[redacted]

STATISTICAL ANALYSIS PLAN

AN OPEN-LABEL, MULTINATIONAL STUDY OF THE EFFICACY AND SAFETY OF EX VIVO, LENTIVIRAL VECTOR-MEDIATED GENE THERAPY AVR-RD-01 FOR TREATMENT-NAÏVE SUBJECTS WITH CLASSIC FABRY DISEASE

PROTOCOL NO.: AVRO-RD-01-201

PREPARED FOR: AVROBIO, Inc.
100 Technology Square
6th floor
Cambridge, MA, 02139
USA

PREPARED BY: [redacted]

DATE OF ISSUE: July 26, 2023

VERSION/STATUS: Final version 3.0

The information in this document is confidential and proprietary to [redacted] and its clients. The information may only be used by the entities to which it was disclosed for the purpose it was disclosed. To the maximum extent permissible by law, the information must not be disclosed to any third parties without the prior written consent of [redacted].

SAP APPROVAL

By my signature, I confirm that this SAP has been reviewed by AVROBIO, Inc. and has been approved for use on the AVRO-RD-01-201 AVRO-RD-01-201:

Name	Title / Company/Role	Signature	Date
[redacted]	Biostatistician/ Novotech/ Co-Author	[redacted]	26-Jul-2023
[redacted]	Head of Biostatistics for AVROBIO, Inc./ Co-Author	[redacted]	26-Jul-2023
[redacted]	[redacted] for AVROBIO, Inc./ Approver	[redacted]	26-Jul-2023
[redacted]	Senior Director, Clinical Development for AVROBIO, Inc. / Approver	[redacted]	27-Jul-2023

TABLE OF CONTENTS

LIST OF TABLES

List of Abbreviations

Abbreviation	Term
AE	Adverse Event
AGA	Alpha-galactosidase A
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutical Chemical (classification)
BMI	Body mass index
BPI-SF	Brief Pain Inventory-Short Form
BSFS	Bristol Stool Form Scale
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
cDNA	Complementary deoxyribonucleic acid
CKD	Chronic kidney disease
CRF	Case Report Form
CRO	Contract Research Organization
CS	Clinically significant
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DIBSS-D	Diary for Irritable Bowel Syndrome Symptoms-Diarrhea
DLCO	Diffusing capacity of the lung for carbon dioxide
DMC	Data Monitoring Committee
DP	Drug Product
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
ERT	Enzyme replacement therapy
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
Gb3	Globotriaosylceramide
G-CSF	Granulocyte-colony stimulating factor

Abbreviation	Term
GI	Gastrointestinal
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSC	Human stem cell
ICH	International Conference on Harmonization
IV	Intravenous
LSD	Lysosomal storage disorder
LV	Lentiviral vector
LVEF	Left ventricular ejection fraction
LVMI	Left ventricular mass index
Lyso-Gb3	Globotriaosylsphingosine
MCS	Mental Component Summary
MedDRA	Medical Dictionary for Regulatory Activities
mGFR	Measured glomerular filtration rate
MH	Medical History
MI	Myocardial infarction
MRI	Magnetic resonance imaging
NCS	Not clinically significant
LOCF	Last Observation Carried Forward
OC	Observed Cases
PCS	Physical Component Summary
PD	Pharmacodynamic
PK	Pharmacokinetic
PT	Preferred Term
PTC	Peritubular capillary
qPCR	Quantitative polymerase chain reaction
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	Red blood cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

Abbreviation	Term
SD	Standard Deviation
SF-36	Short Form 36
SOC	System Organ Class
SOP	Standard Operating Procedure
TIA	Transient ischemic attack
VCN	Vector copy number
VDRL	Venereal Disease Research Laboratory
WBC	White blood cell
WHO	World Health Organization
WHO-DDE	World Health Organization-Drug Dictionary Enhanced

SAP Updates from Version 1 to 2

The SAP is updated from version 1 to version 2 with the following updates:

- The version 1 of the SAP was based on protocol amendment 2, dated May 01, 2018. The version 2 of the SAP is based on protocol amendment 5, dated April 01, 2022.
- Study objectives were updated according to protocol amendment 5. [Note: Protocol amendment 6 was submitted to the Food and Drug Administration; however, this protocol version was not implemented for this study]
- The following protocol terminology has been updated as follows:
  - "AVR-RD-01" replaces "treatment".
  - "AVR-RD-01 infusion" replaces to "transplant".
  - "Pre-AVR-RD-01 Infusion" replaces "Pre-transplant".
  - "Post-AVR-RD-01 Infusion Follow-Up" replaces "Post-transplant Follow-Up."
  - "Preparatory medications" refers to "G-CSF, plerixafor, busulfan/melphalan, or anti-convulsant"
- Extra exploratory efficacy endpoints and their associated descriptive analyses were added:
  - Changes from baseline in peripheral blood cell subpopulations (i.e., T cells [CD3+], natural killer [NK] cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (i.e., hematopoietic stem cells [HSCs] [HSC CD34+CD38-CD90+CD45RA-] and multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-], multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+], common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-], granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+], megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-], and Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells) analyzed by immunophenotyping and scRNAseq over time
  - Average VCN and ISA results in both peripheral blood cell subpopulations (i.e., T cells [CD3+], NK cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (i.e., HSCs [HSC CD34+CD38-CD90+CD45RA-] and multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-], multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+], common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-], granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+], megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-], and Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells) over time
  - Some extra wordings are added/updated to clarify the context.

- Compared with SAP version 1, the version 2 of SAP has been updated for the sections related to statistical summaries:
  - Extra statements were added in Section to clarify the study background.
  - Extra statements were added in Section 5 to clarify the general descriptive analysis methods.
  - Infusion analysis population was added in Section 5.5.3. It was defined to accommodate the analyses for efficacy endpoints. Instead, Efficacy analysis population from SAP version 1 has been removed.
  - In Section 5.7.17, steps and formulas to calculate eGFR were added.
  - In Section 5.7.18, steps and formulas to calculate mGFR were added.
  - In Section 5.7.19, details of assigning AEs into different study periods were added.
  - In Section 6.1, details of displaying outputs by study periods were updated. Accordingly, all mock-up shells for tables were updated to a different format from those in version 1. The data analyses are still descriptive in nature in SAP version 2.
  - In Section 7.5.3, visit windows were added for applicable analyses. In Section 7.5.4, visit labels were added.
  - In Section 8.6, analyses for concomitant medications were updated. The data were to be summarized by study period.
  - In Section 10.1, summaries of AVR-RD-01 infusion drug product characteristics were added.
  - In Section 10.2.7, summaries of AEs by study period were updated according to the updated definitions for study period.
  - Various other exploratory summary analyses and data plots related to efficacy and safety endpoints were added by the sponsor.

SAP Updates from Version 2 to 3

The SAP is updated from version 2 to version 3 with the following updates:

- The version 1 of the SAP was based on protocol amendment 2, dated May 01, 2018. The versions 2 and 3 of the SAP is based on protocol amendment 5, dated April 01, 2022.
- For evaluating subject experience with respect to AVR-RD-01 treatment and perception of changes in Fabry disease burden and quality of life by qualitative interview (for subjects, and caregivers or other qualified observers of subjects, who provide separate written informed consent), no data were captured as the [redacted] qualitative interviews were not validated in Fabry disease. The listing was removed.
- Listings will present all visits and Baseline (Derived), however, figures and tables will only include Baseline (Derived) and post-AVR-RD-01 visits (for efficacy) and post-baseline visits (for safety).
- The All Subjects population will consist of all subjects who consented, i.e. were enrolled.
    - This was changed from enrolled subjects who consented, were confirmed eligible, and entered the baseline period in order to present the total who were enrolled, who were screen failures, and who were rescreened.
        - Subject 101-103 is to be reported as a screen failure.
            - 101-103 was rescreened as 101-105. Where the same data are captured for both subjects, this will only be presented once in listings, tables and figures.
- For subject 101-106, who received Mobilization and Apheresis twice:
    - The study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
    - The study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
    - The study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
    - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2
- For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis.
- Where data are captured on separate individual days during conditioning, e.g., Conditioning (Day 1) etc., subjects who received melphalan (Subjects 101-101, 102-102, and 101-102), busulfan (short washout, Subject 102-104) and busulfan (long washout, Subjects 101-104,

101-105, 102-105, 101-106, and 101-107) will be grouped separately for derivation of summary statistics on all tables and figures.

- The listing of melphalan will include the dose administered, percentage difference between expected and actual dose, the start and end times, total dose (mg), body surface area (m2) and total dose/BSA (mg/m^2).
- The listing of busulfan will include the dose administered each day, percentage dose increased/decreased vs. the previous dose, percentage difference between expected and actual dose, the start and end times, total dose (mg), body surface area (m2), total dose/BSA (mg/m2), AUC and Total AUC.
- The tabulations of AEs by Onset and by TEAEs by Causal Relationship were simplified to include only the tabulation of (TE)AEs by SOC, PT.
- Plots of CFB in ANC and Platelets changed to present absolute values between study day -50 and study day 100, with a line for each subject, and vertical reference lines at study day 1 (solid, black), 35 (dashed, red), 42 (solid, red) and 49 (dashed, red).
    - In order to include the reference lines, the Infused Population will be used.
    - Stacked panel plot of ANC values with SOC of 'Infections and Infestations' replaced by the panel plot of ANC between study day -50 and study day 100.
- General language on inclusion of horizontal reference line(s) for the LLOQ, LLN and ULN, as appropriate, on figures was replaced by endpoint dependent detail of inclusion of horizontal reference line(s).
    - Similarly, endpoint dependent detail was added for when L or H flags are needed on listings.
- Details of LLOQ, LLN and ULN added for AGA enzyme in peripheral leukocytes and in plasma.
- Immunogenicity data will be listed only.
- RCL data will be listed only.
- The frequency table for achievement of hematological reconstitution was removed.
- On the listings of ANC and of platelets, removed requirement for G-CSF and platelet transfusions (will be captured on their own individual listings instead).
    - Added a listing of platelet transfusions.
- Removed panel plots of cumulative AEs for individual subjects.
- Palm provided LLOQ, LLN and ULN for AGA enzyme in peripheral leukocytes and AGA enzyme in plasma.
    - Details added to specify the LLN and ULN to be presented as reference lines on Figures, and the LLN and ULN to be used for L/H flags on Listings.

1. INTRODUCTION

This statistical analysis plan (SAP) for study AVRO-RD-01-201 is developed based on Amendment 5 of the protocol dated April 01, 2020. The purpose of this SAP is to ensure that the data listings and summary tables which will be produced, and the statistical methodologies that will be used, are comprehensive and appropriate for the analysis of the study objectives specified in the protocol.

Any amendments to the SAP will be made prior to database lock. Any additional analyses not described in the final SAP or deviation from the final SAP (e.g., change in the population used, change from statistical method/assumption listed, transformation of data type [e.g., continuous data transformed to categorical], exclusion of planned analysis, etc.) will be documented (and justified) in the clinical study report (CSR). The reason for the changes and the datasets and outputs that are affected will also be documented on a form which is reviewed by the reviewers of this SAP and signed off by the author of the SAP, the Head of Biostatistics (or designated designee) and Clinical Lead.

This SAP supersedes the protocol in all specifications associated with data analyses and statistical methodologies.

Fabry disease is a rare lysosomal storage disorder (LSD) associated with significant morbidity and early mortality caused by deficiency of the alpha-galactosidase A (AGA) enzyme activity due to mutations in the AGA (*GLA*) gene (MIM 301500). This deficiency in enzyme activity leads to accumulation of glycosphingolipids (e.g., globotriaosylceramide [Gb3] and its deacylated form, globotriaosylsphingosine [lyso-Gb3]), resulting in multi-organ damage. Fabry disease is a chronic, progressive disorder that ultimately leads to end-organ damage in the kidneys (end-stage renal disease), heart (hypertrophic cardiomyopathy, diastolic heart failure and arrhythmias) and brain (vascular stroke). Due to end-stage renal disease and other life-threatening complications associated with the disease, average life expectancy in affected males is reduced to approximately 50 years of age.

Study AVRO-RD-01-201 is a multinational, open-label study to assess the efficacy and safety of AVR-RD-01 in approximately 8 to 12 male subjects ≥16 (and post-pubertal) to ≤50 years of age with a confirmed diagnosis of classic Fabry disease based on deficient AGA enzyme activity who have received no enzyme replacement therapy (ERT) and/or chaperone therapy for Fabry disease within 3 years of Screening. It has five study periods: Screening, Baseline, Pre-AVR-RD-01 Infusion, AVR-RD-01 Infusion, and Post-AVR-RD-01 Infusion Follow-Up. Data Monitoring Committee (DMC) will review safety information that may preclude continued study enrollment and/or necessitate changes to the protocol.

The study was prematurely discontinued.

[redacted] will perform all efficacy and safety statistical analyses.

