitro studies demonstrated that GCase variant 85 when presented to human PBMCs or macrophages can be taken up in a dose-dependent manner similar to ERT.

Please refer to the FLT201 Investigator's Brochure (IB) for full details of the nonclinical data.

6.4 Clinical Data

The study represents the first administration of FLT201 to humans. The same AAVS3 capsid and expression cassette (containing a transgene encoding Factor IX [FIX]) is being administered to humans in an ongoing Haemophilia B program (FLT180a) or expression cassette (containing a transgene encoding α-galactosidase (α-GLA)) in an ongoing Fabry disease program (FLT190). Please refer to the IB for details on clinical experience in FLT180a and FLT190.

6.5 Rationale and Risks/Benefits

6.5.1 Rationale

The standard of care therapy for Gaucher disease is long-term parenteral ERT, which aims to increase the level of functional GCase enzyme, or oral SRT, which aims to reduce the amount of GL1 that is produced.

Gaucher disease is a compelling disease for an AAV gene therapy approach, because of the potential for a single administration to enable achievement and maintenance of therapeutic treatment goals. A single administration of FLT201 has the potential to improve the clearance of lyso-Gb1 from tissues and thereby improve patient outcomes, whilst reducing the burden of chronic treatment with standard of care.

This first-in-human clinical study primarily aims to investigate the safety of FLT201 which has the potential to alter the disease phenotype through the endogenous production of GCase following a single administration.

6.5.2 Starting Dose and Dose Escalation

The starting dose (Cohort 1) of FLT201 in this study is 4.5×10^{11} vector genomes (vg)/kg of body weight to be administered IV.

The starting dose for the clinical trial has been based upon the totality of non-clinical data for FLT201 as well as other available safety data for the AAVS3 capsid from similar products to derive a value that is considered safe, tolerated, and expected to provide a potentially therapeutic dose taking into consideration the observed non-clinical safety margins.

The nonclinical strategy to set the starting dose is derived from the following nonclinical components:

- Pharmacokinetic (PK), pharmacodynamic (PD), and efficacy data in the *GBA*-deficient mouse model using the AA2/8 capsid containing the FLT201 genome (LSP-GBA co-variant 85).
- Safety, PK, and PD from the long-term, non-human primate (NHP) investigational study with up to 6-month plasma GCase expression data using the FLT201 clinical product (AAV2/S3 capsid with the genome LSP-GBA co-variant 85).
- GLP toxicology of the transgene product in wildtype mice using the AAV2/8 capsid containing the FLT201 genome (LSP-GBA variant 85).
- GLP NHP toxicology safety data using the same AAV2/S3 capsid with other inserted genomes from other Freeline programs (supplemental).

These nonclinical components are further described below:

1. GBA Murine Pharmacology Model

Hexosylsphingosine substrate concentrations observed in individual *GBA*-deficient mice were plotted relative to the circulating plasma GCase levels and an inverse correlation for accumulated substrate with plasma GCase levels was observed (data on file). For key tissues

(spleen, liver, bone marrow, lung), plasma GCase levels of greater than 250 nmol/h/mL were required for significant (>50%) and consistent reductions in tissue hexosylsphingosine substrate concentrations in *GBA*-deficient mice treated with the FLT201 genome pseudotyped with AAV2/8 capsid (Investigator Brochure, Section 4.4.1.5). The dose of AAV2/S3 that would result in a plasma GCase concentration of greater than 250 nmol/h/mL was calculated by a simple ratio from the available GBA deficient mouse data, and a murine to human scaling factor of 4 applied to account for transduction efficiencies between mouse and human [Dane ASH Abstract 2018], to obtain a predicted pharmacological dose of 4.5 x 10^{11} vg/kg.

2. Extrapolation from GCase Expression in NHP Following Administration of FLT201

NHP plasma data supports that a dose of FLT201 at 2 x 10^{12} vg/kg results in plasma GCase activity in the range of 336–1308 nmol/h/mL at Day 170 post-dose (Investigator Brochure, Section 4.4.6.2).

An investigational study in Rhesus macaques (n=7) received a dose of 2 x 10^{12} vg/kg FLT201 (Figure 2). Plasma GCase levels increased rapidly by Day 8; some fluctuations were noted between Days 21 and 43 and levels plateaued from Day 43. The GCase plasma levels at Day 170 post-dose in this study ranged between 336 to 1308 nmol/h/mL which were consistent with GCase levels that resulted in significant and consistent substrate clearance in the *GBA*-deficient mouse study in the liver, spleen, bone marrow, and lungs. AAV2/S3 products scale well from NHP to human based on other Freeline projects and assuming linear scaling from NHP to human and taking into account the variability observed in the NHP, a dose of 4.5 x 10^{11} vg/kg could result in approximately 76 to 295 nmol/h/mL plasma GCase in patients. The 2 x 10^{12} vg/kg FLT201 dose was well tolerated in NHP with no treatment-related findings noted at the interim analysis at 6 months post-dose and is 4.4-fold greater than the proposed starting dose of FLT201 in the Phase 1/2 study.

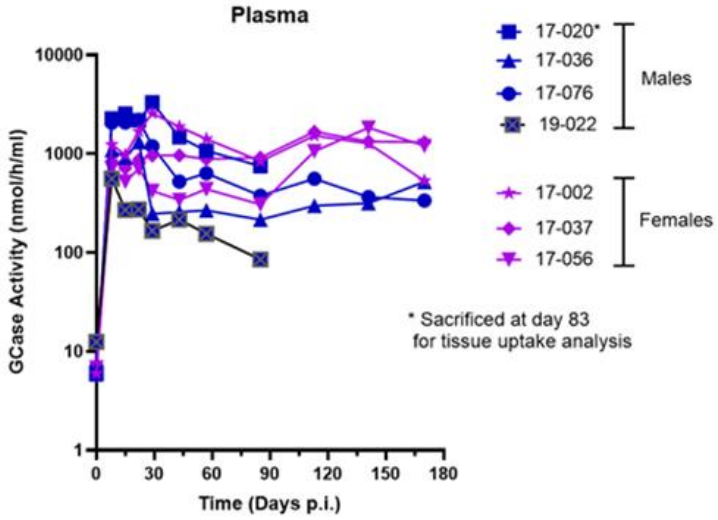


Figure 2: Plasma GCase Activity Levels Post Single IV Infusion of FLT201 at 2 x 10^{12} vg/kg in Rhesus Macaques

3. Murine GLP Toxicology Data

The murine GLP toxicity study identified a NOAEL of 2.57 x 10^{13} vg/kg (highest dose tested) using the genome of FLT201 pseudotyped into the AAV2/8 capsid in C57bl/6 mice and is 57-fold greater than the proposed starting dose of FLT201 in the Phase 1/2 study. AAV2/8 was selected as this capsid possesses a superior ability to transduce murine hepatocytes [Nakai 2005; Gao 2002] compared to AAV2/S3 and was utilized to ensure efficient transduction to assess the impact of high vector genome levels and production of high levels of the transgene

GCase protein. The GCase levels achieved at the NOAEL were far in excess of that expected for FLT201 in humans (>3000-fold the 250 nmol/h/mL discussed in Method 1), which would not be possible with the AAV2/S3 capsid in mice.

Using a 10x margin of safety for the NOAEL, a dose 2.57 x 10^{12} vg/kg using the genome of FLT201 pseudotyped into the AAV2/S3 capsid is supported by the study.

This dose does not exceed the NOAEL 2.44 x 10^{13} vg/kg established for the AAV2/S3 capsid safety in NHP for FLT180a in Method 4.

4. AAV2/S3 NHP Safety Data from Similar Product

Supporting data from an FLT180a GLP toxicology study in Rhesus macaques using the same AAV2/S3 capsid at doses up to the NOAEL of 2.44 x 10^{13} vg/kg FLT180a was well tolerated and is 54-fold greater than the proposed starting dose of FLT201 in the Phase 1/2 study.

In summary, in line with current regulatory guidance, several different methods have been used to estimate the therapeutic FLT201 AAV2/S3 dose since there is no ideal single model that can accurately predict the starting dose. On that basis, considering all the information available a pragmatic starting dose of 4.5 x 10^{11} vg/kg was selected. This dose was considered to be low enough to be well within known safety margins (57-fold for FLT201 genome, 54-fold for AAVS3 capsid safety) and, at the same time offer a potentially therapeutic dose.

Depending on safety/tolerability (including DLT), PK, PD, and clinical data, the dose will be escalated in increments across successive cohorts to a maximum planned dose of 1.1 x 10^{13} vg/kg. If an additional cohort is added, it would not exceed 1.5 x 10^{13} vg/kg, which is below the NOAEL (2.57 x 10^{13} vg/kg).

6.5.3 Potential Risks and Benefits

The overall risk:benefit assessment summary of FLT201 is outlined in the sections below. Please refer to the IB for further details of potential risks associated with FLT201.

6.5.3.1 Potential Risks Associated with FLT201

Raised ALT and/or a Decline in GCase Expression

ALT elevation is a recognised class event following AAV integration into hepatocytes and represents a loss of transduction efficacy rather than a true safety risk because the transaminase elevation and accompanying decline in expressed protein have, to date, been asymptomatic and self-limited.

To mitigate against the risk of vector-associated transaminitis and subsequent loss of GCase expression (activity and concentration) in this study, participants will be intensively monitored over the course of the study. For further details, see Section 12.2.1.

Vector Shedding and Germline Transmission

Following systemic administration of FLT201 there is potential for the spread of vector particles to non-hepatic tissues including the gonads. Therefore, shedding will be assessed in samples taken three times over the first 7-10 days of the study and at each subsequent visit thereafter until no further evidence of AAVS3 is detectable (when samples from all matrices at three consecutive visits are negative). As a precaution, only participants willing to practice birth control methods will be enrolled to avoid the possibility of horizontal, and potentially vertical, transfer of vector. Subjects must refrain from sperm donation for the duration of the trial.

Development of Neutralising Antibodies Against AAV

Experience in animal models indicates that administration of AAV results in a serotype specific stimulation of neutralising antibodies (NAb) production and that such antibodies preclude re-administration of AAV of the same or related, serotype.

Pre-existing anti-AAV humoral immunity is recognised as an important barrier to successful gene transfer through systemic delivery of AAV vectors. To address this challenge, all participants will be screened for pre-existing neutralising antibodies to the AAVS3 vector and excluded if a positive result is returned. A proprietary transduction inhibition assay to measure anti-AAVS3 titres will be performed at a central laboratory.

Development of Anti-GCase Neutralising Antibodies

In Gaucher disease, the presence of anti-GCase antibodies is not generally considered clinically significant and there is no correlation with safety and efficacy in prior Gaucher disease type 1 development programmes [Pastores 2016]. The development of de novo anti-human GCase neutralising antibodies would be unexpected in liver directed AAV gene therapy since such liver directed therapy is reported to be immune tolerising for liver expressed and secreted protein. More than 100 patients with haemophilia B have been given gene therapy to date across multiple studies and no inhibitors have been reported [Arruda 2016]. Therefore, the risk of developing antibodies, with subsequent loss of efficacy from rescue ERT, is thought to be very low, and similar in both previously treated and treatment naïve patients.

Risk of Infusion-related Toxicity

Allergic-type reactions, including anaphylaxis are a rare consequence of administration of biologics. Onset of anaphylaxis is usually rapid following injection of an allergen with 90% beginning within 40 minutes. Patients with a known or suspected intolerance, hypersensitivity, or contraindication to ERT or the investigational product excipients are excluded from the study.

Participants will remain under observation in the investigational centre for at least 8 hours following infusion to minimise the risk associated with acute allergic reaction. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals for the duration of the infusion. Vital signs will be monitored hourly for 6 hours following infusion and then again at 8 hours after the end of the infusion. Infusion reactions should be managed according to Appendix 1: Suggested Management Guidelines for Infusion Reactions.

Myocarditis

Mild, transient myocarditis was observed in the Phase 1/2 study of FLT190 in subjects with Fabry disease. FLT190 consists of the same proprietary AAVS3 capsid however includes a different transgene which encodes αgalactosidase. See the FLT201 Investigator Brochure for further details.

6.5.3.2 Risks Associated with Study Procedures

Risks of Immunosuppressant Use to Prevent and Treat Transaminitis

The long-term use of high dose corticosteroids may be associated with the development of side effects. These include high blood pressure, elevation in blood sugar, weight gain with increased appetite, reduced fertility in men, and fluid retention. Osteoporosis is generally considered a long-term side effect but may, in its most severe form, evolve to aseptic necrosis or fragility fractures.

Cramps and joint pain have also been described. The occurrence of glaucoma and cataracts has been described in patients taking long-term corticosteroids. In addition, gastrointestinal (GI) irritation may occur and could result in GI haemorrhage. Emotional disturbances and mood changes are also described.