2. STUDY OBJECTIVE(S) AND ENDPOINT(S)

2.1. Study Objective(s)

2.1.1. Primary Objectives

The primary objectives of this study are to:

- Evaluate the effect of AVR-RD-01 on substrate (i.e., globotriaosylceramide [Gb3]) in kidney biopsies
- Evaluate the safety and tolerability of AVR-RD-01 including, but not limited to:
  - Evaluation of adverse events (AEs)/serious adverse events (SAEs)
  - Evaluation of immunogenicity of AVR-RD-01
  - Testing for the presence of replication competent lentivirus (RCL)
  - Performance of integration site analysis (ISA) to assess for potential aberrant clonal expansion(s)

2.1.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate AGA enzyme activity in plasma and peripheral leukocytes
- Evaluate the effect of AVR-RD-01 on biomarkers for Fabry disease (i.e., Gb3 and its deacylated form, globotriaosylsphingosine [lyso-Gb3]) in plasma and urine
- Evaluate the effect of AVR-RD-01 on substrate (i.e., Gb3) in skin biopsies
- Evaluate the effect of AVR-RD-01 on other clinical indices of Fabry disease, including:
  - Clinical laboratory measures of renal function, including measured glomerular filtration rate (mGFR), estimated glomerular filtration rate (eGFR) and urine total protein and albumin excretion
  - Cardiac structure assessed by left ventricular mass index (LVMI) on cardiac magnetic resonance imaging (MRI)
  - Abdominal pain and stool consistency assessed by the Diary for Irritable Bowel Syndrome Symptoms-Diarrhea (DIBSS-D)
  - Pain assessed by the Brief Pain Inventory Short Form (BPI-SF) questionnaire
  - Functional status assessed by the Physical Component Summary (PCS) and Mental Component Summary (MCS) scores of the 36-Item Short Form Health Survey (SF-36)
- Assess measures of engraftment of gene-augmented hematopoietic stem cells by determining average vector copy number (VCN) in peripheral blood leukocytes and

bone marrow stem and progenitor cells using quantitative polymerase chain reaction (qPCR) and/or droplet digital polymerase chain reaction (ddPCR) analysis

2.1.3. Exploratory Objectives

The exploratory objectives of the study are to:

- Evaluate the effect of AVR-RD-01 on:
  - Microscopic findings on kidney biopsy
  - AGA enzyme activity in skin and kidney biopsies
  - Podocyturia (shedding of podocytes into urine)
  - Other gastrointestinal (GI) symptoms associated with Fabry assessed by the DIBSS-D, including stool frequency, urgency, recurrent bowel movements, and the abdominal symptom subscale
  - Functional status assessed by the eight subscale scores (i.e., Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning and Mental Health) of the SF-36
  - Exploratory biomarkers for Fabry disease in plasma and urine (for subjects with written informed consent to optional genetic biomarker research) *[Note: Samples were not tested by the end of study]*
- Assess reconstitution dynamics by immunophenotyping the fluorescence-activated peripheral blood subpopulation and bone marrow CD34+ subpopulations, and by studying changes in cell populations composition by single-cell RNA sequencing (scRNAseq)
- Assess engraftment dynamics by determining average VCN, and performing ISA, on fluorescence-activated cell-sorted peripheral blood subpopulations and bone marrow CD34+ subpopulations
- Evaluate subject experience with respect to AVR-RD-01 treatment and perception of changes in Fabry disease burden and quality of life by qualitative interview (for subjects, and caregivers or other qualified observers of subjects, who provide separate written informed consent). *[Note: No data captured as the* [redacted] *qualitative interviews were not validated in Fabry disease]*
- Assess the impact of the conditioning regimen on reproductive potential

2.2. Study Endpoints

2.2.1. Efficacy Endpoints

2.2.1.1. Primary Efficacy Endpoints

The primary efficacy endpoint is the change from baseline in the average number of Gb3 inclusions (i.e., myelinosomes) per kidney peritubular capillary (PTC) per subject at Week 48.

2.2.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- Change from baseline in AGA enzyme activity level in plasma and peripheral leukocytes at Weeks 24 and 48
- Change from baseline in biomarkers for Fabry disease (i.e., Gb3 and its deacylated form, lyso-Gb3) in plasma and urine at Weeks 24 and 48
- Change from baseline in substrate (i.e., Gb3) in skin biopsy (at Weeks 24 and 48)
- Change from baseline in mGFR at Week 48
- Change from baseline in eGFR and urine total protein and albumin at Weeks 24 and 48
- Change from baseline in LVMI assessed by cardiac MRI at Week 48
- Change from baseline in abdominal pain and stool consistency assessed by the DIBSS-D at Weeks 24 and 48
- Change from baseline in BPI-SF questionnaire scores at Weeks 24 and 48
- Change from baseline in physical and mental functioning assessed by the SF-36 PCS and MCS scores at Weeks 24 and 48
- Average VCN in peripheral blood leukocytes assessed by qPCR and/or ddPCR at Weeks 24 and 48
- Average VCN in bone marrow stem and progenitor cells assessed by qPCR and/or ddPCR at Week 48

2.2.1.3. Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include the following:

- Change from baseline in light microscopic findings on kidney biopsy at Week 48
- Change from baseline in AGA enzyme activity in skin biopsy (Weeks 24 and 48) and kidney biopsy (Week 48)
- Change from baseline in the number of podocytes shed into the urine (podocyturia) at Weeks 24 and 48
- Change from baseline in other GI symptoms associated with Fabry disease assessed by the DIBSS-D, including stool frequency, urgency, recurrent bowel movements and the abdominal symptom subscale at Weeks 24 and 48
- Change from baseline in physical and mental functioning assessed by the eight SF-36 subscale scores (i.e., Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning and Mental Health) at Weeks 24 and 48
- Change from baseline in exploratory biomarkers for Fabry disease in plasma and urine at Weeks 24 and 48 (for subjects with written informed consent to optional genetic

biomarker research for consenting subjects) *[Note: Samples were not tested by the end of study]*

- Changes from baseline in peripheral blood cell subpopulations (i.e., T cells [CD3+], natural killer [NK] cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (i.e., hematopoietic stem cells [HSCs] [HSC CD34+CD38-CD90+CD45RA-] and multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-], multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+], common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-], granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+], megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-], and Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells) analyzed by immunophenotyping and scRNAseq over time
- Average VCN and ISA results in both peripheral blood cell subpopulations (i.e., T cells [CD3+], NK cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (i.e., HSCs [HSC CD34+CD38-CD90+CD45RA-] and multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-], multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+], common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-], granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+], megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-], and Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells) over time
- Change from baseline in Fabry disease burden and quality of life, including exploration of the impact of pre-treatment conditioning on participating subjects and perception of changes in disease burden and quality of life, assessed by qualitative interview conducted at Weeks 12, 24, and 48 (for subjects, and caregivers or other qualified observers of subjects, who provide separate written informed consent) *[Note: No data captured as the [redacted] qualitative interviews were not validated in Fabry disease]*

2.2.2. Safety Endpoints

Primary

Primary safety endpoints include the following:

- Incidence and severity of AEs and serious adverse events (SAEs)
- Change from baseline in clinical laboratory values relevant to safety
- Abnormal clinical laboratory values relevant to safety
- Change from baseline in vital signs
- Change from baseline in electrocardiogram (ECG) findings
- Presence of anti-AGA antibodies
- Presence of RCL

- Identification of mononuclear cells with integration site profiles suggestive of aberrant clonal expansion(s)

Exploratory

- The exploratory safety endpoint is change from baseline in sperm count, sperm motility, and sperm morphology.

2.3. Statistical Hypotheses

No formal hypothesis tests or statistical models are planned.

2.4. Pharmacokinetic (PK) and PK/Pharmacodynamic (PD) Hypotheses

Not applicable for this study.

3. STUDY DESIGN

This is a multinational, open-label study to assess the efficacy and safety of AVR-RD-01 in approximately 8 to 12 adults (defined as either ≥18 years of age; or ≥16 and <18 years of age and post-pubertal, where permitted by region) male subjects up to 50 years of age with a confirmed diagnosis of classic Fabry disease based on deficient AGA enzyme activity who are treatment-naïve. For this study, treatment-naïve is defined as having received no enzyme replacement therapy (ERT) and/or chaperone therapy for Fabry disease within 3 years of Screening.

Five study periods (Screening, Baseline, Pre-AVR-RD-01 Infusion, AVR-RD-01 Infusion and Post-AVR-RD-01 Infusion Follow-Up) comprise the study. During the Screening Period (approximately 8 weeks), written informed consent (and assent, if applicable) will be obtained and the subject will complete other Screening procedures to confirm study eligibility. Once study eligibility is confirmed, subjects will enter the Baseline Period (up to 3 days) during which time assessments will be performed to establish a pre-AVR-RD-01 infusion baseline. Once baseline assessments are complete, the subject will enter the Pre-AVR-RD-01 Infusion Period (approximately 6 to 8 weeks) during which time mobilization, apheresis, AVR-RD-01 Drug Product preparation and testing for release and conditioning regimen administration to achieve partial myeloablation will take place. Following completion of the Pre-AVR-RD-01 Infusion Period, the subject will enter the AVR-RD-01 Infusion Period (1 day) during which time AVR-RD-01 infusion will take place. After AVR-RD-01 infusion, the subject will enter the Post-AVR-RD-01 Infusion Follow-Up Period (approximately 48 weeks) during which time periodic safety and efficacy assessments will be performed to assess measures of engraftment, clinical response and safety post-infusion. Post-AVR-RD-01 infusion follow-up will occur at the following time points: Week 1 (Days 1 through 7), Week 2 (Days 10 and 14), Week 4 (Day 28), Week 8 (Day 56), Week 12 (Day 84), Week 24 (Day 168), Week 36 (Day 252) and Week 48 (Day 336).

Individuals who do not meet the criteria for participation in this study (screen failure) may be re-screened once upon discussion between the Investigator and Medical Monitor. Subjects who discontinue from the study prior to infusion of AVR-RD-01 will be replaced.

An independent Data Monitoring Committee (DMC) will be established for the study to review safety information during the Pre-AVR-RD-01 Infusion, AVR-RD-01 Infusion, and Post-AVR-RD-01 Infusion Follow-Up Periods to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol. A safety review meeting will take place to review the first subject's safety data after the subject has received the infusion and completed the Week 4 follow-up visit. Once the DMC has reviewed these Week 4 data and determined that there are no significant clinical safety events (serious adverse events [SAEs] related to AVR-RD-01 infusion), the next 2 subjects can begin the mobilization phase of the study. The DMC will meet again after these 2 subjects have completed the Week 4 follow-up visit. Once the DMC has reviewed the Week 4 data from subjects 2 and 3 and determined that there are no significant clinical safety events (serious adverse events [SAEs] related to AVR-RD-01 infusion), any currently enrolled subjects that have completed the baseline assessments can begin the mobilization phase of the study, and the study will be opened to enrollment. After the 6th subject has completed the Week 4 follow-up visit, the DMC will review all available safety data (i.e., accumulated safety data available from subjects 1 through 6) to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol, as well as make recommendations on the timing of any future periodic DMC meetings. Ad-hoc safety review meetings will also take place if stopping rules for the study

are met, or for any other safety reason(s), at the discretion of the DMC. Further details on the DMC, including its composition and responsibilities, will be outlined in the DMC Charter for the study.

Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 48 assessments (or prematurely discontinue the study).

After study completion, consenting subjects will continue periodic safety and efficacy assessments for approximately 14 years (for a total of 15 years post-infusion follow-up) in a long-term follow-up study to AVRO-RD-01-201.

3.1. Definition of Study Treatment

The Sponsor identifier for the study drug product is AVR-RD-01. The active substance in AVR-RD-01 is autologous CD34+ enriched cell fraction transduced with LV/AGA that encodes for the human AGA cDNA sequence. The finished drug product is presented as a cryopreserved cell solution which is thawed immediately prior to use. The drug product is intended for one time IV infusion of between 3 and 20 x 10^6/kg body weight autologous CD34+ transduced cells.

In addition to AVR-RD-01, in this study subjects will receive the following preparatory medications:

- Four to eight G-CSF and two plerixafor injections for HSC mobilization prior to AVR-RD-01 infusion
- Anti-convulsant medication administration (levetiracetam or others [benzodiazepines or valproic acid]), concurrent with busulfan administration, for seizure prophylaxis
- Five IV infusions of busulfan, one test dose to inform the conditioning dose and four for myeloablative conditioning, prior to AVR-RD-01 infusion
    - In prior versions of the protocol, melphalan was used.

It should be noted that these preparatory medications are not considered IMP.

3.2. Sample Size Considerations

3.2.1. Sample Size Justifications

The sample size of 8 to 12 subjects chosen for this study was selected without statistical considerations but was determined to be adequate to meet the study objectives.

3.2.2. Sample Size Re-estimation

Not applicable for this study.

3.3. Randomization

Not applicable for this study.

3.4. Clinical Assessments

Screening

Demography (date of birth, age, race and ethnicity, height, weight, body mass index (BMI)), medical and surgical history, Fabry diagnosis, historical mutation analysis, mutation analysis, echocardiogram (ECHO) and transmittable disease testing (including HIV).

Efficacy

Globotriaosylceramide (Gb3) and Globotriaosylsphingosine (lyso-Gb3) in plasma and urine, AGA enzyme activity in plasma, serum and peripheral leukocytes, biomarkers for Fabry disease (i.e., Gb3) in skin biopsy and kidney biopsy samples, clinical laboratory measures of renal function (mGFR, eGFR and urine protein/albumin), cardiac function assessed by LVMI on cardiac MRI, GI symptoms assessed by the DIBSS-D, pain assessed by the BPI-SF questionnaire, functional status assessed by the SF-36, engraftment, AGA enzyme activity in skin and kidney from biopsy samples, podocytes in urine and exploratory biomarkers for Fabry disease in plasma and urine (not tested).

Safety

Adverse events and serious adverse events, vital signs (heart rate, systolic and diastolic blood pressure, respiratory rate and temperature), physical examination, 12-lead electrocardiogram (ECG), laboratory assessments (serum chemistry, hematology and urinalysis), pulmonary function test by spirometry, chest x-ray, cardiac MRI, immunogenicity, measures of reproductive potential and concomitant medications.

4. PLANNED ANALYSES

4.1. Interim Analysis

In this open-label study, after AVR-RD-01 infusion, measures of engraftment, clinical response, and safety are analyzed for each individual subject on an ongoing basis. Considering this, a discrete interim analysis of data collected in this study will not be performed.

4.2. Final Analysis

The final database lock will occur when all subjects have completed the study (i.e., the final follow-up visit at Week 48 [or prematurely discontinued the study]) and all data are collected and cleaned.

5. GENERAL CONSIDERATIONS FOR DATA ANALYSES AND HANDLING

5.1. General Summary Table and Individual Subject Data Listing Considerations

Summary tables and listings will be prepared according to ICH Guideline E3.

The All Subjects population will be used for specific outputs, as defined below. Safety data will be summarized using the Safety population, and Efficacy data will be summarized using the Infused population.

Summary tables for any concomitant medications will be coded according to the World Health Organization (WHO) Drug dictionary Version of WHODrug Global B3 March 2022. Adverse event preferred terms and body/organ systems and medical history conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) dictionary Version 25.0.

Missing data will be reported as follows:

- Data captured as 'Unknown', or variations thereof, in the clinical database will be presented as such in the Listings
- Missing data and will be displayed as follows in Listings:
  - 'Not Done' will be presented where a sample was not collected/assessment was not performed (as per EDC)
  - 'No Result' will be presented where the sample was collected/assessment was performed (as per EDC) but no result is available.
  - 'Not Applicable' will be presented when the sample/assessment was not required/expected for that subject/visit, e.g., where a result would only be expected after a protocol amendment but not prior to that amendment.
  - 'Not Calculated' will be presented when no result can be derived, e.g., standard deviation is NC when n<3, or change from baseline (CFB) is NC when no baseline or post-baseline result is available.
- If all data for a specific visit are missing the data are included in the table with N=0.
- Safety assessment values of the form of "< x" (i.e., below the lower limit of quantification (BLQ)) or "> x" (i.e., above the upper limit of quantification) will be imputed as "x" in the calculation of descriptive statistics but displayed as "< x" or "> x" in any listings that are produced.
  - If the calculated mean at a sampling time point is BLQ then the mean value will be reported as 'BLQ' and the SD and CV% will be reported as not calculated (NC) in the summary. Similarly, any calculated BLQ values for median, minimum, maximum and geometric mean will be reported as 'BLQ' in the summary.

Imputed dates and times will NOT be presented in the listings. Completely missing start or end dates/times will remain missing, with no imputation applied. Consequently, time to onset and duration of such events will be missing. Partial dates/times will be displayed as captured in subject listing displays.

5.2. General Post Text Summary Table and Individual Subject Data Listing Format Considerations

The tables and listings will be numbered using a decimal system to reflect main levels of unique tables and listings and sub-levels of replicate tables and listings with two digits per level (e.g., Table XX.YY.ZZ. …).

1. The first level number will be consistent with the corresponding Clinical Study Report (CSR) appendix in which the tables or listings will appear. For example, the post text tables will appear in Appendix 14 (and will be numbered 14.XX.YY) and the individual subject data listings will appear in Appendix 16 (and will be numbered 16.XX.YY). The subject disposition table will be first in the first section of the report and will be numbered Table 14.1. The supportive subject data listing will be Listing 16.1. Any subset table will have the number Table 14.1.2, etc.

2. Table numbering will follow ICH E3. Subject disposition, baseline and demography and prior and concomitant medications tables should appear as the second level number (Table 14.1 series). Efficacy tables will occupy the next sub-level (Table 14.2 series). Safety tables will follow next (Table 14.3 series). Similar conventions will be applied to the subject data listings.

3. Each table and figure title will be complete, accurate and concise. The last line of the title will provide the analysis population being summarized/presented.

4. All analysis and summary tables will include the analysis population sample size (i.e., number of subjects).

5. Each listing title will be complete, accurate and concise. Inclusion in each analysis population will be presented as a flag on selected listings.

6. If possible, variables being summarized and statistics reported will appear in the left most column of a table.

5.3. Data Management

All data will be recorded by the site in individual source documents. An eCRF will be created by the data management group for recording of the required data in the study database. All eCRF information is to be filled in by site staff. If an item is not available or is not applicable, this fact should be indicated. Blank spaces should not be present unless otherwise directed.

All information recorded in the eCRFs for this study must be consistent with the investigator's source documentation for the study participants. The investigative site will make available source documents to any personnel monitoring the study. The study monitor will verify consent of all subjects to participate in the study and will perform 100% source data verification (SDV) of data entered into the eCRF and raise queries for correction by the site. The data entered into the eCRF will be subject to data validation checks for consistency and completeness by the data management group. Data queries will then be generated and sent to the investigational site for response before the database is locked and released for statistical analysis.

Data from any screen failures might be entered into the clinical database. If screen failure data is entered in the clinical database this data might not be cleaned. In order to describe the representativeness of the study, this data should be presented including the reasons for screen failures (failure of individual inclusion/exclusion criteria could be presented if appropriate).

Data storage, data transfer and data cleaning will be conducted according to the relevant [redacted] Standard Operating Procedures (SOPs).

Derived datasets are created using SAS® software. Data analyses and summary tables will be generated using the currently supported version at the time of data analysis.

A [redacted] Clinical Data Associate (CDA) will review the data for discrepancies via programmed electronic consistency checks, data listings, or manually. Any discrepancies discovered via the data review process will be issued as queries in the EDC system to the investigative site for resolution. Once all the source verification is complete, all queries are resolved, and the database has been updated appropriately and data has been signed off by the investigator, the database will be locked and made available to [redacted] Biostatistics for analysis.

All SAS® programs used to create analysis datasets and output will be validated by ensuring that the ".log" files are void of all errors, warnings, and notes indicative of problems. Additionally, each program will be checked to ensure that it performs according to the program specification. All programs are developed and validated through risk-based independent double programming by separate members of the [redacted] Biostatistics Department.

Outputs will include data extraction date. The purpose of the data extraction date is to link the output to the database, either active or archived, that is write-protected for replication and future reference. The program execution date will appear on each output page and will indicate the date the output was generated by the analysis program. Individual source listings will display all the relative values supporting corresponding table(s) and figure(s).

Table 1. Laboratory Data Vendors

Vendor Name	Parameter (Units)	SAP Section (s)	LLOD	LLOQ	LLN	ULN
[redacted]	Gb3 in skin biopsy	9.2.3	N/A	N/A	N/A	N/A
	AGA enzyme in skin biopsy	9.2.3	N/A	N/A	N/A	N/A
	Gb3 in renal biopsy (Gb3 inclusions per PTC; light microscopic findings)	9.1	N/A	N/A	N/A	N/A
	AGA enzyme in renal biopsy (Gb3 inclusions per PTC; light	9.2.1	N/A	N/A	N/A	N/A

Vendor Name	Parameter (Units)	SAP Section (s)	LLOD	LLOQ	LLN	ULN
	microscopic findings)					
[redacted]	Peripheral Blood and Bone Marrow Immunophenotype; Peripheral Blood and Bone Marrow Cell-Sorted VCN	9.3.8	Assays are considered 'research grade' and never qualified or validated so this information, which would be common measures determined for a validated assay, is not available			
[redacted]	Cardiac MRI	9.2.7	The within-subject change is the important variable, so N/A			
[redacted]	Blood and Bone Marrow aspirate for Vector Copy Numbers (VCN)	9.2.11 9.2.12	15 cp/rxn for whole blood and bone marrow	100 cp/rxn	Not part of the validation of the method	
[redacted]	DIBSS data	9.2.8	N/A, refer to scoring algorithm			
[redacted]	Urine podocyte and Urine Creatinine	9.2.6	N/A. LLOD needs to be assessed against a known sample with analyte measured based on a gold standard technique which currently does not exist	N/A. LLOQ needs to be assessed against a known sample with analyte measured based on a gold standard technique which currently does not exist	Urine podocyte: 0 podocyte/g Cr (or 0 podocyte/ mg Cr)	Urine podocyte: 3000 podocyte/g Cr (or 3 podocyte/ mg Cr)
[redacted]	Immunogenicity Testing	9.3.7	N/A	N/A	N/A	1160 RLU

Vendor Name	Parameter (Units)	SAP Section (s)	LLOD	LLOQ	LLN	ULN
[redacted]	AGA enzyme in serum and peripheral leukocytes; Mutation analysis whole blood	9.2.1	Serum: 0 nmol/ min/ mL Leukocytes: Not established Mutation analysis: N/A	Serum: 0 nmol/ min/ mL Leukocytes: Not established Mutation analysis: N/A	Serum: 0.074 nmol/ min/ mL Leukocytes: Not established Mutation analysis: N/A	Serum: 0.457 nmol/ min/ mL Leukocytes: Not established Mutation analysis: N/A
[redacted]	AGA plasma	9.2.1	Not available	0.2 nmol/h/mL	4.2 nmol/h/mL	15 nmol/h/mL
[redacted]	AGA leukocytes (cells)	9.2.1	Not available	0.5 nmol/h/mg protein	24 nmol/h/mg protein	56 nmol/h/mg protein
[redacted]	Biomarker/Gb3 and LGb3 in plasma/Gb3 and LGb3 in urine	9.2.2	Refer to Appendix 1			
[redacted]	ISA on Whole Blood, peripheral blood and bone marrow	9.3.8	ISA reports show the most abundant 10 clones identified in samples while the other identified integrations are combined as 'low abundance'			

LLOD = Lower Limit of Detection; LLOQ = Lower Limit of Quantification; LLN = Lower Limit of Normal; ULN = Upper Limit of Normal

5.4. Data Presentation Conventions

The following conventions will apply for data presentation:

- Continuous safety variables (e.g., clinical laboratory values, vital signs and ECGs) will be reported to the same precision as the source data. Derived variables will be reported

using the same precision as the value(s) from which they were derived. For the reporting of descriptive statistics, the mean, median, standard deviation and CV% will be reported to 1 decimal place more than the source data; the minimum and the maximum values will be presented to the same precision as the source data.

- For categorical/discrete variables, the frequency count and the percentage (of available data) for each category of the variable will be presented and will be displayed in the form XX (XX.X%) where the percentage is in the parentheses. The denominator for all percentages will be the number of subjects with non-missing data within the analysis population of interest, unless otherwise stated. Otherwise, percentages (for example for AEs and Concomitant Medications) will be calculated based on the population total. If the analysis population is not stated in the title then a footnote will clarify the denominator used. A row denoted 'Missing' will be included in count tabulations to account for dropouts and missing values.
- All percentages will be rounded to one decimal place. In the case the numerator is equal to the denominator, the percentage should be presented as (100) instead of (100.0). In the case the numerator is equal to 0, the percentage will not be presented.
- Date variables will be formatted as YYYY-MM-DD for presentation. Time will be formatted in military time as HH:MM for presentation.
- Unscheduled assessments will be considered when applying visit windows and derived study periods and will also contribute to the end of study value, or best/worst case value where required (e.g., shift table).
- When deriving a subject level statistic (e.g., maximum value post-baseline), all values should be included regardless of whether they appear in a corresponding visit-based summary.
- All listings will be sorted for presentation in order of subject, parameter, and date/time of procedure or event.
- Individuals who do not meet the criteria for participation in this study (screen failure) may be re-screened once upon discussion between the Investigator and Medical Monitor.
    - Where a re-screened subject is present in the clinical database under two different identifiers, data will be combined for Tables and Figures, and the identifiers will be concatenated for Listings with a footnote included to describe the details of re-screening. This is for subject numbers 101-103 and 101-105.
        - 101-103 is to be reported as a screen failure.
- For medical/surgical history and AEs, frequency ordering will be used for the summary table presentation (i.e., order by the overall grouping in descending frequency, and then alphabetical order in the case of equal frequency, for each System Organ Class (SOC) and also for each Preferred Term (PT) within each SOC). Columns that are used for grouping in descending frequency will be specified in the footnote.

- Outputs will define Lower Limit of Quantification (LLOQ) values, including the units, if applicable;
    - Values below the LLOQ (BLQ), or not detected or not quantifiable, will be imputed as the LLOQ (where available) or as 0.01 for figures and for summary statistics, and n imp, the number of imputed values, will be reported;
    - Imputed values will be used for Tables and Figures, but Listings will present the original values
- Outputs will define Lower Limit of Detection (LLOD) values, including the units, if applicable;
- Outputs will include reference ranges and Normal Ranges, including the units, if applicable;
- Outputs will include units, if applicable;
- On all plots, the subjects who received melphalan (Subjects 101-101, 102-102, and 101-102), busulfan (short washout, Subject 102-104) and busulfan (long washout, Subjects 101-104, 101-105, 102-105, 101-106, and 101-107) will be identified as follows (a legend will describe the conditioning regime associated with each color):
    - Subjects conditioned with melphalan will be presented in red, with a different symbol (eg, square, circle, triangle) per subject;
    - Subjects conditioned with busulfan with a washout period of 20h (short washout) will be presented in blue, with a symbol (eg, square, circle, triangle)
    - Subjects conditioned with busulfan with a washout period of 68-70h (long washout) will be presented in green, with a different symbol (eg, square, circle, triangle) per subject;
    - Where a mean line is included on plots, a dashed black line (thickness greater than the red/blue/green individual subject lines) will be used;
        - A mean line will be included even when n<3
- Where data are captured on separate individual days during conditioning, e.g., Conditioning (Day 1) etc., subjects who received melphalan (Subjects 101-101, 102-102, and 101-102), busulfan (short washout, Subject 102-104) and busulfan (long washout, Subjects 101-104, 101-105, 102-105, 101-106, and 101-107) will be grouped separately for derivation of summary statistics on all tables and figures;
- A month is counted as 30.4375 days and a year is counted as 365.25 days (30.4375 days × 12 months);
- Summary statistics:
    - Summary statistics for efficacy data (absolute values) will include N, n, geometric mean, coefficient of variation between subjects (CV%), median,

minimum, and maximum will be reported (where n<3, only mean, median, minimum and maximum will be presented);

- Geometric Mean will be presented when at least 3 values (including imputed values) are available;
- %GeoCV will be calculated as: $100 * (e^{\mathrm{sd}(\log(X))} - 1)$;

- Summary statistics for safety data, and for changes from baseline, will include N, n, mean, SD, minimum, median, and maximum will be reported (where n<3, only mean, median, minimum and maximum will be presented);

- For plots of log-normal data, the y-axis may be presented on the log-scale, as required;

Outputs will be delivered as pdf and rtf format.

5.5. Analysis Populations

Subject inclusion into each population will be determined before database lock and prior to commencing the final analysis.

5.5.1. All Subjects Population

The All Subjects population will consist of all subjects who consented, i.e. were enrolled. The All Subjects population will be used for all listings and for summaries of all disposition, demographic, and baseline data.

5.5.2. Safety Population

The Safety population will consist of all enrolled subjects who received any preparatory medication (G-CSF for Mobilization, plerixafor, busulfan/melphalan, or anti-convulsant). This population will be used for the summaries of all safety data, demographic, baseline characteristics and medical history.

5.5.3. Infused Population

The Infused population will consist of all enrolled subjects who received all preparatory medications and AVR-RD-01. This population will be used for the summaries of all efficacy data, and selected summaries of safety data.

5.6. Baseline Definition

For all **safety and efficacy endpoints**, Baseline (Derived) will be defined as the last available, non-missing observation prior to first administration of a preparatory medication (G-CSF for Mobilization, plerixafor, busulfan/melphalan, or anti-convulsant), unless specifically mentioned otherwise.

Non-numeric (i.e., categorical) observations will be considered for baseline calculations, where applicable. In addition, non-missing results from unscheduled assessments prior to first administration of a preparatory medication may also be considered in the calculation of baseline observations.

If more than one assessment is collected before first administration of a preparatory medication and it is not possible to determine which is the closest then the mean of the assessments will be used as Baseline (Derived).

Where baseline values are at or below the LLOQ (BLQ), or not detected or not quantifiable, these will be imputed as the LLOQ (where available) or as 0.01 for figures and for summary statistics.

Baseline (Derived) will be defined in a footnote on each applicable output.