The use of tacrolimus may be associated with the development of side effects. These include lymphoma and other malignancies, susceptibility to infections including polyoma virus and CMV, nephrotoxicity, neurotoxicity, hyperkalaemia, hypertension, hyperglycaemia, insomnia, visual disturbances, headaches, hyperphosphatemia, tremor, myocardial hypertrophy, pure red cell aplasia, haemolytic uremic syndrome, and posterior reversible encephalopathy syndrome. Use of live vaccines for immunisation should be avoided.

Investigators should also consider the risk of cytomegalovirus (CMV) reactivation (Appendix 2: Management Guidelines for CMV Reactivation).

Participants should also avoid prolonged exposure to ultra-violet light and sunlight by wearing protective clothing and sunscreen with a high protection factor (minimum Sun Protection Factor 30) and avoid grapefruit juice/grapefruit during use of tacrolimus.

The risk of these side effects to immunosuppressants will be minimised by participant education, careful monitoring, utilisation of the lowest effective dose, and tapering/cessation in the prophylaxis regimen and in response to break through transaminitis as soon as the evidence of transaminitis begins to subside. The risk of side effects to immunosuppressants are expected to be limited due to the short prophylactic course, though this may be longer if required to treat breakthrough transaminitis.

Patients with a contraindication to immunosuppressants are excluded from the study.

Risk of Blood Tests

The risk of blood tests from a vein includes temporary discomfort at the site of puncture, possibly bruising and swelling around the puncture site, occasionally infection and bleeding into the surrounding muscle and tissues.

Risk of X-ray

The chest X-ray carries the risk of radiation exposure. The radiation exposure from a chest x-ray is low and comparable to exposure from natural sources of radiation in the environment.

6.5.3.3 Potential Benefits

FLT201 has the potential to provide clinically relevant advantages for Gaucher disease participants:

- A single treatment with FLT201 is expected to lead to long-term production of GCase enzyme, avoiding the need of chronic administration of medication.
- The continuous production of functional GCase enzyme will avoid the brief peaks and prolonged troughs in plasma GCase levels that are seen with ERT/SRT and is expected to lead to improved clearance of lyso-GB1 and thereby an improvement in efficacy.
- Based on the fact, that GCase will be generated in the participant's own hepatocytes, the enzyme will have the participant's individualised glycosylation pattern. As such the potential for antibody development including the potential for the development of neutralising antibodies is reduced compared to ERT [Mingozzi 2003].
- Liver expression as a result of AAV gene transfer induces immune tolerance to secreted liver expressed human proteins. Tolerance induction is favoured by higher levels of transgene expression [Mingozzi 2003].

Currently available treatment options include ERT (Cerezyme® (imiglucerase) VPRIV® (velaglucerase) and Elelyso® (taliglucerase)) or SRT (Cerdelga® (eliglustat) and Zavesca® (miglustat)). Despite these treatments, there remains an unmet need due to incomplete efficacy and burden of therapy. All products require chronic, and potentially life-long administration.

6.5.3.4 Overall Risk and Benefit Assessment

The available non-clinical data indicate that a single treatment with FLT201 is expected to lead to long-term production of GCase enzyme, avoiding the need of chronic administration of medication for patients with Gaucher disease type 1. In this clinical study the population has been defined to optimise the likelihood of patients benefiting from treatment. For example, patients with neutralising antibodies to AAVS3 are excluded because this would limit transduction and ultimately the potential efficacy of the ATIMP. Important risks are managed in the study by various measures, for example: exclusion of patients with a history of anaphylaxis or infusion-related reactions to ERT, exclusion of patients with underlying cardiac disease and cardiac monitoring, intensive monitoring for ALT elevation suggestive of vector-associated transaminitis, and recommendation of appropriate contraception.

To mitigate against the risk of vector-associated transaminitis and efficacy loss due to subsequent loss of GCase expression (activity and concentration) in this study, a short prophylactic course of immunosuppression may be recommended. There are risks associated with the use of immunosuppressants; however, these are expected to be limited due to the short course of treatment, though it is acknowledged that longer treatment duration may be required if breakthrough transaminitis occurs.

The risk of developing anti-GCase neutralising antibodies is considered to be very low and may not be associated with efficacy or safety as discussed in Section 6.5.3.1. Participants will be monitored for development of antibodies to GCase in the study.

The starting dose has been carefully selected utilizing data from FLT201 in the mouse and NHP, and NHP and clinical data from other clinical programs utilising the same capsid, (Section 6.5.2) and is anticipated to be efficacious. The maximum potential dose is 1.7-fold below the NOAEL and is also supported by capsid safety data from other Freeline programmes.

Considering the study design, the overall risks, and potential benefits for patients with Gaucher disease receiving FLT201 in this clinical trial is anticipated to be as least as favourable as existing alternative therapies.

7 Study Objectives and Endpoints

Study objectives and endpoints are presented in Table 3.

Table 3: Study Objectives and Endpoints

Type	Objective	Endpoints
Primary		
Safety	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)	Primary safety variable: • incidence of TEAEs (including DLTs) from Day 1 to the last follow-up visit Other safety endpoints: • 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
Secondary		
PK	To investigate the relationship of FLT201 dose to endogenous production of GCase	Change from baseline to each assessment point in plasma and leukocyte GCase activity level
Efficacy	To assess the impact of FLT201 on: • clearance of lyso-Gb1 • spleen size • liver size • haemoglobin • platelet count	Change from baseline to each assessment point in: • lyso-Gb1 in plasma • spleen volume measured by MRI • liver volume measured by MRI • haemoglobin • platelet count
Immunologic	To describe the immune response to GCase transgene product	Change from baseline to each assessment point in total anti-GCase antibody titre and neutralising antibody titre
Shedding	To assess viral shedding after systemic administration of FLT201	Clearance of vg in plasma, urine, saliva, stool, and semen measured by PCR
Exploratory		
Other efficacy/ biomarkers	To assess the impact of FLT201 on: • Gaucher disease severity and progression • bone disease • fatigue • pain • biomarkers • lung disease	Change from baseline to each assessment point in: • Gaucher disease severity measured by the Gaucher Disease Severity Scoring System (GD-DS3) • bone marrow burden score measured by MRI • bone mineral density measured by dual energy x-ray absorptiometry (DEXA) o Z-score and T-score in the lumbar spine (L1-4) and hip (femoral neck) • fatigue measured by FACIT-Fatigue • pain measured by Brief Pain Inventory- Short Form (BPI-SF) • disease activity biomarkers: chitotriosidase, CCL18 • bone biomarkers: bsALP, osteocalcin • chest x-ray and pulmonary function tests

Type	Objective	Endpoints
PK	To characterize the pharmacokinetics of FLT201	• AUC, peak, and steady state GCase activity levels in plasma and leukocytes (baseline to Week 38) • Change from baseline to each assessment point in GCase concentration (antigen levels) • Dose response relationship
Immunologic	To describe the immune response to AAVS3 capsid proteins	• Immune response to AAVS3 capsid (AAVS3 antibody titre and T-cell response) • Change from baseline to each assessment point in immune response biomarkers
HRQoL	To assess the impact of FLT201 on HRQoL	Change from baseline to each assessment point in HRQoL measured by SF-36

Abbreviations: AE=adverse event; AUC=area under curve; DLT=Dose-limiting toxicity; HRQoL=health-related quality of life; PK=pharmacokinetic, MRI=magnetic resonance imaging; SAE=serious adverse event; TEAE=treatment-emergent adverse event.

8 Study Design

8.1 Study Description

This is a first in human, Phase 1/2, open label, safety, tolerability, and efficacy study in adult patients with Gaucher disease type 1.

Participants will undergo the screening assessments described in Section 12.1.1 and Section 12.1.2. for up to 16 weeks prior to Study Day 1 (gene therapy infusion).

In Part 1, eligible participants currently receiving ERT/SRT will maintain their current treatment until a minimum of 2 consecutive results at least 4 weeks post-dosing show leukocyte GCase activity level higher than pre-dose trough GCase activity level, after which ERT/SRT may be withdrawn.

Progression from Part 1 to Part 2 will be by a substantial protocol amendment, based on safety/tolerability (including DLT), PK, PD, and clinical data.

In Part 2, eligible participants will be PUPs. Participants may need to be considered for initiation of ERT/SRT in case of a lack of a trend to normalisation in leukocyte GCase activity level in serial measurements from at least 4 weeks post-dosing, suggestive of low probability of clinical efficacy.

Treatment-eligible participants will report to the infusion study site on the day prior to receiving the gene therapy infusion (Day -1). Participants will either attend their local study site if it has been approved for FLT201 infusion, or if not, an alternative approved infusion study site. On Day 1, FLT201 will be administered as a single-dose, slow IV infusion into a peripheral vein, and the participant will remain in the study centre for at least 8 hours until the investigator has deemed the participant as fit to be discharged.

Participants will be required to undergo study evaluations at intervals over the 9-month post -treatment period. These will take place either at the study infusion site or at their local study site.

The study will be conducted in 2 parts (Figure 3):

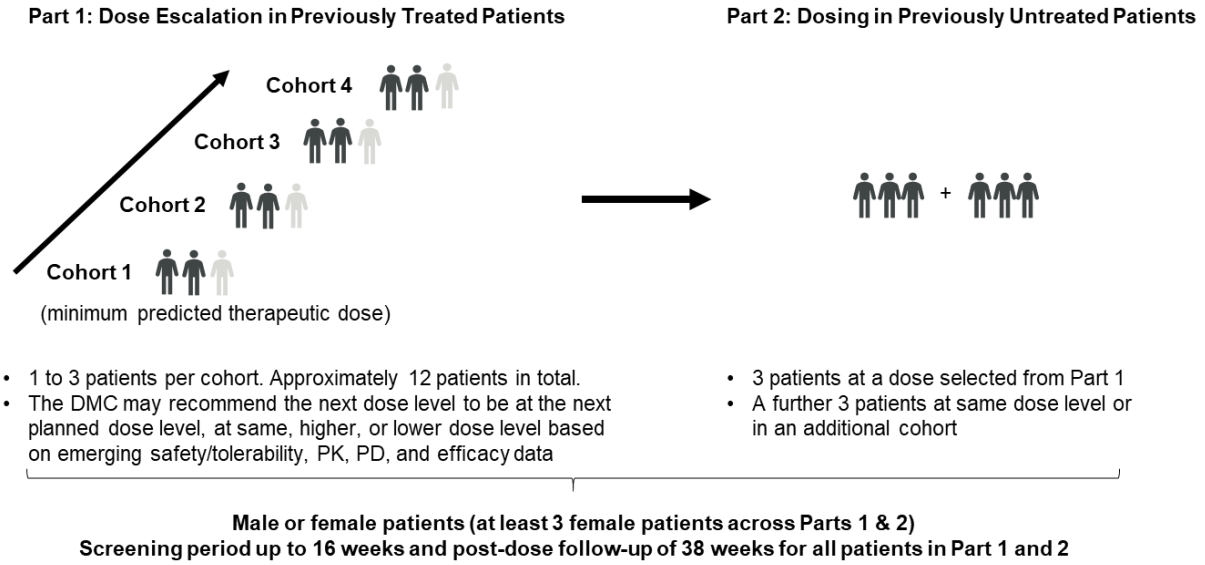


Figure 3: Study Schema

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. Each dose cohort is planned to include at least 2 participants; however, if GCase activity is below the normal range (e.g., <1.5 µmol/L/h, though this value will vary depending on the specific laboratory) and no DLT is observed in the first participant in the cohort, dose escalation to the next dose cohort will occur after this first participant to avoid treating the next participant with a dose predicted to be below the therapeutic range. A third participant may be added to the cohort depending on safety/tolerability.

A minimum of 4 weeks of safety/tolerability (including DLT), PK, PD, and clinical data will be assessed before dosing each participant (both within a dose cohort and before escalating to the next dose level) (Section 13.3).

Dose escalation will be overseen by an independent DMC.

The dose level to be tested in each cohort after Cohort 1 may be an intermediate dose between the current dose and that of the next planned dose cohort based on emerging data but would not be greater than the next planned dose. In addition, based on emerging data, including safety/tolerability, PK, PD, and clinical data, all dose cohorts may not be tested, an additional dose cohort may be added, and/or a dose cohort may be further expanded beyond 3 participants. If an additional dose cohort is added, the dose will not exceed 1.5×10^{13} vg/kg.

When the last participant in Part 1 has completed at least 12 weeks' follow-up post-FLT201 administration, the DMC will be convened to evaluate all available safety/tolerability (including DLT), PK, PD, and clinical data from Part 1. The dose level(s) selected for Part 2 will be informed by DMC recommendation. Progression from Part 1 to Part 2 will be by protocol amendment.

Part 2: Dosing in Previously Untreated Patients (Phase 2)

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

Participants will be dosed at least 4 weeks apart to allow for every participant to contribute to the emerging totality of safety/tolerability (including DLTs), PK, PD, and clinical data used to inform dosing of the subsequent participant(s). If the first 3 participants in Part 2 show acceptable safety profiles, up to 3 further participants may be dosed at this level in the same cohort or a different dose cohort following recommendations from the DMC (Figure 3). If a second dose is selected and is a dose increase, the dose increase would not be greater than 3-fold compared to the first dose cohort in Part 2.