Listings will present all visits and Baseline (Derived), however, figures and tables will only include Baseline (Derived) and post-AVR-RD-01 visits (for efficacy) and post-baseline visits (for safety).

5.7. Derived and Transformed Data

5.7.1. Screening Age

Age, in completed years, at screening will be defined as:

Age (years) = integer value ((Date (first) signed Informed Consent – Date of Birth + 1) / 365.25)

5.7.2. Body Mass Index

Body Mass Index (kg/m^2, to one decimal place) = Body Weight (kg) / (Height x Height (m))

5.7.3. Study Day

Study Day will be defined as:

Study Day = Date of Assessment – Date of AVR-RD-01 infusion (if date of assessment is before Date of AVR-RD-01 infusion)

Otherwise,
Study Day = (Date of Assessment – Date of AVR-RD-01 infusion) + 1

For any population which includes subjects who may not have received AVR-RD-01, e.g., the Safety population, the Study Day will not be derived, except where the SAP indicates date of (first) signing of informed consent is used.

5.7.4. Time since AVR-RD-01 Infusion

Time since AVR-RD-01 infusion will be calculated as**:**

$$Time\ Since\ \text{AVR} - \text{RD} - 01\ \ (months) = \frac{Study\ Day}{30.4375}$$

5.7.5. Age at enrollment

Age at enrollment will be calculated as:

$$Age\ at\ Enrollment\ (months) = \frac{(Date\ of\ (first)\ Informed\ Consent - Date\ of\ Birth)}{30.4375}$$

5.7.6. Age at AVR-RD-01 infusion

Age at AVR-RD-01 infusion will be calculated as:

$$Age\ at\ \text{AVR} - \text{RD} - 01\ Infusion\ (months) = \frac{Date\ of\ Infusion - Date\ of\ Birth}{30.4375}$$

5.7.7. Adverse event onset time relative to AVR-RD-01 infusion

Adverse event onset time relative to AVR-RD-01 infusion will be calculated as:

> Adverse event onset time relative to AVR-RD-01 infusion = Onset Date and Time of AE – AVR-RD-01 infusion Date and Time, if onset date of AE is before Date of AVR-RD-01 infusion
>
> Otherwise, Adverse event onset time relative to AVR-RD-01 infusion = (Onset Date and Time of AE – AVR-RD-01 infusion Date and Time) + 1

Where time is missing, only dates will be used. Refer to Section 5.7.19 for handling of incomplete dates.

5.7.8. Duration of Adverse Events

Duration of Adverse Events (in days) will be calculated as:

> Duration of Adverse Event = (Resolution Date and Time) – (Onset Date and Time)

Where time is missing, only dates will be used. If a resolution or onset date is missing or incomplete, no duration will be derived.

5.7.9. Change from Baseline

For efficacy, CFB will be calculated as (post-AVR-RD-01 result – baseline result).

For safety, CFB will be calculated as (post-baseline result – baseline result).

Changes from baseline will use Baseline (Derived), including where Baseline (Derived) uses the imputed LLOQ value.

Change from baseline comparisons for categorical variables will use Baseline (Derived) and will be displayed using shift-tables that display the frequency and percentage of subjects within each category at baseline and at any specified time points.

If Baseline (Derived) is missing then the CFB will also be set to missing, unless otherwise stated. Only subjects with a value at both the Baseline (Derived) visit and the specific post-baseline visit will be presented.

5.7.10. Observed Cases (OC) and Last Observation Carried Forward (LOCF) and Outliers

Not applicable for this study. Missing data will not be imputed.

Outliers will be included in all outputs.

5.7.11. Completers

Not applicable for this study.

5.7.12. Treatment Compliance

Not applicable for this study (as AVR-RD-01 is administered once, treatment compliance is not pertinent).

5.7.13. Kidney Biopsy

The number of myelinosomes per kidney peritubular capillary (PTC) per subject will be evaluated by two independent renal pathologists. The renal pathologists will score the average number of Gb3 inclusions (i.e., myelinosomes) per kidney PTC per subject, based on electron microscopic images of kidney biopsy samples. The primary efficacy measure will be based on the mean of the average number of Gb3 inclusions (i.e., myelinosomes) per kidney PTC, across the two independent reviewers.

5.7.14. Diary for Irritable Bowel Syndrome (DIBSS-D)

The DIBSS-D produces item-level scores across 2 domains (bowel movements and abdominal symptoms) as well as an abdominal symptoms domain score. At least 4 of 7 days must be completed to calculate a weekly score for either the bowel movements (BM) or the abdominal symptom items.

Scores for bowel movements (stool consistency, stool frequency, and urgency), for abdominal symptoms (abdominal pain, abdominal discomfort, abdominal bloating, and abdominal cramping), and for the abdominal symptoms domain score, will be obtained at each protocol scheduled time point.

5.7.15. BPI-SF

Two scores are calculated from the Brief Pain Inventory.

The pain severity score is calculated as the mean of the non-missing values for questions 3 (worst pain), 4 (least pain), 5 (average pain) and 6 (current pain).

The pain interference score is calculated as the mean of the non-missing values for questions 9a, b, c, d, e, f and g, which represent pain interference with general activity, mood, walking ability, normal work, relations with other people, sleep, and enjoyment of life. Subjects who answer fewer than four questions will not have a pain interference score derived.

5.7.16. SF-36

SF-36 version 2 will be used in the study. There are eight health domains (Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role

Functioning, Social Role Functioning and Mental Health) and psychometrically-based physical component summary (PCS) and mental component summary (MCS) scores. Scoring will be undertaken using the commercial software by QualityMetric. The scores provided by QualityMetric will be used for the data summary.

5.7.17. Estimated GFR

Estimated GFR (eGFR), used to estimate how well the kidneys are filtering certain agents produced by the body, will be calculated using the CKD-EPI Creatinine Equation as follows:

eGFR Calculation

Glomerular filtration rate (GFR) is the best overall index of kidney function. Normal GFR varies according to age, sex, and body size, and declines with age. Research has recommended using the CKD-EPI Creatinine Equation to estimate GFR (1). The CKD-EPI formula, expressed as a single equation, will be used for calculating eGFR:

$$eGFR = 141 * \min\left(\frac{SCr}{\kappa}, 1\right)^{\alpha} * \max\left(\frac{SCr}{\kappa}, 1\right)^{-1.209} * 0.993^{Age} * Sex * Race$$

Abbreviations / Units

- Estimated Glomerular Filtration Rate (eGFR) = mL/min/1.73m^2
- Serum Creatinine (SCr) = mg/dL or SCr/88.4 (if unit=µmol/L)
- Kappa coefficient (κ) = 0.7 (females) or 0.9 (males)
- Alpha coefficient (α) = -0.329 (females) or -0.411 (males)
- Min = minimum of [(SCr/κ) or 1]
- Max = maximum of [(SCr/κ) or 1]
- Age = years
- Sex coefficient = 1.018 (females) or 1 (males)
- Race coefficient = 1.159 (black) or 1 (white or other)

NOTE: eGFR should only be calculated if SCr, Age, Sex, and Race values are non-missing.

5.7.18. Measured GFR

Measured GFR (mGFR), a measurement of how well the kidneys are filtering certain agents not produced by the body, will be assessed using Iohexol clearance following the process described by Brøchner–Mortensen correction, which can be applied independent of age, namely "JBM" (Brøchner-Mortensen & Jødal, 2009), shown in formula 3 below. The JBM-corrected mGFR values will be used for the analysis.

Definitions:

C_{Io} = Dose of Iohexol in mg

A = Intercept of measurements [ln concentration vs. time] [mcg * min / mL]

α = Slope of measurements [ln concentration vs. time] [mcg * min / mL]

CL_{Io} = Clearance of Iohexol

Cor_CL_{Io} = Corrected clearance of Iohexol

BSA = Body Surface Area

Formulas:

1) Body surface area (Du Bois formula)
BSA = $0.007184 \times W^{0.425} \times H^{0.725}$), where W is weight in kg, and H is height in cm.

The height and weight collected closest to the date of Iohexol injection will be used.

2) Calculation for Iohexol Clearance

$$\mathrm{CL_{Io}} = \frac{C_{Io} * -1000}{\frac{\exp(A)}{\alpha}}$$

3) JBM correction

$$\mathrm{Cor\_CL}_{Io} = \frac{CL_{Io} * \frac{1.73}{BSA}}{1 + \left(f * CL_{Io} * \frac{1.73}{BSA}\right)}$$

$$f = 0.00185 * BSA^{-0.3}$$

Notes:

- Formula 2) calculates Administered dose divided by (Intercept / Slope of Iohexol Concentration dynamics = Area under the curve (AUC, approximate) of Iohexol concentration dynamics);
    - The time [HH:MM] of Iohexol injection and sample times [HH:MM] of the 3-hour and 4-hour samples will be used.
- Units: administered dose = [mg]; AUC = [mcg * min / mL]
- Therefore, in formula 2)
    - $\frac{mg}{\frac{mcg*min}{mL}} = \frac{mcg}{\frac{mcg*min}{mL}} * 1000 = \frac{mL}{min} * 1000$
    - Multiplication by 1000 is required for unit conversion from mcg to mg
    - Results from the calculation will always be negative as the marker depletes over time; clearance implies decay, therefore multiplication by -1 (if unit is in mg) or -1000 (if unit is in mcg)

5.7.19. Derivations for Adverse Events

A treatment-emergent adverse event (TEAE) will be any adverse event occurring after a subject has started preparatory medications, i.e., started mobilization with G-CSF. This includes: i) any AE that emerges after start of mobilization with G-CSF (having been absent before start of mobilization with G-CSF) or ii) any AE that although present before G-CSF, worsens after start of mobilization with G-CSF (in this case, there will be a new AE entered after worsening).

Where the start date/time of an AE is partial or missing and the end date/time is on or after the date/time of start of mobilization with G-CSF, or both the start and end dates/times are missing, then a "worst-case" scenario will be assumed, thus the AE is assumed to have occurred after the start of mobilization with G-CSF (i.e., treatment-emergent) unless a partial start or end date indicates otherwise. When partial dates are present in the data, both a partial start date and/or a partial end date will be evaluated to determine whether it can be conclusively established that the AE started prior to the start of mobilization with G-CSF or ended prior to the start of mobilization with G-CSF. If it cannot be conclusively established that the AE started prior to the start of mobilization with G-CSF based on the partial start and/or end dates, then the AE will be considered as treatment-emergent.

The following table presents an algorithm for the determination of whether a given AE will be considered treatment-emergent or not:

Table 2. Algorithm for Treatment Emergent Adverse Events

Start date of AE	End date of AE	Action
Known	Known, Partial or Missing	If start date/time < start date/time of mobilization with G-CSF, then not TEAE Otherwise, if start date/time ≥ mobilization with G-CSF start date/time, then TEAE
Partial, but known components show that it cannot be on or after mobilization with G-CSF start date/time	Known, Partial or Missing	Not TEAE
Partial, could be on or after mobilization with G-CSF start date/time OR Missing	Known	If end date/time < mobilization with G-CSF start date/time, then not TEAE If end date/time ≥ mobilization with G-CSF start date/time, then TEAE
	Partial	Temporarily impute end date/time as latest possible date/time (i.e., 23:59 on last day of month if day and time unknown or 31st

Start date of AE	End date of AE	Action
		December 23:59 if day and month and time are unknown), then: If imputed end date/time < mobilization with G-CSF start date/time, then not TEAE If imputed end date/time ≥ mobilization with G-CSF start date/time, then TEAE
	Missing	Assumed TEAE

AEs will be tabulated by the following study periods based on the onset date/time of AEs:

- Pre-Mobilization: started (or worsened) before first dosing of G-CSF for Mobilization
- Preparatory Medications:
    - Mobilization:
        - G-CSF: started (or worsened) on or after first dosing of G-CSF for Mobilization until start of apheresis
            - For subject 101-106, the study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
    - Apheresis:
        - started (or worsened) on or after start of the first apheresis until the end of the first apheresis
            - For subject 101-106, the study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
    - Post-Apheresis: started (or worsened) after the end of the apheresis and before first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
        - For subject 101-106, the study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
            - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2

        - For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis
    - Conditioning: started (or worsened) on or after first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan) and before infusion of AVR-RD-01
- AVR-RD-01 Infusion: started (or worsened) on or after the start of infusion of AVR-RD-01
- Post-AVR-RD-01 Infusion Follow-Up: started (or worsened) after the end of AVR-RD-01 infusion

The date and time of AEs are captured, as well as date and time for G-CSF, plerixafor, apheresis, levetiracetam or others [benzodiazepines or valproic acid], busulfan/melphalan and AVR-RD-01 infusion. Therefore, when assigning AEs to a study period, the time (HH:MM) of the onset (or worsening) of the AE will be compared to the time of the preparatory medication or AVR-RD-01, e.g., on Day 1, an AE with onset time prior to the start time of AVR-RD-01 infusion will be assigned to the Conditioning study period. Where time is missing or incomplete, the AE will be assigned to the study period using the date alone, e.g., on Day 1, an AE on Day 1 with onset time missing will be assigned to the AVR-RD-01 infusion study period.

If an adverse event continues into subsequent study periods it will only be counted in the study period in which it first occurred, in order to avoid double counting.

6. TREATMENT COMPARISONS

6.1. Data Display Treatment and Other Sub-Group Descriptors

The Analysis Population used will be presented on all tabulations.

Adverse Events will be summarized by System Organ Class (SOC) and Preferred Term (PT). Additionally, AEs will be summarized by study period (Pre-Mobilization, Preparatory Medications, AVR-RD-01 Infusion, and Post-AVR-RD-01 Infusion Follow-Up). The Preparatory Medications study period will be further broken down into Mobilization, Apheresis, Post-Apheresis, and Conditioning. The following labels for study period will be used on applicable AE tabulations:

- All Adverse Events
- Pre-Mobilization
- Preparatory Medications
  - Mobilization
    i. Mobilization #2 (for 101-106)
  - Apheresis
    i. Apheresis #2 (for 101-106)
  - Post-Apheresis
    i. Post-Apheresis #2 (for 101-106)
  - Conditioning
- AVR-RD-01 Infusion
- Post-AVR-RD-01 Infusion Follow-Up

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

The listings and summary tables for the disposition, safety and efficacy data will be the responsibility of the study Biostatistician.

The currently supported version of SAS® software will be used to perform all data analyses. The actual SAS® version used will be presented in the Clinical Study Report.

All data in the database will be presented in the data listings. Unless otherwise stated, all listings will be sorted by subject number and assessment date/time. In the listings, subject number will be of the format 101-001, etc.

The study aims to explore the possibility of any indications of an efficacy response signal, as well as to assess safety and tolerability of AVR-RD-01. As such, all continuous efficacy endpoints will be summarized descriptively at each protocol-scheduled time point and as change from baseline at each post-AVR-RD-01 time point, and all continuous safety and tolerability endpoints will be summarized descriptively at each protocol-scheduled time point and as change from baseline at each post-baseline time point.

7.1. Multicenter Studies

Data from all participating sites will be pooled.

7.2. Other Strata and Covariates

Not applicable for this study.

7.3. Examination of Subgroups

Due to protocol amendments regarding the conditioning regimen, safety data will be presented overall and by the three subgroups of i) subjects who received melphalan, ii) subjects who received busulfan, and iii) subjects who received busulfan (long washout).

7.4. Multiple Comparisons and Multiplicity

Not applicable for this study.

7.5. Data handling conventions

7.5.1. Premature Withdrawal and Dropouts

For subjects who are withdrawn or dropout from the study prior to study completion, all data collected will be listed and included in data summaries. Early termination data will be mapped using visit windows.

7.5.2. Additional/Unscheduled Assessments

Generally, missing dates/times will not be imputed. Adverse events with missing or partial dates will be handled such that in the absence of contradictory information an AE is classified based on the study period closest to the AE onset. Adverse events will be assigned to each study period according to Section 5.7.19

Unscheduled assessments will be considered when applying visit windows and for derived study periods and will also contribute to the end of study value, or best/worst case value where required (e.g., shift table).

When deriving a subject level statistic (e.g., maximum value post-baseline), all values should be included (including scheduled, unscheduled, retest and early discontinuation data) regardless of whether they appear in a corresponding visit-based summary.

Listings will include scheduled, unscheduled, retest and early discontinuation data.

7.5.3. Assessment Windows

For by-visit summaries, visit windows as defined below will be applied:

Table 3. Visit Window Assignment

Assigned Study Day (Inclusive)		**Visit Assigned**	**Target Study Day**
From	To		
1	1	Day 0 (AVR-RD-01)	1
2	2	Day 1 (W1)	2
3	3	Day 2 (W1)	3
4	4	Day 3 (W1)	4
5	5	Day 4 (W1)	5
6	6	Day 5 (W1)	6
7	7	Day 6 (W1)	7
8	9	Day 7 (W1)	8
10	12	Day 10 (W2)	11
13	21	Day 14 (W2)	15
22	42	Day 28 (W4)	29
43	70	Day 56 (W8)	57
71	126	Day 84 (W12)	85
127	210	Day 168 (W24)	169
211	294	Day 252 (W36)	253
295	379	Day 336 (W48)	337

In the case where two or more measurements fall within the same visit window, the available measurement closest to the planned visit date will be used. If two measurements are equidistant from the planned visit date then the earlier value will be used. If multiple measurements occurred on the same date, and the time (HH:MM) of each assessment is available, then these will be reviewed on a case-by-case basis. If time is unavailable, the mean of the measurements will be taken. In the case of a retest where there is clear evidence to suggest that the retest was done due to erroneous data, then the retest value will be used. Otherwise, the mean of the measurements will be used.

Scheduled, unscheduled, retest and early discontinuation data will be included when mapping to visit windows.

7.5.4. Visit/Study Period Mapping and Labels

Within **Tables**, **Figures** and **Listings**, the mapping and derived visit labels in Table 3 will be used from date of AVR-RD-01 infusion onwards.

Prior to the date of AVR-RD-01 infusion, the below mapping and derived study period labels will be used in **Listings**:

- Pre-Mobilization: date is before first dosing of G-CSF for Mobilization
- Mobilization:
    - Date is on or after first dosing of G-CSF for Mobilization and before start of apheresis
        - For subject 101-106, the study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
- Apheresis:
    - Date is on or after start of apheresis until the end date of the last apheresis
        - For subject 101-106, the study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
- Post-Apheresis: date is after the end of the last apheresis and before date of first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
    - For subject 101-106, the study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
        - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2
    - For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis
- Conditioning: date is on or after first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan) and before date of infusion of AVR-RD-01

Listings will present both protocol visit/study day and also derived study period/study day. Baseline (Derived) will also be included. Unscheduled, retest and early discontinuation data prior to the day of AVR-RD-01 infusion will be included when mapping to the above study period labels for **Listings**.

In **Tables** and **Figures**, the following mapping and study period labels will again be used prior to the day of AVR-RD-01 infusion:

- Pre-Mobilization: date is before first dosing of G-CSF for Mobilization
- Mobilization:
    - Date is on or after first dosing of G-CSF for Mobilization and before start of apheresis
        - For subject 101-106, the study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
- Apheresis:
    - Date is on or after start of apheresis until the end date of the last apheresis
        - For subject 101-106, the study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
- Post-Apheresis: date is after the end of the last apheresis and before date of first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
    - For subject 101-106, the study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
        - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2
    - For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis
- Conditioning: date is on or after first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan) and before date of infusion of AVR-RD-01
    - Where data are captured on separate individual days during conditioning, e.g., Conditioning (Day 1) etc., subjects who received melphalan (Subjects 101-101, 102-102, and 101-102), busulfan (short washout, Subject 102-104) and busulfan (long washout, Subjects 101-104, 101-105, 102-105, 101-106, and 101-107) will be grouped separately

For **Tables** and **Figures**, scheduled, unscheduled, retest and early discontinuation data will be included when mapping to study periods. In the case where daily measurements were not scheduled per protocol, but two or more measurements still fall within the same study period,

then these will be reviewed on a case-by-case basis. In the case of a retest where there is clear evidence to suggest that the retest was done due to erroneous data, then the retest value will be used.

In the case where daily measurements were scheduled per protocol, e.g., daily measurements during Mobilization, Apheresis or Conditioning, then each individual scheduled assessment will be included separately in **Tables** and **Figures** under the appropriate study period label, e.g. Mobilization (Day 1).

For subjects who are withdrawn or dropout from the study prior to study completion, all data collected will be listed and included in data summaries. Early termination data will be mapped using visit windows to the derived visits in Table 3 or to the derived study periods described above.

7.6. Derived and Transformed Data

The required endpoints and variables will be derived by the statistical programmers at [redacted] using the derivations specified in Section 5.7 of this SAP.

8. STUDY POPULATION

All disposition, baseline and demographic data will be summarized using the All Subjects population. Baseline and demographic data will also be summarized using the Safety population and Infused population.

8.1. Disposition of Subjects

A disposition listing will present date of informed consent, date of enrollment, date of study completion or early termination, the primary reason for early discontinuation, if applicable and whether included in each population and reasons for exclusion, for each subject.

A listing of whether or not all inclusion and exclusion criteria were met and if not, which criteria were not met, by subject, will also be presented.

The following will be summarized:

- Number of subjects enrolled
- Number of subjects treated with each preparatory medication (G-CSF for Mobilization, plerixafor, melphalan, anti-convulsant, or busulfan) or AVR-RD-01
  - 8 subjects had mobilization/apheresis once and received AVR-RD-01
  - 1 subject (101-106) had mobilization/apheresis twice (due to suboptimal CD34+ cells for making IP) and received AVR-RD-01
  - 2 subjects had mobilization/apheresis once and did not receive AVR-RD-01, namely 503-102 (the second planned mobilization/apheresis did not happen due to study closure) and 504-101 (did not receive AVR-RD-01 due to study closure)
- Number of subjects completing the study
- Number of subjects withdrawn and reason for withdrawal
- Number of subjects in each analysis population
- Duration of follow-up (using Time since AVR-RD-01 infusion)

8.2. Protocol Deviations

A protocol deviation is any divergence from the protocol. Protocol deviations should be defined as either “Important protocol deviations” (IPDs) per the ICH E3 guidance “Structure and content of clinical study reports” or “Other Study Deviations”. Important protocol deviations (IPDs) are defined as a subset of protocol deviations that may significantly affect the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject’s rights, safety, or well-being, and include:

- Any clinical condition that in the Investigator’s opinion could become dangerous for the subject and prevent the good conduct of the clinical trial;
- Protocol violation that could compromise the quality of study data such as: use of erythropoietin, use of biological agents potentially modifying erythropoiesis, enrolment into interventional clinical trials, bone marrow transplantation;

- Occurrence of new diseases that could influence the treatment efficacy, for which AVRO-RD-01 is contraindicated or that are treated with a medication that is not permitted as a concomitant medication.

The process of identifying protocol deviations as well identification of IPDs are specified in the Monitoring Plan.

Protocol deviations will be reviewed on an ongoing basis and the final review and classification of protocol deviations will be performed prior to database lock.

The need for any supplementary analyses resulting from protocol deviations will be determined following review of the protocol deviations ahead of database lock and will be documented prior to the primary analysis being conducted.

All protocol deviations will be listed. The type of protocol deviation, the description, and the date it occurred will be included. The potential impact of notable protocol deviations deemed important by the study team will be discussed in the CSR.

A frequency table will present the number of protocol deviations and IPDs per subject and per site, with percentages included to capture the proportion of the total numbers of PDs or IPDs.

8.3. Demographic and Baseline Characteristics

All baseline and demographic data recorded at screening and prior to dosing will be listed.

Subject demographic and baseline variables (age at enrollment, age at AVR-RD-01 infusion, gender, race, ethnicity, height, body weight, BMI, Gb3 in plasma, lyso-Gb3 in plasma, AGA enzyme in peripheral leukocytes, and average number of Gb3 inclusions (ie, myelinosomes) per kidney PTC) will be summarized.

Disease diagnosis (age at time of diagnosis and age at onset of symptoms) will be listed and summarized.

Historical mutation analysis, mutation analysis, transmittable disease testing and ECHO will be listed only.

8.4. Medical History and Medical Conditions Present at Entry

All medical history (MH) conditions, concomitant diseases and surgical procedures will be classified by system organ class (SOC) and preferred term (PT) using MedDRA® v25.0 . The version will be labelled in the applicable outputs.

The number and percentage of subjects with each medical history condition, concomitant disease and surgical procedure will be presented by SOC and PT.

Past and current medical conditions will be listed, including whether the subject has experienced any past concomitant diseases or surgeries, start date, end date or whether it is ongoing, the disease or symptom, the SOC and PT, the maximum CTCAE Grade, and whether it resulted in hospitalization.

8.5. Treatment Compliance

All preparatory medication administration data (G-CSF for Mobilization, plerixafor, melphalan, anti-convulsant and busulfan) and AVR-RD-01 infusion will be listed. Apheresis data (e.g., total cell count, CD34+ cell count) will also be listed.

The listing of melphalan will include the dose administered, percentage difference between expected and actual dose, the start and end times, total dose (mg), body surface area (m^2) and total dose/BSA (mg/m^2).

The listing of busulfan will include the dose administered each day, percentage dose increased/decreased vs. the previous dose, percentage difference between expected and actual dose, the start and end times, total dose (mg), body surface area (m^2), total dose/BSA (mg/m^2), AUC and Total AUC.

The listing of AVR-RD-01 infusion will include start date and time of infusion, end date and time of infusion, subject weight (kg), total volume infused (mL) and date of initial hospital discharge. If more than one batch of gene therapy was manufactured and administered to the subject, then the sum will also be presented for total volume infused (mL).

A listing will include the dates and derived study day of:

- G-CSF for Mobilization
    - Excludes any G-CSF administered for hematological reconstitution due to neutrophils < 1.5 (10^9/L), which will be listed separately
- Plerixafor
- Apheresis
- Anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
- Conditioning with melphalan or busulfan
- AVR-RD-01 infusion

Since all the above-mentioned administrations will be done at the investigational site, treatment compliance will not be determined for this study.

8.6. Prior and Concomitant Medications

The end dates of medications will be used to assign medications into different categories:

- Prior medication: Prior medications are defined as any medication where the use was stopped prior to the first administration of G-CSF for Mobilization.
- Concomitant medication: Concomitant medications are defined as any medication (other than the preparatory medications and AVR-RD-01) that was used at least once after the first administration of G-CSF for Mobilization. Medications that were stopped on the same date as G-CSF for Mobilization will be defined as concomitant medications. The answer to the question “Ongoing?” as recorded on the eCRF pages will also be taken into consideration to determine if a medication is classed as concomitant.

Table 4 below shows the algorithm for Prior/Concomitant Medications. For the purposes of the below algorithm, note that if the start date is partial then the start date should be temporarily imputed as the earliest possible date (i.e., first day of month if day unknown or 1st January if day and month are unknown) and if the end date is partial then the end date should be temporarily imputed as the latest possible date (i.e., last day of month if day unknown or 31st December if day and month are unknown):

Table 4. Algorithm for assignment of timing for medication use

Start date	End date	Action
Known	Known, Partial or Missing	If end date < start date of mobilization with G-CSF, then not Concomitant Otherwise, if end date ⩾ mobilization with G-CSF start date, then Concomitant
Partial, but known components show that it cannot be on or after mobilization with G-CSF start date	Known, Partial or Missing	Not Concomitant
Partial, could be on or after mobilization with G-CSF start date OR Missing	Known	If end date < mobilization with G-CSF start date, then not Concomitant If end date ⩾ mobilization with G-CSF start date, then Concomitant
	Partial	Temporarily impute end date as latest possible date (i.e., last day of month if day unknown or 31st December if day and month are unknown), then: If imputed end date < mobilization with G-CSF start date, then not Concomitant If imputed end date ⩾ mobilization with G-CSF start date, then Concomitant
	Missing	Assumed Concomitant

The start dates and end dates of prior and concomitant medications will be used to further split prior and concomitant medications into the following study periods:

- Pre-Mobilization: ended before first dosing of G-CSF for Mobilization
- Preparatory Medications:
- Mobilization:
    - Date is on or after first dosing of G-CSF for Mobilization and before start of apheresis
        - For subject 101-106, the study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
- Apheresis:
    - Date is on or after start of apheresis until the end date of the last apheresis
        - For subject 101-106, the study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
- Post-Apheresis: date is after the end of the last apheresis and before date of first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
    - For subject 101-106, the study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
        - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2
    - For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis
- Conditioning: started on or after first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan) and before date of AVR-RD-01 infusion
- AVR-RD-01 Infusion: started on or after the start of infusion of AVR-RD-01
- Post-AVR-RD-01 Infusion Follow-Up: started after the end of AVR-RD-01 infusion

Note since only dates are captured for medications, and not times, that the start/end dates will be used to assign medications to a study period, e.g., a medication starting on Study Day 1 will be assigned to the AVR-RD-01 infusion study period because it is not known if the medication started before or after the start time of the AVR-RD-01 infusion on Day 1.