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

9 Selection of Participants

Participant enrolment at a site will only commence once the study has documented Independent Ethics Committee (IEC)/Institutional Review Board (IRB), competent authority, and local institution approval, and been initiated on behalf of the Sponsor. Participants may only be enrolled at approved study sites.

9.1 Inclusion Criteria

Patients will only be eligible to participate in the study if they meet the following inclusion criteria:

1. Adult ≥ 18 years of age.
2. Diagnosis of Gaucher disease type 1 with deficient GCase enzyme activity ≤30% of normal in leukocytes at diagnosis.
3. All female patients of childbearing potential must not be lactating and must have a negative serum pregnancy test at screening and confirmed negative by urine testing prior to dosing on Day 1.
4. Female patients of childbearing potential and male patients must be willing to follow protocol guidelines for barrier protection/contraception.
5. Able to give full informed consent for the trial.

Part 1 only: previously treated patients:

6. Treatment status at screening (screening period is 16 weeks):

   Treated with either enzyme replacement therapy (ERT) or substrate reduction therapy (SRT) and started this treatment at least 2 years prior to dosing with no change in regimen for the prior 3 months. ERT dose ≥15 U/kg and ≤60 U/kg every other week.

Part 2 only: previously untreated (naïve i.e., never received ERT/SRT) patients:

7. Haemoglobin (Hb) level ≥1 g/dL below the lower limit of normal adjusted for age and sex, and at least one of the following at screening:
   a. Platelet count <120,000/mm^3.
   b. Hepatomegaly on abdominal magnetic resonance imaging (MRI).
   c. Splenomegaly on abdominal MRI.

9.2 Exclusion Criteria

Patients will not be eligible to participate in the study if they meet any of the following exclusion criteria:

1. Diagnosed or suspected type 2 or type 3 Gaucher disease (including any patient with eye movement abnormality on clinical examination).
2. Positive for neutralising antibodies to AAVS3 at screening.
3. Evidence of significant and persistent liver dysfunction at Screening defined as >1.5 x upper limit of normal (ULN) in alinine aminotransferase (ALT), aspartate aminotransferease (AST) or total bilirubin.
4. Evidence of any of the following at screening:
   a. Hb <8 g/dL.

   b. Platelets <45,000/mm^3.
   c. Pulmonary hypertension.
   d. New osteonecrosis within 12 months of screening.
   e. Fragility fracture or bone crisis within 12 months of screening.
5. Hepatitis B surface antigen (HBsAg) positive at screening.
6. Hepatitis C antibody (Hep C Ab) positive and hepatitis C RNA polymerase chain reaction (PCR) (as follow-up test if Hep C Ab-positive)-positive at screening.
7. Cytomegalovirus (CMV) immunoglobulin G (IgG) and CMV DNA PCR-positive at screening.
8. Human immunodeficiency virus (HIV)-1 or -2 antibody positive at screening.
9. Receipt of live attenuated vaccination within 12 weeks prior to screening or intends to receive such vaccination during the study.
10. History of clinically-advanced liver disease e.g., cirrhosis, portal hypertension.
11. History of bone marrow transplant.
12. History of splenectomy (partial or total).
13. History of splenic infarct within 12 months of screening.
14. History of receiving any gene transfer medicinal product.
15. History of receiving any investigational therapy for Gaucher disease within 60 days of screening.
16. Participation in any other clinical study of an investigational medicinal product (IMP), and/or receiving any other IMP during the study.
17. History of idiopathic thrombocytopaenic purpura, thrombotic thrombocytopaenic purpura, thrombocytopaenia, anaemia, hepatomegaly, splenomegaly, and/or osteoporosis, unrelated to Gaucher disease.
18. History of, or active neoplastic disease within 5 years of screening (except for basal or squamous cell carcinoma of the skin or carcinoma in situ which has been definitively treated).
19. History of uncontrolled cardiac failure, unstable angina, or myocardial infarction or other acute cardiac conditions requiring clinical management in the past 6 months.
20. History of acute myocarditis or presence of acute myocarditis during screening.
21. History of substance abuse, including alcohol abuse or alcohol dependence.
22. Known or suspected intolerance, hypersensitivity or contraindication to the investigational medicinal product (IMP) and non-investigational medicinal products (NIMPs) or their excipients.
23. History of anaphylaxis or infusion related reactions to ERT.
24. Contraindication(s) to MRI. (e.g., ferromagnetic metallic implants, some types of pacing and defibrillator devices, nerve stimulators).
25. Any clinical condition (medical or psychiatric) that, in the opinion of the investigator, could jeopardise safety or compromise ability of the patient to participate in this study.

9.3 Restrictions on Participants

9.3.1 Contraception

Male subjects must practice a reliable barrier method of contraception, until the results from three consecutive negative semen samples taken at separate visits (at least 1 week apart) after FLT201 administration are negative.

Semen samples may be omitted at the discretion of the investigator for religious or other personal reasons expressed by the subject. If the subject does not provide semen samples, barrier contraception must be continued until the results from three consecutive serum samples taken at separate visits (at least 1 week apart) after FLT201 administration are negative. In programs to date with our proprietary capsid and in the literature, semen samples become negative before serum samples.

Subjects must refrain from sperm donation for the duration of the trial.

Female participants of childbearing potential must be willing and able to use highly effective birth control methods until the results from three consecutive serum samples taken at separate visits (at least 1 week apart) after FLT201 administration are negative.

Highly effective methods of birth control are methods that achieve failure rate of less than 1% per year when used consistently and correctly:

- Combined (oestrogen- and progesterone- containing) hormonal contraception (oral, implant, injectable) associated with inhibition of ovulation (which must be stable for at least 1 full month prior to screening).
- Progesterone-only hormonal contraceptives (oral, implant, injectable) associated with inhibition of ovulation (which must be stable for at least 1 full month prior to screening).
- Progesterone-releasing intrauterine systems or the copper intrauterine device.
- Vasectomized partner.

Female participants not agreeing to use birth control must be of non-childbearing potential, defined as:

- Postmenopausal for at least 1 year before screening.
- Permanently sterilised (e.g., bilateral tubal occlusion, hysterectomy, bilateral salpingectomy).
- Or congenitally sterile.

9.3.2 Alcohol

Participants will be advised to moderate their intake of alcohol during the study and, specifically, to abstain from alcohol from 1 week prior to vector infusion and for approximately 3 months following vector infusion.

9.3.3 Other

Participants should be up to date with local vaccination policy (e.g., seasonal flu, COVID-19 vaccination).

10 Investigational Medicinal Products and Non-investigational Medicinal Products

10.1 FLT201

The ATIMP in this protocol is FLT201 Solution for Infusion.

FLT201 is a replication-incompetent ss recombinant AAV vector. The vector is composed of a ss DNA genome packaged in an AAV-derived protein capsid (see Section 6.2).

FLT201 drug product is supplied as a sterile Solution for Infusion in 10 mL Crystal Zenith® vials, each vial containing 5 mL extractable volume. The vials are sealed with rubber stoppers and aluminium seals with plastic flip tops. The product is formulated as an approximately isotonic, aqueous solution at neutral pH. The formulation also contains 0.25% w/v recombinant human albumin.

FLT201 Solution for Infusion is stored frozen at ≤ 60°C until thawed for the preparation of the infusion. Detailed instructions for the preparation of infusion solutions will be provided in the FLT201 Pharmacy Manual.

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).

Instructions for the preparation of FLT201 infusion solutions are also included in the FLT201 Pharmacy Manual.

10.2 Source of FLT201 Manufacture, Distribution, and Storage

10.2.1 Secondary Packaging, Labelling, and Distribution

The drug product is shipped using companies specialising in cold chain transportation of biopharmaceuticals. Vials will be packed in validated shippers for transportation and are maintained at ⩽ -60ºC during shipment. All shipments include temperature monitoring devices so that temperature control during transit can be confirmed.

The FLT201 Solution for Infusion is supplied in single vial cartons. Vials and cartons are labelled in accordance with local regulatory requirements (for example, Annex 13 [EudraLex Volume 4] in the EU). Label text is provided in the language of each territory where dosing is to be performed, either as a multi-language booklet label or a single-panel label.

10.2.2 Infusion Sites

Administration of FLT201 will be restricted to study sites that have the capacity to store the FLT201 at the specification storage temperature and to prepare and administer the infusion solutions with the required level of control (see FLT201 Pharmacy Manual). In addition, as part of the study site selection process, the study site's ability to meet the required safety oversight of the participant will be assessed (e.g., site team emergency response capabilities/procedures and access to the required emergency response equipment/departments). If a site is not able to administer FLT201 the Sponsor will attempt to identify an alternative site for the infusion week only.

10.2.3 Receipt Storage and Handling of FLT201 at Site

All FLT201 aspects of the study at participating sites are the responsibility of the investigator, who may delegate this responsibility to the local pharmacist or other appropriately trained personnel. The delegation of duties must be documented.

Detailed instructions for the receipt, storage, and handling of FLT201 drug product are contained in the FLT201 Pharmacy Manual. All local requirements related to activities involving genetically modified organisms are adhered to.

10.2.4 Accountability and Traceability of FLT201

There is a system set-up to ensure the traceability of FLT201 from the starting material, through to administration to the participant and destruction or final transfer. A comprehensive FLT201 Pharmacy Manual and associated Standard Operating Procedures and forms are in place to ensure that the required accountability and traceability data is collected and retained. The requirement for the manufacturer and investigator/clinical study site(s) to retain their part of the traceability information is set out in the relevant contractual agreements with the Sponsor.

10.3 Name and Description of Non-investigational Medicinal Products

Prednisolone or prednisone, methylprednisolone and prolonged-release tacrolimus are NIMPs in this study. Prednisolone/prednisone, methylprednisolone, and tacrolimus will be sourced from commercial supply to the study sites. Prednisolone and prednisone are used in the same manner and are equally effective. Therefore, the recommended schedule for prednisolone, equally applies to prednisone. Tacrolimus has a narrow therapeutic index and should be prescribed by brand name to avoid inadvertent switching, and different oral formulations of tacrolimus should not be substituted during the study. Please refer to the Clinical Immune Management Plan (CIMP) and Non-investigational Medicinal Product guidelines for administration details.

Investigational sites are responsible for maintaining a system which allows adequate reconstruction of NIMP movements and evaluation of participant compliance.

Risks associated with use of NIMP as described in their Summary of Product Characteristics (SmPC) or other applicable labelling should be communicated to the participants by the investigator.

The usage of immunosuppression should be recorded in the electronic case report form (eCRF) on the ‘Immunosuppressant Log’. These details include daily dosage and trough tacrolimus levels.

10.4 Immunosuppression Management

As noted in Section 6.5.3.1, liver-directed gene therapy with an AAV-based capsid has been reported to be associated with a likely T-cell mediated immune response to vector-transduced hepatocytes resulting in rises in liver transaminase levels, most notably ALT.

For all participants, LFTs will be closely monitored (Section 12.2.1) to enable timely identification and treatment of episodes of breakthrough ALT increase to preserve GCase expression (activity and concentration).

Participants will receive a prophylaxis regimen (post-dosing with FLT201) starting at approximately Week 3 post-dosing, continuing for a duration of up to approximately 3 weeks at the initial dose followed by a taper over 11 to 13 weeks.

The prophylaxis regimen consists of oral steroid (prednisolone/prednisone) and/or oral tacrolimus. An appropriate quantity of steroid and/or tacrolimus will be dispensed to the participant to cover the period between clinic visits and usage will be monitored via tablet counts.

In the event of breakthrough elevation of liver transaminase, participants will be assessed by the treating physician, and a reactive regimen (consisting of IV methylprednisolone) may be initiated in accordance with the CIMP.

The CIMP provides detail of the prophylaxis and reactive regimens, as well as more detailed guidance regarding the assessment and management in case of observed ALT elevation. The CIMP will be available to the investigator, pharmacy, and key study personnel. If the investigator considers that any systemic immune-modifying medication other than those prescribed in accordance with the CIMP may be necessary, this should be discussed with the Medical Monitor and Sponsor.

Any significant changes in the CIMP will be reviewed by the DMC, and changes may be issued as an update to the CIMP and communicated to all sites. If the DMC considers that significant changes are required, such as addition of other drugs (i.e., additional NIMP), then a protocol amendment may also be submitted.

11 Concomitant Medication

All non-study treatment received within 30 days prior to initial informed consent and for the duration of the study must be recorded on the appropriate eCRF page. 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 as described in the CIMP.

11.1 Gaucher Disease Therapy

Any prior use, recommencement, initiation, or discontinuation of Gaucher disease therapy, including ERT, SRT, or any other medicinal product that may impact GCase levels should be documented in the Gaucher Treatment eCRF page.

11.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the date of investigational product infusion and the Week 38/end of study (EOS) visit, inclusive. All concomitant treatments information must be recorded on the appropriate eCRF page.