Medications will be classified as a prior medication and/or a concomitant medication. The prior and concomitant medications are exclusive with each other. The same concomitant medication, however, could appear in multiple study periods of Mobilization, Apheresis, Post-Apheresis, Conditioning, AVR-RD-01 Infusion, and Post-AVR-RD-01 Infusion Follow-Up, if it was used in two or more of those study periods.

Concomitant medications do not include G-CSF for Mobilization, plerixafor, busulfan/melphalan, and anti-convulsant medications for seizure prophylaxis. However, any G-CSF administered for hematological reconstitution due to neutrophils < 1.5 (10^9/L) will be listed separately.

Similarly, any platelet transfusions will be listed separately.

Prior and concomitant medications will be coded by WHODrug Global B3 March 2022.

Data will be summarized by Anatomical Therapeutic Chemical (ATC) system (Level 4) and drug preferred name. The summary tables will show the number and percentage of subjects taking each medication by ATC Level 4 and preferred name.

Where a subject may have taken two or more medications, the subject may be counted only once within an ATC classification, if appropriate. Also, the same subject may contribute to two or more preferred terms in the same classification but will only be counted once within the preferred term.

Frequency ordering will be used for the table presentation (i.e., order by the overall grouping in descending frequency for each ATC, and additionally for each preferred term within each ATC; in each case where there are equal frequencies items would be ordered alphabetically).

Summary tables will be presented for concomitant medications over the course of the study for each study period (Pre-Mobilization, Preparatory Medications, AVR-RD-01 Infusion, and Post-AVR-RD-01 Infusion Follow-Up). The Preparatory Medications study period will be further broken down into Mobilization, Apheresis, Post-Apheresis, and Conditioning. Summary tables will be produced for the Safety Population and for the Infused Population.

Prior medications and concomitant medications will be listed and summarized, separately. The study period and within study period study day for medication start and stop and all study periods where the medication has been used will be provided. For concomitant medications, medication name, product name, start dates and end dates, dose, dose unit, frequency, route, and indication (including details of any associated MH or AE) will be presented. Concomitant medications which started prior to a subject's first preparatory medication will be flagged.

The preparatory medication (G-CSF for Mobilization, plerixafor, melphalan, anti-convulsant and busulfan) will be listed separately (see Section 8.6).

Listings will be sorted by subject identification number and start date. If the start date is completely missing, then these events will be presented first. If the onset date is missing a month or a day then partial dates for medications will be imputed as per Table 4 to allow medications to be sorted appropriately. Imputed dates will not be listed.

9. EFFICACY ANALYSES

All efficacy analyses will be conducted on the Infused population.

Absolute values and CFB will be listed.

9.1. Primary Efficacy Analysis

The primary efficacy measure is globotriaosylceramide (Gb3) inclusions in peritubular capillaries on kidney biopsy. Electron microscopic images of kidney biopsy samples will be read centrally by two independent renal pathologists. The renal pathologists will score the average number of Gb3 inclusions (i.e., myelinosomes) per kidney PTC per subject.

The primary efficacy endpoint will be the change from baseline in the average number of Gb3 inclusions (i.e., myelinosomes) per kidney PTC at Week 48 (the mean from two independent reviewers will be used when deriving CFB). This efficacy measure will also be summarized at the other protocol-scheduled time points.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2. Secondary Efficacy Analyses

9.2.1. AGA Enzyme Activity

Observed values and absolute change from baseline in AGA enzyme activity level values will be summarized at each protocol scheduled time point, separately for plasma, serum and peripheral leukocytes.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

For the figure displaying observed values of AGA enzyme in plasma, horizontal reference lines will be added at the LLN of 4.2 and ULN of 15 nmol/h/mL.

For the listing of observed values of AGA enzyme in plasma, values below the LLN of 4.2 and above the ULN of 15 nmol/h/mL will be flagged.

For the figure displaying observed values of AGA enzyme in serum, horizontal reference lines will be added at the LLN of 0.074 and ULN of 0.457 nmol/min/mL.

For the listing of observed values of AGA enzyme in serum, values below the LLN of 0.074 and above the ULN of 0.457 nmol/min/mL will be flagged.

For the figure displaying observed values of AGA enzyme in peripheral leukocytes, horizontal reference lines will be added at the LLN of 24 and ULN of 56 nmol/h/mg protein.

For the listing of observed values of AGA enzyme in peripheral leukocytes, values below the LLN of 24 and ULN of 56 nmol/h/mg protein will be flagged.

9.2.2. Biomarkers

All observed values and absolute changes from baseline in Gb3 and isoforms, and its deacylated form lyso-Gb3 and analogs, will be listed at each protocol scheduled time point, separately for plasma and urine. On the listing of observed values, any values above the ULN will be flagged (refer to Appendix 1).

Gb3 isoforms and lyso-Gb3 analogs will not be included in summaries or plots.

Observed values and absolute change from baseline in total Gb3 and its deacylated form, lyso-Gb3, will be summarized at each protocol scheduled time point, separately for plasma and urine.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, in total Gb3 and its deacylated form, lyso-Gb3, will also be provided.

Figures displaying observed values over time will include horizontal reference lines at the LLOD, LLOQ and ULN, as appropriate (refer to Appendix 1).

9.2.3. Skin Biopsies

Observed values and absolute change from baseline in substrate (i.e., Gb3) values will be summarized at each protocol scheduled time point for skin biopsies.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.4. mGFR

A listing of Iohexol injection will include date/time of Iohexol injection, volume (mL) of Iohexol injection, dose (mg/mL) of Iohexol injection, total dose (mg), the date/time and plasma concentrations (mg/mL) of the 3h and 4h samples, and the number of minutes between time of Iohexol injection and the time of the 3h and 4h samples.

Observed values and absolute change from baseline in mGFR values will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided. On the figure displaying observed values, a horizontal reference line will be added at the LLN of 90 mL/min/1.73 m^2.

On the listing of mGFR, values <90 mL/min/1.73 m^2 will be flagged as lower than normal.

9.2.5. eGFR

Observed values and absolute change from baseline in eGFR and calculated eGFR values will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided. On the figure displaying observed values, a horizontal reference line will be added at the LLN of 90 mL/min/1.73 m^2.

On the listing of eGFR, values <90 mL/min/1.73 m^2 will be flagged as lower than normal.

9.2.6. Urine Protein/Albumin Excretion

Observed values and absolute change from baseline in urine total protein/albumin excretion values will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.7. Cardiac MRI

Observed values and absolute change from baseline in LVMI values assessed by the cardiac MRI will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.8. DIBSS-D

Abdominal pain and stool consistency scores and change from baseline will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.9. BPI-SF

Observed values and absolute change from baseline in BPI-SF questionnaire scores for pain severity and pain interference will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.10. SF-36

Observed values and absolute change from baseline in physical and mental functioning assessed by the SF-36 Physical Component Summary (PCS) and Mental Component Summary (MCS) scores will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.2.11. VCN in Peripheral Blood

VCN in peripheral blood leukocytes assessed by qPCR and/or ddPCR will be summarized at each protocol scheduled time point, using either summary statistics or frequency tabulations, as appropriate for the type of data. Note if values are obtained by qPCR and also ddPCR, these will be reported separately.

Figures displaying observed values over time, along with arithmetic or geometric means, will also be provided.

Scatter plots will be produced for the comparison of VCN in peripheral blood leukocytes (x-axis) with each of the below parameters (y-axis). A log-scale for x and/or y will be considered. To be included, data needs to be available for a subject for x and for y at the same visit:

- AGA enzyme in peripheral leukocytes
  - Reference lines will be added at the LLN of 24 and ULN of 56 nmol/h/mg protein
- AGA enzyme in plasma
  - Reference lines will be added at the LLN of 4.2 and ULN of 15 nmol/h/mL
- AGA enzyme in serum
  - Reference lines will be added at the LLN of 0.074 and ULN of 0.457 nmol/min/mL
- AGA enzyme in skin biopsies
- AGA enzyme in kidney biopsies
- Gb3 concentration in Leukocytes
- Gb3 concentration in Plasma
- Gb3 concentration in Urine
- Average number of Gb3 inclusions (i.e., myelinosomes) per kidney peritubular capillary (PTC)
- Lyso-Gb3 concentration in Leukocytes
- Lyso-Gb3 concentration in Plasma
- Lyso-Gb3 concentration in Urine

9.2.12. VCN in Bone Marrow Aspirate

VCN in bone marrow stem and progenitor cells assessed by qPCR and/or ddPCR will be summarized at each protocol scheduled time point, using either summary statistics or frequency tabulations, as appropriate for the type of data. Note if values are obtained by qPCR and also ddPCR, these will be reported separately.

Figures displaying observed values over time, along with arithmetic or geometric means, will also be provided.

Scatter plots will be produced for the comparison of VCN in bone marrow aspirate (x-axis) with each of the below parameters (y-axis). A log-scale for x and/or y will be considered. To be included, data needs to be available for a subject for x and for y at the same visit:

- AGA enzyme in peripheral leukocytes
  - Reference lines will be added at the LLN of 24 and ULN of 56 nmol/h/mg protein
- AGA enzyme in plasma
  - Reference lines will be added at the LLN of 4.2 and ULN of 15 nmol/h/mL
- AGA enzyme in serum
  - Reference lines will be added at the LLN of 0.074 and ULN of 0.457 nmol/min/mL
- AGA enzyme in skin biopsies
- AGA enzyme in kidney biopsies
- Gb3 concentration in Leukocytes

- Gb3 concentration in Plasma
- Gb3 concentration in Urine
- Average number of Gb3 inclusions (i.e., myelinosomes) per kidney peritubular capillary (PTC)
- Lyso-Gb3 concentration in Leukocytes
- Lyso-Gb3 concentration in Plasma
- Lyso-Gb3 concentration in Urine

9.3. Exploratory Efficacy Analyses

9.3.1. Light Microscopic Findings on Kidney Biopsies

Observed values and absolute change from baseline in light microscopic findings on kidney biopsies will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.3.2. AGA Enzyme Activity in Skin and Kidney Biopsies

Observed values and absolute change from baseline in AGA enzyme activity values will be summarized at each protocol scheduled time point, separately for skin and kidney biopsies.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.3.3. Podocyturia

Observed values and absolute change from baseline in the number of podocytes shed into the urine (podocyturia) will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

For the figure displaying observed values, horizontal reference lines will be included at the LLN of 0 podocyte/g Cr (or 0 podocyte/mg Cr), and at the ULN of 3000 podocyte/g Cr (or 3 podocyte/mg Cr).

On the listing of podocyturia, values below the LLN of 0 podocyte/g Cr (or 0 podocyte/mg Cr) and above the ULN of 3000 podocyte/g Cr (or 3 podocyte/mg Cr) will be flagged.

9.3.4. DIBSS-D

Observed values and absolute change from baseline in other GI symptoms associated with Fabry disease assessed by the DIBSS-D, including stool frequency, urgency, abdominal discomfort, abdominal bloating, abdominal cramping and the abdominal symptom domain score will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.3.5. SF-36

Observed values and absolute change from baseline in the eight SF-36 subscale scores (i.e., Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning, and Mental Health) will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.3.6. Exploratory Biomarkers for Fabry Disease

A listing will include dates and times of samples for any subjects for whom written informed consent to optional genetic biomarker research was received. Note these samples will not be tested by the end of study.

9.3.7. Peripheral Blood Cell Subpopulations and Bone Marrow CD34+ Subtypes

Observed values and absolute change from baseline in peripheral blood cell subpopulations and bone marrow CD34+ subtypes will be summarized at each protocol scheduled time point .

- Peripheral blood cell subpopulations
  - T cells [CD3+]
  - Natural killer [NK] cells [CD56+]
  - B cells [CD19+]
  - Granulocytes [CD15+],
  - Monocytes [CD14+]
- Bone marrow CD34+ subtypes
  - HSCs [HSC CD34+CD38-CD90+CD45RA-]
  - Multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-],
  - Multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+],
  - Common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-]
  - Granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+]
  - Megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-]
  - Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells)

Figures displaying observed values and CFB over time, along with arithmetic or geometric means, will also be provided.

9.3.8. Average VCN and ISA in Peripheral Blood Cell Subpopulations and Bone Marrow CD34+ Subtypes

Observed values in average VCN and ISA results in peripheral blood cell subpopulations and bone marrow CD34+ subtypes will be summarized at each protocol scheduled time point.

- Peripheral blood cell subpopulations
  - T cells [CD3+]
  - Natural killer [NK] cells [CD56+]
  - B cells [CD19+]
  - Granulocytes [CD15+],
  - Monocytes [CD14+]
- Bone marrow CD34+ subtypes
  - HSCs [HSC CD34+CD38-CD90+CD45RA-]
  - Multipotent progenitors [MPP CD34+CD38-CD90-CD45RA-],
  - Multilymphoid progenitors [MLP CD34+CD38-CD90-CD45RA+],
  - Common myeloid progenitors [CMP CD34+CD38+CD7-CD10-CD135+CD45RA-]
  - Granulocytes/monocytes progenitors [GMP CD34+CD38+CD7-CD10-CD135+CD45RA+]
  - Megakaryocyte/erythroid progenitors [MEP CD34+CD38+CD7-CD10-CD135-CD45RA-]
  - Pre-B/NK progenitors [CD34+CD38+CD7-CD10+] cells)

Figures displaying observed values over time, along with arithmetic or geometric means, will also be provided.

9.3.9. Perception of changes in disease burden and quality of life, assessed by qualitative interview

No data captured as the [redacted] qualitative interviews were not validated in Fabry disease.

10. SAFETY ANALYSES

All safety analyses will be conducted on the Safety population.

No inferential statistical testing will be performed for safety variables.

Due to protocol amendments regarding the conditioning regimen, safety data will be presented overall and by the three subgroups of i) subjects who received melphalan, ii) subjects who received busulfan, and iii) subjects who received busulfan (long washout).