Participants should be instructed not to start taking any new medications, including non-prescription drugs and herbal preparations, unless they have received permission from the investigator, time allowing, i.e., investigator permission not required for emergency medical care. Other therapy considered necessary for the participant's welfare may be given at the discretion of the investigator. All such therapy must be recorded in the eCRF. No other ATMP/IMP may be used concomitantly with the study treatment. The participants are not allowed to participate concurrently in another clinical study.

Any systemic immune-modifying medication other than immune suppression prescribed in accordance with this protocol should be discussed with the Medical Monitor and Sponsor. Local or topical steroids, e.g., use of inhaled corticosteroids to manage chronic respiratory conditions are permitted.

In the event of an emergency, any needed medications may be prescribed without prior approval, but the Medical Monitor must be notified of the use of any excluded medications immediately thereafter.

11.3 Prior Treatment

Prior treatment includes all non-Gaucher disease therapy received from 30 days prior to the initial informed consent up until the time of FLT201 infusion. Prior treatment information must be recorded on the appropriate eCRF page.

11.3.1 Discontinuation/Resumption/Commencement of ERT/SRT

ERT/SRT will be discontinued once GCase activity is in the normal range.

If, after the discontinuation of ERT/SRT, haemoglobin or platelet levels start to decline or if disease-specific biomarkers or other clinical signs and symptoms significantly worsen compared to baseline, then ERT/SRT will be resumed. This information will then inform future decisions for subjects as to the level of GCase activity at which ERT/SRT is to be withdrawn.

In addition, GCase expression loss as evidenced by decline in serial GCase activity measurements to baseline (pre-FLT201 dosing) levels, ERT/SRT will be resumed.

The decision to withdraw or restart ERT/SRT should be discussed with the Medical Monitor and Sponsor.

12 Study Procedures

12.1 Study Schedule

12.1.1 Screening

The following study-specific procedures will be carried out after the informed consent form (ICF) is signed to assess the participant's eligibility. Screening evaluations will be performed at the study site. Screening evaluations may take place over a number of visits, as necessary, to complete all assessments. A window of up to 16 weeks (from initial consent to Day 0) is allowed for screening. At least two screening visits will be performed to complete all necessary assessments. Participants may be rescreened should this time window elapse.

- Demography and medical history
- Prior and concomitant medication (at both screening visits)
- Gaucher disease history (including ERT/SRT history)
- Gaucher disease severity (GD-DS3) (within 4 weeks of dosing only)
- Physical examination including measurement of the participant's height and weight.
- Vital signs (pulse, blood pressure, respiration rate, and temperature)
- 12-lead ECG
- AEs during the screening period from the time of initial consent
- Abdominal and bone MRI (within 4 weeks of dosing only)
- DEXA Z-score and T-score; lumbar spine and hip (within 4 weeks of dosing only)
- Participants will be asked to complete an electronic diary, answering questions relating to:
    - BPI-SF questionnaire: pain during the last 24 hours – daily over the 7 days following each Screening visit
    - FACIT Fatigue during the 7 days prior to each Screening visit

  Participants will be issued with instructions on electronic diary completion.
- HRQoL evaluated using the SF-36 questionnaire (within 4 weeks of dosing only)
- Chest x-ray and pulmonary function tests (within 4 weeks of dosing only)
- Serum pregnancy test for female participants (local laboratory analysis) (at both screening visits)
- Blood samples to evaluate (central laboratory analysis) (Screening laboratory assessments can be repeated on one further occasion if the initial test result falls outside the protocol eligibility criteria):
    - AAVS3 immune response. Note: A blood sample drawn within 6 weeks of dosing must be confirmed as negative for AAVS3 neutralising antibodies before a participant can be considered for dosing. It is acceptable to take repeat samples during the screening period, as necessary.
    - Haematology and chemistry (including LFTs and hs troponin-T, vitamin D, Fe2+, and vitamin B12) (at both Screening visits), and viral serology
    - Lyso-Gb1
    - GCase immune response

- GCase activity level
- GCase concentration
- Genotyping (GBA1 and CHIT1); only if previously not determined
- Chitotriosidase, CCL18, bsALP, osteocalcin (at both Screening visits)

Alanine amino transferase (ALT) levels will guide treatment for breakthrough transaminitis during the study and alcohol intake can cause ALT levels to rise, making it difficult to determine whether transaminitis is alcohol or vector associated. As such, participants will be advised to moderate their intake of alcohol as described in Section 9.3.2.

12.1.2 Participant Re-screening

Participants may be rescreened under the following conditions:

- Marginal laboratory screening failure, as listed in inclusion/exclusion criteria
- Transient or erroneous out-of-range laboratory values
- Mild or moderate illnesses e.g., cold at time of testing
- Outside screening window period

Decisions for rescreening should be discussed with the Sponsor.

Participants that are rescreened into the study will receive a new participant number and will be required to reconsent for participation in the study.

As part of the participant rescreen study visit the assessments must be repeated as described in Section 12.1.1 to ensure the participant remains eligible for the study.

Genotyping does not need to be repeated. Chest x-ray, pulmonary function tests, abdominal and bone MRIs, and DEXA Z-score/T-score (lumbar spine and hip) only need to be repeated if more than 6 months has elapsed since the assessment was completed during the participant's initial screening period and the participant's planned dosing date.

Participants will be required to complete BPI-SF questionnaire, FACIT-Fatigue and SF-36 during the rescreening period up until Day -1.

12.1.3 Pre-infusion (Day -1)

Day -1 assessments may be conducted as early as Day -3 for logistical reasons, if required:

- Physical examination (including confirmation of weight).
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- AE and concomitant medication status.
- Urine pregnancy test (serum confirmation if positive urine test)
- SARS-CoV-2 (COVID-19) test
- LFTs including albumin, alkaline phosphatase, direct/indirect and total bilirubin, ALT, and AST (local laboratory analysis)
- Blood samples to evaluate (central laboratory analysis):
  - Haematology, chemistry (including hs troponin-T and LFTs)
  - GCase immune response
  - GCase concentration

  - AAVS3 immune response (to retrospectively check status of anti-AAVS3 neutralising antibodies immediately prior to dosing)
  - Research plasma sample
- Plasma, saliva, urine, stool, (and semen, male participants only) samples for PCR of vg.
- BPI-SF and FACIT-Fatigue questionnaires.

12.1.4 Infusion (Day 1)

The following assessments will be conducted:

- Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored -1 hours before the infusion and at 15-minute intervals (± 5 mins) from the start to completion of infusion. Vital signs will continue to be monitored hourly from the end of infusion (± 10 mins) i.e., +1, +2, 3, 4, 5, and 6 hours. Following 8-hour post-dosing monitoring, additional 2-hourly monitoring can occur, if deemed necessary by the investigator.
- IV catheter insertion into a suitable peripheral vein (e.g., the median cubital vein).
- The IV catheter will be flushed with saline.
- The vector will be thawed and prepared in accordance with the instructions in the FLT201-01 Pharmacy Manual
- Prepared drug will be kept at room temperature prior to administration.
- At approximately 1 hour prior to vector infusion, the investigator or designee will assess the participant's vital signs and blood samples will be collected to analyse:
  - LFTs (local laboratory analysis).
  - GCase activity level (central laboratory analysis).
- The prepared drug containing the calculated vector dose will be infused through the catheter using an appropriate syringe driver, according to the FLT201-01 Pharmacy Manual
- On completion of the infusion, the catheter will again be flushed with saline.
- AEs and concomitant medications will be captured throughout the day.
- LFTs (local laboratory analysis): will be repeated 6 hours after infusion.
- Participants will remain at the infusion centre for at least 8 hours post-infusion to observe any immediate toxicity of the procedure. If the vital signs are stable, the catheter will be removed prior to participant discharge from the centre.
- Participants may be discharged from the study site. All participants will receive a medical alert card.

12.1.5 Post-infusion (Days +2 and +3)

The study visits procedures scheduled on Days +2 and +3 following infusion will be performed at the infusion centre where the participant received their vector infusion.

The following assessments will be conducted at each visit (or as specified):

- Physical examination.
- Vital signs (pulse, blood pressure, respiration rate, and temperature).

- 12-lead ECG on Day +2 only.
- AE and concomitant medication status.
- LFTs (local laboratory analysis).
- Blood samples to evaluate (central laboratory analysis)
  - Haematology and chemistry (including hs troponin-T and LFTs).
  - GCase activity (Day +3 only).
- Plasma, saliva, urine, stool, (and semen, male participants only) samples for PCR of vg on Day +3 only.

12.2 Follow-up Visits (Weeks 1-32)

The following assessments will be conducted at each visit (or as specified):

- Concomitant medication status.
- Gaucher disease severity (GD-DS3) – Weeks 12 and 24.
- Physical examination.
- Vital signs (pulse, blood pressure, respiratory rate, and temperature).
- 12-lead ECG – Weeks 1, 4, 12, and 24.
- Urine pregnancy test (serum confirmation if positive urine test) – Weeks 1, 4, 8, 12, 16, 20, 24, and 32
- AE status.
- LFTs (local laboratory analysis).
- Abdominal and bone MRI – Weeks 12 and 24.
- Chest x-ray and pulmonary function tests – Weeks 12 and 24.
- Participants will be asked to complete an electronic diary commencing at Weeks 4, 12, and 24, completing the following questionnaires:
  - BPI-SF during the last 24 hours –daily for 7 consecutive days commencing at the start of each specified week.
  - FACIT-Fatigue completed at the end of the specified weeks.

  Participants will be issued with instructions on electronic diary completion.
- For the duration a participant is on immunosuppression:
  - tacrolimus trough levels should be measured twice weekly until desired levels are established and weekly thereafter in conjunction with immunosuppressant dosing. See the Immunosuppression Management Plan for further information.
  - For participants positive for CMV IgG at screening, weekly CMV PCR testing should be conducted. Management guidelines in the case of CMV reactivation can be found in Appendix 2: Management Guidelines for CMV Reactivation.
- Blood samples to evaluate (central laboratory analysis):
  - AAVS3 immune response – Weeks 1, 2, 6, 12 and 24.
  - Haematology, chemistry (including hs troponin-T and LFTs).

- Lyso-Gb1 – Weeks 4, 8, 12, and 24.
- Chitotriosidase, CCL18, bsALP, osteocalcin – Weeks 4, 12, and 24.
- GCase activity level.
- GCase concentration – Weeks 1, 2, 3, 6, 12, and 24.
- GCase immune response – Weeks 1, 2, 3, 6, 12, and 24.
- Research plasma sample.

- Plasma, saliva, urine, stool, (and semen, male participants only) samples for PCR of vg (three times within 7-10 days after FLT201 infusion then at separate visits). Collection of a particular matrix sample, e.g., plasma, can be stopped once negative results have been reported in that matrix at three consecutive visits at least 1 week apart after Week 1. Collection of the remaining matrices should continue until each delivers negative results at three consecutive visits at least 1 week apart after Week 1.

12.2.1 Additional Laboratory Monitoring During Weeks 1-38/EOS

In addition to the main study site visits, participants will need to have additional laboratory assessments conducted from Week 1 until the end of Week 38/EOS to monitor for transaminitis (LFTs) and CMV reactivation, and to monitor tacrolimus levels, if required. In the event of suspected transaminitis (i.e ALT elevation), additional local LFT samples should be taken until the episode has resolved. In this situation, one additional sample for GCase activity level should be taken.

Frequency

- LFTs
  - From Week 1 to the end of Week 9, and from Week 28 to the end of Week 38/EOS, the additional laboratory assessments will be conducted once per week if there is already a scheduled study visit during the week, and twice per week if there is no scheduled study visit during the week.
  - From Week 10 to the end of Week 27 the additional LFT laboratory assessments will be conducted twice per week if there is already a scheduled study site visit during the week, and 3 times per week when there is no scheduled study site visit during the week.
  - The study site visit (when applicable) and additional laboratory assessments should be evenly spaced through the week.
- GCase plasma activity
  - In the event of raised LFTs, an additional GCase sample should be taken once per week during the additional laboratory assessments until the episode has resolved.

Sample Collection

These additional samples may be taken at study site or at an alternative location (e.g., participant's home) by a home nursing vendor, in these instances the participant's vital signs will be measured prior to completing the blood draw.

12.3 Week 38/End-of-Study Visit

The following assessments will be conducted at Week 38. Should the participant discontinue the study at an earlier time point, every attempt should be made to conduct the Week 38 assessments to ensure adequate safety follow-up.