10.1. Extent of Exposure

A listing of AVR-RD-01 infusion drug product characteristics will be provided and will include:

- Total nucleated cells (x10^6)
    - Derived as Nucleated cells/kg (x10^6 cells/kg) multiplied by weight (kg) at time of (most recent) apheresis
- Nucleated cells/kg (x10^6 cells/kg)
- CD34+ cells/kg (x10^6 cells/kg)
- CD34+ (%)
- Number of colony-forming unit-granulocyte, monocyte (CFU-GM) cells administered (x10^6)
- Transduction efficiency (colony-forming unit cell [CFU-C]) (%)
- Total CFU (%)
- Vector copy number (VCN/cell)
- Percentage cell viability (%)
- AGA (transduced)

In addition, the following drug product characteristics will be derived:

- CD34+ cells/kg (x10^6 cells/kg) * VCN (/cell)
- CD34+ (%) * Total CFU (%)
- CD34+ (%) * TE (%)
- CD34+ (%) * VCN (/cell)
- CD34+ (%) * TE (%) * VCN (/cell)

Scatter plots will be produced for the comparison of each drug product characteristic and derived drug product characteristic (x-axis) with each of the following parameters (y-axis). A log-scale for x and/or y will be considered, as required:

- VCN in peripheral blood leukocytes
- VCN in bone marrow aspirate

- AGA enzyme in peripheral leukocytes
  - Reference lines will be added at the LLN of 24 and ULN of 56 nmol/h/mg protein
- AGA enzyme in plasma
  - Reference lines will be added at the LLN of 4.2 and ULN of 15 nmol/h/mL
- AGA enzyme in serum
  - Reference lines will be added at the LLN of 0.074 and ULN of 0.457 nmol/min/mL
- AGA enzyme in skin biopsies
- AGA enzyme in kidney biopsies
- Gb3 concentration in Leukocytes
- Gb3 concentration in Plasma
- Gb3 concentration in Urine
- Average number of Gb3 inclusions (i.e., myelinosomes) per kidney peritubular capillary (PTC)
- Lyso-Gb3 concentration in Leukocytes
- Lyso-Gb3 concentration in Plasma
- Lyso-Gb3 concentration in Urine
- Days to neutrophil reconstitution
- Days to platelet reconstitution

10.2. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the IMP. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the IMP, regardless of whether the event is causally related to the IMP.

The definition of an AE also covers:

- Medication errors and uses outside what is foreseen in the protocol, including accidental overdose, if an AE results from the error;
- Any Grade 3 or higher platelet count, grade 3 or higher WBC count, and grade 2 or higher neutrophil count (grade determined by CTCAE) regardless of whether considered clinically significant by Investigator report;
- Fertility-related events, considered an AE of special interest (AESI) for the study.

Adverse events will be coded using MedDRA® version 25.0.

10.2.1. Treatment-emergent Adverse Events

Any AE that started prior to mobilization with G-CSF is considered a pre-treatment AE and is listed, but not included in tabulations (except for tabulations using study periods where Pre-Mobilization is included). However, pre-treatment AEs that worsen/increase in severity after the subject started mobilization with G-CSF will be recorded as new AEs and consequently will be regarded as treatment-emergent and thus will be included in the tabulations.

10.2.2. Serious Adverse Events

An SAE is any untoward medical occurrence or effect that:

- Results in death
- Is life threatening, that is any event that places the subject at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death
- Results of in-patient hospitalisation or a prolongation of existing hospitalisation, excluding admission for social or administrative reasons (see below)
- Results in a persistent or significant disability/incapacity, where disability is a substantial disruption of a person's ability to conduct normal life functions
- Results in congenital anomaly/birth defect in the offspring of a subject who received AVR-RD-01
- Is an important medical event that may not result in death, be life threatening, or require hospitalisation, but that, based upon appropriate medical judgment, may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalisation, or the development of drug dependency or drug abuse.

10.2.3. Adverse Drug Reaction or Adverse Reaction

All untoward and unintended responses to an investigational medicinal product. The phrase responses to an investigational medicinal product means that a causal relationship between the investigational medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

10.2.4. Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unauthorized AVR-RD-01 or summary of product characteristics for an authorized product).

10.2.5. Adverse Event Severity

The intensity or severity of AEs will be defined according to CTCAE as:

- **Grade 1** (Mild): Symptoms causing no or minimal interference with usual social & functional activities.
- **Grade 2** (Moderate): Symptoms causing greater than minimal interference with usual social & functional activities.
- **Grade 3** (Severe): Symptoms causing an inability to perform usual social & functional activities.
- **Grade 4** (Life threatening): Symptoms causing an inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

- **Grade 5** (Death)

10.2.6. Adverse Event Relationship

The relationship between an AE and AVR-RD-01, mobilization (G-CSF or plerixafor), apheresis, conditioning (busulfan/melphalan), anti-convulsant medication(s), study procedures or underlying disease will be assessed based on clinical experience and classified according to the following:

- Definite
  - Event or laboratory test abnormality, with plausible time relationship to drug intake
  - Cannot be explained by disease or other drugs
  - Response to withdrawal plausible (pharmacologically, pathologically)
  - Event definitive pharmacologically or phenomenologically (i.e., an objective and specific medical disorder or a recognized pharmacological phenomenon)
  - Rechallenge satisfactory, if necessary
- Probable
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Unlikely to be attributed to disease or other drugs
  - Response to withdrawal clinically reasonable
  - Rechallenge not required
- Possible
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Could also be explained by disease or other drugs
  - Information on drug withdrawal may be lacking or unclear
- Unlikely
  - Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
  - Disease or other drugs provide plausible explanations
- Unrelated
  - Event or laboratory test abnormality is plausibly related to the subject's clinical state, underlying disease, or the study procedure/conditions
  - Time relationship to drug intake makes a relationship unreasonable
  - Other obvious causes for event or laboratory test abnormality exist
- Unknown
  - Report suggests an AE; however, cannot be judged at this time because information is insufficient or contradictory
  - More data for proper assessment is needed, or additional data is under examination
- Not applicable (N/A)
  - Report suggests an AE; however, the need to assess causality is not practical or of value, due to the event itself, or to the circumstances surrounding the event

Adverse events classified as "Not applicable" will not be included in tabulations.

10.2.7. Adverse Event Tabulations

All AEs (serious and non-serious) occurring after completion of the informed consent process and before the end of study will be included and classified by system organ class (SOC) and preferred term (PT) using MedDRA® v25.0. Date imputation algorithm from Section 5.7.19 will be employed to partial dates.

A data listing of all AEs, including pre-treatment AEs (as defined in Section 5.7.19), will be provided including verbatim term, SOC, PT, toxicity, relationship to mobilization (G-CSF or plerixafor), relationship to apheresis, relationship to conditioning, relationship to AVR-RD-01, relationship to study procedure, relationship to underlying disease, date/time and day of onset, date/time and day of resolution, duration, possible co-medication, treatment given to treat the adverse event, the outcome, whether the event was an SAE, and whether it led to withdrawal. AEs prior to start of treatment (Mobilization) will be flagged on this listing. In addition, a separate listing of all serious AEs will be provided, with any SAEs prior to treatment (Mobilization) flagged.

Deaths, if any, will be listed for subject, date/time, primary cause and autopsy finding and tabulated with frequency counts by SOC and PT.

Listings will be sorted by subject identification number, onset date/time, SOC, and PT. If the onset date is completely missing, then these events will be presented first. If the onset date is missing a month or a day then partial dates for AEs will be imputed as per **Error! Reference source not found.** to allow AEs to be sorted appropriately. Imputed dates will not be listed.

The AE tabulations, unless stated otherwise, will be based on TEAEs. Therefore, all pre-treatment AEs will be excluded from the tabulations, unless stated otherwise. However, pre-treatment AEs that worsen/increase in severity after the start of treatment will be recorded as new AEs and consequently will be regarded as treatment-emergent and thus will be included in the tabulations.

Descriptive statistical methods will be used to tabulate the TEAE data including both the number of subjects and number of events. TEAE tables including the number of events will be presented in the following order: number of subjects, (percentage of subjects) and [number of events].

When it is necessary to calculate percentages, the denominator will be the total number of subjects in the analysis population, unless otherwise stated, and the numerator will be the total number of subjects reporting a TEAE within the relevant category.

For tabulations by SOC and PT, when tabulating the number of subjects, if a subject has repeated episodes of a particular TEAE, all episodes will be counted in the tabulation, however, a subject with multiple events will only be counted once within each SOC and PT. A subject with more than one type of AE in a particular SOC will be counted only once in the total of subjects experiencing AEs in that particular SOC. Since a subject could have more than one type of AE within a particular SOC, the sum of subjects experiencing different AEs within the SOC could appear larger than the total number of subjects experiencing AEs in that SOC. Similarly, a subject who has experienced an AE in more than one SOC will be counted only once in the total

number of subjects experiencing AEs in all SOCs. Furthermore, SOC and PT will be presented in descending order of frequency (i.e., order by the overall grouping in descending frequency for each SOC and also for each PT within each SOC; in each case where there are equal frequencies items would be ordered alphabetically).

For tabulations by severity, the maximum severity for each subject will be used. A missing severity will not be imputed, but a row labelled 'Missing' will be included. If a subject has multiple events with the same SOC or PT, they will be counted under the worst severity for the tabulation, but the events will be counted under the applicable severity category.

If a subject has repeated episodes of a particular TEAE, only the most severe episode, or the episode with the strongest causal relationship, will be counted in the tabulations.

Plots of cumulative AE counts over time will be produced, with study day on the x-axis presented relative to date of AVR-RD-01 infusion (Study Day 1) for the Infused Population and relative to date of (first) signing of ICF for the Safety Population. One plot will include all subjects with a vertical reference line at Study Day of ICF or AVR-RD-01 infusion. These cumulative AE plots will be repeated for AEs ≥ Grade 3, and for AEs that are possibly, probably or definitely related to busulfan/melphalan. Where a single AE is captured multiple times due to changing severity, each change in Grade will contribute to the cumulative number of AEs for that subject. If it is possible to identify such AEs, the plots will be repeated with the multiple events collapsed into a single AE occurrence (with the causality and maximum grade considered appropriately).

- **Overall Summary of TEAEs**

The summary will include number (%) subjects with:

- At least one TEAE
- TEAEs related* to G-CSF for Mobilization
- TEAEs related to Plerixafor
- TEAEs related to Apheresis
- TEAEs related to Melphalan
- TEAEs related to Anti-Convulsant
    - Levetiracetam or others (benzodiazepines or valproic acid) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
- TEAEs related to Busulfan
- TEAEs related to Preparatory Medication(s) or associated procedure
    - Combine G-CSF for Mobilization, Plerixafor, Apheresis, Melphalan, Anti-Convulsant and Busulfan
- TEAEs related to AVR-RD-01 infusion
- TEAEs related to G-CSF for Hematological Reconstitution
- TEAEs related to Study Procedures
- TEAEs related to Underlying Disease
- TEAEs leading to Withdrawal
- TEAEs leading to Discontinuation of the Study

- TEAEs leading to Death
- Each CTCAE Grade
- Any severe (CTCAE Grade ≥ 3) TEAE
- Maximum CTCAE Grade
- At least one Treatment-Emergent SAE (TESAE)

  * 'related' includes those with a deemed possible, probable or definite causal relationship

- **TEAEs by SOC, PT**

TEAEs will be tabulated for both SOC and PT.

- **TEAEs by SOC, PT and CTCAE Grade**

A tabulation of TEAEs by SOC, PT will be presented for each CTCAE Grade 1 to 5, Grade ≥2 and ≥Grade 3. The column for ≥Grade 3 will be used to determine decreasing order of frequencies.

- **Treatment-Emergent Serious Adverse Events**

TESAEs will be tabulated for both SOC and PT.

- **TEAEs by Causal Relationship**

The tabulation of AEs by SOC, PT by Onset will be repeated selecting TEAEs with possible, probable or definite causal relationship to each preparatory medication (G-CSF for Mobilization, plerixafor, melphalan, anti-convulsant or busulfan), apheresis, AVR-RD-01, or study procedure, i.e., the AEs by SOC, PT by Onset will be repeated once for each preparatory medication (G-CSF for Mobilization, plerixafor, melphalan, anti-convulsant or busulfan), apheresis, AVR-RD-01, or study procedure.

TEAEs by Causal Relationship will be produced for the Safety Population and for the Infused Population.

- **AEs Related to Underlying Disease**

A tabulation of number and percentage of subjects experiencing an AE with a deemed causal relationship (possible, probable or definite) to underlying disease will be presented by SOC and PT.

- **TEAEs Related to Fertility**

Fertility-related events are considered an AE of special interest (AESI).

A tabulation of number and percentage of subjects experiencing a TEAE with any SOC or PT potentially associated with fertility will be presented by SOC and PT.

- **AEs Leading to Study Discontinuation**

Incidence of AEs leading to discontinuation of the study, as well as the duration, severity, relationship to treatment, outcome and actions taken will be listed for each subject.

- **AEs by Onset**

The tabulation of AEs by SOC, PT will be repeated using the onset date/time of each adverse event to assign each AE into a study period according to Section 5.7.19. AEs by Onset will be produced for the Safety Population and for the Infused Population. Note since Pre-Mobilization is included that these tabulations include all AEs as well as TEAEs:

- Pre-Mobilization: started before first dosing of G-CSF for Mobilization
- Preparatory Medications:
    - Mobilization:
        - G-CSF: started (or worsened) on or after first dosing of G-CSF for Mobilization until start of apheresis
            - For subject 101-106, the study period between the start of the second Mobilization until the start of the second apheresis will be captured as Mobilization #2
    - Apheresis:
        - started (or worsened) on or after start of the first apheresis until the end of the first apheresis
            - For subject 101-106, the study period between the start of the second apheresis until the end of the second apheresis will be captured as Apheresis #2
    - Post-Apheresis: started (or worsened) after the end of the apheresis and before first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan)
        - For subject 101-106, the study period between the end of the first apheresis and the start of Mobilization #2 will be captured as Post-Apheresis
            - The study period between the end of the second apheresis and the first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan will be captured as Post-Apheresis #2
        - For subjects 503-102 and 504-101, who had Mobilization but did not proceed to receive conditioning or AVR-RD-01, the study period after the end of Apheresis will be captured as Post-Apheresis

    - Conditioning: started (or worsened) on or after first dosing of melphalan (for subjects conditioned with melphalan) or first dosing of anti-convulsant medication (levetiracetam or others [benzodiazepines or valproic acid]) given for prophylaxis ahead of busulfan (for subjects conditioned with busulfan) and before date of AVR-RD-01 infusion
- AVR-RD-01 Infusion: started (or worsened) on or after the start of infusion of AVR-RD-01
- Post-AVR-RD-01 Infusion Follow-Up: started (or worsened) after the end of AVR-RD-01 infusion

- **AEs by Presence/Absence of anti-AGA antibodies**

If applicable, AEs will additionally be classified and tabulated by SOC, PT based on presence of anti-AGA antibodies, i.e., Positive result at any time post-treatment vs. Negative result at all post-treatment assessments.