- AE and concomitant medication status.
- Gaucher disease severity (GD-DS3).
- Physical examination.
- Vital signs (pulse, blood pressure, respiration rate, and temperature).
- 12-lead ECG.
- Urine pregnancy test (serum confirmation if positive urine test).
- Local laboratory analysis LFTs.
- Abdominal and bone MRI.
- Chest x-ray and pulmonary function tests.
- DEXA Z-score and T-score; lumbar spine and hip.
- Participants will be asked to complete an electronic diary commencing at week 37 for 7 consecutive days, completing the following questionnaires:
    - BPI-SF during the last 24 hours – daily for 7 days prior to this visit
    - FACIT-Fatigue during the last 7 days recorded on Day 7 of the diary completion

  Study sites will contact participants at the beginning of Week 37 to remind them to complete the questionnaires during Week 37.
- Blood samples will be collected to evaluate (central laboratory analysis):
    - AAVS3 immune response.
    - Haematology, chemistry (including hs troponin-T and LFTs).
    - GCase activity level.
    - GCase concentration.
    - GCase immune response.
    - Lyso-Gb1.
    - Chitotriosidase, CCL18, bsALP, osteocalcin.
    - Research plasma sample.
- Plasma, saliva, urine, stool, (and semen, male participants only) samples will be taken for PCR of vg. Collection of a particular matrix sample, e.g., plasma, can be stopped once negative results have been reported in that matrix at three consecutive visits at least 1 week apart after Week 1. Collection of the remaining matrices should continue until each delivers negative results at three consecutive visits at least 1 week apart after Week 1.
- HRQoL will be evaluated using the SF-36 questionnaire.

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

12.4 Unscheduled Visits

Unscheduled visits may be performed during the study if safety concerns arise or at the discretion of the investigator, e.g., for evaluation of AEs and/or laboratory abnormalities, or to assess vector shedding for the release from contraception requirements (in this case, vector shedding samples must be taken at least 1 week apart).

In addition, if the participant is not able to enrol immediately into the separate long-term follow-up study, e.g., due to logistical reasons, the assessments listed under the unscheduled visit (as a minimum) should be performed monthly until the participant is able to enter the long-term follow-up study.

Table 1 indicates the minimum assessments which should be performed at an unscheduled visit; however, any of the protocol-defined assessments may be performed, as necessary, at the discretion of the investigator. A pregnancy test will be required if both (a) the unscheduled visit is 4 weeks or longer from previous pregnancy test and (b) viral shedding results are not negative at three consecutive visits taken at least 1 week apart after Week 1 in all matrices.

CRFs will be completed for all unscheduled visits.

12.4.1 Continuance of Participant Care During Exceptional Circumstances

In exceptional circumstances, such as restrictions in movement due to COVID-19, where the participant is unable to attend the study site for scheduled visits, in order to maintain participant safety and ensure continued care the assessments may be conducted at an alternative location (e.g., participant's home, or local medical facility) either by study site personnel, local doctor, or by a home nursing vendor. Implementation of any such arrangements must be agreed in advance by the Sponsor.

12.5 Study Procedures/Evaluations

All study assessments will be conducted in accordance with the study schedules.

- Table 1: Schedule of Assessments
- Table 2: Detailed Schedule of Assessment for Day -1 to Day 3 (Infusion Week)

For all central laboratory samples, the procedures for collection, processing, storing, and transporting to the central laboratory are fully described in the study Laboratory Manual.

12.5.1 Demographic and Baseline Assessments

Informed Consent

Informed consent should be obtained prior to any study related assessments are started.

It is the responsibility of the investigator, or a suitably qualified co-investigator delegated by the investigator, to obtain written informed consent from each participant before any study-related procedures are undertaken. The investigator or designee will explain the aims, methods anticipated benefits, and potential hazards of the studies, that the participants are under no obligation to participate and that they can withdraw at any time, without having to give a reason.

A copy of the signed ICF will be given to the participant. The original signed ICF will be retained at the study site and a copy placed in the medical notes.

As part of the consent process, participants will be informed that in the event of death, no matter what cause, permission for an autopsy will be requested of their families. Participants will be asked to advise their families of this request and of its scientific and medical importance should they choose to participate.

Participant Identification Code

On enrolment to the study, the participant will be given a unique participant identification code Allocation of the participant identification code will be handled through the eCRF.

Demography

The following demographic information will be collected for each participant at the screening visit:

- Year of birth
- Gender
- Ethnicity

Medical History

A 5-year medical history should be taken during the screening period. Details of any clinically relevant abnormalities should be noted on the eCRF.

AE and concomitant medication status will be recorded.

Gaucher Disease and ERT/SRT History

The participant's Gaucher disease medical history and ERT/SRT history (if applicable) will be recorded in the eCRF. Including the following information:

- Age at diagnosis
- Clinical symptoms (including onset dates)
- Genotyping GBA1 and CHIT1
- ERT/SRT history and any associated infusion reactions (Part 1 participants only)

Freeline AAVS3 Neutralising Antibody Test

During the screening period, a blood sample will be taken from the subject to determine the presence or absence of neutralising antibodies to the AAVS3 serotype.

A negative result within 6 weeks of dosing is required to confirm eligibility. It is acceptable to take repeat samples during the screening period.

These samples will be analysed at a central laboratory. See laboratory manual for further details.

Serology Screening

A blood sample will be taken at screening to assess the following and analysed at a central laboratory, (refer to laboratory manual for details on sample collection and processing):

- HBsAg
- HepC status (HepCAb and HCV RNA test only indicated for participants with a positive HepCAb Screen)
- HIV 1 and 2 (anti-HIV1/2) antibodies,
- CMV IgG antibodies and CMV PCR. (CMV PCR only indicated if participants are positive on CMV IgG).

12.5.2 Efficacy

GCase Activity Level

Blood samples will be drawn for analysis of plasma and leucocyte GCase activity level. In addition, a bloodspot will be created by spotting whole blood onto a DBS test card. Samples will be analysed at a central laboratory. Refer to the laboratory manual for details of sample collection and processing.

Lyso-Gb1 Levels

A bloodspot will be created by spotting whole blood onto a DBS test card for assessment of Lyso-Gb1. Samples will be analysed at a central laboratory. Refer to laboratory manual for details of sample collection and processing.

Haematology: Haemoglobin and Platelet Count

Blood samples for assessment of haemoglobin and platelet count will be collected. Samples will be analysed at a central laboratory. Refer to the laboratory manual for details of sample collection and processing.

MRI

Abdominal MRI will be conducted to assess liver and spleen volumes. Bone MRI will be conducted to assess bone marrow burden.

Images will be analysed by a central imaging vendor. MRI will be conducted as outlined in the separate study imaging manual. Refer to the imaging manual for the full list of bone and abdominal parameters to be measured.

DEXA

DEXA will be conducted to assess Z-score and T-score of the lumbar spine (L1-4) and hip (femoral neck). Any clinically significant deviations from screening should be reported as an AE. Images will be analysed by a central imaging vendor. DEXA will be conducted as outlined in the imaging manual.

Chest X-ray

Standard posterior, anterior and lateral x-ray of the chest will be analysed locally.

Pulmonary Function Tests

Measures of lung volume using the nitrogen wash-out technique, diffusion measured by carbon monoxide transfer coefficient corrected for lung volume and haemoglobin (KCO) and spirometry, capturing forced vital capacity (FVC), forced expiratory volume in one second (FEV1), peak expiratory flow rate (PEFR), total lung capacity (TLC), functional residual capacity (FRC), forced expiratory flow over the middle half of the vital capacity (FEF25–75), forced expiratory flow at 25, 50, and 75% of vital capacity (FEF25, FEF50, FEF75, respectively), residual volume (RV) and KCO, expressed as a percentage of predicted values for height and sex. Analysis will be performed locally at study sites.

12.5.3 Safety

Any clinically significant changes for any safety measure from screening should be reported as an AE.

Physical Examination

The following sites will be examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes.

The following systems will be assessed: musculoskeletal and neurological.

The participant's height (screening only) and weight will also be measured.

Laboratory Safety Assessments

The following laboratory tests will be performed at a **central** laboratory to assess safety (refer to laboratory manual for details on sample collection and processing):

- Haematology: complete blood count with differential, platelet count. International normalised ratio (INR), partial thromboplastin (PTT).
- Chemistry: sodium, potassium, chloride, phosphate, CO2, glucose, blood urea nitrogen, serum creatinine, C-reactive protein, hs troponin-T.
- LFTs: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, and AST.
- The following laboratory tests will be performed at a local laboratory to assess safety:
- LFTs: albumin, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, ALT, and AST.

Local LFT laboratory analysis will be utilised to ensure rapid turnaround of results.

Blood samples for tacrolimus levels and CMV testing will be drawn and analysed locally. On days where other clinical assessments are not required these samples may be taken at study site or an alternative location (e.g., participant's home). Management guidelines in the case of CMV reactivation can be found in Appendix 2: Management Guidelines for CMV Reactivation.

If immunosuppressants are implemented at any other time, monitoring for tacrolimus levels and CMV testing (if required) should be undertaken in conjunction with the immunosuppressant dosing and for two weeks following cessation of immunosuppressants (see immunosuppression management plan for further details).

GCase Immune Response

Blood samples will be drawn to assess total anti-GCase antibody titre and neutralising antibody titre. The samples will be analysed at a central laboratory (refer to the laboratory manual for details on sample collection and processing).

AAVS3 Immune Response

Blood samples will be drawn for the assessment of AAVS3 antibody titre and T-cell response, the samples will be analysed at a central laboratory, (refer to the laboratory manual for details on sample collection and processing

Vital Signs

Vital sign measurements including blood pressure, pulse rate, respiratory rate, and temperature (°C/°F), and should be taken after the participant has been resting supine or upright for 5 minutes.

ECG

A 12-lead ECG will be conducted and analysed locally at the study sites.

AE Collection

Participants will be questioned in a general way at each study visit to establish whether AEs have occurred since the previous visit (e.g., "How have you been feeling since your last

visit?"). Additionally, the investigator will evaluate other collected data (e.g., questionnaires, clinical evaluations) to ascertain whether and AE has occurred. The duration of capture for AEs is as outlined in Section 14.2.1.

Concomitant Medications

Concomitant medication status will be recorded.

12.5.4 Vector Shedding

Plasma, saliva, urine, stool, and semen (for males only) samples will be taken for PCR of vg (three times within 7-10 days after FLT201 infusion then at separate visits [at least 1 week apart] until negative vector shedding results are obtained in that matrix at three consecutive visits after Week 1). Collection of a particular matrix sample, e.g., plasma, can be stopped once negative results have been reported in that matrix at three consecutive visits at least 1 week apart after Week 1. Collection of the remaining matrices should continue until each delivers negative results at three consecutive visits at least 1 week apart after Week 1.

Samples will be analysed at a central laboratory (refer to laboratory manual for details of sample collection and processing).

12.5.5 Health-related Quality of Life

HRQoL will be evaluated using the SF-36 questionnaire.

12.5.6 Other Assessments

Pain and Fatigue Assessment

Participants will answer questionnaires relating to pain (BPI-SF) and fatigue (FACIT-Fatigue) using an electronic diary.

GD-DS3

An integrated assessment of Gaucher disease type 1 burden based on bone, hematologic and visceral domains will be performed using the Gaucher Disease Type 1 Severity Scoring System (GD-DS3).

Research Plasma

Plasma samples will be taken for research purposes. Research samples will be retained beyond the end of the study and may be analysed alongside samples taken as part of the long-term follow-up study as part of future research which has been ethically approved.

The samples will be analysed at a central laboratory.

12.6 Volume of Blood to be Drawn from Each Participant

During this study, it is expected that approximately 900 mL of blood will be drawn from each participant for central laboratory assessments.

The protocol allows for monitoring of certain parameters through local laboratories. Blood volume requirements are likely to vary between study sites; however, it is estimated that approximately an additional 500 mL will be drawn from each participant for local laboratory assessments over the course of the study.

Note: The amount of blood to be drawn for each assessment is an approximation. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment.

12.7 Study Withdrawal

Participants are free to withdraw from the study, at any time, without prejudice to their continued care. The reason for discontinuation/withdrawal must be determined by the investigator and recorded in the participant's medical record and in the eCRF. If a participant is withdrawn for more than one reason, each reason should be documented in the source document and the most clinically relevant reason should be entered in the eCRF.

Reasons for withdrawal include but are not limited to:

- Protocol violation
- Withdrawal by participant
- Death
- Lost to follow-up
- Other (If "other" is selected, the investigator must specify the reason on the eCRF).

If a participant expresses their wish to withdraw from the study, the investigator should explain the importance of remaining on study follow-up, or failing this, of allowing routine follow-up data to be used for study purposes.

In the event of early withdrawal/discontinuation, every effort should be made to collect data in line with Week 38/EOS assessments.

If a participant relocates from the area, every effort should be made for the participant to be followed up at another participating study site and for this new site to take over the responsibility for the participant, or for follow-up via the participant's general practitioner.

The investigator should contact the Sponsor to discuss the withdrawal of any participant.

12.8 Definition of End of Study

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.

13 Dose Progression, Dose Escalation, and Temporary Halt Rules

The Sponsor will retain final responsibility for decision making on all aspects of dose progression, dose escalation, temporary halt, and stopping rules. Progression of dosing within a cohort, dose escalation/reduction, communication between active sites and the Sponsor, and temporary halt and stopping rules are detailed below and in study specific plans. The following elements will be included.

13.1 Oversight Committees

13.1.1 Independent Data Monitoring Committee

The role of the DMC is to provide independent advice on data and safety aspects of the study. The DMC will review participant data prior to a dose escalation decision, dose expansion or in the case of arising safety concerns. The DMC will be governed by a charter that dictates constitution and decision making on dose escalation/study modification and study temporary halt and stopping rules.

13.2 Dose-limiting Toxicity

A Dose-limiting Toxicity (DLT) is 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. Transaminase increases should be investigated to exclude other causes but it is expected that such transaminase increases will be cases of vector-associated transaminitis which is an event anticipated with liver directed AAV gene therapy (see Section 6.5.3.1). In the case of vector-associated transaminitis, where ALT or AST levels are classed as severe, changes to the prophylactic immunosuppressive regimen will be considered for ongoing participants and before treating further participants.

The study may be stopped before completion at any time on the recommendation of the DMC or decision by the Sponsor.

13.3 Dose Escalation

13.3.1 Dose Cohorts and Dose Interval Between Participants

The planned dose escalation scheme is as follows (if subject weight is >90 kg, the dose will be calculated based on 90 kg weight):

- Cohort 1: 4.5×10^{11} vg/kg of body weight.
- Cohort 2: 1.3×10^{12} vg/kg.
- Cohort 3: 3.9×10^{12} vg/kg.
- Cohort 4: 1.1×10^{13} vg/kg.

The dose level to be tested in each cohort after Cohort 1 may be an intermediate dose between the current dose and that of the next planned dose cohort based on emerging data but would not be greater than the next planned dose. In addition, based on emerging data, including safety/tolerability, PK, PD, and clinical data, all dose cohorts may not be tested, an additional dose cohort may be added and/or a dose cohort may be further expanded beyond 3 participants. If an additional dose cohort is added the dose will not exceed 1.5×10^{13} vg/kg, which is below the NOAEL (2.57×10^{13} vg/kg).

The dose to be given is made in line with the escalation/decision rules defined in Sections 13.3.1.1, 13.3.1.2, 13.3.1.3. In all cases, safety will be assessed first followed by evaluation of efficacy.

A minimum of 4 weeks of safety/tolerability (including DLT), PK, PD, and clinical data will be assessed before dosing each participant (both within a dose cohort and before escalating to the next dose level).

All cohorts will contain at least two participants (except as described in Section 13.3.1.2). Depending on assessment of safety/tolerability (including DLT), PK, PD, and clinical data, additional participants may be treated.

Dose escalation decisions will be overseen by a DMC and will be made in accordance with the following specific rules:

13.3.1.1 Safety Assessment Based on DLT

- If 1 participant experiences a DLT, that cohort will be expanded to 3 participants
- If 2 out of 3 participants in the same cohort experiences a DLT, the DMC will be convened to consider a dose reduction. The DLT rate is expected to be less than or equal 33%.

13.3.1.2 GCase Activity Level

If GCase activity is below the normal range (e.g., <1.5 µmol/L/h though this value will vary depending on the specific laboratory) and no DLT is observed in the first participant in the cohort then dose escalation to the next dose cohort would occur after this first participant to avoid treating the next participant with a dose predicted to be below the therapeutic range.

13.3.1.3 Safety Rules Based on NOAEL

The dose levels planned for Cohorts 1 to 4 are below the NOAEL of the FLT201 GLP toxicology study (2.57×10^{13} vg/kg).

If deemed necessary by the Sponsor, and in consultation with the DMC, up to 3 further participants may be enrolled in an additional cohort. The dose level for this cohort may be a previously tested dose level, intermediate dose level, or escalated based on all available safety/tolerability (including DLT), PK, PD, and clinical data when the final participant in the Cohort 4 has completed at least 4 weeks' post-dose follow-up. The highest dose that may be tested will not exceed 1.5×10^{13} vg/kg, which is below the NOAEL (2.57×10^{13} vg/kg), except by protocol amendment.

13.4 Dosing Previously Untreated 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.

Progression from Part 1 to Part 2 will be by protocol amendment only, based on all available safety/tolerability (including DLT), PK, PD, and clinical data, evaluated in discussion with DMC.

For the dose evaluation decisions in Part 2, the total number of participants that experience DLTs in both Parts (Part 1 + Part 2) will be considered.

13.5 Temporary Halt

Once a temporary halt is applied to the study a review by the DMC will take place. If the Sponsor deems it appropriate to restart the study; this can be done only following approval by the relevant competent authorities, IECs/IRBs, and any local approvals required.

13.6 Temporary Halt Rules

Further enrolment into the study will be put on hold in the event of any of the following:

- Death of a participant related to FLT201
- Development of malignancy related to FLT201
- Development of neutralising anti-GCase antibodies, defined as a positive result from the central laboratory in conjunction with a decrease in GCase activity levels and an increase in Gaucher disease biomarkers (e.g., lyso-Gb1). *
- Severe AE with life-threatening consequences or urgent intervention indicated in a subject related to FLT201

*The investigator should contact the Medical Monitor if there is a suspicion of neutralising anti-GCase antibodies. Transient antibodies, defined as a negative GCase antibody test after a positive result, with no significant drop in plasma GCase activity level, would not trigger the temporary halt rules.

Participants who have already received the vector infusion will continue to be followed per the protocol. If, 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.

13.7 Communication Plan for Dissemination of Safety Data

The independent DMC will review participant data prior to any dose escalation decision, dose expansion or in the case of any arising safety concerns (Section 13.1.1). All recommendations will be based on a thorough risk-benefit assessment based on all available data at each review. The DMC recommendation regarding both within-cohort decisions and dose escalation decisions will be disseminated to all sites by the Sponsor.

Investigators are to immediately report AEs that meet the criteria for a temporary halt of the study (Section 13.6), Adverse Events of Special Interest (AESI) (Section 14.2.9), Serious Adverse Events (Section 14.3), or Other Important Medical Events (Section 14.3.9).

If the investigator and Sponsor agree that temporary halt criteria have **not** been met, then dosing of the study may continue as per current protocol. Discussion between the investigator and Sponsor should be documented.

In all other circumstances, the Sponsor will convene an urgent meeting (to include relevant Clinical Development and Safety representatives) to determine if temporary halt criteria have been met. If it is determined that temporary halt criteria have been met, the Sponsor will immediately contact all investigators to notify them of the temporary halt to dosing in the study, and, in addition, the relevant competent authorities and IECs/IRBs will be informed.

To re-start the study, the independent DMC must complete a benefit-risk review and be satisfied that any proposed amendments are appropriate, and such amendment must be approved by all relevant competent authorities, IECs/IRBs, and any other local approvals prior study re-start.

14 Safety Definitions, Reporting, and Follow-up

An AE is any untoward medical occurrence in a clinical investigation participant administered a pharmaceutical product. An AE can, therefore, be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not related to the medicinal investigational product (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guidance E2A 1995).

14.1 Adverse Event Definitions

Adverse event (AE) definitions are provided in Table 4.

Table 4: Adverse Event Definitions

Term	Definition
Adverse event	Any untoward medical occurrence in a clinical investigation participant administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment
Adverse reaction	All untoward and unintended responses to an investigational medicinal product related to any dose administered
Serious adverse event	Any AE that: • results in death, • is life-threatening*, • requires hospitalisation** or prolongation of existing hospitalisation, • results in persistent or significant disability or incapacity or consists of a congenital anomaly or birth defect. • is considered an important medical event (defined below)
SUSAR	A suspected unexpected Adverse Reaction which is also categorised as serious
Important medical event	These events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered "serious." See Section 14.3.8 for study-specific events that should also be considered as important medical events

Abbreviations: AE=adverse event; SUSAR=suspected unexpected serious adverse reaction.
* A life-threatening event refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE.

14.2 Adverse Events

14.2.1 Adverse Event Collection Timeframe

The time-period for reporting AEs will be from the time the initial ICF is signed by a participant until completion of the study. This includes events occurring during the screening phase of the study, regardless of whether FLT201 is administered.

14.2.2 Assessments of Adverse Events

Each AE will be assessed in terms of severity, causality, expectedness, and seriousness.

14.2.3 Severity

The medical assessment of severity will be determined by using the definitions specified in Table 5.

Table 5: Adverse Event Severity

Category	Definition
Mild	The AE does not interfere with the participant's daily routine and does not require intervention; it causes slight discomfort
Moderate	The AE interferes with some aspects of the participant's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort
Severe	The AE results in alteration, discomfort or disability which is clearly damaging to health

14.2.4 Causality

The investigator must make the assessment of relationship to ATIMP (FLT201) and NIMP for each AE (prednisolone (prednisone), methylprednisolone and tacrolimus are considered to be NIMPs in this study). The investigator should decide whether there is a reasonable possibility that the event may have been caused by the ATIMP/NIMP. 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 ATIMP/NIMP and the occurrence of the AE, then the AE should be considered "related." The causality assessment must be documented in the source document(s).

14.2.5 Seriousness

Any AE that meets the criteria for seriousness (see Table 4) will be documented as such within the source document(s). Hospitalisation for pre-existing conditions, including elective procedures, or a study related procedure (e.g., administration of IV methylprednisolone) does not constitute an SAE.

14.2.6 Expectedness

The reference safety document to be used to assess expectedness against the ATIMP (FLT201) is the IB. Expectedness of AEs are defined in Table 6.

Where an AE is determined as "related" and therefore constitutes an adverse reaction an assessment of expectedness will be determined using the following definitions:

Table 6: Adverse Event Expectedness

Category	Definition
Expected	An AE that is consistent with the information about the ATIMP listed in the IB or clearly defined in this protocol.
Unexpected	An AE that is not consistent with the information about the ATIMP listed in the IB*

Abbreviations: AE=adverse event; IB=investigator's brochure; ATIMP=advanced therapy investigational medicinal product.

* This includes listed events that are more frequently reported or more severe than previously reported.

14.2.7 Follow-up of Adverse Events

All AEs must be followed to conclusion regardless of whether the participant is still participating in the study. Conclusion can be that the event has resolved or until, in the opinion of the investigator, the event has stabilised or been determined to be chronic. The follow-up must be documented in the source document(s).

14.2.8 Reporting of Adverse Events

All AEs and SAEs are collected from the time the ICF is signed until Week 38/EOS visit.

Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the investigational product indication should also be captured on the AE eCRF.

14.2.9 Adverse Events of Special Interest (AESI)

The following will be considered as AESIs:

- Any of the following related to ALT elevation:
    - Increase of ALT above normal on one occasion (if normal at baseline)
    - >50% increase of ALT from baseline on one occasion followed by a further increase from the prior level at the next blood test (at same laboratory)
    - Increase in ALT associated with unexpected fall in plasma GCase activity level.
- Unplanned increase in immunosuppression- an increase in immune suppression includes an increase in dose or duration or number of drugs used above the existing prophylactic and reactive regimens.
- A positive test result for SARS-CoV-2 (COVID-19) infection.
- An increase in hs troponin-T to greater than twice the upper limit of normal (normal troponin-T is <14 pg/mL).
- A change in 12-lead ECG deemed clinically significant by the investigator.
- Myocarditis.

Any events meeting the definition of an AESI should also be reported by the investigator to PPD Pharmacovigilance Department via the same process as reporting SAE, see Section 3 (SAE Hotline: +44 (0) 1223 374 240, SAE Faxline +44 (0) 1223 374 102) within 24 hours of the first awareness of the event. In the event of that Medidata RAVE project database is not available, use the SAE form and highlight that the event is an AESI. The CRF of Medidata RAVE must be updated as soon as it is available.

14.3 Serious Adverse Events

14.3.1 Serious Adverse Event Reporting Procedures

Any AE that meets the criteria for seriousness (Table 4) will be recorded as such within the eCRF. All initial and follow-up SAE reports must be reported by the investigator to PPD Pharmacovigilance Department within 24 hours of the first awareness of the event/information by recording all relevant SAE information in the CRFs of the Medidata RAVE project database. In the event of that Medidata Rave is not available, the investigator should utilise the back-up paper SAE report form to report the Initial SAE or follow-up information within 24 hours. See Emergency Contact Information in Section 3. The SAE Form needs to be transmitted to the PPD Pharmacovigilance Department. The CRF of the Medidata RAVE must be updated as soon as it is available. Applicable fax numbers and email addresses can be found on the form.

14.3.2 Serious Adverse Event Collection Timeframe

The time-period for reporting SAEs will be from the time the ICF is signed by a participant until completion of the study. This includes events occurring during the screening phase of the study, regardless of whether FLT201 is administered.

In addition, any SAE(s) considered "related" to the FLT201 and discovered by the investigator at any interval after the study has completed must be reported to PPD Pharmacovigilance Department, see Emergency Contact Information in Section 3.

14.3.3 Serious Adverse Event NIMP Causality Assessment

As per Section 14.2.4, the investigator must make the assessment of causal relationship for any SAE to the NIMP that the participant has received.

14.3.4 Follow-up of Serious Adverse Events

All SAEs must be followed to conclusion regardless of whether the participant is still participating in the study. Conclusion can be that the event has resolved or until, in the opinion of the investigator, the event has stabilised or been determined to be chronic. The follow-up must be documented in the source document(s).

14.3.5 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalisations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the participant after signing the ICF or leading up to the onset date of the SAE, or following the resolution of the SAE, must be recorded as an AE, if appropriate.