10.3. Pregnancies

Not applicable for this study.

10.4. Clinical Laboratory Evaluations

The details of measurement of laboratory parameters are described in the study protocol. International System (SI) units will be used in all outputs.

All hematology, clinical chemistry and urinalysis data including CFB will be listed. In these listings, sample collection data (e.g., collection times) for laboratory analysis and urinalysis data will be included, and observed data will be flagged with an “H” or an “L” for values that are higher or lower than their normal ranges, respectively.

For the listings of ALT and of AST, the following values will be flagged to indicate possible evidence of Drug-Induced Liver Injury:

- ALT or AST > 8×ULN
- ALT or AST > 5×ULN
    - In the CSR, an assessment will be made of whether ALT or AST remained above 5×ULN for more than 2 weeks
- ALT or AST > 3×ULN
    - In the CSR, an assessment will be made of whether ALT or AST remained above 3×ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

For each laboratory parameter with a defined normal range, absolute values will be defined at each protocol scheduled time point as low, normal, or high, and post-baseline values will be compared to the baseline value through shift tables.

In addition, one flag will be derived to identify whether the parameter had at least one value above the normal range at any time post-treatment, and another to identify whether the parameter

had at least one value below the normal range at any time post-treatment. Subjects having both High and Low values relative to normal ranges at post-treatment visits for laboratory parameters will be counted in both the High and Low categories of the "Any visit post-treatment" row of related summary tables.

The denominator used in laboratory shift table summaries will only include evaluable subjects (i.e., those who have both a baseline and at least one post-baseline value recorded).

For continuous parameters, absolute values and CFB will be summarized at each protocol scheduled time point. For any parameter where multiple laboratory normal ranges, which may differ by subject and/or visit, have been applied, then data from each laboratory will be summarized separately.

For categorical parameters, frequency tabulations of the number of normal and abnormal (low and high) records will also be summarized at each protocol scheduled time point, as well as the number considered clinically significant (CS) and not clinically significant (NCS), if captured.

Box plots will be presented separately for the ratio of value to LLN (Hgb, HCT, Platelets and Leukocytes) and for the ratio of value to ULN (ALT, ALP, AST, Bilirubin and GGT) vs. scheduled visit. A horizontal reference line will be added at 1 to indicate where the value equals the LLN or ULN.

Individual subject data for the ratio of value to LLN for Hgb, HCT, Platelets, and Leukocytes will be plotted vs. study day, with a line for each parameter on the same plot and one plot for each subject. Vertical reference lines will be included to represent the study periods of Mobilization, Apheresis, Post-Apheresis and Conditioning. A horizontal reference line will be included at 1.

Individual subject data for the ratio of value to ULN for ALT, ALP, AST, Bilirubin and GGT will be plotted vs. study day, with a line for each parameter on the same plot and one plot for each subject. Vertical reference lines will be included to represent the study periods of Mobilization, Apheresis, Post-Apheresis and Conditioning. A horizontal reference line will be included at 1.

An Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) plot will be presented at each scheduled visit. The ratio of Bilirubin to ULN will be plotted on the log-scale against the ratio of ALT to ULN on the log-scale, with reference lines indicating Hy's law thresholds, i.e., ALT = 3×ULN and Bilirubin = 2×ULN.

This eDISH plot will be repeated using AST instead of ALT, and repeated for ALT and AST using INR = 1.5×ULN instead of Bilirubin = 2×ULN.

Events meeting the Hy's law criteria will be considered SAEs (the assessments of ALT/AST and Bilirubin/INR must all have been made on the same date to assess Hy's law criteria).

Urinalysis results will be summarized at each protocol scheduled time point, using frequency tabulations.

Any available microscopic urinalysis will be listed.

Partial thromboplastin time (PTT) and international normalized ration (INR) will be listed.

Blood group information, and T and B cell counts will be listed.

10.5. Vital Signs Evaluations

Observed vital signs results (systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate and body temperature) will be presented in data listings.

Observed values and absolute change from baseline for vital signs parameter values will be summarized at each protocol scheduled time point. Change from baseline will only be derived when the position (e.g., sitting or supine) is the same at baseline and post-baseline.

10.6. ECG Evaluations

Observed ECG parameter results (heart rate, PR interval, RR interval, QRS interval, QT interval, QT interval corrected using Bazett's formula (QTcB) and Fridericia's formula (QTcF)) and the clinical assessment of the ECG will be presented in data listings.

Observed values and absolute change from baseline for ECG parameter values will be summarized at each protocol scheduled time point. Change from baseline will only be derived when the position (e.g., sitting or supine) is the same at baseline and post-baseline.

Clinical assessment of the ECG (Normal, Abnormal NCS, Abnormal CS) will be summarized at each protocol scheduled time point, using frequency tabulations.

10.7. Pulmonary Function Test

Observed pulmonary function test results by spirometry (FVC, FEV1, FEV1/FVC ratio, FEV1 % of predicted, FVC % of predicted and FEV1/FVC ratio % of predicted) will be presented in data listings.

Observed values and absolute change from baseline for spirometry parameter values will be summarized at each protocol scheduled time point.

10.8. Chest X-Ray

All chest x-ray data will be presented in data listings.

Interpretation of the chest x-ray (Normal, Abnormal) will be summarized at each protocol scheduled time point, using frequency tabulations.

10.9. Immunogenicity

Observed immunogenicity data will be presented in data listings, including the presence or absence of anti-AGA antibodies (and Titer results).

In case of presence/increase of anti-AGA antibodies, any investigation of the presence of neutralizing anti-AGA antibodies, as well as the clinical relevance of their presence with regards to (i) reduction of enzyme activity in blood, plasma and cells; (ii) reduction in transduced progenitor cells and VCN; (iii) autoimmune-mediated cytopenias will be discussed in the CSR.

If neutralizing anti-AGA antibodies are detected, safety and/or efficacy outputs may be presented separately for subjects with a Positive result at any time post-treatment vs. subjects with a Negative result at all post-treatment assessments.

10.10. Physical Examination

Physical examination data will be listed.

10.11. Rescue Medication

The use of rescue medication will be listed.

10.12. Reproductive Potential

Measures of reproductive potential include sperm count, sperm motility, and sperm morphology. Observed values and absolute change from baseline will be summarized at each protocol scheduled time point.

Figures displaying observed values and CFB over time, along with arithmetic means, will also be provided.

10.13. Achievement of Hematological Reconstitution

The occurrence of hematological reconstitution is defined as the first day of absolute neutrophil count (ANC) ≥ 0.5 (10^9/L) and of platelets ≥ 20 (10^9/L) (in the absence of platelet transfusions), i.e. time from date of AVR-RD-01 infusion to the first date of the three consecutive values.

All ANC and platelet values will be listed. Flags will indicate where ANC < 0.5 (10^9/L) or platelets < 20 (10^9/L).

For the Infused Population, panel plots of individual subjects will be presented for ANC (10^9/L) between study day -50 and study day 100, with study day on the x-axis. Vertical reference lines will be included at study day 1 (solid, black), 35 (dashed, red), 42 (solid, red) and 49 (dashed, red) and to represent the study periods of Mobilization, Apheresis, Post-Apheresis and Conditioning. A horizontal reference line will be included at 0.5 (10^9/L).

For the Infused Population, panel plots of individual subjects will be presented for platelet counts (10^9/L) between study day -50 and study day 100, with study day on the x-axis. Vertical reference lines will be included at study day 1 (solid, black), 35 (dashed, red), 42 (solid, red) and 49 (dashed, red) and to represent the study periods of Mobilization, Apheresis, Post-Apheresis and Conditioning. A horizontal reference line will be included at 20 (10^9/L).

10.14. Presence of Replication Competent Lentivirus (RCL)

Results of the serum and whole blood tests will be listed, with absence of RCL identified by a negative result. In case of confirmed positivity, any resulting RCL culture test results will be listed.

10.15. Identification of mononuclear cells with integration site profiles suggestive of aberrant clonal expansion(s)

Monitoring for aberrant clonal expansion (ACE) requires the clinical review of multiple datasets (e.g., routine clinical and laboratory surveillance, repertoire study, bone marrow examination, integration site analysis). Therefore, no summary tables focused solely on ACE will be produced.

The results of the manual clinical review for ACE will be described in the summary documents with cross-reference to the relevant study listings and a separate integration site analysis report.

10.16. Other Safety Measures

All other safety data will be listed.

11. REFERENCES

Bröchner-Mortensen, J. (1972). A Simple Method for the Determination of Glomerular Filtration Rate. *Scandinavian Journal of Clinical and Laboratory Investigation*, *30*(3), 271–274. https://doi.org/10.3109/00365517209084290

Brøchner-Mortensen, J., & Jødal, L. (2009). Reassessment of a classical single injection 51Cr-EDTA clearance method for determination of renal function in children and adults. Part II: Empirically determined relationships between total and one-pool clearance. *Scandinavian Journal of Clinical and Laboratory Investigation*, *69*(3), 314–322. https://doi.org/10.1080/00365510802653680

Du Bois D, Du Bois EF (Jun 1916). A formula to estimate the approximate surface area if height and weight be known. Archives of Internal Medicine 17 (6): 863-71. PMID 2520314, cited at https://www.calculator.net/body-surface-area-calculator.html, retrieved June 18, 2022.

APPENDIX 1

Reference ranges - [redacted] in Clinical Mass Spectrometry

Abbreviations:

ULN: Upper limit of normal. It is defined as the 95th percentile of the healthy control analysis and as the reference value between normal and elevated levels of analytes.

LLN: Lower limit of normal. It is not applicable, because the lower limit of normal is "Not detected".

LLOD: Lower limit of detection of the analyte in the UPLC-MS/MS methodology developed

LLOQ: Lower limit of quantification of the analyte in the UPLC-MS/MS methodology developed

N/A: Not applicable

ND: Not detected

Important note: In the Final Excel results sent, all values between the LLOD and LLOQ are marked in red.

Globotriaosylceramide (Gb_3) and isoforms - Biomarkers

Gb_3 plasma	Gb_3(C16:0)	Gb_3(C16:1)	Gb_3(C18:0)	Gb_3(C22:0)	Gb_3(C24:0)	Gb_3(C24:1)	Total Gb_3
Upper limit of normal – ULN (nM)	3232	332	283	323	483	662	4961
Lower limit of normal – LLN (nM)	ND	ND	ND	ND	ND	ND	ND
Limits of detection – LLOD (nM)	21	13	23	17	19	26	N/A
Limits of quantification – LLOQ (nM)	71	44	78	58	64	87	N/A

Gb_3 urine - Methylated	Gb_3 (C16:0)Me	Gb_3 (C18:0)Me	Gb_3 (C20:0)Me	Gb_3 (C22:1)Me	Gb_3 (C22:0)Me	Gb_3 (C24:1)Me	Gb_3 (C24:0)Me	Total Methylated Gb_3
Upper limit of normal – ULN (nmol/mmol creatinine)	0,2	0,2	0,2	0,1	0,3	0,0	0,1	0,7
Lower limit of normal – LLN (nmol/mmol creatinine)	ND	ND	ND	ND	ND	ND	ND	ND
Limits of detection – LLOD (pmol) *	0,4	0,5	0,7	0,5	1,1	0,6	0,5	N/A
Limits of quantification – LLOQ (pmol) *	1,3	1,5	2,5	1,5	3,7	2,2	1,8	N/A

*** Please note that the limits of detection and limits of quantification are in pmol, thus were not normalized to creatinine. But reference values are normalized to creatinine.**

Gb_3 urine - Non-Methylated	Gb_3 (C16:0)	Gb_3 (C18:0)	Gb_3 (C20:0)	Gb_3 (C22:1)	Gb_3 (C22:0)	Gb_3 (C24:1)	Gb_3 (C24:0)	Gb_3 (C24:OH)	Total Non-Methylated Gb_3
Upper limit of normal – ULN (nmol/mmol creatinine)	1,7	0,4	0,7	0,3	1,4	1,0	1,5	1,3	6,5
Lower limit of normal – LLN (nmol/mmol creatinine)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Limits of detection - LLOD (pmol) *	3,1	0,6	1,0	0,6	1,6	2,2	3,8	1,5	N/A
Limits of quantification – LLOQ (pmol) *	10,3	2,2	3,3	2,0	5,3	7,5	12,7	5,0	N/A

*** Please note that the limits of detection and limits of quantification are in pmol, thus were not normalized to creatinine. But reference values are normalized to creatinine.**

Globotriaosylsphingosine (Lyso-Gb_3) and analogs - Biomarkers

Lyso-Gb_3 plasma	Lyso-Gb_3	Lyso-Gb_3 (-28)	Lyso-Gb_3 (-2)	Lyso-Gb_3 (+16)	Lyso-Gb_3 (+18)	Lyso-Gb_3 (+34)	Lyso-Gb_3 (+50)
Upper limit of normal – ULN (nM)	2,4	nd	0,9	nd	nd	0,3	nd
Lower limit of normal – LLN (nM)	ND	ND	ND	ND	ND	ND	ND
Limits of detection – LLOD (nM)	0,2	0,1	0,3	0,2	0,1	0,2	0,1
Limits of quantification – LLOQ (nM)	0,8	0,2	1,0	0,7	0,5	0,8	0,3

Lyso-Gb_3 urine	Lyso-Gb_3	Lyso-Gb_3 (-28)	Lyso-Gb_3 (-12)	Lyso-Gb_3 (-2)	Lyso-Gb_3 (+14)	Lyso-Gb_3 (+16)	Lyso-Gb_3 (+34)	Lyso-Gb_3 (+50)
Upper limit of normal – ULN (pmol/mmol creatinine)	nd	nd	nd	nd	nd	25	20	85
Lower limit of normal – LLN (pmol/mmol creatinine)	ND	ND	ND	ND	ND	ND	ND	ND
Limits of detection – LLOD (pM) *	18	14	8	15	17	18	22	14
Limits of quantification – LLOQ (pM) *	60	48	27	49	56	60	72	46

*** Please note that the limits of detection and limits of quantification are in pM, thus were not normalized to creatinine. But reference values are normalized to creatinine.**