14.3.6 Documentation and Reporting of Serious Adverse Events

As well as the expedited reporting noted in Section 14.3.1, SAEs should be captured on the appropriate AE pages in the eCRF and in source document(s).

14.3.7 Fatal Outcome

Any SAE that results in the participant's death (i.e., the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the participant's death, the outcome should be considered not resolved, without a resolution date recorded.

14.3.8 Other Important Medical Events

For this study the following will be considered as important medical events and will be reported in line with SAE reporting procedures.

- Development of a malignancy.
- Development of suspected anti-GCase neutralising antibodies defined as a positive result from the central laboratory in conjunction with a drop in GCase activity levels. (The investigator should contact the Medical Monitor if there is a suspicion of anti-GCase neutralising antibodies [see Section 13.6 for further details].)
- A participant develops a severe allergic reaction related to administration of FLT201.

14.3.9 Pregnancy

If a female participant becomes pregnant the pregnancy should be immediately reported on a pregnancy reporting form and submitted to the PPD Pharmacovigilance Department (Section 3). Progression of the pregnancy and the eventual outcome must be documented on the

pregnancy reporting form. Every reasonable attempt should be made to follow the health of the child for 30 days after birth.

If the female partner of a male participant becomes pregnant before the results from three consecutive semen (or serum, if semen not provided) samples taken at separate visits (at least 1 week apart) after FLT201 administration are negative, consent to report information regarding the pregnancy must be obtained from the participant's pregnant partner. A study-specific pregnancy monitoring information sheet and ICF for partners of study participants must be used for this purpose. If consent if given, the pregnancy should be reported on a pregnancy reporting form and submitted to the PPD Pharmacovigilance Department (Section 3). The site team will request for permission to keep in touch with the participant's partner to follow-up the pregnancy to its conclusion. The PPD Pharmacovigilance Department must be kept informed of any new developments involving the pregnancy.

A pregnancy becomes an SAE in the following circumstances:

- Miscarriage
- Termination (elective or spontaneous)
- Ectopic pregnancy
- Foetal demise
- Congenital anomalies or any birth defect

Such additional SAEs must be reported using the SAE form and should be reported to the Sponsor immediately.

14.3.10 Expedited Reporting of Other Safety Events

New events related to the conduct of the study or the development of the FLT201 which are likely to affect the safety of the participants should be reported according to the existing timelines for expedited reporting.

This includes:

- SAEs which could be associated with the study procedures and which could require modification of the conduct of the study.
- Events which pose significant hazard to the participant population.
- SAEs related to mandatory concomitant medication, product application process (surgical or other) and product failure (including lack of efficacy) should all be considered.
- Events (as described above) which are fatal or life-threatening, will be notified to the IEC/IRB and competent authority by the Sponsor/CRO within 7 days of the Sponsor/CRO learning of them. Events falling into one of the other categories of serious (Table 4) will be reported by the Sponsor/CRO to the IECs/IRBs and competent authority within 15 days after the Sponsor/CRO has learned of them.

Expedited reporting and reporting of AEs will be in accordance with EudraLex Volume 10, Chapter II (Detailed Guidance on the collection, verification and presentation of AE/adverse reaction reports arising from clinical trials on medicinal products for human use ('CT-3'; 2011/C 172/01), June 2011) in the EU and with 21 Code of Federal Regulation Part 312 to the Food and Drug Administration.

Should an event also be considered as an urgent safety measures, it must be reported in line with the requirements set out in Section 14.4.

14.4 Urgent Safety Measures

Urgent safety measures can be put in place by the Sponsor with immediate effect without needing to gain prior authorisation by the IEC/IRB (and competent authority where applicable), to protect clinical study participants from any immediate hazard to their health and safety. The IEC/IRB and national competent authorities will be notified of the new event(s), the measures taken, and the plan for further action as soon as possible.

Implementation of urgent safety measures by the investigator should be notified immediately to the Sponsor.

15 Statistical Analysis

A Statistical Analysis Plan (SAP) will be written and finalised prior to database lock. This plan will give a detailed description of all summaries and analyses that will be presented. All study data will be listed and all relevant data will be tabulated and summarised by cohort/dose level and overall, where applicable. Statistical analysis will be performed using SAS® (SAS Institute Inc., Cary, NC, United States of America) statistical software (Release 9.4 or later). Continuous variables will be summarised using number of observations, mean and standard deviation, median, minimum, and maximum values. Categorical values will be summarised using number of observations and percentages. Any deviations from the analyses planned in the protocol will be detailed in the SAP, and deviations from the original statistical plan will be captured in the clinical study report. Analyses will be reported separately by each study part and, where appropriate, combined (e.g., for safety data).

15.1 Demography and Baseline Characteristics

Baseline parameters and demography (e.g., age, gender, ethnicity) will be summarised. Gaucher disease history, ERT/SRT history, and medical history will be summarised using number of observations and percentages of participants reporting each category. Exposure to investigational product (i.e., total amount of study drug received) will be listed for all participants by cohort/dose level.

15.2 Definition of Analysis Sets

Safety Population: The safety analysis set will include all participants who received any dose of FLT201. This population will be used to report both Part 1 and 2.

15.3 Analysis of Primary Safety Endpoints

Dose-limiting Toxicities

All DLTs will be summarised by cohort/dose level.

Although the dose escalation is based on an algorithm, the final data may be used, for example, to model the DLTs as a function of dose, adjusted for any potential confounders, to further refine any estimate of the MTD (e.g., to determine the probability of toxicity closest to a given threshold toxicity at some observed dose level). The uncertainty around the estimate of the true DLT rate may be provided in terms of 95% confidence intervals or 95% credible intervals.

Adverse Events

Frequency of treatment-emergent AEs and SAEs will be calculated for each body system and preferred term, and by dose level, for number of events and number of participants reporting the event. The severity of the AEs and the relationship to study medication will be summarised for each body system and preferred term by cohort/dose level.

Other Safety Endpoints

Safety endpoints including the following will be summarized descriptively by cohort/dose level.

- Change from baseline in laboratory data
- Change from baseline in 12-lead ECG data
- Change from baseline in physical examination data
- Change from baseline in vital signs data.

15.4 Analysis of Secondary Endpoints

Efficacy

The following secondary efficacy endpoints will be summarised by cohort/dose level.

- Change from baseline to each assessment point in lyso-Gb1 in plasma.
- Change from baseline to each assessment point in spleen volume by MRI.
- Change from baseline to each assessment point in liver volume by MRI.
- Change from baseline to each assessment point in haemoglobin.
- Change from baseline to each assessment point in platelet count.

Pharmacokinetic

Change from baseline in plasma and leukocyte GCase activity will be summarised by cohort/dose level and plotted by participant and overall. The area under curve (AUC) of GCase activity will be calculated from baseline to Week 38 and summarised. The details of derivations of the AUC using a linear trapezoidal rule will be provided in the SAP.

Shedding

Clearance of vg in plasma, saliva, urine, stool, and semen will be summarised by cohort/dose level.

Immune Response to Transgene Product

Immune response to the GCase transgene product measured as change from baseline in total anti-GCase antibody titre and neutralising antibody titre will be summarised by cohort/dose level and plotted.

15.5 Exploratory Endpoints

Efficacy

The following exploratory efficacy endpoints will be summarised by cohort/dose level:

- Change from baseline to each assessment point in bone marrow burden score measured by MRI.
- Change from baseline to each assessment point in bone mineral density measured by DEXA.
    - Z-score and T-score in the lumbar spine (L1-4) and hip (femoral neck).
- Change from baseline to each assessment point in Gaucher disease severity measured by GD-DS3.
- Change from baseline to each assessment point in fatigue measured by FACIT-Fatigue.
- Change from baseline to each assessment point in pain measured by BPI-SF.
- Change from baseline to each assessment point in Gaucher disease activity biomarkers: chitotriosidase, CCL18.
- Change from baseline in bone biomarkers: bsALP, osteocalcin.
- Change from baseline to each assessment point in lung disease measured by chest x-ray and pulmonary function tests.

Pharmacokinetic

AUC, peak, and steady state plasma and leukocyte GCase activity levels will be summarised for each participant and cohort/dose level, as appropriate.

Change from baseline in GCase concentration (antigen levels) will be summarised by cohort/dose level and plotted for each participant and overall.

Steady state may be determined using a model-based approach to determine the slope and whether it is statistically zero. The dose-response relationship will be characterised, using a model-based approach, as appropriate.

Immune Response to AAVS3 Capsid

Immune response to AAVS3 capsid (AAVS3 antibody titre and T-cell response) will be summarised by cohort/dose level.

Change from baseline to each assessment point in immune response biomarkers will be summarised by cohort/dose level.

15.6 Handling of Missing Data

Missing data will not be imputed. All analyses will be based on observed cases, unless otherwise stated.

15.7 Protocol Deviations

There will be no Per Protocol analysis and as such protocol deviations will be listed. The impact of any major protocol deviations will be noted in the summary statistics for specified outcomes that will be defined in the SAP.

All protocol deviations are to be recorded with the indication of whether they are major as determined by the study management team, in cooperation with data management, medical monitoring, and the Sponsor. These data will be imported into SAS, including the assignment of minor or major. A review of the protocol deviations will be performed before database lock. Assessment windows for observations will be defined in the SAP.

15.8 Interim Analysis

On completion of Part 1, an interim analysis will be conducted to inform the decision to start a registrational study. Analysis of 3 months of data from the final participant in Part 1 and longer-term data (up to several years in the follow-up study) in prior participants will enable assessment of durability, adverse events, and clinical parameters that may change over a longer period, such as lung function and bone disease. Planned interim analyses will be detailed in the SAP.

16 Data Management

16.1 Data Collection Tools and Source Document Identification

16.1.1 Source Data

ICH E6 Section 1.51 defines source data as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies)."

The basic concept of source data is that it permits not only reporting and analysis but also verification at various steps in the process for the purposes of confirmation, quality control, audit, or inspection.

16.1.2 Source Documents

ICH E6 1.52, defines source documents as "Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, participants' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, participant files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study)."

The investigator is responsible for maintaining accurate medical records (source documents) from which information is recorded in eCRFs.

A source document identification list will be implemented prior to the start of the study to identify which data is recorded firstly into source documents, such as medical notes, and then transcribed into the eCRF.

The eCRF will not be utilised as source for any data in this study.

16.1.3 Electronic Case Report Form

Data for each participant will be recorded in a study-specific eCRF by sites.

Source data contained in source documents must be accurately transcribed into the eCRF.

All eCRFs must be completed by staff that are listed on the site staff delegation log and authorised by the investigator to perform data collection and handling. It is the responsibility of the investigator to ensure the accuracy of all data entered in the eCRF. All data sent to the Sponsor will be endorsed by the investigator.

16.1.4 Data Handling and Analysis

A study-specific data management plan will be in place for the study. This will contain details of the software to be used for the database, the process of database design, coding, data entry, data quality checks, data queries, data security (including data base access), database lock, and data transfers.

17 Monitoring, Audit, and Inspection

17.1 Monitoring

The Sponsor will determine the appropriate level and nature of monitoring required for the study. Risk will be assessed on an ongoing basis and adjustments made accordingly.

The degree of on-site monitoring (if required) will be proportionate to the objective, purpose, phase, design, size, complexity, blinding, endpoints, and risks associated with the study.

A study-specific monitoring plan will be in place for the study. The study will be monitored in accordance with the agreed monitoring plan which may include elements of remote monitoring in cases where appropriate.

The investigator must permit authorised representatives (study monitors) of the Sponsor access to the site to inspect the facilities, essential documents, study data including source data. The study monitor (and auditors, IEC/IRB, or regulatory inspectors) may check the eCRF entries against the source documents.

17.2 Audit and Inspection

To ensure compliance with relevant regulations, essential documents and data generated by this study must be available for inspection upon request by representatives of, for example, the US Food and Drug Administration (as well as other United States national and local regulatory authorities), the European Medicines Agency the Medicines and Healthcare Products Regulatory Agency, other regulatory authorities, the IEC/IRB for each site and the Sponsor or its representatives.

The Sponsor and investigator must permit authorised representatives of the respective national, local, or foreign regulatory authorities, the IEC/IRB, auditors, and Sponsor to inspect facilities and to have direct access to essential documents, study data and original source records relevant to this study, regardless of media.

18 Ethics and Regulatory Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
- Applicable ICH GCP Guidelines and European Commission Guideline on GCP specific to Advanced Therapy Medicinal Products 2019.
- Applicable laws and regulations, including privacy laws.

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be reviewed and approved by the Sponsor and submitted to an IEC/IRB by the investigator and reviewed and approved by the IEC/IRB before the study is initiated.

Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The protocol and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IEC/IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IEC/IRB
- Notifying the IEC/IRB of SAEs or other significant safety findings as required by IEC/IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IEC/IRB, European regulation 536/2014 for clinical studies (if applicable), EU directive 2001/20/EC, and all other applicable local regulations

Sponsor (or its representative) will communicate safety information to the appropriate regulatory authorities and all active investigators, in line with all applicable regulatory requirements. The IEC/IRB will also be informed by Sponsor or the investigator, as required by the applicable local regulatory requirements. Where the investigator has provided IEC/IRB notification, evidence of IEC/IRB notification should be provided to Sponsor.

18.1 Informed Consent Process

The Investigator or authorised designee will explain the nature of the study to the participant and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. It should be emphasized that the participant may refuse to enter the study or may choose to withdraw from the study at any time, without consequences for their further care or penalty or loss of benefits to which the participants are otherwise entitled. Participants who refuse to give or who withdraw written informed consent should not be included or continue in the study.

The Investigator or authorised designee must ensure that each participant is fully informed about the objectives, methods, sharing of data related to the study, anticipated benefits, and potential risks, including the risks associated with the processing of the participant's personal data and inconveniences of the study. The participant should be given every opportunity to

ask for clarification of any points they do not understand and, if necessary, ask for more information. The participant will be given ample time to consider the study.

Participants will be required to sign a statement of informed consent that meets the requirements of local regulations, the IEC/IRB or study centre, ICH guidelines, and where applicable Health Insurance Portability and Accountability Act (HIPAA) requirements and 21 CFR 50, prior to any study related procedures.

Participants can give electronic consent where local regulations allow.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorised person obtaining the informed consent must also sign the ICF. Signed consent forms must remain in site file and must be available for verification at any time.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant.

The original signed form will be retained at the study site, and a copy will be placed in the participant's medical notes.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorised designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate in this optional research will not provide this separate signature.

The investigator will provide the Sponsor with a copy of the ICF, where applicable, which was reviewed by the IEC/IRB and which received their favourable opinion/approval. A copy of the IEC/IRB written favourable opinion/approval of these documents must be provided to the Sponsor before the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (i.e., Sponsor or coordinating investigator) is responsible for this action. Additionally, if the IEC/IRB requires modification of the sample participant information and consent document provided by the Sponsor, the documentation supporting this requirement must be provided to the Sponsor.

18.2 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those participants who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at a site, the investigator will promptly inform the Sponsor and the IEC/IRB and provide them with a detailed written explanation. The investigator will also return all study materials to the Sponsor. Upon study completion, the investigator will provide the Sponsor, IEC/IRB, and regulatory agencies with final reports and summaries as required by (inter)national regulations.

Communication with local IEC/IRBs to ensure accurate and timely information is provided at all phases during the study and may be done by the Sponsor, applicable CRO, Investigator, or,

for multicentre studies, the Coordinating Investigator according to national provisions and will be documented in the Investigator agreement.

18.3 Notification of Serious Breaches to Good Clinical Practice and/or the Protocol

A "serious breach" is a breach which is likely to effect to a significant degree:

a) the safety or physical or mental integrity of the participants of the study; or
b) the scientific value of the study.

If required by the national regulations, the Sponsor of a clinical study shall notify the licensing authority in writing of any serious breach of GCP within the timeframe, as required by the national regulations.

The Sponsor and CRO will be notified immediately of any case where the above definition applies during the study conduct phase.

18.4 Indemnity/Liability and Insurance

Insurance coverage will be handled according to local requirements.

18.5 Public Posting of Trial Information

The Sponsor is responsible for posting appropriate study information on applicable websites, as required. Information included in clinical study registries may include participating Investigators' names and contact information.

18.6 Trial Suspension, Termination, and Completion

The Sponsor may suspend or terminate the study at any time for any reason. If the study is suspended or terminated, the Sponsor will ensure that applicable sites, regulatory agencies, and IECs/IRBs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The Sponsor will make an end-of-study declaration to relevant competent authority/authorities as required by Article 10 (c) of EU Directive 2001/20/EC.

18.7 Submission of Summary Clinical Study Report to Competent Authorities and Ethics Committees

The Sponsor will provide a summary of the clinical study report to the competent authority and IECs/IRBs, as required by applicable local regulations, and will comply with the European Commission Guideline — Guidance on posting and publication of result-related information on clinical trials (2012). This requirement will be fulfilled within 1 year as required for non-paediatric studies.

18.8 Record Keeping and Archiving

Essential documents are those documents that individually and collectively permit evaluation of the conduct of the study and quality of the data produced with the principles of GCP and all applicable regulatory requirements. Essential documents relating to the study will be kept in an orderly manner, and remain current, by the Sponsor and investigators in secure study files during the study. At the end of the study, all essential documentation and study data must be archived securely by the Sponsor and study sites according to ICH GCP requirements and Advanced Therapy Regulations (1394/2007/EC) for a minimum of 30 years. These files will be available for inspection by the regulatory authorities or Sponsor and its representatives.

Essential documents and study data must be maintained and may not be destroyed without written permission from the Sponsor. It is the responsibility of the Sponsor to inform study sites when these no longer need to be retained. An investigator must contact the Sponsor before destroying any study -related documentation. In addition, all participant medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

18.9 Privacy and Confidentiality

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the Sponsor, by appropriate IEC/IRB members, and by inspectors from regulatory authorities.

19 Publication Policy

All manuscripts, abstracts, or other models of presentation arising from the results of the trial must be reviewed and approved by the Sponsor, in advance of submission. The review is aimed at protecting the Sponsor's proprietary information either existing at the date of the commencement of the trial or generated during the trial. Authorship will follow guidelines established by the International Committee of Medical Journal Editors (ICMJE 2015).

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21 Appendices

21.1 Appendix 1: Suggested Management Guidelines for Infusion Reactions

Management of Hypersensitivity and Anaphylaxis

Participants will be informed in advance of potential early symptoms and signs of hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnoea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. Participants will remain under observation in the investigational centre for at least 8 hours following infusion to minimise the risk associated with acute allergic reaction. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals from the start of infusion. Vital signs will be monitored hourly for 6 hours following infusion and then every 2 hours for 6 hours.

Should anaphylaxis occur, then initial management will consist of standard acute control measures - epinephrine, antihistamine, and steroid, followed by intensification of immune suppression by means of continued steroid, cyclosporine A and cyclophosphamide.

Suggested Management of Immune Complex Disease

Immune complex disease may be managed by immune suppression with agents detailed in Houssiau 2013.

Management of Infusion Reaction

Whilst acute infusion reaction is deemed highly unlikely in view of its absence in over 50 infusions of varying concentrations and amounts of AAV vector for gene therapy, participants will remain under observation in the investigational centre for at least 8 hours following infusion. Vital signs (pulse, blood pressure, respiration rate, and temperature) will be monitored at 15-minute intervals from the start of infusion. Vital signs will be monitored hourly for 6 hours following infusion and then every 2 hours for 6 hours. Should it occur, infusion reaction will be managed in the same way as transfusion associated reaction.

21.2 Appendix 2: Management Guidelines for CMV Reactivation

Human cytomegalovirus (CMV) can result in opportunistic infection in participants requiring immunosuppression for their underlying immune disorders such as systemic lupus erythematosus but at a much lower frequency than organ transplantation and haematopoietic stem cell transplantation (Atabani 2012; Lim 2019). Nevertheless, surveillance for CMV reactivation is necessary in these individuals so that treatment can be instituted early. Natural history studies have demonstrated a correlation between replication kinetics, peak and cumulative viral load with CMV end-organ disease (Atabani 2012). Systematic review and meta-analysis have also demonstrated the validity of viral load, as determined by real-time PCR (qPCR), as an appropriate surrogate endpoint for predicting the development of CMV disease and guiding pre-emptive therapeutic intervention for the prevention of CMV disease (Griffiths 2016; Natori 2018).

A CMV PCR titre > 3000 genomes/mL (or locally agreed on cut-off) should lead to firstly a re-evaluation of the immunosuppression regimen, regardless of any observed clinical symptoms. Secondly, treatment is clinically indicated if there are any symptoms suggesting CMV infection (Ljungman 2017). CMV treatment is continued until two negative DNA results are obtained. If a subsequent episode is detected after treatment has been stopped, treatment will be reinitiated only if viral load is greater than 3000 genomes/mL.

21.3 Appendix 3: Summary of Protocol Changes

Previous Versions

Version	Date
1.0	11 December 2020
2.0	08 February 2021
3.0	25 October 2021

Note: Versions 4.0-7.0 are country-specific versions of the protocol and, as such, are not described here.

Version 3.0 to 8.0

Section(s)	Summary of Change
12.1.3, 12.2, 12.3, 12.5.6, Schedule of Assessments	Deletion of research mononuclear cells, research serum, and research urine sampling.
12.4, Schedule of Assessments	Addition of unscheduled visits which may be performed during the study or in the event of a delay in enrolling into the long-term follow-up.
12.5.4, Schedule of Assessments	Clarification that collection of a particular matrix sample, e.g., plasma, can be stopped once negative results have been reported in that matrix at three consecutive visits at least 1 week apart after Week 1. Collection of the remaining matrices should continue until each delivers negative results at three consecutive visits at least 1 week apart after Week 1.
12.6	Volume of blood to be drawn from each participant reduced from 1100 mL to 900 mL.
Throughout document	Minor editorial and typographical corrections and administrative changes.

Version 2.0 to 3.0

Section(s)	Summary of Change
6.5.2	Starting dose rationale updated.
6.5.2, 8.1, 13.3, Synopsis	Changes to the dose escalation approach: • The dose escalation assessment will include consideration of PK, PD, and other emerging clinical data as well as safety/tolerability (including DLT) and GCase activity levels. • Qualification related to GCase activity level added to avoid exposure of participants to a dose predicted to be below the therapeutic range.
6.5.3	Addition of a restriction on sperm donation during the trial.
6.5.3.1	Addition of myocarditis as a risk factor for FLT201.
6.5.3.2	Addition of x-ray as a risk factor for the study procedures.
7, 15.6, Synopsis	Addition of lung disease to exploratory objectives. Addition of chest x-ray and pulmonary function tests to exploratory endpoints.
7, 12.1.1, 12.1.2, 12.2, 12.3, 12.4.2, 15.6, Synopsis, Schedule of Assessments	Addition of chest x-ray and pulmonary function tests at baseline, and Weeks 12, 24, and 38/EOS.
8.1, Synopsis	Changes to the number of participants.
8.1, 13.4, Synopsis	Dose selection for Part 2 will be based on 12 (rather than 4) weeks of safety/tolerability and efficacy data from Part 1.
9.1, Synopsis	Clarification to the definition of previously treated patients (inclusion criterion #7).
9.2, Synopsis	Addition of exclusion criteria related to acute cardiac conditions, and myocarditis.

9.3, Synopsis	New section for restrictions on participants. Relocation and clarification of text contraception requirements, as well as relocation of existing text on alcohol and other restrictions.
10.2.1	Addition of option to provide label text as a single-panel label as well as a multi-language booklet.
10.3	Non-investigational product guidelines referenced.
11.3.1	Clarification of the approach to discontinuing/restarting previous therapy (ERT/SRT).
12.1.1, 12.1.2, 12.1.5, 12.2, 12.3, 12.4.3, Schedule of Assessments	Addition of hs troponin-T assessment at each visit.
12.1.3, 12.1.5, 12.2, 12.4.4, Schedule of Assessments	Statement added that semen samples may be omitted at the discretion of the investigator for religious or other personal reasons expressed by the subject.
12.5	Total blood volume to be drawn from participants increased from 900-934 mL to 1100 mL
13.2, 14.2.3, 15.3, Synopsis	AE severity assessment system changed from CTCAE to mild/moderate/severe
13.3.1, Synopsis	Changes to the FLT201 doses and addition of body weight dose cap of 90 kg.
14.2.5	Addition that hospitalisation for pre-existing conditions, including elective procedures, or a study related procedure (e.g., administration of IV methylprednisolone) does not constitute an SAE.
14.2.9	Addition of further AESIs: • An increase in hs troponin-T to greater than twice the upper limit of normal (normal troponin-T is <14 pg/mL). • A change in 12-lead ECG deemed clinically significant by the investigator. • Myocarditis.
14.3.9	Relocation of contraception text to new Section 9.3.
15.3, 15.4, 15.5	Clarification of endpoint analyses.
15.8	Addition of interim analysis on completion of Part 1 to inform decisions regarding a planned Phase 3 study.
Throughout document	Minor editorial corrections and administrative changes.

Version 1.0 to 2.0

Section(s)	**Summary of Change**
Throughout document	Administrative change to consistently reference participant and not subject.
Title Page	Updated Version number
List of Abbreviations	Added Clinical Immune Management Plan (CIMP)
Synopsis	Administrative correction for consistency with Table 3
6.5.4	Added cross-reference to section 10.4 where further details of the Clinical Immune Management Plan are presented
10.4	Revised to reflect key aspects of immunosuppression management (including when it starts, its duration, and what the immunosuppression regimen consists of). Also outlines what is contained within the Clinical Immune Management Plan and confirms the remit of the DMC in relation to any changes in the Clinical Immune Management Plan.
10.3	Changing wording, reflecting the supply of NIMPs
13.7	New section added relating to communication plan for dissemination of safety data from the Sponsor to the investigational sites.